Brain neurochemicals in migraine and pain

A thesis presented by

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CANDIDATE'S CERTIFICATE

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A/Prof. Trudy Rebbeck
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ABSTRACT

Migraine is the leading cause of neurological disability worldwide. It is also the leading cause of years lived with disability in those under 50 years of age. Despite the high prevalence of migraine, the underlying mechanisms are still not fully understood. Therefore, first-line treatments are generally not migraine-specific and, as a result, have limited effectiveness and a high incidence of side-effects. Gaining a better understanding of mechanisms responsible for migraine has therefore become an international research priority to improve outcomes of those living with migraine by aiding the development of more effective and tolerable migraine-specific treatments.

Brain neurochemicals and their relative concentrations are one potential mechanism responsible for migraine. Specifically, GABA, the main inhibitory neurometabolite of the central nervous system, is thought to mediate the excitatory state of the brain produced by glutamate. Elevated GABA levels have been reported in people with migraine compared to controls, but not in people with other pain conditions, suggesting that elevated GABA levels may reflect a unique biomarker or mechanism of migraine. Advances in magnetic resonance imaging (MRS), such as MEGA-PRESS, have enabled direct measurement of GABA in the human brain, although there is no agreed best practice for data acquisition. Therefore, to determine the potential of GABA levels to represent a biomarker or mechanism of migraine or pain, best-practice methods need to be agreed to ensure high-quality GABA measurement.

The two main aims of the thesis were, therefore:

1) To advance the understanding of the role of GABA in migraine (and pain conditions)

2) To improve the standardisation of methods for quality assessment, accurate measurement and the reporting of GABA in studies using MEGA-PRESS.

To address the first main aim, this thesis used research designs consistent with the first three stages of a biomarker validation framework: i) a systematic review and meta-analysis to gain proof of concept that elevated GABA levels might be a unique biomarker or mechanism of migraine (Chapter 2); ii) a head-to-head comparison to directly compare GABA+ levels in people with migraine with other headache and pain conditions to determine if GABA+ changes are specific to migraine or observed in other headache and pain conditions (Chapter 3); and iii) a longitudinal study to determine if GABA levels change in response to change in clinical condition (Chapter 4).

Elevated GABA levels appeared unique to migraine when results were pooled for meta-analysis (Chapter 2). GABA levels were elevated in participants with migraine compared to controls (Hedge's G 0.5, 95% CI: 0.2 to 0.8), whereas GABA levels in musculoskeletal pain conditions (Hedge's G 0.19, 95% CI: 0.53 to 0.15) and chronic pain syndromes (Hedge's G 0.08, 95% CI: 1.61 to 1.46) did not differ from controls. In addition, glutamate levels were significantly higher in people with migraine, and Glutamate and Glutamine (Glx) were elevated in those with chronic pain syndromes. Although these results suggest that brain neurochemical levels may be specific to different pain conditions, it was unclear whether these differences in neurochemical levels were true biological findings or a result of the heterogeneity in the quality of methods used across the studies.

Therefore, to directly compare GABA+ levels across pain conditions, a head-to-head comparison using a GABA+ optimised study design was conducted (Chapter 3). A cross-sectional study was used to measure GABA+ levels of people with migraine (n = 20), whiplash-headache (n = 17) and low back pain (n = 19) compared to a pool of age-sex-

matched controls (n = 22). GABA+ levels in the posterior cingulate gyrus (PCG) were elevated across pain conditions compared with controls (e.g. back pain 4.88 IU ± 0.44 vs controls 4.6 IU ± 0.32; P = .01). This study concluded that elevated GABA+ levels might not reflect a unique biomarker or mechanism of migraine as suggested in Chapter 2 but more likely associated with chronic pain in general.

To determine whether GABA+ levels changed as clinical characteristics or pain status of a condition changed, one group (migraine n = 19) were followed over time (Chapter 4). An increase in ACC GABA+ was found to be associated with a decrease in clinical characteristics of migraine, such as migraine frequency ($\rho = -0.51$, $p = 0.03$). These findings led to the speculation that ACC GABA+ might not be an underlying biomarker or mechanism of migraine. Rather it could be a protective mechanism attempting to suppress further migraine attacks.

To address the second main aim of the thesis, that is, to improve the standardisation of methods for quality assessment, accurate measurement and the reporting of GABA in studies using MEGA-PRESS, it was necessary to develop a quality assessment tool. To establish the quality of methodology currently being used in the field (Chapter 2), the first quality assessment tool was developed and implemented for use in MRS studies (MRS-Q). The MRS-Q critically appraises three areas considered by experts as vital for producing high-quality studies of GABA, including i) sequence/acquisition parameters; ii) the reporting of quality metrics; and iii) reporting of study design and analysis. A wide range of methodology was being used in the field (Chapter 2), resulting in highly variable quality of acquisition and reporting within the field. This finding further supported the need for standardisation in GABA measurement and led to the development of the Comprehensive guide to MEGA-PRESS (Chapter 5).

Advances in MRS, such as MEGA-PRESS, have led to significant improvements in accuracy for measuring GABA. However, the rapidly developing nature of the field, lack of standardisation and poor reporting has led to substantial heterogeneity in the quality of methods used. Therefore, a translational framework was used to develop evidence-based clinically-orientated guidelines for the measurement of GABA using MEGA-PRESS (Chapter 5). This work was the first of its kind in the field of MRS to use a translational framework to develop robust guidelines for the use of MEGA-PRESS to measure GABA. The approach involved first establishing a working party that included experts in the field of MRS and experts in guideline development and implementation in addition to end-users. A systematically conducted scoping review was used to synthesise all that is known in the field. The quality of evidence was assessed, and recommendations were developed based on the evidence. Finally, the recommendations were externally validated by an international panel of 21 renowned MRS experts using a modified Delphi process. The final product was a guideline consisting of 23 recommendations that serve to inform those new to the field of MEGA-PRESS on how to accurately measure GABA in clinical and research populations.

Together, the findings reported in this thesis advance what is known about the role of GABA in migraine and other pain conditions. The findings suggest that elevated GABA levels are not unique to migraine but more likely reflect chronic pain in general. Further, work from this thesis shapes future research into GABA through providing methods to assess quality, guide reporting and provide guidelines which present agreement on what constitutes best practice for the accurate measurement of GABA using MEGA-PRESS.

TABLE OF CONTENTS

RESEARCH DISEMINATION

Parts of the work presented in this thesis have been published and/ or presented as follows:

Published peer-reviewed papers forming part of this thesis

Chapter Two:

- **Peek AL**; Rebbeck T; Puts N; Watson J; Aguila M and Leaver AM. GABA and glutamate levels across pain conditions: A systematic literature review and meta-analysis of 1HMRS studies. *NeuroImage,* 2020, 210, 116153

Chapter Three:

- **Peek AL**; Leaver AM; Foster S; Oeltzschner G; Puts NA; Galloway G; Sterling M; Ng K; Refshauge K; Aguila ME and Rebbeck T. Increased GABA+ in people with migraine, headache and pain conditions- A potential marker of pain. *Journal of Pain,* 2021 22 (12): 1631-1645

Chapter Four:

- **Peek AL**; Leaver AM; Foster S; Puts NA; Oeltzschner G; Henderson L; Galloway G; Ng K; Refshauge K and Rebbeck T. Increase in ACC GABA+ levels correlate with decrease in migraine frequency, intensity and disability over time *Journal of Headache and Pain,* 2021, 22 (150): 1-13

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Chapter Five:

- **Peek AL;** Rebbeck T; Leaver AM; Puts NA; Foster S; Refshauge K and Oeltzschner G. MRS Expert Panel. A Comprehensive Guide to MEGA-PRESS for GABA Measurement. *(Submitted NeuroImage 12/11/21)*

Additional publications by author relevant to this thesis

- Lin A….**Peek A**…et al. Minimum Reporting Standards for in vivo Magnetic Resonance Spectroscopy (MRSinMRS): Experts' consensus recommendations. *NMR Biomedicine* 2021; doi 10.1002/nbm.4484
- Demayo M, Morley K, Logge W, Hunt G, **Peek A**. Glutamate alterations in alcohol use disorder: A systematic review and meta-analysis of magnetic resonance spectroscopy studies. PROSPERO 2020; CRD42020144746 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020144746

Invited Presentations

- Peek AL. Syd MSK Mentees of 2019: share and celebrate achievements. Sydney Musculoskeletal Health Alliance Mentoring Closing Workshop, Sydney, 18th September 2020
- **Peek AL** and Puts NA. Standard Practice: Study Reporting. 5th International Symposium of GABA and Advanced MRS, Park City, Utah. 20th November 2019

Conference presentations- Oral

- **Peek AL;** Leaver AM; Foster S; Puts NA; Oeltzschner G; Henderson L; Galloway G; Ng K; Refshauge K and Rebbeck T. GABA+ levels correlate with change in migraine frequency; a longitudinal cohort study. Thrive Australian Physiotherapy Association, Brisbane, 23rd October 2021 (POSTPONED until March 2022)

- **Peek AL**; Leaver AM; Foster S; Oeltzschner G; Puts NA; Galloway G; Sterling M; Ng K; Refshauge K; Aguila ME R; and Rebbeck T. GABA+ a potential marker of pain- A step closer towards a better understanding of pain neurochemistry. Sydney Musculoskeletal Health Alliance Meeting, 31 July 2020

- **Peek AL**; Rebbeck T; Puts NA; Watson J; Aguila ME and Leaver AM. Brain GABA and glutamate concentration across pain conditions A systematic literature review and meta-analysis of 1H-MRS studies. 5th International GABA and Advanced MRS Symposium, Park City Utah. 19th November 2019

- **Peek AL**; Rebbeck T and Beales D. How to assess for pain sensitization: an update for clinicians. Australian Physiotherapy Association National Conference, Adelaide, 17-19 October 2019

- **Peek AL**; Leaver AM and Rebbeck T. Brain neurochemicals in headaches and pain- A New Mechanism. Whiplash Symposium. University of Queensland, 3rd-4th October 2019

- **Peek AL;** Rebbeck T; Puts N; Watson J; Aguila M and Leaver A. Brain GABA and glutamate concentration across pain conditions A systematic literature review and meta-analysis of 1H-MRS studies. Sydney Musculoskeletal Health Alliance Meeting, 2nd August 2019

meta-analysis of 1H-MRS studies. Sydney Musculoskeletal Health Alliance Meeting, 2nd August 2019

- **Peek AL;** Rebbeck T; Puts N; Watson J; Aguila M and Leaver A. GABA and glutamate levels across pain conditions: A systematic literature review and meta-analysis of 1HMRS studies. Australian Pain Society 39th Annual Scientific Meeting. Gold Coast, 7-10th April 2019

- **Peek AL**; Leaver A; Galloway G; Rebbeck T. Development and feasibility of a cross sectional protocol to investigate brain neurochemicals in people with Migraine and Pain. Sydney Musculoskeletal Health Alliance Annual Scientific Meeting. Sydney, 9th November 2018.

- **Peek AL**; Leaver A; Galloway G; Rebbeck T. An investigation of brain neurochemicals in migraine, whiplash associated disorder (WAD) with persistent headache and low back pain. CRE Symposium. Brisbane 7th November 2017.

Conference presentations- Poster

- **Peek AL**; Leaver A and Rebbeck T. GABA+ a potential marker of pain- A step closer towards a better understanding of pain neurochemistry. [Poster presentation online with oral commentary], Australian Pain Society, 21st April 2021
- **Peek AL**; Leaver A; Galloway G and Rebbeck T. Brain Neurochemicals in Pain Conditions: A Cross-Sectional Study, JWCRR Forum, 30th July 2019

Invited media engagement

- Migraine Australia-Invited interview – Research Update, Available from https://www.youtube.com/watch?v=UNHwfGJ_JTQ, 30th June 2021

- Migraine Australia- Panel Discussion- Research in migraine, Available from https:// www.youtube.com/watch?v=f-cb5zmB-og, 30th June 2020
- Migraine Australia-Invited interview- Targeted treatment in migraine, Available from https://www.youtube.com/watch?v=C4sdxw0UpBI, 2nd June 2020

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- Judge's choice best lightening presentation- Sydney Musculoskeletal Health Alliance Meeting, 31 July 2020
- Gold Standard Phantoms- Best Student Presentation Award- 5th International Symposium of GABA and Advanced MRS, Park City, Utah- 20th November 2019
- Deans Research Scholar Award- "In recognition of an outstanding presentation in the 3MT Competition" Runner Up Faculty Final, 3rd July 2019
- University of Sydney- 3MT- Highly Commended- July 2018

CHAPTER ONE

Introduction

INTRODUCTION

Migraine: incidence and definitions

Migraine is the leading neurological cause of disability worldwide[1], with an estimated global prevalence of 14.7%[2]. In Australia alone, more than 4.9 million Australians are affected by migraine with total economic costs of $35.7 billion annually[1]. In addition to economic costs, consisting of health system costs, loss of productivity and other costs, including welfare payments, migraine also has a substantial personal cost to the individual and their family financially and in terms of quality of life[3]. The impact of migraine on individuals, their families and society is emphasised in that migraine is most prevalent amongst those who are of working age[4]. As a result, migraine is the top cause of years lived with disability in those under 50 years of age[5,6].

The term migraine is an umbrella term that describes a heterogeneous condition enveloping numerous subclassifications[7]. Historically, migraine has been categorised principally into episodic and chronic migraine, which are differentiated through the number of headache days experienced per month[8]. Episodic migraine is defined as having fewer than 15 headache days a month, compared to chronic migraine, which is defined as headaches that persist for more than or equal to 15 days a month, with at least 8 of those days having features of migraine and persisting for at least 3 months[8]. While chronic migraine represents only 7.5% of the total migraine population[9], it is associated with far greater healthcare utilization[10,11], work disability[12,13] and reduction in quality of life[11,14] than episodic migraine. Therefore,

improving the outcome of people with the chronic form of migraine is considered a health priority[6].

Migraine: Current diagnosis and treatment

Migraine lacks a diagnostic biological marker or clinical test and therefore diagnosis relies on classifying headaches based on signs and symptoms. The current internationally recommended[15] system is the International Classification of Headache Disorders-3 (ICHD-3)[8]. The ICHD-3 is a classification system that details clinical features of over 170 migraine and headache types to allow clinicians and researchers to determine the most likely diagnosis based on the presenting signs and symptoms (*Table 1*). The ICHD-3 has been validated in different headache populations and has been shown to predict migraine in 65.7 % of adolescents with migraine, and 62.6% with tension-type headache[16]. However, the complexity of migraine and headache populations with inter-individual variance challenges the specificity of diagnosis.

Differential diagnosis of migraine and headache is further complicated by the considerable overlap in signs and symptoms between common headache types. The most common primary headache types presenting to primary care are tension-type headache (TTH) (78%)[17], migraine (16%[17] to 45%[18]), and cervicogenic headache (4%, but represents around 20% of those with chronic headache)[19,20]. The diagnostic challenge is that 60-70% of people with migraine also have neck pain, a fundamental sign associated with cervicogenic headache[21,22]. In addition, nausea, photo and phonophobia, considered distinguishing features of migraine, have also been reported in 17% of people with cervicogenic headache and 18% of those with TTH[17,23,24]. As these headache types progress to chronicity, differentiation becomes more challenging, with many people presenting with mixed headache types. For example, the reported prevalence of TTH and cervicogenic headache in a migraine population is 94% and

75% respectively[25,26]. These data highlight the limitations of the current ICHD classification system in assisting with differential diagnosis of migraine from other common headache types.

Table 1: The International Classification of Headache Disorders 3rd edition[8].

Migraine without Aura

A At least five attacks fulfilling criteria B-D
B Headache attacks lasting 4-72 hours (untreated or unsuccessfully treated)
C Headache has at least two of the following characteristics
   1. unilateral location
   2. pulsating quality
   3. moderate or severe pain intensity
   4. aggravation by or causing avoidance of routine physical activity (e.g., walking or climbing stairs)
D During headache at least one of the following
   1. nausea and/or vomiting
   2. photophobia and phonophobia
E Not better accounted for by another ICHD-3 diagnosis

Migraine with Aura

A At least two attacks fulfilling criteria B-D
B One or more of the following fully reversible aura symptoms
   1. visual
   2. sensory
   3. speech and/or language
   4. motor
   5. brainstem
   6. retinal
C At least 3 of the following six characteristics:
   1. at least one aura symptom spreads gradually over ≥ 5 minutes
   2. two or more aura symptoms occur in succession
   3. each individual aura symptom lasts 5-60 minutes
   4. at least one aura symptom is unilateral
   5. at least on aura symptom us positive
   6. the aura is accompanied, or followed within 60 minutes, by headache
D Not better accounted for by another ICHD-3 diagnosis

Chronic Migraine

A Headache (migraine-like or tension-type-like) on ≥ 15 days/month for > 3 months, and fulfilling criteria B and C
B Occurring in a patient who has had at least five attacks fulfilling criteria B-D for *Migraine without aura* and/or criteria B and C for *Migraine with aura*
C On ≥ 8 days/month for > 3 months, fulfilling any of the following
   1. criteria C and D for *Migraine without aura*
   2. criteria B and C for *Migraine with aura*
   3. believed by the patient to be migraine at onset and relieved by a triptane or ergot derivative
D Not better accounted for by another ICHD-3 diagnosis

Tension-type headache (TTH)

A At least 10 episodes of headache occurring on <1 day/month on average (<12 days/year) and fulfilling criteria B-D)
B Lasting from 30 minutes to seven days
C At least two of the following four characteristics
   1. bilateral location
   2. pressing or tightening (non-pulsating) quality
   3. mild or moderate intensity
   4. not aggravated by routine physical activity such as walking or climbing stairs
D Both of the following
   1. no nausea or vomiting
   2. no more than one of photophobia or phonophobia
E Not better accounted for by another ICHD-3 diagnosis

Cervicogenic Headache

A Any headache fulfilling criterion C
B Clinical and /or imaging evidence of a disorder or lesion within the cervical spine or soft tissues o the neck known to be able to cause headache
C Evidence of causation demonstrated by at least two of the following
   1. headache has developed in temporal relation to the onset of the cervical disorder or appearance of the lesion
   2. headache has significantly improved or resolved in parallel with improvement in or resolution of the cervical disorder or lesion
   3. cervical range of motion is reduced and headache is made significantly worse by provocative manoeuvres
   4. headache is abolished following diagnostic blockade of a cervical structure or its nerve supply
D 1. Not better accounted for by another ICHD-3 diagnosis

The criteria for the diagnosis of migraine without and with aura, chronic migraine, tension type headache and cervicogenic headache. *(Reprinted from Cephalgia, 38 (1), International Headache Society, International Headache Classification of Headache Disorders 3rd ed, 2018, through open source licence.)*

Limitations in the specificity of differential diagnosis in chronic migraine have been reflected in the approach to treatment, also being non-specific. Historically, guidelines and systematic reviews have recommended that treatment for chronic migraine is selected based on the pattern of migraine, tolerability, and patient factors. For example, clinical practice and funding guidelines from the USA[27], Germany[28], UK[29], Europe[30,31] and Australia[32] universally recommend a stepped approach to management. First-line treatment commences with trialling repurposed medications, including anti-inflammatories, betablockers, anti-depressants and anti-convulsants (*Table 2*)[29,30]. These medications typically target mechanisms that are non-specific to migraine. Guidelines recommend escalation to newer, more migraine-specific medications only after trial and failure of at least 3 different classifications of first-line medications[29,31,32]. As a result, people with migraine typically trial multiple medications until they find one that helps[33].

The non-specific nature of first-line medications creates two main problems in the management of chronic migraine. Firstly, evidence suggests non-specific medications have limited effectiveness for the reduction of migraine frequency. Evidence from systematic reviews of randomised controlled trials have demonstrated minimal effects of serotonin uptake inhibitors (3 studies chronic migraine)[34] and calcium channel blockers (4 studies chronic migraine)[35] compared to other medications. Secondly, the non-targeted action of medication can often lead to unwanted side-effects. An example is the anti-convulsant topiramate, which has demonstrated a significant reduction in migraine frequency compared to placebo in two clinical trials[36,37]. However, evidence from two reviews of adherence demonstrated that 53-70% of people discontinue the medication within 12 months due to intolerable side effects[38,39]. The prevalence of migraine, lack of response, and unwanted side effects have led to a global call to better understand the mechanisms underpinning chronic

migraine[6,40]. A better understanding of mechanism can then be used to inform the development of disease-specific, mechanism-based medications specifically for migraine.

Table 2: Stages of preventative migraine treatment

Drug class	Drug	Dosage and route	Contraindications
First-line medication			
Beta blockers	Atenolol	25–100 mg oral twice daily	Asthma, cardiac failure, Raynaud disease, atrioventricular block, depression
	Bisoprolol	5–10 mg oral once daily	
	Metoprolol	50–100 mg oral twice daily or 200 mg modified-release oral once daily	
	Propranolol	80–160 mg oral once or twice daily in long-acting formulations	
Angiotensin II-receptor blocker	Candesartan	16–32 mg oral per day	Co-administration of aliskiren
Anticonvulsant	Topiramate	50–100 mg oral daily	Nephrolithiasis, pregnancy, lactation, glaucoma
Second-line medication			
Tricyclic antidepressant	Amitriptyline	10–100 mg oral at night	Age <6 years, heart failure, co-administration with monoamine oxidase inhibitors and SSRIs, glaucoma
Calcium antagonist	Flunarizine	5–10 mg oral once daily	Parkinsonism, depression
Anticonvulsant	Sodium valproate[a]	600–1,500 mg oral once daily	Liver disease, thrombocytopenia, female and of childbearing potential
Third-line medication			
Botulinum toxin	OnabotulinumtoxinA	155–195 units to 31–39 sites every 12 weeks	Infection at injection site
Calcitonin gene-related peptide monoclonal antibodies	Erenumab	70 or 140 mg subcutaneous once monthly	Hypersensitivity Not recommended in patients with a history of stroke, subarachnoid haemorrhage, coronary heart disease, inflammatory bowel disease, chronic obstructive pulmonary disease or impaired wound healing

Stages of preventative migraine treatment as endorsed by the European Headache Federation. *(Reprinted from Nature Reviews Neurology, 17 Eigenbrodt A et al. Diagnosis and management of migraine in ten steps, 2021[30], through open source licence available here http://creativecommons.org/licenses/by/4.0/.)*

Understanding mechanisms of migraine to improve outcomes

Over the past decade, advances in the understanding of mechanisms of migraine have led to the development of medications targeting a migraine-specific mechanism. The program of research commenced in the 80s, initiated by Edvesson *et al.* [41] and was progressed by

Goadsby *et al.*[42]. The research first identified increased levels of the amino acid neuropeptide Calcitonin Gene-Related Peptide (CGRP) to be associated with migraine in animal models[41,43,44]. A substantial increase in CGRP levels in the external jugular vein during a migraine attack (usual level <40 pmol/litre to mean ± SD 92 ± 11 pmol/litre) which decreased with pain reduction following administration of a triptan (a medication for acute migraine relief) mean difference ± SE −2.2 ± 0.7 ($p = 0.03$) was then observed in humans[45]. Furthermore, infusion of CGRP has been shown to trigger a migraine in people with episodic migraine[42,46]. This comprehensive investigation of CGRP led to the development and approval of four monoclonal antibodies (CGRP-mAbs) for the treatment of migraine. One targets this mechanism by directly binding to the CGRP receptors (*erenumab*)[32] and the others inhibit the molecule itself (*fremanezumab, eptinezumab, galcanezumab*)[47-49].

Targeting a migraine-specific mechanism appears to have significantly improved response rates in both episodic and chronic migraine. CGRP-mAbs have been progressed through phase I to phase III clinical trials[50-62]. Here we focus on trials and reviews of treatment for *chronic migraine*[50,57,61,63,64]. Results of phase II and III trials of CGRP-mAbs have demonstrated > 50% reduction in migraine frequency in 25%[50], 40%[57,61] and 60%[64] of participants with chronic migraine and 34%[63] of participants with hard to treat migraine, defined as those who have failed 2-4 classes of preventative medication. A systematic review[65] reporting the likelihood of help and harm (LHH) demonstrated that CGRP-mAbs have a favourable LHH ratio, ranging from 1.4 to 5.1, compared to non-specific migraine medication such as onabotulinumtoxinA; LHH ratio 1.1 and topiramate LHH 0.7, where LHH <1 indicates a greater risk of experiencing an unwanted side-effect than help[65]. Together, the favourable treatment profile of CGRP-mAbs and lower NTT (e.g. CGRP-mAbs [5 CI 95% 3 to 16] vs. onabotulinumtoxinA [9 CI 95% 6 to 15]) shows great promise in developing migraine-specific treatments. However, the results from phase III trials in chronic

migraine still demonstrate 40-75% of people with chronic migraine will also fail to respond to these medications[50,57,61,64]. Therefore, ongoing research is required to identify further mechanisms of chronic migraine and discover mechanisms associated with recovery in this population.

Discovering unknown mechanisms that either cause persistent chronic migraine or are associated with recovery, therefore, has become a research priority. This understanding can help improve outcomes for people with chronic migraine in several ways. Firstly, the identification of potential therapeutic targets enables the development of mechanism-specific treatments, an aim frequently proposed in the field of migraine by professional bodies, working groups and experts alike[6,66,67]. As demonstrated through the given example of CGRP-mAbs, mechanism-specific treatments can improve outcomes and reduce unwanted side-effects from off-target actions, which together should improve adherence[39,68]. Secondly, the identification and validation of a diagnostic biomarker would assist the diagnosis of subclassifications of the disorder which may reveal varying biological subgroups. Better diagnosis could lead to the more specific provision of mechanism-based medication[69]. Finally, elucidating the characteristics of responders might provide insight into those likely to respond to mechanism-specific treatments to better guide treatment and the allocation of resources, bringing us a step closer towards individualised treatment[66,69-71].

Understanding of the mechanisms that result in the development of chronic migraine has evolved over time, leading to the theory that migraine is a complex disorder underpinned by multiple mechanisms[67,72]. It was initially proposed that migraine was the result of vascular changes demonstrated in extracranial vasodilation and intercranial vasospasm of the cerebral arteries[73]. However, this theory evolved to become the neurovascular theory due to the inability of clinical evidence to fully explain migraine by vascular changes alone[74-76]. The

neurovascular theory[77] is considered the prevailing theory, with the most recent understanding being initiation through cortical spreading depression[78,79] and trigeminovascular sensitisation[77]. Whilst these theories have led to many proposed mechanisms, it is the interplay between brain activity, inflammatory mediators and brain neurochemistry that have arisen as promising mechanisms.

Brain neurochemicals (GABA) as a potential mechanism of migraine and pain

This thesis will focus on understanding brain neurochemicals as a proposed mechanism of migraine, particularly the potential role of Gamma-amino butyric acid (GABA). To date, research into mechanisms of migraine has largely focused on brain structure [e.g. 80,81], brain activity [e.g. 82-86], neuropeptides [e.g. 87-89], and inflammatory mediators [e.g. 90,91]. However, relatively little attention has been given to brain neurochemistry. Gamma-amino butyric acid (GABA) is a neurochemical of interest because it is the main inhibitory neurochemical of the central nervous system and is widely distributed throughout the neuraxis[92]. Inhibitory interneurons make up 20-30% of all neurons within the brain, with 70% of these acting via GABA[93]. The role of GABA is understood to mediate the excitatory state of the brain produced by glutamate[92,94,95]. A homeostatic balance between GABA and glutamate is therefore required for coordinated, healthy brain function[96,97]. Consequently, an imbalance between GABA and glutamate has been proposed as a mechanism driving pathological conditions of the central nervous system, including neurodevelopmental disorders[98] such as autism, psychological disorders such as depression[99,100], and pain conditions including migraine and chronic pain[101-103].

Research in migraine and pain populations collectively implies that an imbalance between GABA and glutamate may be involved in the manifestation of such conditions, although

several different hypotheses have been proposed. Studies in migraine populations consider alterations in GABA and glutamate levels might underpin the migraine-specific mechanism of cortical spreading depression[103,104]. Cortical spreading depression, which is characterised by a wave of cortical excitation followed by inhibition, has been demonstrated in neuroimaging studies[105,106]. The proposed association between brain neurochemistry and cortical spreading depression is plausible given the role of GABA and glutamate in inhibition and excitation, respectively. Alternatively, it has been proposed that altered GABA levels in people with migraine might be responsible for hyperexcitability in the trigeminovascular system[107,108].

In contrast to migraine, it is hypothesised that chronic pain conditions such as fibromyalgia and neuropathic pain are caused by an imbalance between GABA and glutamate, leading to a 'loss of inhibition' and resultant heightened neuronal activity[97,102,109]. The theory of 'loss of inhibition' is supported through multiple lines of enquiry demonstrating the involvement of the GABAergic system in pain conditions. Examples include studies demonstrating a reduction in GABA level in people with fibromyalgia, which was also associated with a reduced pressure pain threshold (Spearman's $\rho = 0.63$; $p = 0.02$)[97]. Further, activation of GABA-A or GABA-B receptors have demonstrated an anti-nociceptive effect[110,111]. In addition, suppression of inhibitory GABAergic inputs onto output neurons have been proposed to explain the analgesic response of cannabinoids and opioids in forming the descending analgesic pathway[94]. Together, this body of evidence implies the involvement of the GABAergic system in migraine and pain, although the exact mechanisms remain unclear.

Until recently, direct and accurate measurement of GABA levels in the brain has not been possible. Early studies of GABA in humans used indirect measures of brain GABA levels by measuring them in saliva, blood and cerebral spinal fluid or post-mortem studies[112-116].

However, it has not been established how circulating GABA levels or *in-vitro* GABA levels correlate with *in-vivo* GABA levels in specific regions of the living human brain. Advanced brain imaging techniques, such as magnetic resonance spectroscopy (MRS), have provided the technology to directly and non-invasively measure GABA levels in the living human brain[117]. MRS has seen rapid advancement, and as a result, methods improving the accuracy of GABA detection have been established and reflect current best practice[118]. Hence from here on in this thesis, the measurement of GABA will refer to the measurement of brain GABA levels in human subjects using MRS. The term 'GABA' will be used when referring to concepts involving the neurotransmitter Gamma-aminobutyric acid or in discussing MRS measurements of GABA using a macromolecule suppressed sequence. The term 'GABA+' (GABA+ macromolecules) will be used to acknowledge the macromolecule component of the signal in conventional MEGA-PRESS data. MRS will be discussed in more detail in the section *The measurement of GABA using Magnetic Resonance Spectroscopy.*

Brain neurochemicals (GABA) as a potential biomarker for migraine and pain

In addition to potentially providing knowledge of mechanisms, GABA could also be a relevant biomarker for migraine or pain. Biomarkers can be defined as any objective measure that reflects a normal biological process, pathological process or response to intervention [119]. Models of biomarker validation from other fields use a stepwise approach to evaluate the potential of candidate biomarkers[120]. To elucidate the potential of GABA as a biomarker for migraine or pain, research designs consistent with the first three stages of a biomarker validation framework are used[120]. The stages addressed in this thesis are: i) a systematic review and meta-analysis to gain proof of concept that elevated GABA levels might be a unique biomarker of migraine (Chapter 2); ii) a head-to-head comparison to directly compare

GABA+ levels in people with migraine with other headache and pain conditions to determine if GABA+ changes are specific to migraine or observed in other headache and pain conditions (Chapter 3); and iii) a longitudinal study to determine if GABA levels change in response to change in clinical condition (Chapter 4).

The first step aimed to establish whether altered GABA levels (measured with MRS) are present in people with migraine (or pain) compared with controls. At the time of this thesis, one study using a GABA-optimised MRS sequence observed elevated GABA+ levels in people with migraine compared to controls[103]. The elevated GABA+ levels had yet to be observed in other studies of migraine or in other pain conditions. Previous studies of migraine had not been optimised to detect GABA and had reported either a decrease in GABA level[121] or no difference[122]. In contrast to migraine, GABA-optimised studies reported a decrease in GABA+ levels in fibromyalgia[97] and pelvic pain[123], whilst studies not optimised to detect GABA reported either a decrease in GABA level[101,102] or no difference[124] compared to controls. Together, this suggests elevated GABA/GABA+ levels could be unique to migraine, forming one hypothesis of this thesis.

Robust synthesis of studies reporting GABA levels across pain conditions has not been undertaken. To ensure reliable conclusions can be drawn from the pooled data, the quality of data acquisition for individual studies needs to be considered. Therefore, Chapter 2 aimed to evaluate the difference in GABA levels between migraine and other pain conditions and controls through systematic review and meta-analysis; and determine whether the quality of data acquisition (i.e. use of a GABA-optimised study design) influenced metabolite levels.

The second step in the thesis aimed to establish whether elevated GABA levels are specific to migraine or are seen in other headache and pain conditions when compared using optimised testing procedures. Prior to undertaking this thesis, a direct comparison between

neurochemical levels in migraine and other pain conditions had yet to be made. Two small studies found no difference in GABA levels between migraine with and without aura[122,125]. Further, one study found GABA levels were decreased only in those with spinal cord injury with pain but not those who were pain-free or in healthy controls[101]. Together, these studies support the hypothesis that GABA levels are relevant to pain rather than reflective of other factors such as visual field disturbance in migraine or reduced function in spinal cord injury.

To determine if the previously reported elevated GABA levels could reflect a unique biomarker of migraine, a GABA-optimised sequence is required to provide a direct comparison of headache and pain conditions. Therefore, GABA levels are measured and compared across people with migraine, whiplash-headache (a chronic headache condition with known aetiology) and low back pain (a chronic condition independent of headache features) and compared to healthy controls (Chapter 3).

The third step investigated whether the proposed biomarker is sensitive to change in the clinical condition. In this instance, if GABA levels were reflective of migraine or pain, we would expect a change in GABA level, as clinical characteristics of migraine change over time. Clinical characteristics that form core constructs important for the measurement of migraine have been established by guidelines for clinical trials in migraine[15]. The recommendation states that migraine frequency should be considered the primary outcome measure of interest in such studies[15]. Accordingly, frequency of migraine has been implemented as a primary outcome measure in many RCTs of migraine[57,126,127]. Additional recommended outcome measures should capture migraine pain and disability. An outcome measure recommended for migraine is HIT-6, which shows good reliability in detecting change in migraine populations[128]. To date, the correlation between GABA levels and the core outcomes measures of migraine or pain has yet to be fully explored. Although one single

cross-sectional study has reported a fair correlation between elevated GABA levels and higher scores on the central sensitisation inventory (Spearman's ρ = .48; P = .03) but a negligible correlation with the HIT-6 (Spearman's ρ = .06; P = .79)[108]. Therefore, to determine if GABA levels are associated with change in migraine status, the minimal constructs to consider should be migraine frequency and headache disability.

To determine if GABA levels are sensitive to *change* in migraine status, the core constructs need to be measured longitudinally over a reasonable time frame. Prior to this thesis, there had been no longitudinal studies of GABA levels in migraine. While evidence provided through cross-sectional studies have demonstrated proof of concept that GABA levels are altered in migraine and pain compared to controls, they do not inform the direction or magnitude of these changes. Therefore, to further elucidate the relationship between GABA levels and migraine, a longitudinal study that examines the association between *change* in core characteristics of migraine (e.g. frequency, pain and disability) and *change* in GABA levels over time is required. A three-month (12-week) period has been recommended as a suitable timeframe to observe treatment response (i.e. change in clinical characteristics) in interventional studies for migraine[15]. Therefore, change in clinical characteristics of migraine and neurochemicals is measured over a three-month period and their associations explored (Chapter 4).

The measurement of GABA using magnetic resonance spectroscopy

Magnetic resonance spectroscopy

Magnetic resonance spectroscopy (MRS) allows the non-invasive investigation of neurochemicals such as GABA in the living human brain[129]. MRS uses magnetic resonance

imaging to quantify neurochemicals based on their chemical structure and surrounding chemical environment[130].

MRS detects radiofrequency signals from hydrogen proton-spins when placed in a magnetic field. The precession of these protons will depend on the degree of electromagnetic shielding surrounding the hydrogens within the molecule[129]. As a result, hydrogen spins will occur at different frequencies and will present at different points along an x-axis known as the chemical shift axis[130]. The strength of signal is roughly proportionate to metabolite concentration and is displayed on the y-axis, which is later used for quantification (*Figure 1*). Single voxel MRS allows for the estimation of the concentration of metabolites, including NAA, Cr, Cho, Glutamate, and GABA, which are all of interest in the understanding of typical and atypical neuronal functioning. However, the accuracy of measuring complex and low concentration neurometabolites such as GABA at clinical field strengths (e.g. 1.5-3T) is problematic due to crowding of the chemical shift axis and strong overlap of more highly concentrated metabolites.

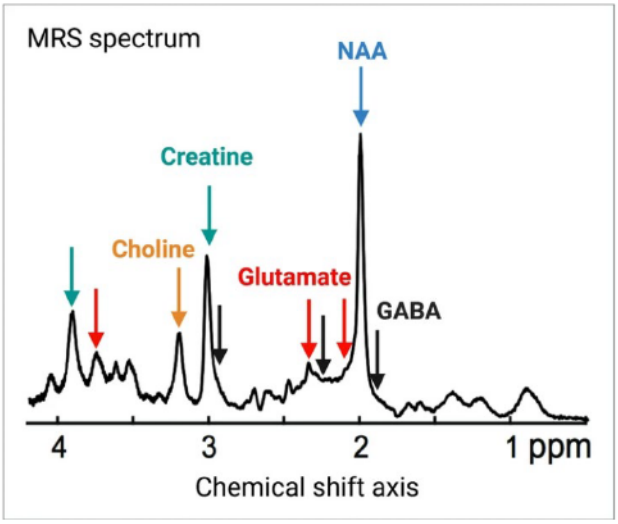

Figure 1: example of an un-edited single-voxel MRS spectra acquired using a PRESS sequence at 3T. The MRS spectrum shows metabolite peaks along the x-axis (chemical shift

axis) plotted against their relative concentration (y-axis). Metabolites are identified using prior knowledge of their chemical properties, which determines their positions along the chemical shift axis. This example demonstrates the overlap of the GABA signal by other more abundant metabolites when using an un-edited sequence. *Reprinted from Brain Structure and Function, Ip I, Bridge H. doi:10.1007/s00429-021-02273-0*[131]*. Investigating the neurochemistry of the human visual system using magnetic resonance spectroscopy, 2020, through open source licence.*

GABA levels have traditionally been challenging to measure using conventional single voxel techniques due to spectral overlap. GABA is presented at low concentrations in the human brain (1 mM)[117]. Therefore, the GABA signal is substantially overlapped by more abundant neurochemicals such as NAA and Cr at 3 ppm. The measurement of the GABA signal is further challenged due to the complex nature of the molecule, resulting in the signal being split over several sub-peaks, known as multiplets. As a result of these factors, the GABA signal is more challenging than other neurochemicals to accurately detect[117,130]. To overcome these limitations, J-difference editing techniques such as MEGA-PRESS (MEscher-Garwood Point-RESolved Spectroscopy)[132] have been developed and widely implemented[133,134].

MEGA-PRESS

MEGA-PRESS overcomes the limitation of single-voxel spectroscopy through the use of editing techniques to remove overlapping signals. MEGA-PRESS runs two simultaneous experiments (Figure 2); one experiment uses an editing pulse applied at 1.9ppm to selectively refocus the coupling evolution of GABA at 3ppm (Edit ON). The second experiment allows the free evolution of the spin system throughout the echo time (Edit OFF). GABA is measured through the difference spectra; here the Edit OFF experiment is subtracted from the Edit ON experiment, which maintains the GABA signal whilst editing out the stronger

overlapping signal[118,132]. Whilst, other metabolite signals are edited out, the signal at 3 ppm is still contaminated by macromolecular signal, which can account for up to 50% of the edited signal[135,136]. Therefore, conventional MEGA-PRESS studies should report the output as GABA+ co-edited macromolecules (GABA+). Although attempts have been made to suppress macromolecule contamination, these methods are highly susceptible to experimental instability[137]. As a result, at present conventional MEGA-PRESS, reporting GABA+ is considered the most reliable method to measure GABA accurately.

MEGA-PRESS is widely implemented and has been recommended over other editing techniques, given its improved accuracy, relative experimental stability, ease of implementation into conventional PRESS sequences and the multi-vendor compatibility of the sequence[118,132].

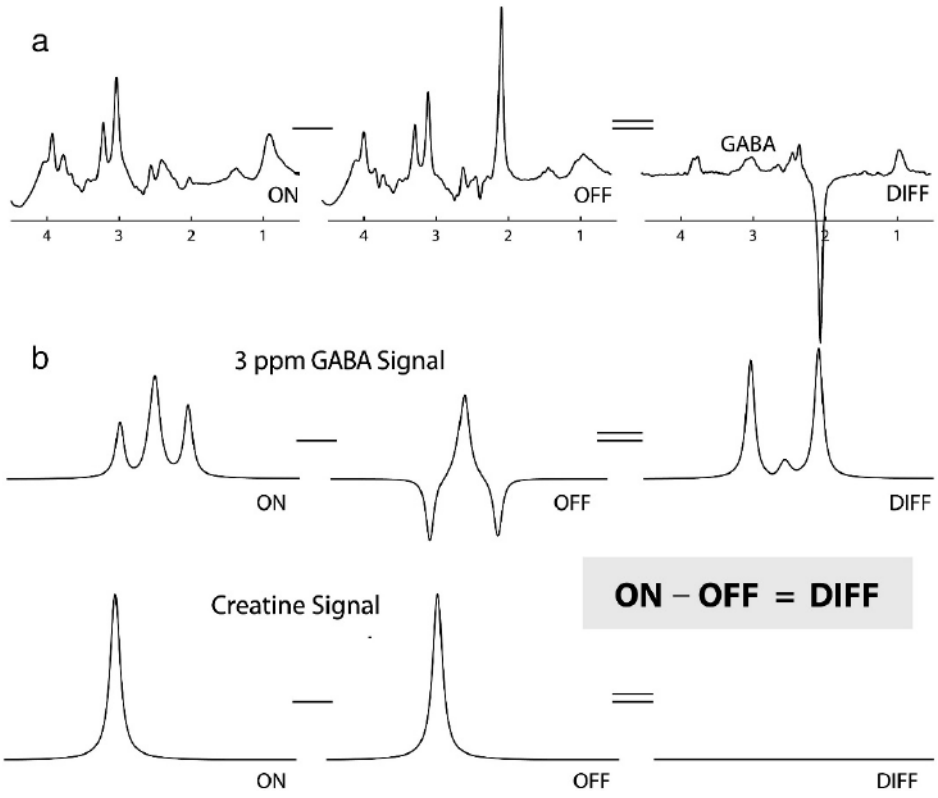

Figure 2: MEGA-PRESS editing for GABA, a) editing pulses applied at 1.9 ppm modulate the shape of pulses at 3 ppm, subtracting scans acquired without these pulses

(OFF) from scans acquired with editing pulses (ON) removes overlying creatine signals from the edited spectrum, revealing GABA signal in the difference spectrum (DIFF) J-difference editing to edit out overlying creatine signals. B) shows the effect of the editing pulses on signals at 3ppm only. *Reprinted from Neuroimage, 86, Mullins P et al. Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA, 43-52, Copyright (2014), with permission from Elsevier.*

Quality assessment of methods used to measure GABA

The MEGA-PRESS sequence has significantly improved the separability of GABA signal from other neurometabolites in the human brain, but study design and methodology can significantly affect the quality of data. The quality of data acquisition has a substantial impact on the accuracy of GABA measurement[138,139]. Prior to this thesis, there were no formal methods or tools for assessing the quality of the methodology used to measure GABA. To address this gap, the work in Chapter 2 developed and implemented the first evidence-based quality assessment tool in MRS (MRS-Q). The quality assessment tool appraises three domains considered critical for determining the quality of GABA acquisition in MRS studies. 1) sequence/acquisition parameters, 2) the reporting of quality metrics, and 3) the reporting of study design/analysis procedures.

The first domain to consider for the accurate measurement of GABA is the selection of appropriate sequence/acquisition parameters to produce sufficient signal-to-noise ratio (SNR). SNR reflects the measure of true signal compared to random background noise[140,141], and therefore high SNR is important for the accurate detection and measurement of GABA. Achieving optimal SNR for GABA experiments requires use of an edited sequence such as MEGA-PRESS and a balance of three inter-related parameters; scanner strength, voxel volume and scan time (number of transients). To date, expert opinion has suggested at a

scanner strength of 3T, a voxel volume of 27 ml ($30 \times 30 \times 30$ mm^3) and scan time of 10 minutes would be sufficiently optimised for GABA[118]. However, demands of clinical research to reduce scan time and improve specificity have resulted in compromises such as shorter scan times and smaller voxel sizes (e.g. scan time 4 minutes 16 seconds[139] voxel size 8 ml ($2 \times 2 \times 2$ mm^3)[101,102]. Reducing scan time improves patient comfort, thus reducing the chance of movement artefact, thereby improving data quality. However, the reduced scan time results in lower SNR and consequently reduces the separability of the GABA signal from noise, thus reducing the quality of data acquisition. One option to overcome the lower SNR is to increase the voxel size[118]. However, the larger voxel size reduces the specificity of the voxel and the quality of the shim, thereby reducing the quality of the data[117]. This example demonstrates the complex nature of choosing acquisition parameters for the given research population, highlighting the importance of achieving balance in acquisition parameters to acquire high-quality data. Failure to do so can result in unreliable or unusable data, particularly in clinical populations who might not tolerate long scanning protocols.

The second domain critical for quality assessment is the reporting of quality metrics. Documentation of quality metrics provides evidence that the method was sufficient to detect the GABA signal accurately. This is important given the rapidly developing nature of the field and the pressure to investigate smaller brain regions in shorter scan times. Traditionally one quality metric typically reported was Cramér-Rao lower bounds (CRLBs)[142]. CRLBs reflect the maximum trust that can be associated with an area (concentration) estimated in model fitting[143]. However, this statistical method subsequently demonstrated the inability to differentiate between outliers resulting from poor quality data and clinically relevant findings[143]. A more robust approach is to report several metrics covering different aspects of quality, such as shim values, full-width half maximum of the water peak and fit error, and to provide visualisation of all spectra[144]. Therefore, the robust reporting of quality metrics

allows for the quality of data to be demonstrated to the readership, particularly when using new sequences or parameters or when scanning challenging brain regions.

The third domain important for quality assessment is the reporting of study design and analysis methods used to quantify GABA (including stages of pre- and post-processing). Reporting study design and analysis is essential to improve transparency and facilitate reproducibility. One consideration is the selection of appropriate analysis methods, given there is large variability in available analysis packages and toolkits. There are four common analysis methods used, LC model[145], Tarquin[146], JMRUI[147]and Gannet[148]. Whereas each analysis method individually has demonstrated good test-rest reproducibility of GABA concentrations[149], considerable variation exists when comparing the output across analysis methods. A recent comparative analysis of the four main analysis methods demonstrated mean estimates of GABA concentration differed by between 4 and 106% when analysing the same dataset[150]. Given the impact the analysis method can have on GABA quantification, it is important that details of analysis methods are clearly recorded.

A second consideration is the use of post-processing procedures such as partial volume correction (PVC). PVC accounts for the variable concentration of GABA dependent on tissue type within the region of interest, considering the concentration of GABA is higher in grey matter than white matter and negligible in cerebrospinal fluid[151]. A number of methods for PVC have been proposed. However, the approaches result in significantly different measures of GABA[151,152]. For example, in data taken from the PCG of a control participant processed using Gannet[148], GABA levels estimated from the same data set could be reported as 3.56 IU, 4.97 IU or 4.72 IU, depending on which PVC correction model was used. The lack of standardisation of practice in PVC is emphasised in a meta-analysis of GABA levels in people with schizophrenia. In this review, 20% of studies used some method for PVC, 45%

corrected for PVC statistically, and 35% did not use or mention PVC. Therefore, for transparency and reproducibility, use of pre- and post-processing procedures such as PVC should be clearly recorded[118].

The three quality assessment domains, sequence/acquisition parameters, reporting of quality metrics and reporting of study design/analysis methods presented in the MRS-Q are important to determine data quality and thereby the accuracy of data in MRS studies. Given there was no tool for quality assessment of MRS methodology prior to the commencement of this thesis, and that accuracy of data is essential to determining GABA levels, the development of a quality assessment tool formed part of the early work in this thesis undertaken as part of Chapter 2.

Methods to improve standardisation to improve accuracy of GABA measurement

In addition to a quality assessment tool to appraise the quality of studies, standardisation of methods to accurately measure GABA is essential for the field to move forward. Prior to the commencement of this thesis there were no standardised methods or guidelines for the measurement of GABA using MEGA-PRESS. Given GABA is a key neurochemical in the central nervous system, many clinician researchers are drawn to the field to understand the role of GABA as a potential biomarker in many clinical conditions (e.g. chronic pain[102,153], psychological disorders[100], autism[98]). This rapid expansion of the field at a time when there is a lack of standardisation of methods has led to the use of a wide variety of different methods of data acquisition and analysis. This means in some instances data has been acquired without observing MEGA-PRESS-specific caveats. Further, the lack of methods for critically appraising and reporting studies means there is no way of ensuring data accuracy and thereby accuracy of results. On a data synthesis level, the variability in methods and variable data

quality means study outcomes cannot be reliably pooled, thus impeding the advancement of the field as a whole.

Many of the common mistakes that lead to poor quality data could be avoided if there was a simple guide to follow when planning an MRS study. In addition to domains outlined by the MRS-Q, such as sequence and acquisition methods, several other domains can affect the quality of the data. Examples of these include scanner drift or failure to control for confounders such as age. Such oversights have commonly occurred in published studies, resulting in a large number of publications having used methods now considered insufficient to detect GABA reliably. First are the studies that have used unedited sequences to measure GABA[121,154]. Second are the studies that used an edited sequence but did not use suitable acquisition parameters, for example, the use of a small voxel volume [101,102]. Third are studies that were unable to sufficiently control for external factors resulting in substantial frequency drift, which resulted in the exclusion of data[133]. Finally, are the studies that did not sufficiently address confounders such as age and sex[122,155], or tissue composition[156]. Many of these pitfalls have been discovered through trial and error and have not been comprehensively recorded to date. Navigating the caveats and pitfalls specific to MEGA-PRESS is particularly challenging for clinician-researchers who may not have direct access to expert MRS physicists on site. Further, mistakes made at the time of planning or data acquisition typically cannot be corrected for by using post-processing procedures. Therefore, highlighting the need for a clinically-orientated, translatable guide to assist the planning of MEGA-PRESS experiments to accurately measure GABA and avoid the commonly occurring pitfalls of MEGA-PRESS.

At the time of this thesis, there were no comprehensive clinically orientated guides for designing and running a MEGA-PRESS experiment to measure GABA accurately. One

document reported expert opinion from a consensus meeting on current practice for the use of MEGA-PRESS [118]. However, this document was written by experts with a high level of technical knowledge. Therefore, interpretation of this document for implementation into practice by those without a background in magnetic imaging physics is challenging. Together, the lack of comprehensive guidelines and observable mistakes in published data provides considerable evidence for the need for an evidence-based, clinically orientated, user-friendly, comprehensive guide to MEGA-PRESS for the measurement of GABA.

Development of translatable guidelines to improve standard practice for GABA measurement

To create a credible and easily translatable guideline in Chapter 5, both guideline development processes and research translation frameworks should be applied. Firstly, guideline development processes used in other fields can be applied to provide a stepwise process to develop an MRS guideline from a robust evidence base. Key steps include i) evidence identification and synthesis through a systematically conducted scoping review; ii) formation of the recommendations followed by quality assessment using the Grading of recommendations assessment development and evaluation (GRADE) tool; and iii) where no recommendations exist, use of expert consensus to develop recommendations, *de novo,* ('from scratch'). The adoption of this approach is novel in the field of MRS but well suited to the development of a guideline for MEGA-PRESS as it ensures recommendations are evidence-based rather than developed in an ad-hoc fashion, which can be freely influenced by personal opinion and bias.

Secondly, research translation frameworks provide guidance on the processes required to ensure the guideline is used and translatable to standard practice. Common to all frameworks (e.g. ADAPTE[157], RE-AIM[158], CFIR[159], Sax Institute[160]) is appropriate stakeholder

engagement throughout the development process. In the case of this thesis, key stakeholders and end-users included pain clinician-researchers, radiographers, MRS mentors and early career researchers. It was important to engage these key stakeholders in the design, synthesis and writing of the guide to ensure the guideline remained fit for purpose. Further, the engagement of both MRS experts and translational experts ensured guidelines were credible and easily translatable. The engagement of MRS experts to gauge the suitability of the guide for implementation in standard practice offered an additional step to ensure the guidelines would be well accepted and implemented in the field. Hence, both guideline development and research translation frameworks were followed to develop a translatable guideline for the measurement of GABA in Chapter 5. The process aimed to address the gap of translating knowledge created by expert MRS physicists into clinically implementable recommendations to assist clinician-researchers to progress the understanding of the role of GABA in clinical conditions.

Aims of the thesis

The two main aims of this thesis are:

1) To advance the understanding of the role of GABA in migraine (and pain conditions)

2) To improve the standardisation of methods for quality assessment, accurate measurement and the reporting of GABA in studies using MEGA-PRESS.

Specifically, this thesis set out to:

1) Investigate neurochemical levels in people with pain compared to controls

2) Compare GABA levels in people with migraine, whiplash-headache and backpain to age-sex matched controls

3) Explore the association between change in neurochemical levels in response to change in clinical characteristics of migraine over time;

4) Develop a quality assessment tool for the critical appraisal of MRS studies; and

5) Develop an evidence-based clinically-orientated guideline to standardise the measurement of GABA.

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CHAPTER TWO

Brain GABA and glutamate levels across pain conditions: A Systematic Review and Meta-analysis of 1H-MRS studies using the MRS-Q quality assessment tool

PREFACE

Chapter 2 presents a meta-analysis and systematic review to determine brain neurochemical levels across pain conditions when compared to controls. It also presents the MRS-Q (Supplement One) and two associated publications; an expert consensus which recommends the MRS-Q and a published commentary in response to the meta-analysis (Supplement Two).

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- **Peek AL;** Rebbeck T; Puts N; Watson J; Aguila M and Leaver A. Brain GABA and glutamate concentration across pain conditions A systematic literature review and meta-analysis of 1H-MRS studies. Sydney Musculoskeletal Health Alliance Meeting, 2nd August 2019
- **Peek AL;** Rebbeck T; Puts N; Watson J; Aguila M and Leaver A. GABA and glutamate levels across pain conditions: A systematic literature review and meta-analysis of 1HMRS studies. Australian Pain Society 39th Annual Scientific Meeting. Gold Coast, 7-10th April 2019

Impact

The paper has received 36 citations within the first year of publication. The MRS-Q (Supplement one) has been adopted by a clinical consensus document on reporting in MRS (Lin et al 2021; *NMR Biomed*, Supplement Two). The systematic review also attracted an invitation to collaborate on a project to use the MRS-Q to perform a meta-analysis investigating neurochemical levels in alcohol use disorder (DeMayo et al; Appendix 4). The systematic review also received a favourable commentary (Cruz-Almeida and Porges. 2021; *NeuroImage,* 224, 117392, Supplement Two).

AUTHORSHIP ATTRIBUTION STATEMENT

The co-authors of the paper *'Brain GABA and glutamate levels across pain conditions: A Systematic Review and Meta-analysis of 1H-MRS studies using the MRS-Q quality assessment tool'* confirm that Aimie Laura Peek has made the following contributions:

- Design of the work
- Collection and extraction of the data
- Analysis and interpretation of the data
- Manuscript preparation, revision and critical appraisal for important intellectual content

As the primary supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

[redacted]

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Brain GABA and glutamate levels across pain conditions: A systematic literature review and meta-analysis of 1H-MRS studies using the MRS-Q quality assessment tool

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ABSTRACT

Background: A proposed mechanism of chronic pain is dysregulation between the main inhibitory (GABA) and excitatory (glutamate) neurometabolites of the central nervous system. The level of these neurometabolites appears to differ in individual studies of people with pain compared to pain-free controls across different pain conditions. However, this has yet to be systematically investigated.

Aims: To establish whether GABA, glutamate, glutamine and Glx levels differ across pain conditions when compared to pain-free controls.

Methods: Five databases were searched. Studies were included if they investigated: 1) A pain condition compared to control. 2) Reported GABA, glutamate, glutamine or glutamate/glutamine level. 3) Used 1H-Magnetic Resonance Spectroscopy (Prospero Project ID CRD42018092170). Data extracted included neurometabolite level, pain diagnosis, and spectroscopy parameters. Meta-analyses were conducted to establish the difference in neurometabolite level between participants with pain and pain-free controls for different pain conditions. The MRS-Q was developed from existing clinical consensus to allow for the assessment of quality in the included studies.

Results: Thirty-five studies were included investigating combinations of migraine (n = 11), musculoskeletal pain (n = 8), chronic pain syndromes (n = 9) and miscellaneous pain (n = 10). Higher GABA levels were found in participants with migraine compared to controls (Hedge's G 0.499, 95%CI: 0.2 to 0.798). In contrast, GABA levels in musculoskeletal pain conditions (Hedge's G −0.189, 95%CI: 0.530 to 0.153) and chronic pain syndromes (Hedge's G 0.077, 95%CI: 1.612 to 1.459) did not differ from controls. Results for other brain neurometabolites revealed significantly higher levels for glutamate in participants with migraine and Glx in chronic pain syndromes compared to controls.

Conclusion: These results support the theory that underlying neurometabolite levels may be unique in different pain conditions and therefore representative of biomarkers for specific pain conditions.

1. Introduction

Two key neurometabolites implicated in the pathophysiology of pain are glutamate and gamma-aminobutyric acid (GABA). Glutamate is the principal excitatory neurometabolite in the central nervous system, and is involved in many metabolic pathways (Rae, 2014; Ramadan et al., 2013; Zhou and Danbolt, 2014). GABA is the most abundant inhibitory neurometabolite in the central nervous system (Enna and McCarson, 2006; Rae, 2014) and is considered an important regulator of the balance between excitation and inhibition in the brain (Petroff, 2002). Both glutamate and GABA are critical for many centrally regulated physiological functions, including pain processing and pain modulation.

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Dysfunctions in glutamate and GABA metabolism, resulting in too much or too little of the neurometabolite, have been implicated in clinical conditions, such as chronic pain (MacDermott, 2001; Zunhammer et al., 2016).

In-vivo quantification of these neurometabolites is possible through proton magnetic resonance spectroscopy (1H-MRS). 1H-MRS is a non-invasive neuroimaging technique that allows for the separation of neurometabolites based on their chemical structure. Separation of spectra is possible through observing the radiofrequency signal detected from hydrogen nuclei spins and their chemical environment when placed in a magnetic field (Puts and Edden, 2012). Accordingly, neurometabolites can be separated along an x-axis dependent on their chemical specific radiofrequency, otherwise known as their chemical shift. The strength of this signal is reflective of the level of neurometabolite. Whilst 1H-MRS has been helpful in quantification of many neurometabolites, the measurement of GABA and glutamate have their own specific challenges.

Quantification of GABA is problematic due to its low concentration (1–2 mM (Govindaraju et al., 2000; Petroff, 2002)), and spectral overlap with other more abundant neurometabolites such as creatine at 3 ppm (Puts and Edden, 2012). To resolve GABA, J-difference editing can be used to selectively edit the signal of interest. J-difference editing of GABA uses frequency selective 'editing' pulses, applied to the 1.9 ppm GABA signal, which in turn, selectively refocused the GABA signal at 3 ppm, but not the creatine signal. The most widely used sequence for measuring GABA is MEGA-PRESS (Mikkelsen et al., 2017; Mullins et al., 2014). Here, editing pulses are applied on-resonance (edit-ON) at 1.9 ppm in half the acquisition, and not in the other half (edit-OFF). The difference spectrum contains only those signals affected by the editing pulses, revealing a quantifiable GABA signal at 3 ppm (for a review, see Puts and Edden, 2012). However, various implementations of the sequence exist, utilizing different radio-frequency pulses and timings (Mikkelsen et al., 2017, 2019; Saleh et al., 2019). One limitation of J-difference editing is macromolecule contamination meaning that studies using this editing report GABA + rather than measures of GABA only. This can be overcome with symmetrical editing or measured macromolecule baselines which reflect a more refined measure of GABA (Edden et al., 2012; Mikkelsen et al., 2018a).

There is little consensus as to the best way to measure glutamate. Glutamate is present at higher concentrations (12 mM (Choi et al., 2006)) than GABA, however, difficulties separating it from glutamine (1–4 mM) and glutathione (2–3 mM (Rae, 2014)) have been highlighted. Whilst some studies estimate glutamate alone (Schubert et al., 2004), others choose to estimate Glx, the combined measure of glutamate and glutamine, although the signal also contains some glutathione. Glx is measured either from edited MRS (Sanaei Nezhad et al., 2017) or from short echo time PRESS (≈30 ms) (Gonzales de la Aleja et al., 2013). Other techniques specific to measuring glutamate (separating it from glutamine), including TE-averaging, also exist (Hurd et al., 2004).

Several 1H-MRS studies to date have demonstrated changes in GABA and glutamate in pain conditions compared to controls. However, the direction of concentration change is inconsistent across pain conditions. For example, Aguila et al. (2015) demonstrated an increase in GABA levels in individuals with migraine compared to controls. In contrast, GABA levels were decreased in people with fibromyalgia (Foerster et al., 2012) and chronic pelvic pain (Harper et al., 2018). Similarly, glutamate levels were higher in people with migraine compared with controls (Gonzales de la Aleja et al., 2013; Zielman et al., 2017), however, lower in people with low back pain (Gussew et al., 2011). The variability in these data suggest that there may be a unique neurometabolite signature for each pain condition. However, to-date these data have not been systematically appraised.

An alternative explanation for the variability of neurometabolite levels between pain conditions and MRS studies could be reflective of the quality of the magnetic resonance (MR) acquisition and analysis. This includes the 1H-MRS sequence utilised. For example Bridge et al. (2015) used an unedited sequence and demonstrated a 10% decrease in GABA level in individuals with migraine compared to controls (Bridge et al., 2015). Conversely, Aguila et al. (2015) used an edited sequence and demonstrated a significant increase in GABA level in people with migraine compared to controls. More importantly, it is well-established that reporting of acquisition parameters is important for allowing of interpretation and reproducing prior studies.

The role of different brain regions in pain processing have been extensively studied using a variety of imaging and in vitro methods in both humans and animals It is therefore possible that the level of neurometabolites differ between brain regions. Differences have been demonstrated in people with fibromyalgia (Foerster et al., 2012), where the same individuals demonstrated an increase in GABA level in the insula but not the anterior cingulate cortex (ACC). Alternative explanations for these differences include; variation in signal to noise ratio dependent on location of brain region being scanned, the composition of the voxel in terms of grey and white matter, and the distribution of GABA and glutamate receptors in that specific brain region. Advances in analysis techniques and the introduction of volume-based correction allows better understanding of these factors, however, they have not been uniformly applied across studies, and therefore their impact must be considered when synthesizing results from studies.

The primary aim of this review therefore was to determine whether GABA, glutamate, glutamine and Glx levels differ across pain conditions compared to pain-free controls. The secondary aim was to report on the quality of the MR data acquired in the literature in this field and then, to determine whether the quality of reporting, or brain region, influences brain neurometabolite levels. Assessing appropriate acquisition parameters necessitated us to develop the MRS-Q tool for systematic review of 1H-MRS acquisitions, as no such tool previously existed. The determinants are based on prior consensus.

2. Methods

2.1. Protocol registration

This review was conducted in adherence with the Preferred Reporting Items for Systematic Reviews and Meta-analysis statement (PRISMA) (Moher et al., 2015) and was registered prospectively on Prospero (CRD42018092170).

2.2. Eligibility criteria

Studies were included if they used 1H-MRS of the brain to report measures of GABA, glutamate, glutamine or the combination measure of glutamate and glutamine i.e. Glx. Among these studies were Spectroscopic Imaging (MRSI) studies. Studies were required to have recruited human participants who had a pain condition that was compared with healthy pain-free controls. Included studies were of primary research design, such as cross-sectional, longitudinal, interventional or case series, and written or translated into English via Google Translate.

Studies were excluded if they used other forms of spectroscopy e.g. phosphorous MRS or examined other tissues, such as the spinal cord. Studies that investigated animals or conditions which were primarily psychological disorders without pain as a predominant feature (e.g. post-traumatic stress) were also excluded, as were literature reviews or conference proceedings.

2.3. Search methods for identification of studies

A comprehensive search strategy was derived and piloted with assistance from the University's librarian. The full search strategy and search terms are attached in Appendix 1. In brief we combined MeSH headings and key words for **magnetic resonance spectroscopy** (for example: magnetic resonance imag*, NMR- Spectroscopy, MEGA-PRESS) AND **neurometabolites** (for example: GABA or glutamate or glutamine or Glx, brain neurochemical*, metabolite*) AND **pain** (for example:

chronic pain (expanded), musculoskeletal pain).

OVID MEDLINE, EMBASE, WEB of SCIENCE, CINAHL and Pubmed were electronically searched without any restrictions to date, study design or language (inception to September 4, 2019). Reference lists of included studies and systematic reviews in this field were searched to ensure key studies had not been missed.

2.4. Study selection

A two stage approach was used to screen studies for inclusion (Furlan et al., 2009). In the first stage, two reviewing teams (AP) and (MA or AL or TR or JW) independently screened titles and abstracts to identify titles for full text retrieval. Where there was uncertainty, the full text was requested. In the second stage, two reviewers (AP) and (MA or AL or TR or JW) independently assessed the full text of all studies to determine their eligibility. Disagreements were discussed and resolved by a third independent reviewer (AL or TR). Reasons for exclusion were documented and duplicates were removed.

2.5. Data extraction

Data were extracted in duplicate by 2 reviewers (AP, JW) using a standardised form (Appendix 2). Authors were contacted for missing data or raw data when data was only presented graphically. When authors failed to respond, graphical results were extracted using on-screen callipers (Screen Callipers Version 4.0). Where non-parametric statistics were reported, means and SD were imputed using methods recommended in the Cochrane handbook (Higgins et al., 2011). Only primary analyses were extracted from the included studies. In the case of longitudinal or interventional studies, only baseline data were extracted. Data from different brain regions of the same individual was interpreted as being independent, and therefore extracted separately for each brain region (Aoki et al., 2012; Schur et al., 2016). A secondary meta-analysis (not shown) averaged across brain regions of the individual.

Data were extracted into 4 tables: 1) spectroscopy parameters, where data extracted included scanner make, acquisition parameters e.g. voxel size and location, TR, TE and post processing details such as software and fitting methods (see Appendix 2); 2) neurometabolite levels, where the primary outcome of interest (mean (SD)) of GABA, glutamate, glutamine or Glx levels for subjects with pain and control subjects; 3) participant characteristics, including age, sex, pain condition, excluded comorbidities, and 4) Bibliometric data, including authors, year of publication, country, funding sources and if prospectively registered (Appendix 2).

2.6. Quality metrics

2.6.1. AXIS

Two quality measures were used. Firstly, the modified Appraisal tool for Cross-sectional studies (AXIS) (Downes et al., 2016) was used to determine the methodological quality of the research design (Appendix 3). The modified AXIS was piloted on 1H-MRS studies prior to inception of the review (AL, AP, JW, NP). Two reviewers (AP, NP or JW) independently assessed the quality of each included paper. Disagreements were subsequently discussed and resolved by a third reviewer (AL).

2.6.2. MRS-Q

Secondly, the quality of the 1H-MRS acquisition method was assessed. Although two recent 1H-MRS white papers suggest that researchers use standardized acquisition and analysis metrics (Mullins et al., 2014; Wilson et al., 2019) to-date there are no published standardized tools to objectively evaluate the quality of spectroscopic acquisition. For assessment, this necessitated the development of a new quality appraisal tool (MRS-Q) for this review, based upon consensus papers and expert opinion on best-practice (Harris et al., 2017; Mikkelsen et al., 2017, 2019; Mullins et al., 2014; Wilson et al., 2019). The MRS-Q evaluates 12 and 13 parameters for unedited and edited studies respectively. The MRS-Q has three parts, Part 1 checks whether appropriate sequences and adequate parameters were used to accurately detect the neurometabolite of interest. The criteria in Part 1 include quality parameters that are considered fundamental to producing good quality spectra. Two of these parameters, *appropriate sequence and adequate parameters,* were also used to determine the quality of acquisition for the purpose of our sensitivity analysis. Part 2 evaluates whether sufficient quality checks were utilised such as reporting full width at half maximum (FWHM) and the visualisation of data. In Part 3, details of study design such as sample size calculations and post processing methods were appropriate and explicitly reported are evaluated. As such, this tool reports on both acquisition and the adequate reporting of this information (e.g. for allowing reproducibility of such studies). Each study was assessed independently by 2 reviewers (AP,NP). The cut-off points and rationale for each of the criteria are displayed in Table 1. Only studies that reported using adequate spectroscopy parameters for the neurometabolite of interest were considered high-quality and used in the sensitivity analysis (see Data synthesis: secondary aims).

2.7. Data synthesis and analysis

2.7.1. Primary aim

In line with the review's primary aim; to determine if brain neurometabolites are different across pain conditions, it was decided a priori to categorise studies into one of four pain categories for analysis. The categories were migraine, musculoskeletal pain, chronic pain syndromes or miscellaneous pain for studies that did not fit into the above categories. The migraine group was inclusive of any form of painful migraine or headache listed in the ICHD 3b. Musculoskeletal pain was defined as any condition diagnosed from a single anatomical site, and likely to be driven by a nociceptive input e.g. low back pain, knee osteoarthritis. Conversely, chronic pain syndromes were defined as any widespread chronic pain condition affecting multiple regions with a non-specific musculoskeletal diagnosis, that is predominately associated with central processing abnormalities (Arnold et al., 2016) e.g. fibromyalgia, complex regional pain syndrome, the remaining studies were considered as miscellaneous pain and encompassed any other non-musculoskeletal pain conditions such as abdominal pain, spinal cord injury with painful neuropathy. From here-on the group name will be used to refer to individuals who experience these particular conditions e.g. people with migraine (migraine).

Data was labelled according to brain region investigated. It was decided a priori that labelling would be regardless of hemisphere investigated, unless a single study contributed both a left and right data set. Nomenclature of brain region was simplified in terms of region, for example the dorsolateral prefrontal cortex was labelled and identified as the prefrontal cortex.

Meta-analysis was performed using Comprehensive Meta-analysis software (Borenstein et al., 2005) on studies pooled by neurometabolite, and sub-grouped by pain condition to allow comparison of neurometabolite level between pain conditions. Because the “miscellaneous” group were a heterogeneous category, these results in this group were not pooled in the meta-analysis. Standardised mean differences and 95% confidence intervals (Hedge's G) were used to compare the pain groups to the painfree controls to allow for data presented in different units (mmol, IU, ratios).

Results were analysed by neurometabolite and sub-grouped by pain type (migraine, musculoskeletal pain, chronic pain syndromes) regardless of brain region studied. Where multiple results were presented for the same neurometabolite preference was given to results of actual concentration or institutional units over ratios. Heterogeneity was assessed using the i^2 test and a random-effects model was implemented to compensate for variation in acquisition parameters, voxel location and the selective use of partial volume correction.

2.7.2. Secondary aims

To investigate the review's secondary aims, firstly summary measures

Table 1
Criteria and rationale of the MRS-Q tool[a].

	Criteria	Setting	Rationale
1. Parameters	Scanner Strength	**Edited for GABA:** Over 3T **Unedited:** 3T preferable	Scanning at 3T and above provides a higher signal-to-noise ratio, with increased spatial and temporal resolution. Reducing spectral overlap of Glu, Gln, and GABA (Di Costanzo et al., 2007; Wilson et al., 2019)
	Appropriate Sequence:	**Edited for GABA:** MEGA-PRESS, MEGA-semi LASER, other editing	To accurately quantify GABA a specific editing sequence must be implemented (Mullins et al., 2014). EDITINGSCHOOL [b], Expert opinion [c]
	***Used to determine quality**	**Unedited:** PRESS or vendor specific PRESS, semi-LASER or STEAM	Common clinical implementation agreed through consensus opinion (Wilson et al., 2019)
	Adequate Parameters:	**Edited for GABA:** Averages over 240	This number of averages are required due to the low amplitude of signal due to the typically low concentration of GABA and splitting due to coupling (Mikkelsen et al., 2018b) EDITINGSCHOOL [b]
	***Used to determine quality**		
		TE: GABA+ 68, GABA 80 (68 S)	*See Appropriate TE below
		Voxel Size ~27 ml	The voxel size required to quantify GABA as a compromise between localization and adequate signal to noise (Mullins et al., 2014)
		Unedited: 128 Av, 15 × 15 × 15mm^3 voxel, 3T; 64 Av, 20 × 20 × 20 mm^3 voxel, 3T; 256 Av, 15 × 15 × 15 mm^3 voxel 1.5T; 128 Av, 20 × 20 × 20 mm^3 voxel, 1.5[33]	In order to produce adequate SNR, the number of averages need to be increased when using lower strength scanners, or smaller sized voxels (Wilson et al., 2019) Common clinical implementation agreed through consensus opinion (Wilson et al., 2019)
		MRSI: 3T, 16 × 16 matrix, voxel 15 mm^3 TR 1500,	–
	Data Points	**Edited for GABA:** NA **Unedited:** 1024 complex data points from 2000Hz	Common clinical implementation agreed through consensus opinion (Wilson et al., 2019)
	Appropriate TE	**Edited for GABA:** 68 ms or 80 ms	68 ms is optimal for GABA- due to complete inversion in the ON acquisition. (Rothman et al., 1993) 80 ms for macromolecule editing (Edden et al., 2012) Mullins et al., 2014 [e]. EDITINGSCHOOL [b]
		Unedited: 20/30 ms	Common clinical implementation agreed through consensus opinion (Wilson et al., 2019)
2. Quality Measures	Quality measure	Reported Shim or FWHM (Full Width Half Maximum)	Poor shimming leads to aberrant quantification. Linewidth is known to affect fitting and be an index of data quality (Wilson et al., 2019)
	Quality measure	Fit Error Calculation reported	While the format is less-important, fit error reports on the quality of the spectra and/or appropriate fitting methods. While fit-error cut offs are proposed (e.g. <20% CRLB for LC model analysis (Cavassila et al., 2001)) we did not stipulate specific cut offs here.
	Data visualisation	A visual display of at least one data set	Recommendations are that visual display of spectra (e.g. an example spectrum, all spectra) are reported in a figure (e.g. Zielman et al., 2017)
	Partial volume correction	Partial Volume Corrected- not just for grey matter	For water-referenced data, partial volume can substantially affect data quantification and could be a prominent driver of group differences. In addition, only correcting for grey matter is deemed inappropriate (Gasparovic et al., 2006; Harris et al., 2015; Mikkelsen et al., 2016; Porges et al., 2017) EDITINGSCHOOL [b]
	Scanner drift	Frequency Drift Reported	This has been shown to be of particular importance for edited MRS (Harris et al., 2014; Mikkelsen et al., 2017; Near et al., 2015)
3. Study Design	Power calculation	Report how sample size was determined	Allows demonstration of whether the study is adequately powered to detect between group difference- reducing the chance of type I and II error (Nayak, 2010)
	Frequency/phase corrected	Reported either frequency or phase correction	Frequency and phase correction prior to fitting is strongly recommended, and is key for edited MRS (Mullins et al., 2014; Wilson et al., 2019) EDITINGSCHOOL [b], Expert opinion [d]

[a] Template available for use from http://doi.org/10.17605/OSF.IO/8S7J9.
[b] EDITINGSCHOOL was held in December 2018 and focused on edited MRS. Expert instructors attended (http://www.gabamrs.com/blog/2018/10/12/editingschool-final-schedule).
[c] Co-author NP.
[d] Wilson et al., 2019 Consensus document agreed on by 49 MRS experts.
[e] Mullins et al., 2014 Consensus document written from a meeting of a number of specialist groups in 2011 in the UK documenting current "minimal best practice".

of spectroscopy parameters, and brain region, were tabulated (Tables 2–4) (University of York, 2009) Secondly, a sensitivity analysis was conducted, where the primary meta-analysis was repeated only on the studies that satisfied the use of minimal best practice in terms of appropriate sequence and adequate spectroscopy parameters as determined by the *appropriate sequence and adequate parameters* subsections of the MRS-Q (Table 1, rows 2, 3). To determine the impact of brain region on neurometabolite levels, studies were grouped broadly by brain region. Results were pooled where there were two or more homogeneous studies of a particular brain region within a pain condition. Finally, post-hoc meta-analyses were conducted, where data from multiple brain regions of the same individual were averaged and included within the analysis.

3. Results

3.1. Study selection

An initial search retrieved 8022 studies. Following removal of

Table 2
Study characteristics.

	Strength (scanner)/Sequence/(TR/TE)/Avs/Processing	Region/Voxel size (ml)	Neurometabolites	Participants: Number/Pain/%Female/Age/Duration	Controls: Number/%Female/Age
Aguila et al. (2015)	3T (GE)/MEGA-PRESS/(1800/68)/256/Gannet (GABA), Tarquin (Glu, Gln)	PCG/27 ml	GABA+, Glu, Gln	n = 19/Migraine (ICHD II), 1 attack a month+/70%F/33 IQR (28.2–47.2) yrs/180 months IQR (60–288)	n = 19/70%F/30 IQR (26.5–47.5) yrs
As-Sanie et al. (2016)	3T (GE)/PRESS/(3000/3*)/-/LC model	R ant Ins, R post Ins/ 12 ml	Glx *(NAA)*	**Group 1:** n = 15/Chronic pelvic pain with endometriosis/100%F/26.7 (6.6) yrs/5.5 (3.5–9.5) yrs **Group 2:** n = 6/Chronic pelvic pain without endometriosis/100%F/24.2 (4.6) yrs/3.75 (0.9)yrs	**Matched to Group 1:** n = 14/100%F/26.5 (6.6) yrs **Matched to Group 2:** n = 11/100%F/24.2 (4.0) yrs
Bathel et al. (2018)	3T (P)/MEGA-PRESS/(2000/68)/320/Gannet PRESS/(2000/30)/32/LC model	Occ/27 ml, R Thal/22.5 ml	GABA+ Glx *(NAA, Cr)*	n = 15/Migraine without aura (ICHD-II), >2 a month, pain-free 72 h prior and 48 h after scanning/80%F/35.2 (10.8) yrs/-	n = 15/80%F/33.4 (8.5) yrs
Bednarska et al. (2019)	3T (P)/MEGA-PRESS/(2000/68)/-/LC Model	L ant Ins, R ant Ins/24 ml	GABA+, Glx	n = 39/IBS/100%F/32.1 (18–57) yrs/-	n = 21/100%F/32.1 (20–55) yrs
Bigal et al. (2008)	4T (V)/3D-LASER**/(2000/72)**/-/-	Occ/13.5 ml	GABA	**Group 1:** n = 9/Migraine with aura/84.7%F/34.1 (95% CI 27.9–40.2) yrs/-**Group 2:** n = 10/Migraine without aura/84.7%F/41.4 (95% CI 30.5–52.2) yrs/-	n = 9/84.7%F/26.5 (95% CI 22.4–30.5) yrs
Bridge et al. (2015)	3T (Sie)/SPECIAL/(11.2/4.68)/128/LCModel	Occ/8 ml	GABA, Glu	n = 26/Migraine with aura (IHS)/100%F/33 (8) yrs/-	n = 13/100%F/30 (6) yrs
Chan et al. (2019)	3T (Sie)/MEGA-PRESS/(1500/68)/192/Gannet	Occ/15 ml	GABA, Glx	**Group 1:** n = 9/Migraine with aura/88.9%F/31 (95% CI 21–42) yrs/- **Group 2:** n = 7/Migraine without aura/71.4%F/31.1 (95% CI 20–49) yrs/-	n = 16/50%F/27.1 (95% CI 20–34) yrs
Di Pietro et al. (2018)	3T (P)/MEGA-PRESS/(2000/68)/200/jMRUI, AMARES (GABA), QUEST (Cr)	Thal/8 ml	GABA, *(Cr, Other neurometabolites)*	n = 20/Chronic orofacial neuropathic pain/65%F/50.1 SEM (4.4) yrs/>3yrs	n = 20/65%F/42.2 SEM (2.9) yrs
Fayed et al. (2010)	1.5T (GE)/Probe P/(2000/35)/128/LC model	L SM1, L and R Thal, L and R hippoc, PCG/8 ml	Glx, *(Cr, Cho, mI, NAA)*	n = 10/Fibromyalgia (ACR)/80%F/40 (6.2) yrs/1.6 (0.3) yrs	n = 10/80%F/37.8 (8.7) yrs
Fayed et al. (2012)	1.5T (GE)/Probe P/(2000/35)/128/LC model	PCG, pos Ins, L and R hippoc/8 ml	Glx, Glu, *(NAA, Cr, mI, tChol, tNAA)*	**Group 1:** n = 10/Fibromyalgia (ACR)/90%F/38.94 (5.56) yrs/2.13 (0.52) yrs **Group 2:** n = 10/Somatoform disorder (SCID-I)/80%F/43.93 (9.96) yrs/3.82 (0.76) yrs	n = 10/80%F/39.52 (11.32) yrs
Fayed et al. (2014)	1.5T (GE)/PRESS/(2000/35)/128/LC model	V PCG/8 ml	Glu, Glx *(Cr, mI, NAA, tCho)*	**Group 1:** Migraine/n = 33/63.6%F/45.2 yrs/- **Group 2:** Fibromyalgia/n = 54/90.7%F/45.1 yrs/-**Group 3:** Somatoform disorder/n = 10/80%F/44.1 yrs/-**Group 4:** Trigeminal cervical neuralgia/n = 8/75%F/46.9yrs/-	n = 193/60.6%F/53.2yrs
Feraco et al. (2011)	3T (GE)/PRESS/(2000/35)/128/LC model	Thal/5.8 ml VL PFC/9.2 ml	Glu, Glx *(tNAA, Cho, mI)*	n = 12/Fibromyalgia (ACR)/92%F/43.2 range (30–54) yrs/-	n = 12/92%F/41.3 range (28–56) yrs
Foerster et al. (2012)	3T (GE)/MEGA-PRESS/(1800/68)/256/In house-Matlab program with Gaussian curve fitting (GABA) PRESS/(2000/35)/32/LC model (NAA)	ACC, Occ, R ant and R post Ins/18 ml	GABA *(NAA)*	n = 16/Fibromyalgia (ACR), >1 yr/100%F/37.2 (12.8) yrs/>1yr	n = 17/100%F/36.1 (11.7) yrs/>1yr
Gerstner et al. (2012)	3T (GE)/PRESS/(3000/30)/-/LC model	L and R Ins/12 ml	Glu, Gln, Glx *(NAA, Cho)*	n = 11/Temporomandibular disorder-RDC-1- (ongoing pain >3 tender muscle sites ipsilateral to palpation pain)/91%F/25.8 (2.33) yrs/range 0.5–7 yrs	n = 11/91%F/24.8 (1.20) yrs

(*continued on next page*)

Table 2 (*continued*)

	Strength (scanner)/Sequence/(TR/TE)/Avs/Processing	Region/Voxel size (ml)	Neurometabolites	Participants: Number/Pain/%Female/Age/Duration	Controls: Number/%Female/Age
Gonzales de la Aleja et al. (2013)	3T (GE)/Probe P/(2000/28)/160/LCModel	Occ/27 ml, APC/8 ml	Glu, Gln, *(NAA, Cr, Cho)*	n = 28/Migraine or Migraine with aura (ICHD2) >2 attacks a month in the last 3 months, >3 yr history/100%/31.74 (8) yrs/>3yrs	n = 19/100%F/31.79 (4.5) yrs
Grachev et al. (2000)	1.5T (GE)/STEAM, Probe-S, PSD/1500/30/-/Direct from the scanner	DLPFC/8.1 ml Thal/8/1 ml, CC/8 ml (Ins/8 ml, SM1/7.7 ml OFC/8 ml, Vis cort 8 ml)	Glu, Gln, GABA, *(tmI, Glc, Lac, Cr, NAA, Cho)*	n = 9/Low back pain >1yr/22.2%F/45 (6) yrs/9 (5) yrs	n = 11/18.2%F/44 (3) yrs
Gussew et al. (2011)	3T (Sie)/PRESS/(2500/40)/-/LC model	L ant Ins/3 ml, ACC/3.9 ml, L Thal 3.5 ml	Glu, Gln, *(NAA, Cr, tCho, mI)*	n = 10/Low back pain >1yr/80%F/range (22–52 yrs)/range (1–5 yrs)	n = 10/80%F/range (22–52 yrs)
Gustin et al. (2014)	3T (P)/MEGA-PRESS/(2000/68)/100/jMRUI, AMARES (GABA) -/(2000/29)/-/jMRUI, QUEST	R Thal/8 ml	GABA Glu, Gln, *(NAA, Cr, Asp, mI, GroPCho)*	n = 12/Spinal cord injury (SCI) with neuropathic pain, (IASP-SCI)/33.3%F/57 (4) yrs/182 SEM 42 months	n = 21/38.1%F/31 (2) yrs
Harfeldt et al. (2018)	3T (Sie)/-/(2000/30)/-/LC model	R and L post Ins/2 ml	Glu, Glx *(NAA, tCr, Cho, m)*	**Group 1:** n = 19/Temporomandibular disorder with generalised pain/100%F/43 IQR (40–56) yrs/>3months **Group 2:** n = 17/Temporomandibular disorder with local pain/100%F/40 IQR (30–44) yrs/>3months	n = 10/100%F/36 IQR (26–51)
Harper et al. (2018)	3T (P)/MEGA-PRESS/(1800/68)/256/LC model PRESS/(2000/33)/32/LC model	R ant Ins, R post Ins, Mid ACC, Mid Occ (Control Region)/18 ml	GABA Glx, Glu	n = 18/UCPPS- urological chronic pelvic pain syndrome including interstitial cystitis, and bladder pain/100%F/34.8 yrs SD 11/5.9 (6.5) yrs	n = 20/100%F/34.7 (12.3) yrs
Harris et al. (2009)	3T (GE)/-/(3000/30)/-/LC model	R ant Ins, R post Ins/12 ml	Glu, Glx, *(NAA, MI, Cho, Cr, mI)*	n = 19/Fibromyalgia (ACR), >1 year/100%F/45.2 (15) yrs/>1yr	n = 14/100%F/45.9 (11.1) yrs
Henderson et al. (2013)	3T (P)/MEGA-PRESS/(2000/68)/200/jMRUI, AMARES	Contra Thal, (R-controls)/8 ml	GABA	n = 23/Painful trigeminal neuropathy (Liverpool criteria)/82.6%F/46.6 SEM (2.4)/6.1 SEM (4.6) yrs	n = 43/72%F/49.1 SEM (2.5) yrs
Ito et al. (2017)	3T (GE)/-/(2000/30)/96/LC model	ACC/20 × 20 × 40 16 ml	Glu, Gln, Glx *(tCr, mI, NAA)*	n = 56/Chronic pain: neuropathic pain-narrowing of the spinal canal, trigeminal neuralgia, intercostal neuralgia, postoperative neuropathy, radiculopathy, plexus injury, peripheral nerve injury, reflex sympathetic dystrophy, diabetic neuropathy, non-neuropathic-fibromyalgia, cephalgia, somatoform, unidentified general or partial pain/67.9% F/58 range (45–67) yrs/36.5 range (13.5–74.5) months	n = 60/63.3%F/40 range (28–48) yrs
Janetzki et al. (2016)	3T (Sie)/MEGA-PRESS/(2000/68)/-/- PRESS/(1800/30)/-/-	ACC, L Ins/-	GABA Glx *(NAA, mI, tCr, tCho)*	n = 19/Low back pain >3 months/68.4% F/55.3 yrs/42.1% > 5 years, 52.6% < 5 years, 5.3% unknown	n = 19/68.4%F/53.8yrs
Kameda et al. (2018)	3T (GE)/PRESS/(2000/30)/96/LC model	ACC/16 ml	Glu, Glx *(NAA, Cr, mI)*	n = 60/Low back pain >6 months/61.7% F/58.8 (16) yrs/-	n = 56/62.5%F/39.5 (12.8) yrs
Niddam et al. (2011)	3T (Sie)/PRESS/(2000/30)/128/LC model	L and R Hippoc/3 ml	Glu, Glx *(Cho, Cr, mI, NAA)*	n = 15/Irritable bowel syndrome (ROME III), 53%F/36.6 (11.6)/7.2 (6.8) yrs	n = 15/66.7%F/33 (9) yrs
Niddam et al. (2018)	3T (Sie)/MRSI- Proton echo planar spectroscopic imagine sequence/(1500/30)/-/LC model	Whole Brain (ACC, Thal, Occ/8 × 8)	Glx *(Cho, Cr, mI, NAA)*	**Group 1:** n = 24/Episodic migraine (ICHD-II)/80%F/37 (7) yrs/17.2 (9.1) yrs **Group 2:** n = 25/Chronic migraine (ICHD-II)/70.8%F/33.8 (10) yrs/13.3 (9.1) yrs	n = 25/75%F/32.6 (8.3) yrs
Prescot et al. (2009)	4T (V)/2DJ resolved/(2000/30–260)/16 per TE/LC model	ACC, L Ins/8 ml	GABA, Glu, Gln *(Full basis set)*	n = 12/Acute episodic migraine/43 (11) yrs/70%F/23 yrs	n = 8/70%F/41 (9) yrs

(*continued on next page*)

Table 2 (*continued*)

	Strength (scanner)/Sequence/(TR/TE)/Avs/Processing	Region/Voxel size (ml)	Neurometabolites	Participants: Number/Pain/%Female/Age/Duration	Controls: Number/%Female/Age
Reckziegel et al. (2016)	3T (GE)/PRESS/(2500/105)/128/LC model	ACC/8–12 ml	GABA, Glu, Glx, *(NAA, tNAA, mI, tCho)*	n = 20/Knee osteoarthritis on X-ray, pain mostly constant in the last month/45%F/67 (9) yrs/7.7 (4.9) yrs	n = 19/42.1%F/59 (9) yrs
Sharma et al. (2011)	3T (Sie)/MRSI- PRESS/(1500/30)/-/LC model	L, R and Mid SSC/matrix size 16 × 16; FOV = 160 mm^2	Glx *(NAA, Cho)*	n = 11/Chronic low back pain over 4/10/-%F/33.6 (10.6) yrs/>3months	n = 11/-%F/31.4 (13.9) yrs
Siniatchkin et al. (2012)	3T (P)/PRESS/(2000/37)/128/-8 ml	Primary and Secondary Vis Cort/	Glx, GABA (GABA values not reported) *(NAA, Cr)*	n = 10/Migraine with aura (ICHD-II)/60%F/19.3 (3.4) yrs/4.2 (4.1) yrs	n = 10/60%F/20.3 (3.2) yrs
Valdes et al. (2010)	1.5T (GE)/PRESS/(1500/35)/-/LC model	L and R Amyg/3.37 ml L and R Thal/2.25 ml, L and R PFC/3.37 ml	Glu, Gln *(NAA, Cho, Cr, mI)*	n = 30/Fibromyalgia (AMR)/100%F/42.62 (8.76) yrs/151 (120) months	n = 30/100%F/43.86 (10.60) yrs
Widerstrom-Noga et al. (2013)	3T (Sie)/PRESS/(2000/30)/256/LC model	ACC/8.75 ml	Glx *(NAA, tCr, Cho, mI)*	**Group 1:** n = 31/SCI with low neuropathic pain/16.12%F/37.5 (13.4) yrs/10.6 (9.07) yrs **Group 2:** n = 19/SCI with high neuropathic pain/26.3%F/40.4 (11.8) yrs/12 (9.85) yrs	n = 24/20.8%F/34.4 (8.6) yrs
Widerstrom-Noga et al. (2015)	3T (Sie)/2D chemical shift imaging using PRESS/(2000/30)/4/LC model	L and R Thal/matrix size 8 × 8; FOV 160 mm	Glx *(NAA, Cho, mI)*	**Group 1:** n = 35/SCI with low neuropathic pain/20%F/35.7 (12.4) yrs/13.1 (9.7) yrs **Group 2:** n = 19/SCI with high neuropathic pain/15.8%F/43 (12.5) yrs/12 (9.66) yrs	n = 24/20.8%F/34.4 (8.6) yrs
Zielman et al. (2017)	7T (P)/Semi-LASER/(5000/30)/32/LC model	Vis Cor (Occ)/12 ml	Glu, Gln, Glx *(tNAA, tCr, Ins, tCho, PE, Asp)*	**Group 1:** Migraine without aura (ICHD-3b)/n = 27/51.9%F/35.1 (8.2) yrs/20.9yrs **Group 2:** Migraine with aura (ICHD-3b)/n = 23/47.8%F/35 (9.3) yrs/20.6yrs	n = 24/50%F/34.8 (8.7) yrs

P- Phillips, Sie- Siemens, GE- General Electric, V- Varian, *Likely Typo, **Not stated-taken from reference, - Not stated, L- Left, R- Right, Ant- Anterior, Post- Posterior, Mid- Midline, V- Ventral, PCG- Posterior Cingulate Gyrus, Ins- Insula, Occ- Occipital, Thal- Thalamus, APC- Anterior paracingulate cortex, Hippoc- Hippocampus, VL PFC- Ventrolateral Prefrontal Cortex, DLPFC- Dorsolateral Prefrontal Cortex, SM1- Primary sensorimotor cortex, CC- Cingulate Cortex, OFC- Orbital frontal cortex, Vis cort- Visual Cortex (Occipital Lobe), Amyg- Amygdala, SSC- Somatosensory cortex, GABA-gamma-aminobutyric acid, Glu-glutamate, Gln-glutamine, NAA- N-acetylaspartate, Cr- Creatine, mI- myoinositol, Cho- Choline, Glx-combined glutamate and glutamine, mI- myoinositol, tCho-total choline, tNAA-total N-acetylaspartate and N-acetylaspartyl glutamate, Glc-glucose, tmI- total myo-and scyllo-inositol, Lac- Lactate, Asp- Aspartate, GroPCho- Glycerophosphocholine, tCr-total creatine and phosphocreatine, phosphorylethanolamine, SCI- spinal cord injury, ICHD- The international classification of headache disorders 3 beta, IHS- International headache society, ACR- American college of Rheumatology, ROME III- Diagnostic criteria for irritable bowel syndrome.

Table 3
Assessment of spectroscopy quality using the MRS-Q tool: un-edited studies.

	Parameters					Utilisation of quality checks			Study design/Post processing				QUALITY
	>3T	Sequenceϕ	Data points	TE	ParametersΔ	Shim or FWHM	Fit error	Data visualised	Power calc	Frequency drift	Partial vol correction˅	Frequency/ Phase corrected	
As Sanie et al. (2016)	Y	Y	<i>	?	<i>	<i>	N	Y	N	N	Y	N	UNSURE
Bathel et al. (2018)	Y	Y	Y	Y	Y (Thal #)	<i>	Y	Y	N	N	NA	Y	HIGH (Thal #)
Bigal et al. (2008)	Y	<i>	<i>	Y*	<i>	<i>	<i>	N	N	N	N	<i>	UNSURE
Bridge et al. (2015)	Y	N	<i>	Y	Y	Y	Y	Y	N	Y	Y	Y	LOW
Di Pietro et al. (2018)	Y	Y	Y	Y	<i>	<i>	N	Y	N	N	NA	N	UNSURE
Fayed et al. (2010)	N	Y	<i>	Y	Y	<i>	N	Y	N	N	N	N	HIGH
Fayed et al. (2012)	N	Y	<i>	Y	Y	<i>	Y	Y	N	N	N	N	HIGH
Fayed et al. (2014)	N	Y	<i>	Y	Y	<i>	N	Y	N	N	N	N	HIGH
Feraco et al. (2011)	Y	Y	<i>	Y	N	Y	N	Y	N	N	NA	N	LOW
Forester et al. (2012)	Y	Y	<i>	Y	Y	<i>	Y	Y	N	N	Y	N	HIGH
Gerstner et al. (2012)	Y	Y	<i>	Y	<i>	<i>	N	Y	N	N	N	N	UNSURE
Gonzales de la Aleja et al. (2013)	Y	Y	<i>	Y	Y	<i>	Y	Y	N	N	Y	<i>	HIGH
Grachev et al. (2000)	N	Y	<i>	Y	<i>	<i>	N	Y	N	N	NA	N	UNSURE
Gussew et al. (2011)	Y	Y	Y	Y	<i>	Y	Y	Y	N	Y	Y	Y	UNSURE
Gustin et al. (2014)	Y	Y	Y	Y	<i>	Y	N	Y	N	N	NA	Y	UNSURE
Harfeldt et al. (2018)	Y	<i>	<i>	Y	<i>	<i>	Y	N	N	N	N	N	UNSURE
Harper et al. (2018)	Y	Y	N	Y	Y	<i>	Y	Y	N	N	Y	N	HIGH
Harris et al. (2009)	Y	Y	<i>	Y	<i>	<i>	N	Y	N	N	Y	N	UNSURE
Ito et al. (2017)	Y	Y	<i>	Y	Y	<i>	N	Y	N	N	NA	<i>	HIGH
Janetzki et al. (2016)	Y	Y	<i>	Y	<i>	<i>	N	Y	N	N	N	<i>	UNSURE
Kameda et al. (2018)	Y	Y	<i>	Y	Y	Y	Y	N	N	N	N	N	HIGH
Niddam et al. (2011)	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	N	HIGH
Niddam et al. (2018)	Y	Y	<i>	<i>	N**	Y	Y	Y	N	Y	Y	N	UNSURE
Prescot et al. (2009)	Y	Y~	Y	Y	Y	Y	Y	Y	N	N	Y	Y	HIGH
Reckziegal et al. (2016)	Y	N (Not for GABA)	N	<i>	N (Not GABA)	N	Y	Y	Y	Y	Y	N	LOW
Sharma et al. (2011)	Y	Y	<i>	Y	Y**	Y	Y	N	N	Y	Y	N	HIGH
Siniatchkin et al. (2012)	Y	Y	<i>	N	Y	<i>	N	Y	N	N	NA	Y	HIGH
Valdes et al. (2010)	N	Y	<i>	Y	<i>	<i>	Y	Y	N	N	Y	Y	UNSURE
Widerstrom et al. (2013)	Y	Y	N	Y	Y	<i>	N	N	N	N	NA	Y	HIGH
Widerstrom et al. (2015)	Y	Y	N	Y	N**	<i>	N	Y	N	N	NA	N	LOW
Zielman et al. (2017)	Y	Y	Y	Y	Y	<i>	Y	Y	Y	N	Y	Y	HIGH
% Yes	83.3	87	23	85	52	29	52	81	6	19	41	33	

ϕ = MEGA-PRESS, MEGA-semi-LASER, other GABA editing; **Data points** 1024 complex data points from 2000Hz. **Short TE** = ~20/30 ms, ***Δ*** = ***Adequate parameters 128 Av, 15 × 15 × 15 voxel, 3T; 64 Av, 20 × 20 × 20 voxel, 3T; 256 Av, 15 × 15 × 15 voxel 1.5T; 128 Av, 20 × 20 × 20 voxel, 1.5[33]. Partial volume correction*** ˅ not just grey matter correction, not required for data presented as ratios (NA), *Y = Yes, N = No, <i> insufficient information, * = extracted from cited paper, ** = MRSI, ~ = PRESS based 2DJ, ? = likely error in reporting, # = criteria not fully met.*

duplicates, 5505 titles and abstracts were screened for eligibility, with 162 studies deemed eligible for full text screening. Following full text screening, 127 studies were excluded leaving 35 studies to be included in the analysis (Fig. 1). Two of which were translated from German and Japanese prior to inclusion. The 35 studies contributed a total of 140 data sets for inclusion within the study.

3.2. Study characteristics

3.2.1. Spectroscopy

Twenty-eight studies used 3-T scanners, six studies used 1.5 T, and two single studies used 4T and 7T respectively. Some studies used both editing and non-editing: A PRESS sequence or vendor specific variation was used in 30 analyses including, three of which were implemented using 2D MRSI (Niddam et al., 2018; Sharma et al., 2011; Widerstrom-Noga et al., 2015), whilst MEGA-PRESS was used in ten analyses (Aguila et al., 2015; Bathel et al., 2018; Bednarska et al., 2019; Chan et al., 2019; Di Pietro et al., 2018; Foerster et al., 2012; Gustin et al., 2014; Harper et al., 2018; Henderson et al., 2013; Janetzki et al., 2016). Individual studies used 2DJ resolved (Prescot et al., 2009), semi-LASER (Zielman et al., 2017), STEAM (Grachev et al., 2000), SPECIAL (Bridge et al., 2015), and 3D LASER. (Bigal et al., 2008) (Table 2).

3.2.2. Neurometabolites

GABA was reported in 14 studies (Aguila et al., 2015; Bednarska et al., 2019; Bigal et al., 2008; Bridge et al., 2015; Chan et al., 2019; Di Pietro et al., 2018; Foerster et al., 2012; Grachev et al., 2000; Gustin et al., 2014; Harper et al., 2018; Henderson et al., 2013; Janetzki et al., 2016; Prescot et al., 2009; Reckziegel et al., 2016), glutamate in 16 (Bridge et al., 2015; Fayed et al., 2012, 2014; Feraco et al., 2011; Gerstner et al., 2012; Gonzales de la Aleja et al., 2013; Grachev et al., 2000; Gussew et al., 2011; Harfeldt et al., 2018; Harper et al., 2018; Harris et al., 2009; Ito et al., 2017; Kameda et al., 2018; Niddam et al., 2011; Prescot et al., 2009; Zielman et al., 2017), glutamine in eight (Gerstner et al., 2012; Gonzales de la Aleja et al., 2013; Grachev et al., 2000; Gussew et al., 2011; Harris et al., 2009; Harper et al., 2018; Prescot et al., 2009; Zielman et al., 2017) and Glx in 21 (As-Sanie et al., 2016; Bathel et al., 2018; Bednarska et al., 2019; Chan et al., 2019; Fayed et al., 2010, 2012, 2014; Feraco et al., 2011; Gerstner et al., 2012; Gussew et al., 2011; Harper et al., 2018; Harris et al., 2009; Ito et al., 2017; Janetzki et al., 2016; Kameda et al., 2018; Niddam et al., 2011, 2018; Reckziegel et al., 2016; Sharma et al., 2011; Siniatchkin et al., 2012; Valdes et al., 2010; Widerstrom-Noga et al., 2013, 2015; Zielman et al., 2017). None of the included studies used macromolecular suppression and therefore are more likely to reflect GABA+, however for the purpose of this study we refer to this as GABA. The included studies reported level of neurometabolites as either Institutional units, absolute concentration (e.g. mmol/l), ratios relative to Cr, or ratios relative to NAA. (Tables 3 and 4). Raw data was not presented for four studies (Bridge et al., 2015; Kameda et al., 2018; Niddam et al., 2018; Widerstrom-Noga et al., 2015) and therefore required callipers for extraction from graphical representations.

3.2.3. Pain conditions

Migraine was compared to control participants in 11 studies, (migraine sub-classifications studied included two acute episodic migraine (Niddam et al., 2018; Prescot et al., 2009), one chronic migraine (Niddam et al., 2018), four migraine without aura (Aguila et al., 2015; Bathel et al., 2018; Bigal et al., 2008; Zielman et al., 2017), four with aura (Bigal et al., 2008; Bridge et al., 2015; Siniatchkin et al., 2012; Zielman et al., 2017) and three mixed (Chan et al., 2019; Fayed et al., 2014; Gonzales de la Aleja et al., 2013). Musculoskeletal pain (five chronic low back pain (Grachev et al., 2000; Gussew et al., 2011; Janetzki et al., 2016; Kameda et al., 2018; Sharma et al., 2011), one knee osteoarthritis (Reckziegel et al., 2016), two temporomandibular joint pain (Gerstner et al., 2012; Harfeldt et al., 2018)) was compared to control participants in eight studies. Chronic pain syndromes (seven fibromyalgia (Fayed et al., 2010; Fayed et al., 2012; Feraco et al., 2011; Foerster et al., 2012; Harfeldt et al., 2018; Harris et al., 2009; Valdes et al., 2010), two somatoform disorder (Fayed et al., 2012, 2014), one chronic widespread pain (Ito et al., 2017)) were compared to control participants in nine studies and the remaining miscellaneous studies (three spinal cord injury with neuropathic pain (Gustin et al., 2014; Widerstrom-Noga et al., 2013; Widerstrom-Noga et al., 2015), one pelvic pain with and without endometriosis (As-Sanie et al., 2016), one urological chronic pain (Harper et al., 2018), three with facial neuropathic pain (Di Pietro et al., 2018; Fayed et al., 2014; Henderson et al., 2013), two painful irritable bowel syndrome (Bednarska et al., 2019; Niddam et al., 2011) were compared to control participants in nine studies.

3.2.4. Brain regions

Neurometabolites were investigated across 12 brain regions including; amygdala, anterior cingulate cortex (ACC), anterior frontal cortex, cingulate cortex, hippocampus, insula, occipital lobe (including visual cortex), prefrontal gyrus, posterior cingulate gyrus (PCG), sensorimotor-cortex, somatosensory cortex, thalamus (Fig. 2). Thirteen studies reported data from more than one brain region for the review's primary analysis.

3.3. Quality assessment

3.3.1. AXIS

The quality varied from seven studies (Aguila et al., 2015; As-Sanie et al., 2016; Bathel et al., 2018; Di Pietro et al., 2018; Niddam et al., 2011; Valdes et al., 2010; Zielman et al., 2017) satisfying over 80% of the criteria to four studies (Bridge et al., 2015; Fayed et al., 2014; Harper et al., 2018; Prescot et al., 2009) satisfying only 50% of criteria. Quality metrics reported by all studies were the measure used to determine statistical significance, clear aims, and ethical approval or consent. In contrast, few studies justified sample size (5/35, 14.28%) or categorised non-responders (5/35, 14.3%) (Fig. 3). Furthermore, the control of confounding variables such as limiting the inclusion of participants with other comorbidities (25/35, 71.4%), controlling for medications (21/35, 60%), and controlling other confounders (15/35, 42.9%) e.g. smoking, time of day or menstrual cycle were inconsistently addressed across the studies.

3.3.2. Quality assessment: spectroscopy (MRS-Q)

Most sequences used in the studies (n = 21/41 from 35 studies, 51.2%) did not report using adequate spectroscopy parameters. For example, adequate parameters were used in 20% (n = 2/10) of edited, and 52% (n = 16/31) of unedited studies. Of these, 12% (edited) and 35.5% (unedited) studies did not record sufficient details to determine the overall quality of spectroscopy and allow for reproducing these studies. Details not reported included averages, voxel size and scanner strength. Of the 22/41 sequences in studies that did report the parameters used, two (Bridge et al., 2015; Reckziegel et al., 2016) did not use an appropriate sequence to detect all reported neurometabolites of interest. Of the studies using sequences edited specifically for GABA (n = 8/39), 50% (n = 4/8) used the recommended number of averages and 25% (n = 2/8) used an appropriately sized voxel for all regions (Tables 3 and 4).

3.4. Results: primary aim: neurometabolites between pain conditions

3.4.1. GABA level across pain conditions

The level of GABA in migraine was significantly increased compared with controls (Hedge's G 0.394, 95%CI: 0.095 to 0.0.693, $i^2 = 0$). In contrast the level of GABA was significantly decreased in three of the six miscellaneous studies investigating pelvic pain, trigeminal neuralgia and painful spinal cord injury compared to controls. GABA level was not significantly different in musculoskeletal pain (Hedge's G −0.15, 95%CI

−0.44 to 0.15, $i^2 = 0$), or chronic pain syndromes (Hedge's G −0.08, 95% CI −1.61 to 1.46, $i^2 = 89.479$) compared to controls (Fig. 4).

3.4.2. Glutamate level across pain conditions

The level of glutamate in migraine demonstrated a significant increase compared with controls (Hedges G: 0.45, 95% CI 0.17 to 0.73, $i^2 = 56.79$). In contrast glutamate level was significantly decreased in musculoskeletal conditions compared with controls (Hedge's G −0.262, 95%CI −0.481 to −0.043, $i^2 = 0$). There was no significant difference between glutamate level in either chronic pain syndromes or any individual study in the miscellaneous pain category compared with controls (Fig. 5).

3.4.3. Glutamine level across pain conditions

The level of glutamine was not significantly different between any pain condition and controls. Data compared with controls were migraine (Hedge's G: 0.309, 95%CI −0.027 to 0.646, $i^2 = 57.45$) musculoskeletal pain (Hedge's G: −0.124, 95%CI −0.627 to 0.379, $i^2 = 58.87$), chronic pain syndromes (Hedge's G: 0.255, 95%CI −0.035 to 0.857 $i^2 = 36.25$) or the single study in the miscellaneous pain category (Fig. 6).

3.4.4. Glx level across pain conditions

The level of Glx was significantly increased in chronic pain syndromes compared with controls (Hedge's G 0.552, 95%CI: 0.332 to 0.773, $i^2 = 56.97$). This was not evident in any other pain group compared with controls. Data compared with controls were migraine (Hedge's G 0.14, 95%CI: −0.16 to 0.43, $i^2 = 79.14$) musculoskeletal pain (Hedge's G 0.346, 95%CI: −0.169 to 0.861, $i^2 = 79.8$) and studies of miscellaneous pain that had a wide spread of results including a significant decrease of Glx in four studies (two of spinal cord injury, and two of irritable bowel syndrome), and a significant increase in three studies (two studies of pelvic pain with and without endometriosis and one of trigeminal neuralgia (Fig. 7).

3.5. Secondary aims

3.5.1. Does spectroscopy quality influence brain neurometabolite levels

Secondary analysis was performed using 64/137 (47%) data sets from 19/33 (57.6%) studies that reported using adequate spectroscopy parameters (Tables 3 and 4). The analysis using only high-quality studies, demonstrated that GABA remained significantly increased in migraine (Hedge's G 0.394, 95%CI: 0.050 to 0.739, $i^2 = 6.048$) as per the original analysis. Similarly, as demonstrated in the original analysis, there was no difference in GABA levels in people with chronic pain syndromes compared to controls. There were no high-quality spectroscopy studies that investigated GABA levels for musculoskeletal pain.

When only high-quality studies were analysed, glutamate levels remained significantly increased in people with migraine (Hedge's G 0.443, 95%CI: 0.154 to 0.732, $i^2 = 56.79$), and decreased in a single study of musculoskeletal pain (Hedge's G −0.387, 95%CI: −0.752 to −0.022) compared with controls. There remained no differences in glutamate levels in chronic pain syndromes compared with controls in the high-quality studies. Glutamine continued to show no significant level changes in migraine and there were no high-quality studies for musculoskeletal pain, and chronic pain syndromes.

Glx was the only neurometabolite to demonstrate a difference when only high-quality studies were used in the meta-analysis. Whilst the original analysis demonstrated a non-significant trend towards an increase in Glx levels in people with migraine (Hedge's G 0.135, 95%CI: −0.161 to 0.432, $i^2 = 79.14$), the high-quality studies demonstrated a significant increase (Hedge's G 0.657, 95%CI: 0.417 to 0.898, $i^2 = 12.01$). The increase in Glx level in chronic pain syndromes compared to control remained significant when only high-quality studies were considered (Hedge's G 0.508, 95%CI: 0.292 to 0.723, $i^2 = 6.1$). Glx levels in musculoskeletal pain, were not different to the controls in the high-quality studies in line with the original analysis.

Table 4
Assessment of spectroscopy quality using the MRS-Q tool: edited studies.

	Parameters						Utilisation of quality checks			Study design/Post processing				QUALITY
	>3T	Sequence ϕ	Avs	TE	Voxel size	Parameters Δ	Shim or FWHM	Fit error	Data visualised	Power calc	Frequency drift	Partial vol correction˅	Frequency/ Phase corrected	
Aguila et al. (2015)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N (GM)	Y	HIGH
Bathel et al. (2018)	Y	Y	Y	Y	Y (#Occ)	Y (#Occ)	<i>	Y	Y	N	N	NA	Y	HIGH (#Occ)
Bednarska et al. (2019)	Y	Y	<i>	Y	Y	<i>	<i>	N	Y	N	N	N	Y	UNSURE
Chan et al. (2019)	Y	Y*	N	Y	Y	N	<i>	Y	Y	Y	N	Y	Y	LOW
Di Pietro et al. (2018)	Y	Y	N	Y	N	N	<i>	N	Y	N	N	NA	N	LOW
Foerester et al. (2012)	Y	Y	Y	Y	Y	Y	<i>	Y	Y	N	N	Y	N	HIGH
Gustin et al. (2014)	Y	Y	N	Y	N	N	Y	N	Y	N	N	NA	Y	LOW
Harper et al. (2018)	Y	Y	Y	Y	N	N	<i>	Y	Y	N	N	Y	N	LOW
Henderson et al. (2013)	Y	Y	<i>	Y	N	N	Y	N	Y	N	N	NA	Y	LOW
Janetzki et al. (2016)	Y	Y	<i>	Y	<i>	<i>	<i>	N	Y	N	N	NA	<i>	UNSURE
% YES	100	100	40	100	50	30	20	50	100	20	10	30	56	

ϕ = PRESS/semi-LASER (or vendor specific) or STEAM; Δ = Averages over 240, TE GABA+ 68, GABA 80 (Siemens 68) voxel size around 27 ml.
Y= Yes, N = No, <i> insufficient information, (Cr) = Creatine ratio used instead of partial volume, GM = only corrected for grey matter, # = criteria not fully met, * typo-study reported using point resolved spectroscopy but was later apparent that it was MEGA-PRESS.

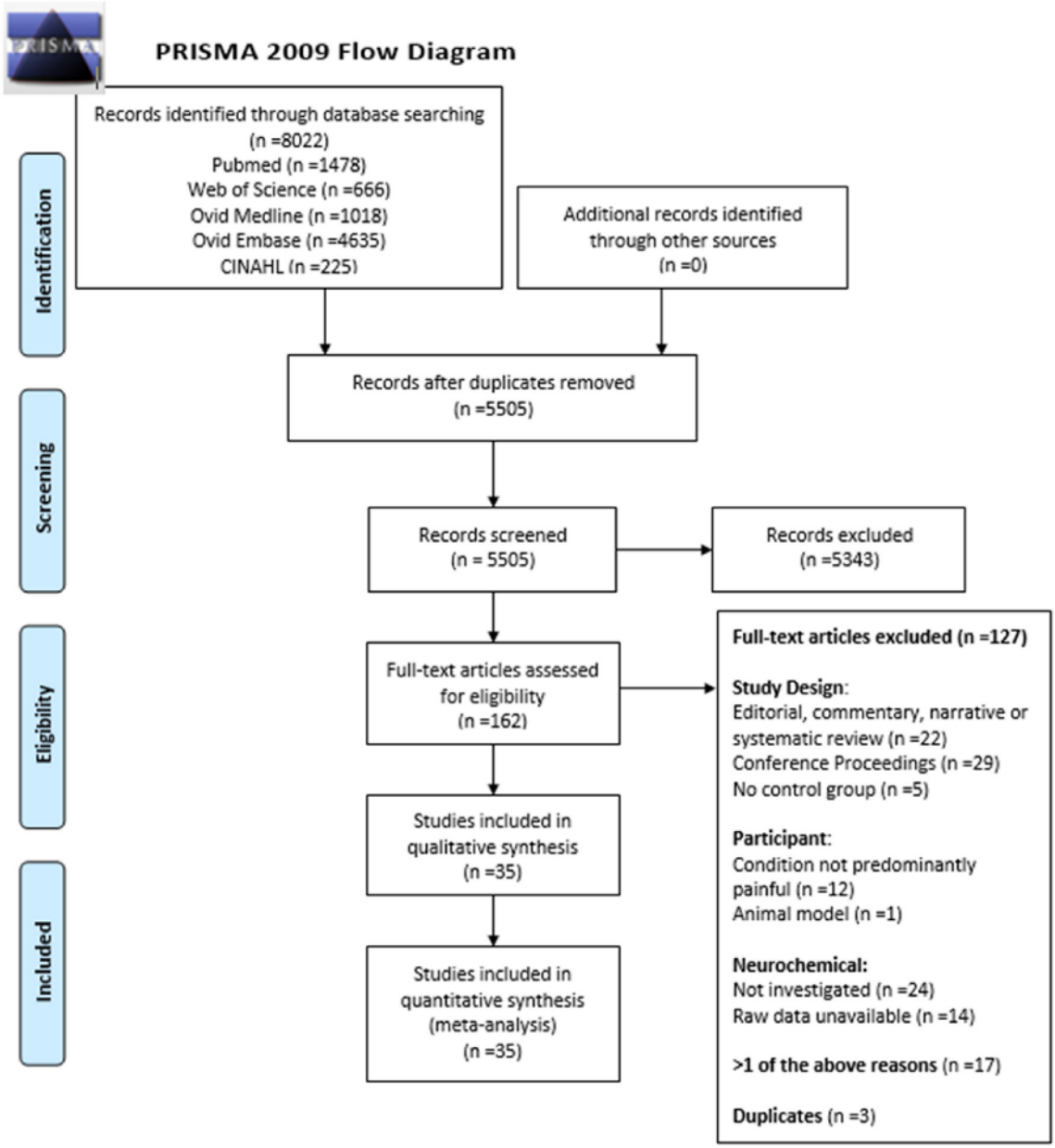

Fig. 1. PRISMA flow diagram (Moher et al., 2015).

3.5.2. Does brain region influence brain neurometabolite levels

There was insufficient data from the majority of brain regions to answer the question; are brain neurometabolite changes influenced by brain region. Across all neurometabolites and pain conditions, six brain regions demonstrated significant differences in neurometabolite level between pain group and control (ACC, PCG, occipital lobe, thalamus, hippocampus, insula). The number of data sets contributing to these results varied from one single data set to 11, with the occipital lobe providing the most comparisons. Pooled data from 11 data sets investigating the occipital lobe demonstrated a significant increase in level of Glx (Hedge's G 0.452, 95%CI: 0.184 to 0.721, $i^2 = 53.12$) and glutamate (Hedge's G 0.572, 95%CI: 0.230–0.904, $i^2 = 46.56$) in people with migraine compared with control. However, there were insufficient data to compare occipital region with other regions in the brain and the occipital region was not studied in any other pain condition other than migraine.

The ACC was the only region to be studied across all neurometabolites and pain conditions. Single studies demonstrated a significant increase in glutamine level in the ACC in migraine (Hedge's G 1.148, 95%CI: 0.214 to 2.083) and conversely a decrease in glutamine level in the ACC in musculoskeletal pain (Hedge's G −1.102, 95%CI: 2.008 to −0.196) compared with controls. Glx levels in the ACC were significantly increased in chronic pain syndromes (Hedge's G 0.308, 95%CI: 0.308 to 1.053) compared with controls. All other neurometabolites in other pain conditions were insignificant. There were insufficient data to compare levels of neurometabolites between the ACC and other brain region.

When brain neurometabolite levels were averaged across brain regions, there was no significant change except glutamine in migraine, which remained increased compared to control but reached statistical significance (Hedge's G 0.350, 95%CI: 0.021 to 0.680).

4. Discussion

The meta-analyses presented here demonstrate that different pain conditions appear to have unique neurometabolite signatures. Individuals with migraine appeared to have generally increased levels of

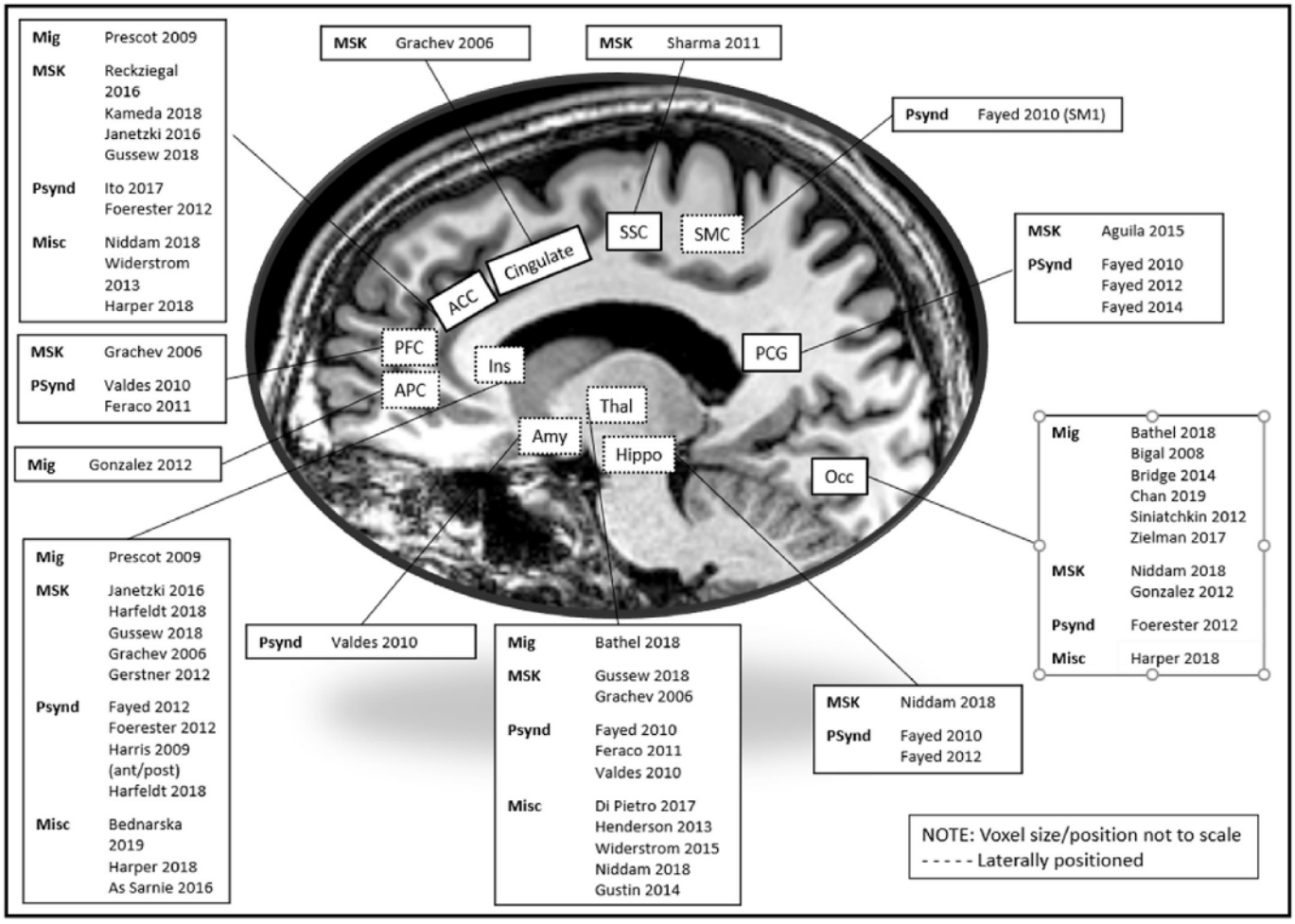

Fig. 2. Brain Regions examined in included studies.

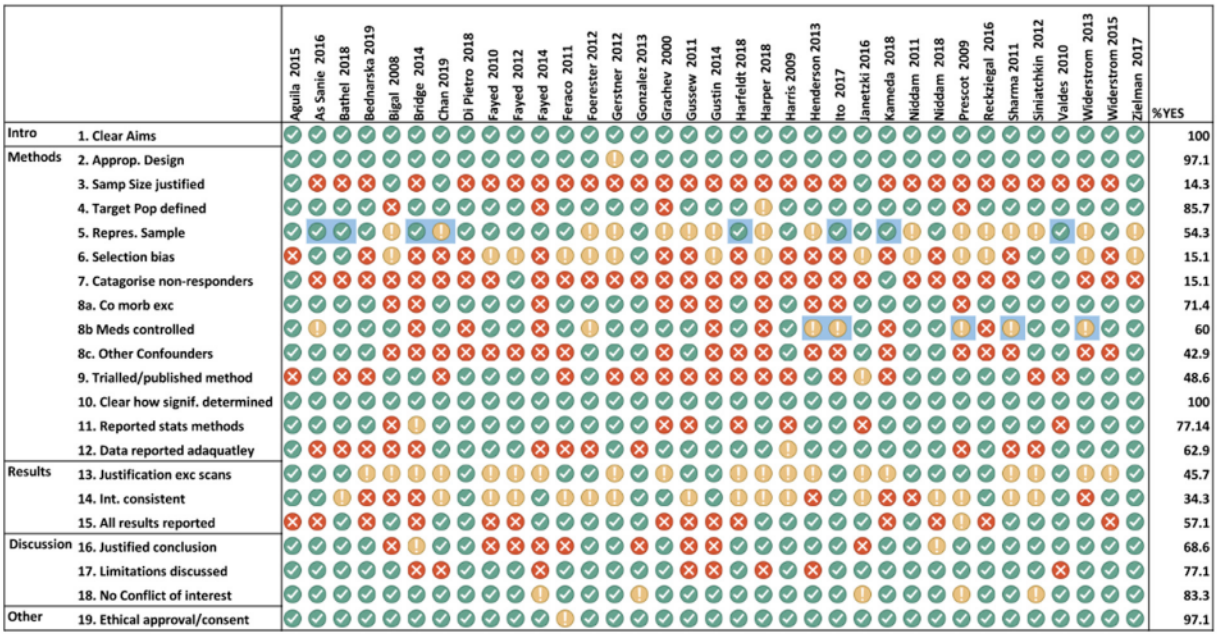

Fig. 3. AXIS methodological quality.

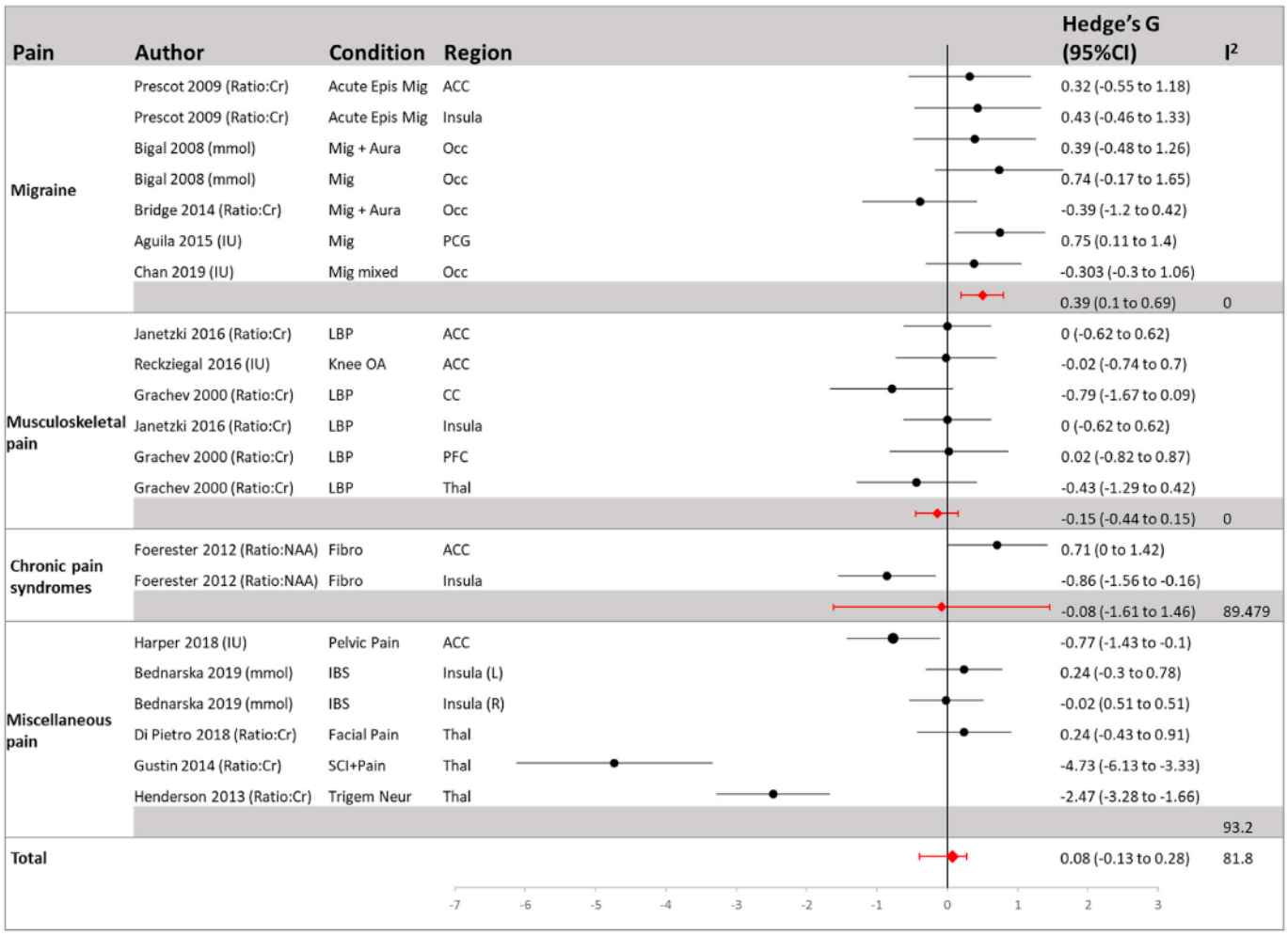

Fig. 4. GABA: analysed by pain conditions.

brain neurometabolites (GABA, Glu. Glx), whilst those with the other pain conditions studied varied in their neurometabolite profile. Four unique neurometabolite signatures were observed across the different pain conditions. Some of these observations are consistent with current theories in chronic pain, others are divergent from them. Hypotheses for these different observations are discussed below. We also discuss how results may be influenced by factors such as the quality of reporting and brain region investigated. This review also highlights that the quality of reporting 1H-MRS acquisition and methods is generally poor and calls for the introduction of a standardized reporting tool.

The neurometabolite signature observed in people with migraine appears to be unique, people with migraine demonstrated increased levels of glutamate and GABA compared to control participants, which was not seen in other conditions. One plausible explanation for higher glutamate levels occurring in migraine and not in other pain conditions could be cortical spreading depression, a process uniquely associated with transient neurological disorders such as migraine and epilepsy (Cozzolino et al., 2018). Cortical spreading depression is characterized as a wave of excitation, followed by inhibition which spreads across the brain. High levels of glutamate have been hypothesized to initiate this process (Charles and Baca, 2013; Cozzolino et al., 2018). The observed increase in inhibitory GABA however is more difficult to explain (Aguila et al., 2015; Bigal et al., 2008). Proposed hypotheses include that GABA has a protective role in suppressing headaches (Bigal et al., 2008), or that increased GABA levels reflect a homeostatic response to the increased glutamate through the GABA metabolic pathway (Pearl et al., 2006). Alternatively, increased GABA may reflect a pathophysiological mechanism of migraine which has yet to be fully explained. For example GABA may have a role in the regulation of vasodilation (Kocharyan et al., 2008), or with neurogenic inflammation seen in migraine (Palmer et al., 1994).

It remains unclear exactly what mechanisms underlie the findings of increased GABA and Glu in migraine. The downside of MRS is that there is no specificity as to what pool of GABA is being measured. MRS measures the presynaptic pool of GABA as a neurotransmitter, and studies have shown that the GABA measured with MRS is most related to GAD1, the gene encoding for GAD67 which is predominantly present in the soma (Marenco et al., 2010). Therefore, GABA is generally thought to reflect 'inhibitory tone' (Rae, 2014). Increased GABA may be a response to increased excitation and indeed, several studies (Diener et al., 2015) suggest drugs targeting GABAA or GABAB-receptor function may be promising as treatment for pain disorders, including migraine. Endogenous increases in GABA could reflect a similar mechanism to increased Glu. However, it is possible that dysfunctional GABA signaling through GABA receptors plays a key role in the emergence of migraine; Studies have implicated polymorphisms in genes encoding for GABA receptor subunits in the migraine (Garcia-Martin et al., 2018). Reduced GABA-receptor function could lead to hyperexcitability of both inhibitory and excitatory neurons and thus, increased neurotransmitter levels.

In contrast, people with chronic pain syndromes (e.g. fibromyalgia) demonstrated an imbalance between the level of the inhibitory and excitatory neurometabolites. An imbalance in neurometabolites have been frequently hypothesized as a mechanism underlying chronic pain (Chang et al., 2013; Sanaei Nezhad et al., 2017). People with chronic

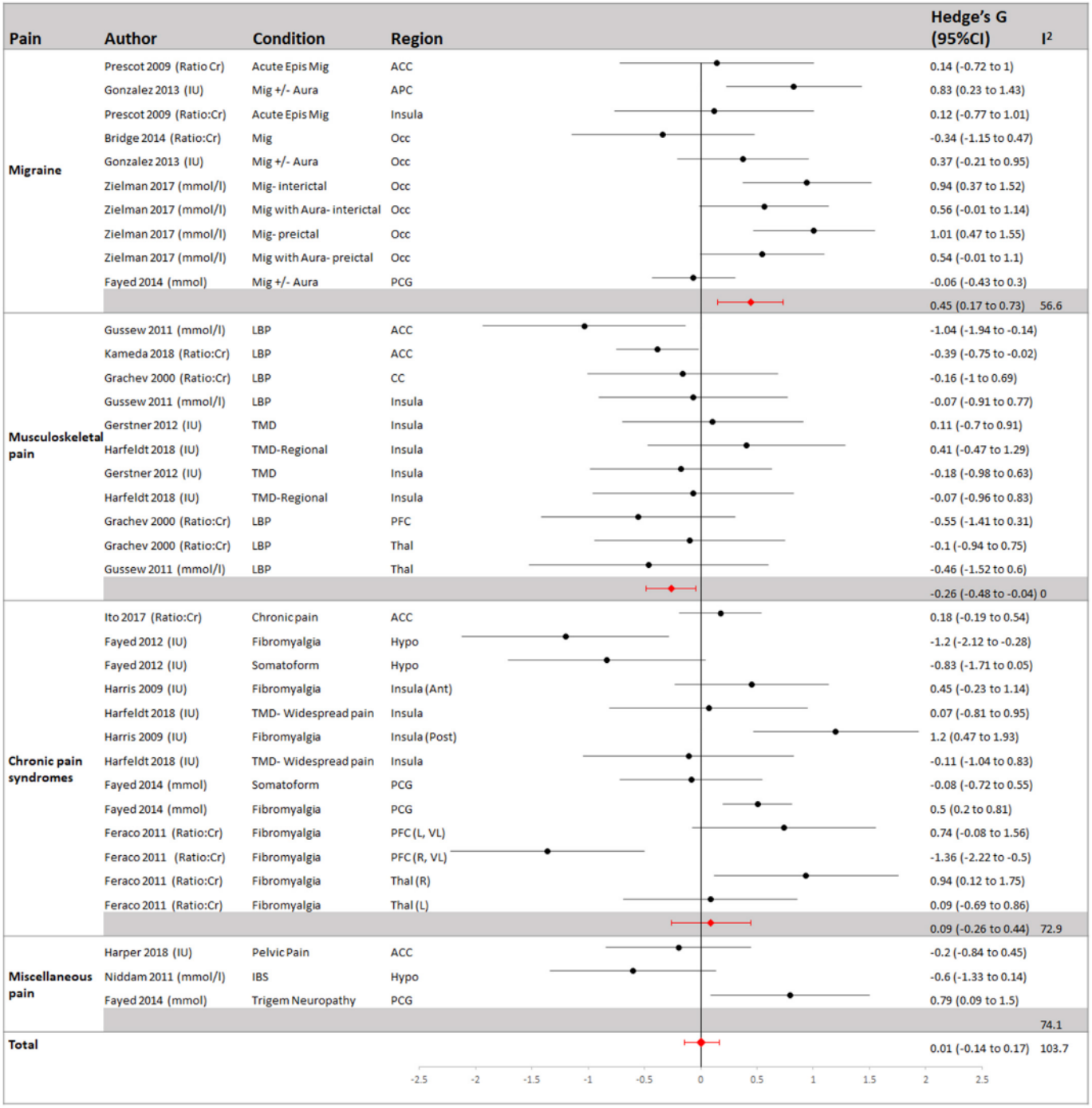

Fig. 5. Glutamate: analysed by pain conditions.

pain syndromes demonstrated an increase in excitatory Glx with no difference in inhibitory GABA. This neurometabolite pattern has been associated with increased pain catastrophizing (Fayed et al., 2012), suggesting that increased Glx in conditions such as fibromyalgia could reflect the psychological aspects of living with a widespread chronic pain syndrome. It has been suggested that the balance of excitatory and inhibitory tone and its relationship with pain could be explored through ratios such as GABA to Glutamate. This was not investigated within this review, but may be considered in future studies, to better understand the relationship between excitation and inhibition in pain conditions.

Musculoskeletal conditions also demonstrated a unique neurometabolite signature, with a significant decrease in glutamate. However, only one of the eleven studies used sufficient acquisition parameters such that this result requires further confirmation. In summary, our observations together with known observations in the literature suggest there are distinct neurometabolite signatures for different pain conditions, which potentially allows for specific disease biomarkers.

Glutamine did not demonstrate significant changes across any of the pain conditions in the primary analysis. Difficulties in quantifying Glutamine have been reported, and therefore it is often not reported alone, except in cases of significant elevation, such as hepatic encephalopathy (Rama Rao et al., 2012). Glutamine's contribution to the Glx signal is not fully appreciated and can be problematic in conditions, where the Glu and Gln levels change in opposite directions (Sanaei Nezhad et al., 2017). To overcome this issue study of the Glu/Gln ratio has been recommended. Whilst this was not within the scope of this

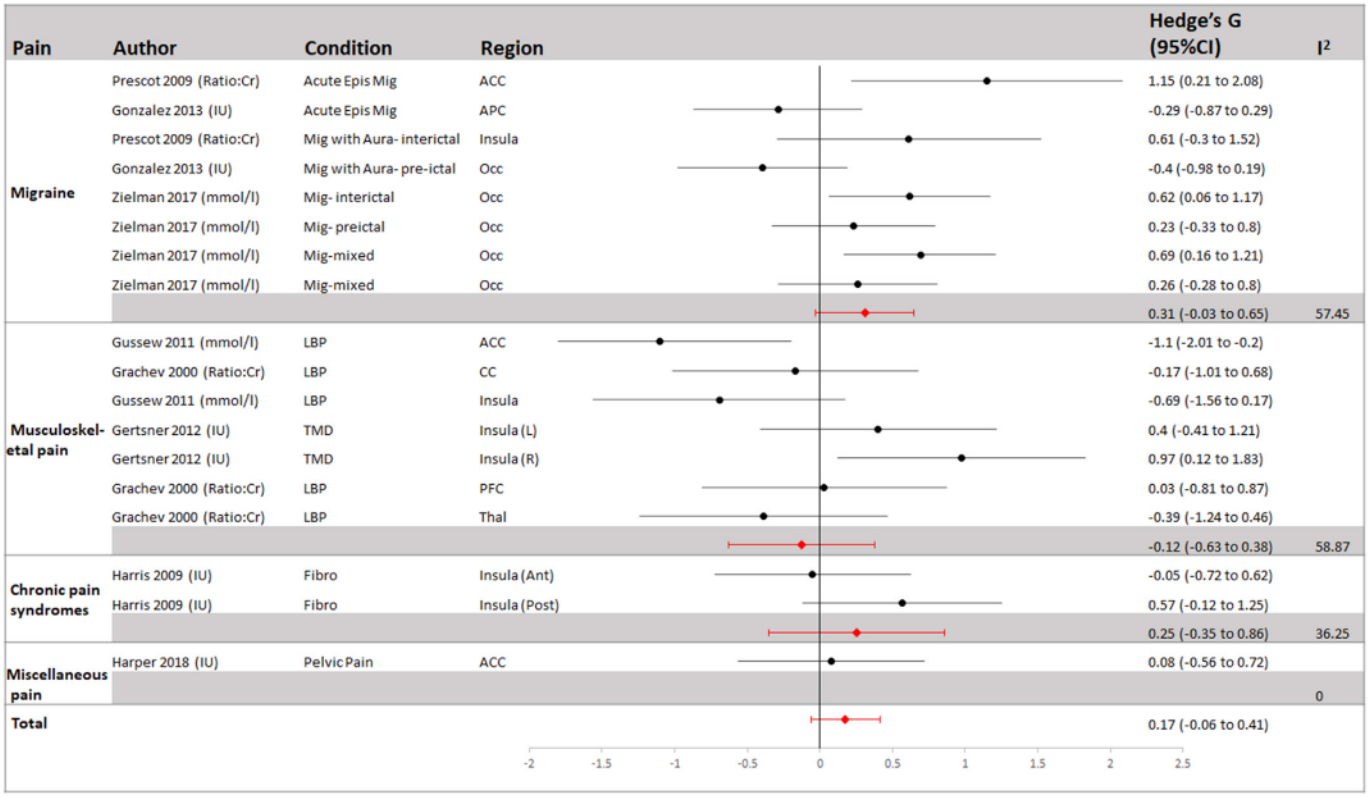

Fig. 6. Glutamine: analysed by pain conditions.

review, future studies may consider this approach to gain a better insight into the nature of this relationship in pain conditions.

Our MRS quality appraisal undertaken in this systematic review suggests that the reporting of MR spectroscopy parameters could be improved. One-third of all studies did not report key MRS parameters including the use of an adequately sized voxel, scanner strength and number of acquisitions. Reporting in studies of GABA in musculoskeletal pain, would particularly benefit from improvement, where none of the included studies documented these three key parameters required to reproduce or evaluate the study. A common methodological limitation in the spectroscopy studies was not controlling or reporting potential confounders such as medication use (Cai et al., 2012; Kuzniecky et al., 2002; Monteleone et al., 1990), smoking status or substance use (Schulte et al., 2017), menstrual phase (De Bondt et al., 2015; Epperson et al., 2002, 2005) or alcohol intake (Meyerhoff et al., 2018). The lack of detail makes it difficult to pool data in meta-analysis such as these and to be certain about accuracy of reported results in individual studies.

Despite the paucity of reporting, our sensitivity analysis suggests that adequate spectroscopy parameters were likely used in the majority of studies. This notion is supported given that results were mostly unchanged in the sensitivity analysis compared with the original analysis. A call to improve reporting has been made in other research designs and imaging modalities. This has led to the successful introduction of checklists such as PRISMA (Moher et al., 2015) in systematic reviews, and the CONSORT (Schulz et al., 2010) in randomized controlled trials and more specifically in functional MRI (Poldrack et al., 2008). Whilst there have been three white papers recommending the optimal spectroscopy parameters for use in MEGA-PRESS (Mullins et al., 2014), PRESS (Wilson et al., 2019) and Universal (Saleh et al., 2019) this has yet to be translated into a standardized methodological reporting tool. We believe the MRS-Q, introduced and developed in this study is an important first step. Both our finding (only 46% of studies reporting using adequate parameters) and the call to improve reporting in other fields suggests the need for the field of MRS to develop a standardized reporting tool. We propose the MRS-Q could be further validated for this purpose.

There was insufficient data to establish whether brain region influenced differences in neurometabolite levels. The results presented here demonstrate that there were inconsistencies in voxel naming, shaping and positioning. An example is in the ACC where several studies positioned a long rectangular voxel dorsally along the corpus collosum (Gussew et al., 2011; Widerstrom-Noga et al., 2013), yet others used a shorter voxel positioned rostrally (Harper et al., 2018; Prescot et al., 2009; Reckziegel et al., 2016), without adjusting the nomenclature accordingly. While we aimed to pool data based on brain region within pain groups, there were insufficient data to do so. The most frequently studied brain region was the occipital lobe in people with migraine. Pooled results for the occipital lobe demonstrated a significant increase in level of Glx and glutamate in migraine compared to controls. The occipital lobe has been frequently studied in both headache and mental health studies partially owing to the high-quality spectra that can be obtained compared with other brain regions (Puts and Edden, 2012). Hence, the significant findings found in people with migraine may be due to the more homogenous field allowing more consistent findings, resulting in narrower confidence intervals, rather than the region being clinically different from other regions. Nonetheless, for people with migraine, the occipital lobe may be relevant to study, due to its's role in migraine with aura (Charles and Brennan, 2010; Hadjikhani et al., 2001). Despite these observations, comparison of brain neurometabolites between brain regions requires further primary studies.

There are several limitations that need to be considered when interpreting the findings of this review. Our meta-analyses pooled results from studies that reported neurometabolite levels using absolute concentrations, institutional units and ratios. This firstly assumes these measures are reflecting the same variable, and in the case of ratios and institutional units assumes the creatine and water remain stable. Whilst there is some evidence that the denominator neurometabolite, most commonly creatine, is indeed stable across various conditions including pain (Chang

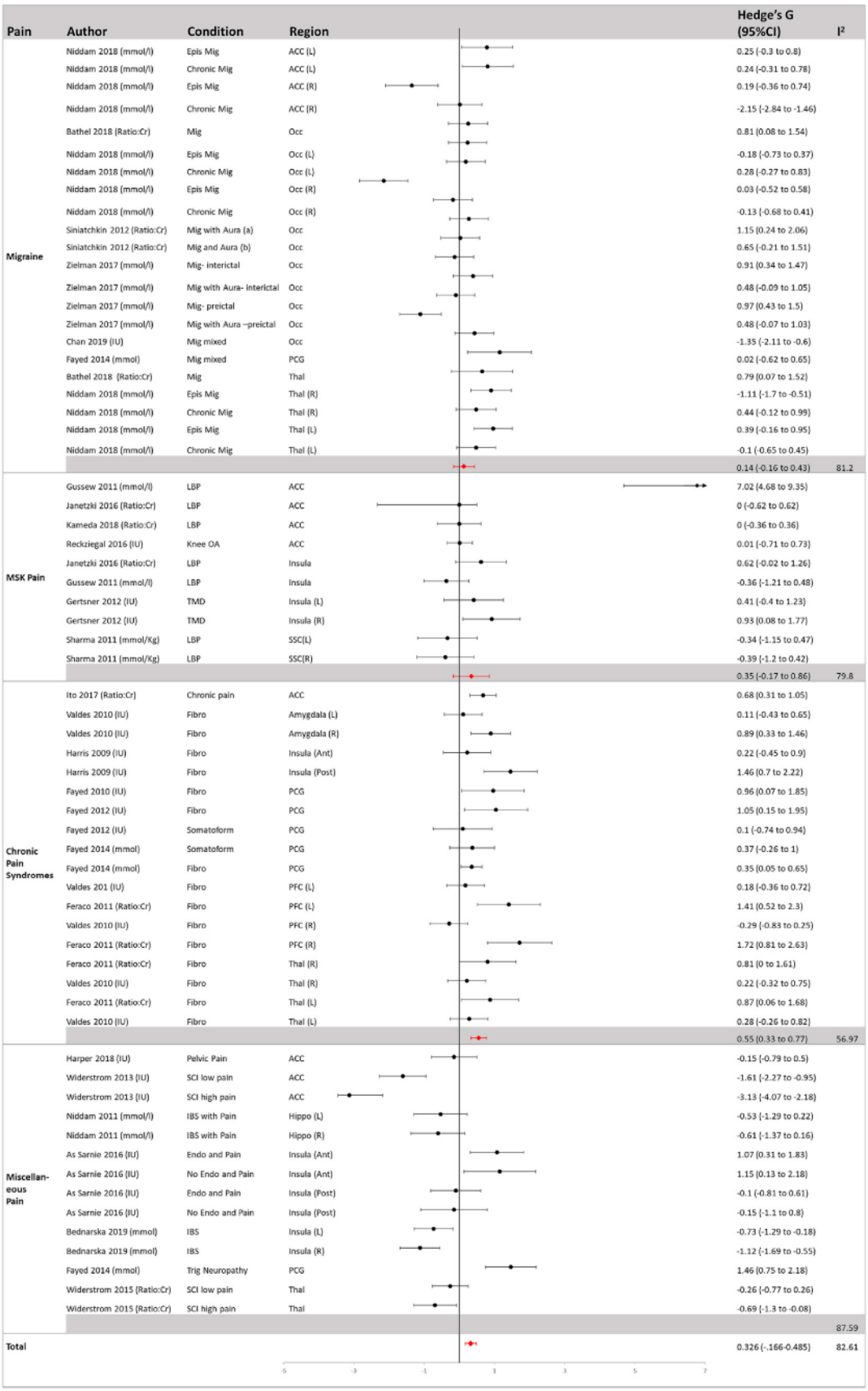

Fig. 7. Glx: Analysed by pain conditions.

et al., 2013; Govindaraju et al., 2000; Gussew et al., 2011) there still remains some uncertainty (Rae, 2014). Steen et al. (2005) were able to demonstrate equivalence between studies measuring both ratios and absolute values, further supporting their inclusion in the meta-analysis. Secondly our primary analysis assumed independence of brain regions and included data from different brain regions of the same participants, which may cause over inflation of results. Therefore, we conducted a post-hoc meta-analysis (not shown) akin to Schur et al. (2016) and Luykx et al. (2012) and demonstrated that averaging brain neurometabolite concentration across all brain regions had minimal effect to the overall results with the exception of glutamine in migraine. Finally, our primary analysis included studies regardless of quality, the sensitivity analysis used only studies that reported using acquisition parameters that satisfied minimal best practice as determined by published clinical consensus (Mullins et al., 2014; Wilson et al., 2019).

The accuracy of quantification of GABA and Glutamate is continually developing, and we can expect to see considerable advances in the field with improved methods of macromolecule suppression, better analysis techniques, and further insight into the application of partial volume correction. Whilst we acknowledge these aspects can create heterogeneity and variation in outcome measures the synthesis of information remains important to help inform future directions in biomarker and pain research.

In conclusion this meta-analysis serves to catalog what is known in the field of excitatory and inhibitory neurometabolites in pain conditions. Furthermore, it provides evidence that unique neurometabolite signatures may exist in different pain conditions. The main limitation in the field of spectroscopy is failure to adequately report acquisition parameters and calls for the development and integration of a standardized reporting tool for magnetic resonance spectroscopy research, allowing for improved reproducibility and validation of prior work.

Declaration of competing interest

Researchers Trudy Rebbeck, Maria Eliza Aguila, and Andrew Leaver were authors of a paper included in the review (Aguila et al., 2015). These three authors had no role in the data extraction or quality assessment of any of the included papers. No other competing interests to disclose.

Author contribution

AP, TR, AL conceived the study idea and designed the study protocol; AP carried out the searches; AP, TR, JW, MA, AL screened studies for inclusion; AP, JW, NP completed data extraction; AP, NP designed and developed the MRS-Q; AP, NP, JW assessed quality of studies using AXIS and MRS-Q; AP conducted meta-analysis; AP, TR, NP, AL wrote and edited manuscript.

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Julia Watson Queensland University of Technology - Translational Research Institute (TRI) Radiographer Research Scholarship, Australia.

Appendix 1. Search strategy from OVID

Mesh	Keyword
gamma-aminobutyric acid	gaba.mp
glutamic acid	glutamate.mp
glutamine	Glx
brain chemistry	brain adj 3 chemistry.mp
neurotransmitter agents	neurotransmitter*
	neurochemical*
	metabolite*
	brain metabolite*
	neurometabolite*
AND	
spectrum analysis	spectroscop*.mp.
magnetic resonance imaging	(magnetic resonance and (imag* or spectroscop*)).mp.
magnetic resonance spectroscopy	magnetic resonance spectroscopy.mp
proton magnetic resonancy spectroscopy	proton magnetic resonancy spectroscopy.mp
	mega press
	1 hmrs
	1h-mrs
	in-vivo mrs
	Mr
	Mrs
	mr-specto*
	nmr
	in-vivo nmr
AND	
pain- exp	pain
migraine disorders	migraine.mp
back pain exp	back pain
low back pain	low back pain
fibromyalgia	fibromyalgia.mp
chronic pain	chronic pain
headache	headache
headache disorders primary exp	

(continued on next column)

(*continued*)

Mesh	Keyword
headache disorders, secondary exp	
migraine with aura	migraine with aura
migraine without aura	migraine without aura
musculoskeletal pain exp	musculoskeletal pain
whiplash injuries	whiplash
cancer pain exp	cancer pain.mp
wounds and injuries exp	
peripheral nervous system exp	
trauma nervous system	

Appendix 2. Data extraction sheets

Data extraction sheet 1: Bibliometric data, and clinical characteristics.

Study #	Study	Country	Funding Source	Prospectively Registered	Mean Age	Age: if other measure used HC (Healthy control):Mean, SD; Patient group: Mean, SD	Female (%)	Pain Category	Description of Pain Condition	Subjects recruited From	Duration of Illness (months)	Excluded Comorbidities	Co-morb Comments	Meds Stopped?	Meds Comments	Pain Score	Other Outcomes	Sx

Data extraction sheet 2: Spectroscopy parameters.

Study	Scanner Make	Scanner Strength	Head Coil	Sequance	Brain Region	Voxel size	TR/TE (ms)	Averages	Number of Points	Spectral Width	Shim (Hz)	Quantification	Voxel based morphometry	Analysis Software	Metabolites

Data extraction sheet 3: Results.

Study	Pain Condition	Metabolite	Region	Pain									Control									Measure	Std diff means	p-value	Comments
				N	Mean	SD	SEM	Median	IQR-Low	IQR-High	Range	Lower 95% CI	Upper 95% CI	N	Mean	SD	SEM	Median	IQR-Lower	IQR-Higher	Range	Lower 95% CI	Upper 95% CI	Unit	

Appendix 3. Modified AXIS marking sheet-adapted from Downes et al., 2016

Questions				
Introduction		Yes	No	Don't Know/Comment
1	Were the aims/objectives of the study clear?			
Methods				
2	Was the study design appropriate for the stated aim(s)?			
3	Was the sample size justified?			
4	Was the target/reference population clearly defined?			
5	Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?			
6	Was the selection process likely to select subject/participants that were representative of the target/reference population under investigation?			
7	Were measures undertaken to address and categorise non-responders?			
8	Co-morbidities Excluded Were meds stopped/restricted/or adjusted for Were other confounders accounted for			
9				

(*continued on next column*)

(*continued*)

Questions	
	Were the risk factors and outcome variables measured correctly using instruments/ measurements that had been trialled, piloted or published previously
10	Is it clear what was used to determine statistical significance and/or precision estimates? (e.g. p-values, confidence intervals)
11	Were the methods (including statistical methods) sufficiently described to enable them to be repeated?
Results	
12	Were the basic data adequately described?
13	Was there a full justification of any scans excluded from the analysis
14	Were the results internally consistent?
15	Were the results presented for all the analyses described in the methods?
Discussion	
16	Were the authors discussions and conclusions justified by the results?
17	Were the limitations of the study discussed?
Other	
18	Were there any funding sources or conflicts of interest that may affect the author's interpretation of the results?
19	Was ethical approval or consent of participants attained?

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CHAPTER TWO: SUPPLEMENT ONE

The MRS-Q

Magnetic resonance spectroscopy quality assessment tool

The MRS-Q was designed for quality assessment of MRS studies and implemented in Chapter 2. The tool has been uploaded onto the data registry for use in further systematic reviews, available from https://mfr.osf.io/render?url=https://osf.io/w3q92/?direct%26mode=render%26action=download%26mode=render

	What to report	Criteria for CA	Y/N/<i>
Acquisition parameters	Scanner strength	**Edited:** 3 T and above **Unedited:** 3 T preferable	
	Sequence	**Edited:** MEGA-editing (SPECIAL?) **Unedited:** PRESS, sLASER, STEAM	
	Scan parameters (averages, voxel size/volume, TE)	**Edited:** 240 averages, ~27 ml voxel, TE 68 – 80 ms **Unedited:** 128 Averages, 15 x 15 x 15 mm^3 voxel, 3T; 64 Averages, 20 x 20 x 20 mm^3 voxel, 3T; 256 Averages, 15 x 15 x 15 mm^3 voxel 1.5T; 128 Averages, 20 x 20 x 20 mm^3 voxel, 1.5 MRSI: 16 x 16 matrix, 15mm^3 voxel, TR 1500, TE 20/30 ms (short)	
	Data points	1024 data points from 2000 Hz (minimum)	
Quality metrics	Quality measures (shim or FWHM and fit error)	Important to report but no formal cut-offs established	
	Data visualization	At least one spectrum	
	Scanner drift	Should be reported but no cut-off established	
Study design/ analysis (pre/post-processing procedures)	Power calculation	Justification of sample size provided. Reduces type I and type II error.	
	Partial volume correction (and approach)	Not GM-only. Predominantly for Water-Referenced data.	
	Frequency and phase correction (and approach)	For edited MRS	
	Analysis	Report software and method used for pre-processing, and post processing	

Y- Yes; N- No; <i> Information missing

[+]For justification and references, please see Peek et al (2020) "Brain GABA and glutamate levels across pain conditions: a systematic literature review and meta-analysis of 1H-MRS studies using the MRs-Q quality assessment tool." NeuroImage. Link to manuscript and justification, see **Table 1.**

Please reference https://doi.org/10.1016/j.neuroimage.2020.116532 when using this tool.

Bibliography of important references:

- EDITINGSCHOOL: Held in December 2018 and focused on edited MRS. Expert instructors attended (http://www.gabamrs.com/blog/2018/10/12/editingschool-final-schedule).
- Wilson et al. 2019 Methodological consensus on clinical proton MRS of the brain: Review and recommendations. *Magnetic resonance in medicine; 82: 527-550.* A consensus document agreed on by 49 MRS experts.
- Mullins et al. 2014 Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. Neuroimage; 86:43-52. A consensus document written from a meeting of a number of specialist groups in 2011 in the UK documenting current "minimal best practice".

Planned future updates

In addition to the template, we aim to include the justification and an operational manual as part of this document

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CHAPTER TWO: SUPPLEMENT TWO

Associated Publications

Lin et al (2020) Minimum Reporting Standards for in vivo Magnetic Resonance Spectroscopy (MRSinMRS): Experts' consensus recommendations. *NMR Biomedicine.* DOI 10.1002/nbm.4484

An expert consensus document which recommends the use of the MRS-Q for reporting studies of MRS. The Author of the thesis was invited to collaborate following the presentation of the MRS-Q in Utah November 2019

Cruz- Almeida Y. and Porges E. (2021) Additional considerations for studying brain metabolite levels across pain conditions using proton magnetic resonance spectroscopy. *NeuroImage.* 117392

A published commentary in response to the meta-analysis presented in Chapter 2.

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SPECIAL ISSUE RESEARCH ARTICLE

NMR IN BIOMEDICINE WILEY

Minimum Reporting Standards for in vivo Magnetic Resonance Spectroscopy (MRSinMRS): Experts' consensus recommendations

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Abbreviations: ^{1}H MRS, proton MRS; 2D, two dimensional; 3D, three dimensional; CRLB, Cramér-Rao lower bound; FOV, field of view; FWHM, full-width at half-maximum; MRSI, magnetic resonance spectroscopic imaging; NA, number of acquisitions per spectrum; NAA, *N*-acetylaspartate; ppm, parts per million; PRESS, point resolved spectroscopy; SD, standard deviation; SNR, signal-to-noise ratio; STEAM, stimulated echo acquisition mode; tCho, total choline; tCr, total creatine; T_E, echo time; T_{E1}, first sub-echo time in PRESS sequence; T_{E2}, second sub-echo time in PRESS sequence; T_M, mixing time; T_R, repetition time; VOI, volume of interest.

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The translation of MRS to clinical practice has been impeded by the lack of technical standardization. There are multiple methods of acquisition, post-processing, and analysis whose details greatly impact the interpretation of the results. These details are often not fully reported, making it difficult to assess MRS studies on a standardized basis. This hampers the reviewing of manuscripts, limits the reproducibility of study results, and complicates meta-analysis of the literature. In this paper a consensus group of MRS experts provides minimum guidelines for the reporting of MRS methods and results, including the standardized description of MRS hardware, data acquisition, analysis, and quality assessment. This consensus statement describes each of these requirements in detail and includes a checklist to assist authors and journal reviewers and to provide a practical way for journal editors to ensure that MRS studies are reported in full.

KEYWORDS
MR spectroscopy (MRS) and spectroscopic imaging (MRSI) methods, reporting guidelines

1 | INTRODUCTION

Despite over 30 years of development and thousands of papers describing the use of in vivo MRS for non-invasive research in health and disease, including diagnosis and treatment monitoring across a broad range of human conditions, MRS has yet to reach full clinical acceptance.[1] While there remain several important technical issues,[2] one of the major problems is the lack of standards for reporting MRS studies. The importance can be described on several levels. First, there is increasing concern in the general scientific community over the lack of rigor and reproducibility of scientific studies.[3] Details of MRS methodologies need to be fully reported for readers to critically evaluate the quality of the published results and to reproduce the experiments. Second, recent meta-analyses and evidence-based reviews of MRS[4] have noted the lack of detail in peer-reviewed publications, which makes it difficult to compare study results. Third, the lack of reporting guidelines for MRS means that new researchers in the field find limited guidance on practice. As a result, MRS studies are sometimes conducted using inappropriate or incorrect methods that may lead to erroneous and/or inconsistent conclusions. Finally, MRS is a versatile method that finds application across fields where there may be insufficient peer expertise to provide critical technical evaluation of methods and analysis. A core set of standards for the rigorous reporting of MRS studies will help to ensure that MRS studies can be adequately reviewed to standards accepted by the specialist MRS community.

This lack of consistency in reporting was highlighted in a recent meta-analysis of MRS studies in chronic pain,[5] leading those authors to propose a minimum quality assessment guide, MRS-Q.[5] To this end, an expanded set of guidelines for minimum and recommended reporting requirements is presented in this paper. The origin of these guidelines was a panel at the 2016 International Society of Magnetic Resonance in Medicine (ISMRM) workshop "MR spectroscopy: from current best practice to latest frontiers." These minimum and recommended requirements were then reviewed and amended by authors selected from the ISMRM Magnetic Resonance Spectroscopy Study Group who have reviewed at least 10 MRS-focused papers for the following journals: *Magnetic Resonance in Medicine*, *NMR in Biomedicine*, *Journal of Magnetic Resonance*, *Radiology*, and *Magnetic Resonance Materials in Physics, Biology, and Medicine*. These are well-established peer-reviewed specialist journals that have focused on MRS-related topics, which ensures that the authors are considered experts in the technical aspects of MRS and experienced in its scientific use. Recognizing the need to include input from less experienced authors, we also included two trainees as authors to review and edit the manuscript to ensure it was clear to authors new to the field. We then formed the *Experts Working Group on reporting standards for MRS*, who support the paper's recommendations with collaborators with more than 5 years of experience in MRS methodology and application, who either have extended years of service as reviewers for the main MRS journals or are editors of those journals. This follows the same pathway to

consensus as the other consensus papers in this special issue.[6–17] These consensus papers provide context to these recommendations, and for further details, as indicated throughout the paper, new authors should reference these papers.

In order to facilitate implementation of these guidelines, a checklist of minimum requirements for the publication of MRS studies was developed (Table 1)—the Minimum Reporting Standards for in vivo Magnetic Resonance Spectroscopy (MRSinMRS) checklist—and exemplar filled in

TABLE 1 MRSinMRS checklist. Additional columns are provided for multisite or multisequence studies if necessary

Site (name or number)
1. Hardware
a. Field strength [T]
b. Manufacturer
c. Model (software version if available)
d. RF coils: nuclei (transmit/receive), number of channels, type, body part
e. Additional hardware
2. Acquisition
a. Pulse sequence
b. Volume of interest (VOI) locations
c. Nominal VOI size [cm^3, mm^3]
d. Repetition time (T_R), echo time (T_E) [ms, s]
e. Total number of excitations or acquisitions per spectrum In time series for kinetic studies i. Number of averaged spectra (NA) per time point ii. Averaging method (eg block-wise or moving average) iii. Total number of spectra (acquired/in time series)
f. Additional sequence parameters (spectral width in Hz, number of spectral points, frequency offsets) If STEAM:, mixing time (T_M) If MRSI: 2D or 3D, FOV in all directions, matrix size, acceleration factors, sampling method
g. Water suppression method
h. Shimming method, reference peak, and thresholds for "acceptance of shim" chosen
i. Triggering or motion correction method (respiratory, peripheral, cardiac triggering, incl. device used and delays)
3. Data analysis methods and outputs
a. Analysis software
b. Processing steps deviating from quoted reference or product
c. Output measure (eg absolute concentration, institutional units, ratio), processing steps deviating from quoted reference or product
d. Quantification references and assumptions, fitting model assumptions
4. Data quality
a. Reported variables (SNR, linewidth (with reference peaks))
b. Data exclusion criteria
c. Quality measures of postprocessing model fitting (eg CRLB, goodness of fit, SD of residual)
d. Sample spectrum

versions are included as appendices (Appendix 1, Appendix 2, Appendix 3, and Appendix 4). The intention is that, for papers utilizing MRS, the authors would complete the table and submit it to the journal, in addition to their manuscript, for review, or use the table to check whether all essential parameters have been listed in the Methods part of the manuscript, with the table subsequently to be included as an appendix to the article. For single site or nucleus studies the first column should be used, and for multisite or multisequence studies it is recommended to complete additional columns as appropriate. Likewise, in the appendices of this paper, several examples have been provided to illustrate how this table should be completed. The model follows checklists such as STARD,[18] CONSORT,[19] PRISMA,[20] and STROBE.[21] This will enable editors, reviewers, and ultimately readers to be sure of the MRS methodology employed in particular studies, and to ensure that all sufficient details are available to those intending to reproduce or extend the studies or use the results for meta-analyses. The checklist (Table 1) will also help to standardize the presentation of MRS information and provide journals less familiar with MRS with a systematic way of certifying the methods used.

2 | REPORTING GUIDELINES

Below we set out in five sections the important pieces of information about an MRS study that are to be considered as either requirements, or recommendations, along with reasons why these are considered important. A more in-depth description of terminology and abbreviations to be used can be found in the work of Kreis et al,[11] while a fuller discussion of several concepts are to be found in other consensus papers in this special issue.[6–17]

2.1 | MRI system description

a. Field strength, *eg 1.5 T, 3 T, 7 T, 9.4 T*
b. Manufacturer, *eg General Electric, Philips, Siemens, Toshiba*
c. Model, eg *General Electric Signa HD/X/T/de, Optima MR450/MR450W, Discovery MR750/MR750W, Signa Premier; Siemens Biograph mMR, Magnetom, Aera, Espree, Prisma, Skyra, Trio, Verio, Magnetom 7 T, Terra; Phillips Ingenia 1.5 T S, 3 T X/S, Elition 3 T X/S, Ambition 1.5 T X/S, Achieva 1.5 T/3 T*. Software version, *eg Siemens VB17A, VD19, VE11C; General Electric 12x-24x; Phillips Release 5, 5.1 (R1-3), 5.6, 6*
d. RF coils used (nuclei, number of channels, type, body part), *eg ^{1}H, ^{31}P, ^{13}C, ^{31}P-^{1}H; type, eg head/neck, torso, knee; if not manufacturer, design, eg butterfly, quadrature etc*
e. Additional hardware, *eg shim inserts, dielectric pads*

Rationale:
Full and accurate description of the MR system and MR hardware allows for appropriate comparison across studies.

a. ***Field strength.*** First and foremost is the field strength of the MR system utilized, expressed in tesla. This allows a reader to position the results in the wider literature. Field strength also has implications for the sensitivity of the MRS approaches employed, and for problems or issues that may exist[2,14,17] (eg increased chemical shift displacement error with higher field strength, differences in spectral dispersion and hence appearance). Specifying the exact resonance frequency in megahertz in addition to the field strength may be useful, in particular for meta-analyses and data sharing purposes, because the field strength is usually indicated with zero- or single-digit precision only. Indicating the resonance frequency is particularly encouraged where the actual field strength deviates considerably from the rounded value indicated for B_0 (eg 123.06 MHz with 2.89 T instead of 127.74 MHz at 3.0 T).
b. ***Manufacturer***. While the physical principles governing MRS are well understood, different vendor approaches can lead to systematic differences in results, and therefore manufacturer information should be included.
c. ***Model***. Hardware differences exist depending on the model of the scanner, for example the bore size and gradient hardware, which impact on B_0 homogeneity and echo-planar spectroscopic imaging performance, respectively.[22] The *software version* is often omitted, but should also be given whenever possible, as some special features such as frequency correction and shimming algorithms may differ between different software versions.
d. ***RF coils***. The RF coil information should include the nuclei the coil is tuned to so that it is clear which nuclei are observed. For double-tuned coils, both nuclei should be indicated with a forward slash in between. As the coil design can have a major impact on the data acquired, it is important to include all the relevant details of the coil such as whether a single coil was used for transmit and receive and/or the number of channels for phased array receive and transmit coils. If it is not a standard manufacturer product coil, further details such as the design of the coil should be included and a reference for a previous publication that may provide more detail.
e. ***Additional hardware***. Finally, details should be included of any additional hardware used, such as shim/gradient inserts, dielectric pads, or any other modification of the hardware used for data acquisition.

2.2 | Acquisition parameters in full

i. Pulse sequence, *eg spin-echo, point resolved spectroscopy (PRESS), stimulated echo acquisition mode (STEAM), semi-LASER, etc.*
ii. Location of volume(s) of interest (VOI(s)), *eg posterior cingulate gyrus, M. tibialis anterior, internal capsule of prostate, etc.* A figure that displays the VOI on anatomic images is recommended.
iii. Nominal VOI size [cm^3, mm^3], *eg* $40 \times 40 \times 10\ mm^3$.
iv. Repetition time (T_R), echo time (T_E) [ms, s]; if STEAM, mixing time (T_M).
v. Total number of excitations per spectrum.
vi. Additional sequence parameters, eg

i. Spectral width [Hz, kHz] and number of data points
ii. Frequency offset (if any)
iii. If magnetic resonance spectroscopic imaging (MRSI): specification of two-dimensional (2D) or three-dimensional (3D) spatial mapping, field of view (FOV), matrix size, acceleration factor, sampling/reconstruction method (eg parallel imaging, compressed sensing, spatial spectral encoding, etc), nominal and effective (ie final) voxel volumes, flip angles for fast MRSI
iv. For multidimensional acquisitions, number of encodings in the second spectral dimension
v. For editing methods, editing pulse information including pulse shape, bandwidth and offset frequency
vi. For multinuclear sequences: details of decoupling or polarization transfer sequences and related parameters.

f. Water suppression method (and any other suppression methods used, eg lipid suppression, outer volume suppression).
g. Shimming method,[10] reference peak used for assessing shim performance, and thresholds for "acceptance of shim" chosen.
h. Triggering method, if used (respiratory, peripheral, cardiac triggering, including device used and delays).
i. Frequency and motion correction methods, if used (prospective or retrospective, external tracker or navigator method).

Rationale:

a. ***Pulse sequence***. The pulse sequence dictates the parameters that need to be described under ***additional sequence parameters***. Citing the original article that first introduced and described this sequence in detail is recommended along with outlining important deviations from the original sequence, and if the sequence is vendor supplied or a customized sequence.
b. ***Location.*** The voxel location is the anatomical position of the VOI selected for single-voxel spectroscopy or the excitation or selection volume in MRSI methods. It should be described in the checklist table in brief and in the manuscript be either shown in a figure or described in detail with anatomical landmarks. It is important to address concerns regarding regional specificity of results and possible tissue-specific effects (ie for brain gray matter, white matter, and cerebrospinal fluid content).
c. ***VOI size***. The VOI size must include the dimensions along the right-left, anterior-posterior, and superior-inferior directions with anatomical referencing if relevant. For MRSI, this should be the excitation volume. Regional analyses for MRSI can use signal averaging over multiple voxels, which should also be described in detail if used.
It is important to show ***example spectra*** obtained from these regions to allow the reviewer and reader to assess the quality of the data. The spectra should be representative, and, if possible, visualize the studied effect by comparison with a reference spectrum (eg healthy subject/tissue versus patient/affected tissue; physiological conditions such as rest versus end of exercise for muscle). See also the quality assurance section. In recognition of the limited space for figures in some journals, a figure containing the VOI and corresponding MR spectra could be placed in the appendix or supplemental data. See Figure 1 for an example.
d. ***Timing parameters (echo time and repetition time)*** are considered essential parameters as these will affect the way spectra appear. T_E and T_R lead to differential T_2 and T_1 relaxation effects, with this effect being present between different metabolites. The importance of this is best illustrated by considering total creatine (tCr) and total choline (tCho). The methyl signal of tCr is a commonly utilized internal reference peak; however, its T_2 relaxation constant is shorter than that for tCho.[23,24] This means that for studies with long T_E (eg 144 ms) the tCho/tCr peak height or area ratio will be larger than for studies with shorter T_E (eg 30 ms). This can lead to a misinterpretation of differences between two different studies if the T_2 relaxation difference is not considered. For STEAM sequences ***mixing time*** (TM; the time between the second and third 90° RF pulses) will affect the evolution of multiquantum coherence,[25] and so may impact quantification even if the effective T_E is the same between two studies. Similar effects can be seen for changes in standard vendor-implemented sequences' timings for other acquisition schemes, and so information on any such changes should always be provided.

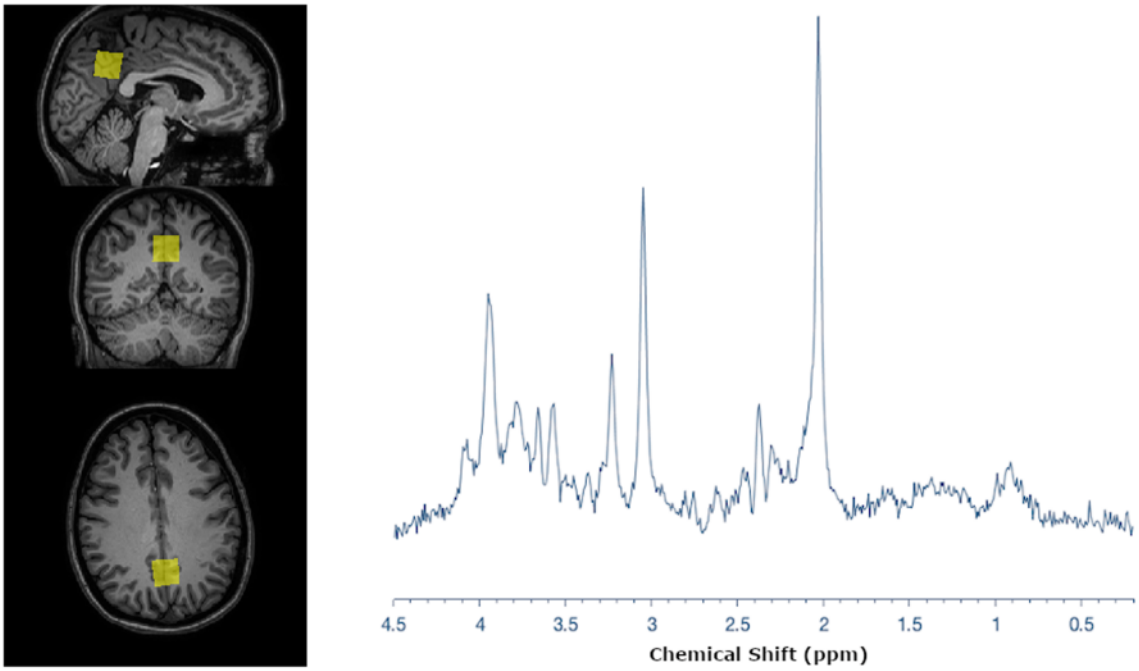

FIGURE 1 Representative spectrum and voxel location. Representative PRESS spectrum from the posterior cingulate acquired on a 3 T Philips Ingenia. In this figure the raw data from one participant are shown; however, mean data with SDs, multiple data sets, and fitted data may also be shown, as long as the raw data are presented in a fashion that allows an assessment of data quality. The chemical shift axis is labeled in ppm units. Data were collected in accordance with the WMA Declaration of Helsinki

e. ***Number of excitations/acquisitions***. The signal-to-noise ratio (SNR) in MRS is dependent on VOI size and the ***number of acquisitions*** (NA; averages or phase encodings in MRSI). More acquisitions lead to an improved SNR, which in turn improves reliability of fitting. Different approaches to data acquisition between vendors and groups mean that MRS data may be acquired as either an average or sum of multiple acquisitions, or as an average of a series of blocks, which themselves contain a set number of acquisitions, or number of excitations. Describing both the number of acquisitions per block/scan, and if present the number of blocks, allows the total number of acquisitions for the entire acquisition to be calculated. It is recommended that this total number of scans per acquisition/analysis be reported. This is of particular importance for kinetic studies, in which the data are acquired as time series. Here the number of excitations per time point, signal averaging method (eg block-wise or moving average), and total number of spectra (acquired/in time series) should be reported (see the consensus paper by Meyerspeer et al[15] in this special issue for details of reporting on kinetic studies).

f. ***Additional sequence parameters***. These will be determined by the sequence used for acquisition. For most methods, however, it would be appropriate to describe the spectral width in hertz and the number of data points acquired. If any frequency offset is used, it should also be described.

   For MRSI methods, the necessary details include the FOV and matrix size so that the nominal volume of MRS voxels can be determined. Acceleration methods (such as parallel imaging, compressed sensing, or spatial-spectral encoding) can be used to reduce the scan times required for MRSI methods and should be described with details of the method and parameters used.[26] Similarly, *k*-space weighting of the acquisition should also be described, such as whether full or elliptical *k*-space sampling is used or retrospective filters used (eg Hamming), and any *k*-space zero-filling factors applied. These factors will impact the effective, ie resultant, voxel volume, which should also be stated if known.[14]

   Additional modifications to the default settings of the pulse sequence should be described. For example, if a frequency offset for excitation of water-suppressed scans is used to address chemical shift differences between the water reference and metabolite scans, this should be specified, either as offset frequency from water or as chemical shift value in parts per million (ppm) on the standard MRS frequency axis. Reporting of sub-echo times (T_{E1}, T_{E2}), if known, is also recommended.

   Edited MRS sequences require reporting of several more advanced parameters, for example the bandwidth and frequency of editing pulses used, co-edited metabolites, and specific timing parameters or acquisition schemes. (For more detail and recommendations on spectral editing, see the consensus paper by Choi et al[8] in this special issue.) Similarly, for multinuclear sequences, details of decoupling, editing, or polarization transfer sequences have to be indicated and related parameters provided.

g. ***Water and fat suppression***. Water suppression is a key element of the data acquisition in proton MRS (^{1}H MRS), as both the method used, and the degree of water suppression, can greatly influence the spectral quality and analysis of the data. The type of water suppression used should be specified if specific water suppression methods are selected. If the authors used the default water suppression method for their choice of pulse sequence, it is acceptable to report "Standard," as manufacturers often do not specify which water suppression method is used. If there are parameters related to water suppression such as "weak water suppression" as specified on Siemens systems, or the bandwidth of the water suppression pulses, this should be listed. For further details see the consensus paper by Tkáč et al[6] in this special issue. As with water, fat suppression techniques may also impact data quality, and if used, specifics should also be listed (eg frequency offset, number and location of outer voxel suppression bands, bandwidth).
h. ***Shimming method***. Similarly, different shimming methods may be selected at the time of acquisition. In most cases, authors will utilize the vendor-provided automated shimming, which usually involves the use of a gradient echo field map to optimize the B_0 field homogeneity, but may employ other methods (eg "pencil beam" VOI in Philips). If a vendor-supplied methodology is used, the authors should state this; if first, second, or third order shims are used; and describe whether or not the resulting linewidth was measured and used for quality assurance. Ideally, studies should measure the linewidth (to be reported as full-width at half-maximum, FWHM) for the unsuppressed water resonance or a specific metabolite peak[11] in each examination and report the threshold at which shimming was considered acceptably achieved, and how this was assessed (eg system reported results for shim, phase or magnitude spectrum, or other). If manual shimming is used this fact should be listed in the checklist table along with the maximum linewidth allowed. More details on shimming are described in the consensus paper by Juchem et al[10] in this special issue.
i. ***Triggering method***. If used it should be mentioned. This can then be considered, along with T_R, to ascertain if any T_1 effects are likely to have an impact on data and SNR. For example, triggering via cardiac measures might cause a shorter T_R in exercise studies during a period of exercise that increases heart rate.
j. ***Frequency and motion correction***. Methods available vary, with the possibility of retrospective or prospective correction for frequency shifts caused by field drift, motion, or other factors. Reporting the methodology used, and at what part of the process it occurred, allows for more accurate replication of future studies, as well as appropriate comparisons between studies. For more details, see the consensus paper on frequency and motion correction by Andronesi et al[7] in this special issue.

2.3 | Spectral quantification methods and parameters

a. Software package used to reconstruct and analyze the MRS data including MR manufacture software (eg *General Electric PROBE*, *Siemens Syngo*, *or Phillips SpectroView*) and/or third-party software packages (eg *LCModel*,[27] *jMRUI*,[28] *TARQUIN*,[29] *SIVIC*,[30] *INSPECTOR*,[31] *FID-A*,[32] *BrainSpec*,[33] *MIDAS*,[34] *GANNET*[35])
b. Deviations in processing steps from quoted reference or product defaults
c. Quantitative output measures
d. Quantification references and assumptions, model fitting assumptions

Rationale:

a. ***Software package***. The software packages used to reconstruct and analyze the MRS data including MR manufacturer software and/or third-party software packages must be described in the table under the ***Analysis software*** section. If the authors used vendor-provided software this should be specified, or if third-party software is used it should be described in the table, and a suitable reference provided. Different analysis packages have different approaches to parameter estimation, which may impact the results.[16] If more than one software package was used, they should all be listed along with the aspects of the analysis for which they were used.
b. ***Deviations in processing steps***. Any automatic and manual processing steps deviating from a software package's default analysis have to be listed: for example, changes to phasing, frequency alignment, eddy current corrections. For phased array coils, any alterations to coil combination should be described. In addition, it should be described if these changes are performed on single acquisitions before averaging. References should be provided that describe the methodology and its specifics rather than publications that simply utilize the method.
c. ***Quantitative output measures***. The output measure of the spectral analysis should be described. There are three main ways that MRS metabolite concentrations can be described.
   First, MRS results are often reported as a ratio of the primary metabolite to another. This can be done using the ratio of the peak area measurements, or ratio of relative concentrations, which accounts for the number of resonant nuclei in each compound. It is important to indicate which metabolite is used as the denominator.
   The second method is to report the metabolites as "institutional units," which is the signal reported by the software, normalized such that measures at different time points or from different subjects can be compared. This normalization usually stops short of all steps required to report

conventional concentration estimates. The most basic measure is based on the peak height of the metabolite, but this is greatly influenced by the linewidth, and therefore reporting the area under the curve (or the equivalent measure for time-domain fitting) is recommended. In either case the baseline fitting method should be described. A common approach for normalization to institutional units in ^{1}H MRS is to take the ratio of the fitted metabolite signal to the fit of the unsuppressed water resonance.

Finally, metabolite concentrations can be expressed in "absolute units" (standard chemical units, such as millimoles per wet weight, molar, or molal) using some conversion methods, which usually rely on multiple assumptions (eg an assumed tissue content for the reference component). In order to provide estimates of metabolite concentration, contributions to the signal from different tissue compartments should be considered. For the brain this means that gray matter, white matter, and cerebrospinal fluid volumes calculated via voxel segmentation should be reported as appropriate, especially if water is used as the internal reference. If relaxation correction is applied, listing T_2 and T_1 values used (and/or a suitable reference) is necessary.

d. ***Quantification references and assumptions, and model fitting.*** Some software packages utilize model-fitting methods for spectral analysis. In those cases, the models used should be described in detail, as the number of metabolites used can greatly impact the result; for example, were the models simulated, and if so using what software (eg VESPA,[36] GAMMA,[37] FID-A,[32] NMR-PROBE or NMRSCOPE in jMRUI,[28] MARRS, etc). The basis set used should be described either as the "default" basis set provided with the software, or if it was modified which metabolites were included in the basis set. In addition, the fitting model also has to be specified in terms of implemented parameter relations and constraints. This must be spelled out in full if deviating from default parameter sets for the specific versions of the fit packages or quoted literature reference.

   Moreover, for brain ^{1}H MRS spectra information on how the macromolecule signals were handled in the fitting procedure is mandatory. This can be done either by using a spectrum of macromolecules acquired in vivo, or by a mathematical approach, which is usually incorporated in the software package. When the mathematical approach is used, details of how it was done also need to be mentioned, ie for QUEST in jMRUI the number of points used or for LCModel number of macromolecules and lipid peaks included. For more detail on macromolecule contributions in MRS see the consensus paper by Cudalbu et al[9] in this special issue.

2.4 | Quality assurance. Studies must include the following

a. Reported variables (SNR, linewidth, and description of how they were obtained)
b. Data exclusion criteria
c. Other quality measures from fitting software are also recommended (eg standard deviation (SD), Cramér-Rao lower bound (CRLB)), and/or the robustness of the measures gained (repeatability measures if known)
d. Figure showing representative spectra

Rationale:

a. ***Reported variables.*** One of the greatest challenges of the MRS literature is the evaluation of spectral quality. There are no agreed standards for data quality. While no single measure is the gold standard of data quality, the primary measures in practice are SNR, spectral linewidth, and CRLB. As SNR can be measured in many different ways, it is important that authors report both the SNR and its measurement method (see the work of Kreis et al[11] and Oz et al[1]).

   Linewidths are typically measured as the FWHM of the fitted resonance. For ^{1}H MRS, this may be done using the water resonance and determined either at the time of acquisition during the pre-scan shimming routine or post hoc through a spectral analysis of the water spectrum. These values should be reported to ensure that spectra are of adequate quality to analyze. In non-^{1}H MRS, usually the most prominent singlet resonance in the spectrum is used to measure linewidth (eg PCr). Linewidths can also be obtained from the output of fitting packages, where they would usually indicate the linewidth of specific metabolite signals. It is important to specify the origin of the linewidth indicated.

b. ***Data exclusion criteria.*** The data exclusion criteria should specifically provide the thresholds for which data were excluded, whether they were based on SNR, linewidth, and/or other quality measures, and the specifics of this measure, as this can bias the overall analysis of the study data.[18] For example, "subjects were excluded if the SNR of tCr was less than 5 or the FWHM was greater than 12 Hz." Note that, to avoid bias,[38] if CRLBs are used as exclusion criteria they should not be in the form of percentage values of a metabolite of interest that can have a small value in an individual subject, but rather be formulated in absolute concentration units (or relative to a stable reference metabolite). It is also important to describe how many subjects or voxels per subject cohort were eliminated based on the specified criteria.

c. ***Quality measures of model fitting.*** Additional measures of goodness of fit, or fit error, should be reported where applicable (eg CRLB for lower bound of the fit error, or SD). If reproducibility or repeatability[11] of a measure has been shown, it is recommended to report it to demonstrate the robustness of single measures.

d. ***Representative spectrum***. Finally, one of the most important methods of quality control is visual inspection of the MR spectrum by experienced users or MRS experts (note: in MRSI visual inspection of metabolic maps becomes equally important[14]). Sample spectra are required so that both reviewers and readers can assess the quality and interpretation of the MRS data (see the 'Acquisition' section in the selection criteria for representative spectra). While a single spectrum may of course not reflect the quality of all of the data, it does provide a general assessment. In contrast, selecting a single spectrum from thousands of spectra in MRSI may not reflect the overall MRSI data quality, and so maps, with details of how they are scaled and exemplar spectra displayed, are more appropriate. The requirements for this spectrum include the following. (1) The raw spectrum must be shown, not the fitted data alone, as the fitted data do not reflect the SNR or potential systematic artifacts. If the baseline is calculated, it is recommended to show it. Fitted data are recommended to be shown in addition as an overlay to reflect quality of model fitting. (2) The *x* axis or chemical shift axis should be displayed with units in ppm. (3) If the spectrum is apodized for display purposes, the apodization parameters should be given in the figure caption. An example is shown in Figure 1. As described above, spectra can be included in a figure that also presents the voxel location to meet space and figure constraints for specific journals. In addition, for a more complete representation of spectral quality in the study, plotting of the average spectrum across all data points and SDs around this average from each studied cohort may be displayed. In the checklist, the figure number should be described so that it can be easily referenced and also serves to indicate its presence.

The details described above should be included in the text of the manuscript or in a supplemental methods section. The MRSinMRS checklist (Table 1) is intended to be a reference for the author and the reviewer as well as the reader, and while it should be included as supplemental material it is not intended to replace the manuscript text. Example checklists based on information from existing publications are provided in the appendices of this paper (Appendix 1, Appendix 2, Appendix 3, Appendix 4) to provide guidance to authors as to the details that should be included in the checklist. Those items in the checklist that are in italics are details that should have been but were not included in these publications, illustrating further the value of including the checklist to ensure that all important details are included in the manuscript.

3 | CONCLUSION

These minimum reporting guidelines for MRS should allow the field to improve the rigor and critical examination of reported results, improve the reproducibility and comparability of studies, and provide new entrants to the field with detailed guidance as to reporting practices. To assist authors in reporting and reviewers in assessing these essential and recommended parameters, we have provided a simplified checklist (Table 1). It is hoped that this checklist will facilitate writing for authors, improve analysis for journal reviewers, and provide an easy way for journal editors to ensure that MRS studies are reported in full. In addition, reporting requirements, if checked early, encourage researchers to consider these aspects ahead of time, hopefully before data collection has commenced. While it is preferred that details of the MRS acquisition and analysis are included in the main text, the MRS reporting checklist can also be provided as part of the appendix or supplementary material of a submission and used in the review process, as with many other manuscript checklists such as PRISMA, STARD, CONSORT, and STROBE. (Researchers may also find the MRS-Q V1 form at Open Science Framework (https://osf.io/8s7j9/) useful.)

Adherence to these minimum requirements and recommended guidelines is expected to ensure that all MRS papers provide the necessary information to reproduce studies as well as provide a basis for comparison for the evaluation of the studies across clinical domains. As with initiatives in other fields of biological and clinical research, it is expected that this will improve reproducibility and validity, and strengthen the field going forward.

EXPERTS' WORKING GROUP ON REPORTING STANDARDS FOR MR SPECTROSCOPY

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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APPENDIX 1

EXAMPLE OF THE MRSinMRS CHECKLIST FOR A SINGLE VOXEL ^{1}H-MRS STUDY

1. Hardware		
a. Field strength [T]		3 T
b. Manufacturer		Siemens
c. Model (software version if available)		Verio (VB17)
d. RF coils: nuclei (transmit/receive), number of channels, type, body part		32 channel head coil
e. Additional hardware		N/A
2. Acquisition		
a. Pulse sequence	3D localized correlated spectroscopy	
b. Volume of interest (VOI) locations	Posterior cingulate gyrus	
c. Nominal VOI size [cm^3, mm^3]	$3 \times 3 \times 3$ cm^3	
d. Repetition time (T_R), echo time (T_E) [ms, s]	T_R 1500 ms, *initial* T_E *30 ms*, 0.8 ms increments	
e. Total number of excitations or acquisitions per spectrum In time series for kinetic studies i. Number of averaged spectra (NA) per time point ii. Averaging method (eg block-wise or moving average) iii. Total number of spectra (acquired/in time series)	64 increments with 8 averages per increment	
f. Additional sequence parameters (spectral width in Hz, number of spectral points, frequency offsets) If STEAM: mixing time (T_M) If MRSI: 2D or 3D, FOV in all directions, matrix size, acceleration factors, sampling method	F1/F2: 2000 Hz/1250 Hz, 1024 points	
g. Water suppression method	WET	
h. Shimming method, reference peak, and thresholds for "acceptance of shim" chosen	Automated B_0 field mapping followed by manual shimming of water to <14 Hz	
i. Triggering or motion correction method (respiratory, peripheral, cardiac triggering, incl. device used and delays)	N/A	
3. Data analysis methods and outputs		
a. Analysis software	Felix-2007	
b. Processing steps deviating from quoted reference or product	F2 domain (skewed sine-squared window, 2048 points, magnitude mode), F1 domain (sine-squared window, linear prediction to 96 points, zero-filling to 512 points, magnitude mode)	
c. Output measure (eg absolute concentration, institutional units, ratio)	Ratio to creatine	
d. Quantification references and assumptions, fitting model assumptions	Each spectrum was calibrated by setting the lysine cross peak (at 3.00-1.67 ppm) and specifying a constant 'number of contour levels' (set to 28), as well as a constant 'level multiplier' (defined as the difference between values of consecutive contour, set to 1.05).	
4. Data quality		
a. Reported variables (SNR, linewidth (with reference peaks))		*SNR and linewidth not described*
b. Data exclusion criteria		*No subjects excluded*
c. Quality measures of postprocessing model fitting (eg CRLB, goodness of fit, SD of residual)		*No QA measures described*
d. Sample spectrum		Figure 1

The example above used the following paper: Lin AP, Ramadan S, Stern RA, et al. Changes in the neurochemistry of athletes with repetitive brain trauma: preliminary results using localized correlated spectroscopy. *Alzheimers Res Ther*. 2015;7(1):13.
Items listed in italics are details that were not included in the paper that served as the source for this example.

APPENDIX 2

EXAMPLE OF THE MRSinMRS CHECKLIST FOR A MULTI-SEQUENCE MULTI-NUCLEAR MRS STUDY (^{1}H, ^{31}P)

Site (name or number)			
1. Hardware			
a. Field strength [T]	3 T	3 T	
b. Manufacturer	Siemens	Siemens	
c. Model (software version if available)	Skyra (VD13)	Skyra (VD13)	
d. RF coils: nuclei (transmit/receive), number of channels, type, body part	32 channel ^{1}H head coil	*10 cm* ^{31}P tuned transmit/receive surface coil	
e. Additional hardware	N/A	Custom-built dynamic knee extension apparatus	
2. Acquisition			
a. Pulse sequence	PRESS		500 μs pulse and acquire
b. Volume of interest (VOI) locations	Posterior cingulate gyrus		N/A
c. Nominal VOI size [cm^3, mm^3]	30 × 30 × 30 mm^3		N/A
d. Repetition time (T_R), echo time (T_E) [ms, s]	T_R/T_E = 2000/30 ms		T_R = 2000 ms
e. Total number of excitations or acquisitions per spectrum In time series for kinetic studies i. Number of averaged spectra (NA) per time point ii. Averaging method (eg block-wise or moving average) iii. Total number of spectra (acquired/in time series)	64 averages		300 total FIDs SIFT used for averaging
f. Additional sequence parameters (spectral width in Hz, number of spectral points, frequency offsets). If STEAM: mixing time (T_M). If MRSI: 2D or 3D, FOV in all directions, matrix size, acceleration factors, sampling method	1200 Hz, 1024 data points		3000 Hz, 2048 data points
g. Water suppression method	CHESS		N/A
h. Shimming method, reference peak, and thresholds for "acceptance of shim" chosen	Automated B_0 field mapping followed by manual shimming of water to <14 Hz		Automated B_0 field mapping
i. Triggering or motion correction method (respiratory, peripheral, cardiac triggering, incl. device used and delays)	None		None
3. Data analysis methods and outputs			
a. Analysis software	LCmodel vers 6.2	jMRUI	
b. Processing steps deviating from quoted reference or product	None	SIFT pre-processing prior to use of jMRUI	
c. Output measure (eg absolute concentration, institutional units, ratio), processing steps deviating from quoted reference or product	Ratios to creatine	PCr amplitude	
d. Quantification references and assumptions, fitting model assumptions	Default basis set	AMARES Gaussian lineshapes	
4. Data quality			
a. Reported variables (SNR, linewidth (with reference peaks))	*SNR: 61.8 ± 6 (51-71) FWHM: 0.043 + 0.006 (0.38-0.57) ppm as reported by LCmodel* *None eliminated*		*SNR and FWHM not reported*
b. Data exclusion criteria	*SNR < 40; CRLB > 20%*		*SD > 10%*
c. Quality measures of postprocessing model fitting (eg CRLB, goodness of fit, SD of residual)	*CRLB of NAA: 2 ± 0(2)%*		*SD: 4.5 ± 1.7%*
d. Sample spectrum	Figure 1		Figure 2

The example above used the following paper: Zhou M, Liao H, Sreepada LP, Ladner JR, Balschi JA, Lin AP. Tai chi improves brain metabolism and muscle energetics in older adults. *J Neuroimaging*. 2018;28(4):359-364. https://doi.org/10.1111/jon.12515.
Items listed in italics are details that were not included in the paper that served as the source for this example.

APPENDIX 3

EXAMPLE OF THE MRSinMRS CHECKLIST FOR AN X-NUCLEAR MRS STUDY (DYNAMIC ^{31}P MRS, MUSCLE)

1. Hardware	
a. Field strength [T]	7 T
b. Manufacturer	Siemens Healthineers, Erlangen, Germany
c. Model (software version if available)	Magnetom 7 T (VB17)
d. RF coils: nuclei (transmit/receive), number of channels, type, body part	Custom-built three channel ^{31}P (d = 15 cm, l = 10 cm), two channel ^{1}H (d = 17 cm, l = 12.5 cm) transceiver coil, shaped to the human calf (Goluch et al. *Magn Reson Med.* 2015;73 (6):1190-1195)
e. Additional hardware	Custom-built pedal ergometer with pneumatic piston and MR-compatible sensors for pedal angle and force
2. Acquisition	
a. Pulse sequence	Semi-LASER
b. Volume of interest and VOI locations	Single voxel placed obliquely in gastrocnemius muscle, avoiding subcutaneous fat, fasciae and adjacent muscles
c. Nominal VOI size [cm^3, mm^3]	Anatomy matched, 27 ± 6 cm^3 *(ca. 2 × 3.5 × 4 cm^3)*
d. Repetition time (T_R), echo time (T_E) [ms, s]	T_R = 6 s, T_E = 29 ms
e. Total number of excitations or acquisitions per spectrum (NA) In time series for kinetic studies i. Number of averaged spectra) per time point (NA) ii. Averaging method (eg block-wise or moving average) Total number of spectra (acquired/in time series)	*1 acquisition per spectrum (NA = 1), except for pH quantification 90 s after exercise, where NA = 4, with block-wise averagingTotal number of spectra in time series was 140, at NA = 1 (or series of 35 spectra at NA = 4)*
f. Additional sequence parameters (spectral width in Hz, number of spectral points, frequency offsets) i. If STEAM: mixing time (T_M) ii. If MRSI: 2D or 3D, FOV in all directions, matrix size, acceleration factors, sampling method	5 kHz, 2048 complex points after removing oversampling
g. Water suppression method	N/A
h. Shimming method, reference peak, and thresholds for "acceptance of shim" chosen	*1st and 2nd order, vendor standard method (DESS sequence in "advanced shim" mode until convergence), line-width of PCr peak was evaluated post hoc*
i. Triggering or motion correction method (respiratory, peripheral, cardiac triggering, incl. device used and delays)	Subjects were instructed to push the pedal only during times without RF excitation or signal reception, *cued by gradient noise. Adherence to the protocol was inspected via data from the force sensors.*
3. Data analysis methods and outputs	
a. Analysis software	^{31}P MR spectroscopy data were processed from raw data exported from the scanner using in-house developed Python scripts (http://www.python.org) for phasing and channel combination. Signals were phased to the highest peak magnitude of PCr in the frequency domain after 7 Hz Lorentzian apodization and 4 × zero-filling. The channel combination was then performed by weighted averaging of the raw data (that is, without apodization and zero-filling). Weights were calculated as proportional to signal, averaged over four resting spectra (excluding the fully relaxed spectrum). Spectra were then fitted in AMARES, as implemented in jMRUI, *version 5.0*
b. Processing steps deviating from quoted reference or product analysis software (vendor, version)	*Gaussian line shapes, soft constraints for frequencies*
c. Output measure (eg absolute concentration, institutional units, ratio) Processing steps deviating from quoted reference or product	Concentrations in institutional units and pH values

(Continues)

3. Data analysis methods and outputs	
d. Quantification references and assumptions, fitting model assumptions	*Quantification relative to total ^{31}P signal, which was assumed to be constant.* End-exercise PCr depletion relative to post-exercise asymptotic value of mono-exponential fit of recovery
5. Data quality	
a. Reported variables (SNR, linewidth (with reference peaks))	SNR was calculated using the partially saturated resting spectra of each time series by dividing the PCr peak amplitude by the SD of the signal in a region containing only noise, 15 ppm off-center across 1/16 of the total bandwidth. Linewidths were taken from the AMARES fit of the PCr peak.
b. Data exclusion criteria	*>10% changes of sum of total ^{31}P signal* *Linewidth of PCr peak > 15 Hz* *Unphysiological pH values (>7.1)* *Splitting of P_i peak*
c. Quality measures of postprocessing model fitting (eg CRLB, goodness of fit, SD of residual)	*SD of residual*
d. Sample spectrum	Figure 2

NAA, *N*-acetylaspartate.

The example above used the following paper: Niess F, Schmid AI, Bogner W, et al. Interleaved ^{31}P MRS/^{1}H ASL for analysis of metabolic and functional heterogeneity along human lower leg muscles at 7 T. *Magn Reson Med*. 2020;83:1909-1919. https://doi.org/10.1002/mrm.28088.

Items listed in italics are details that were not included in the paper that served as the source for this example.

APPENDIX 4

EXAMPLE OF THE MULTI-SEQUENCE MRSinMRS CHECKLIST FOR A COMBINED SINGLE-VOXEL AND MAGNETIC RESONANCE SPECTROSCOPIC IMAGING STUDY

1. Hardware		
a. Field strength [T]	3 T	3 T
b. Manufacturer	Siemens	Siemens
c. Model (software version if available)	Skyra (VD13B)	Skyra (VD13B)
d. RF coils: nuclei (transmit/receive), number of channels, type, body part	32 ch ^{1}H head coil	32 ch ^{1}H head coil
e. Additional hardware	N/A	N/A

2. Acquisition		
a. Pulse sequence	PRESS	Semi-LASER CSI
b. Volume of interest (VOI) locations	Patients: lesion Controls: centrum semiovale	Patients: lesion
c. Nominal VOI size [cm^3, mm^3]	20 × 20 × 20 mm^3	80 × 80 × 15 mm^3
d. Repetition time (T_R), echo time (T_E) [ms, s]	T_R = 2000 ms, T_E = 97 ms	T_R = 1700 ms, T_E = 97 ms
e. Total number of excitations or acquisitions per spectrum In time series for kinetic studies i. Number of averaged spectra (NA) per time point ii. Averaging method (eg block-wise or moving average) iii. Total number of spectra (acquired/in time series)	128 averages	3 averages
f. Additional sequence parameters (bandwidth in Hz or dwell time in ms, number of spectral points, frequency offsets) If STEAM: mixing time (T_M) If MRSI: 2D or 3D, FOV in all directions, matrix size, acceleration factors, sampling method	1200 Hz, 1024 points	2D: 160 × 160 × 15 mm^3 FOV; matrix size 16 × 16, no acceleration factor; weighted distribution sampling
g. Water suppression method	WET	WET
h. Shimming method, reference peak, and thresholds for "acceptance of shim" chosen	Automated 3D B_0 field mapping technique followed by manual adjustment <14 Hz	Automated 3D B_0 field mapping technique followed by manual adjustment <25 Hz
i. Triggering or motion correction method (respiratory, peripheral, cardiac triggering, incl. device used and delays)	N/A	N/A

3. Data analysis methods and outputs		
a. Analysis software	LCmodel 6.2	LCmodel 6.2
b. Processing steps deviating from quoted reference or product	Custom basis set	Custom basis set
c. Output measure (eg absolute concentration, institutional units, ratio), processing steps deviating from quoted reference or product	Ratios to creatine	Ratios to creatine
d. Quantification references and assumptions, fitting model assumptions	*The basis set included spectra of 2HG, NAA, GABA, glutamate, glycine, creatine, myo-inositol, glutamine, lactate, alanine, acetate, aspartate, ethanolamine, glutathione, phosphorylethanolamine, scyllo-inositol, taurine, N-acetylaspartylglutamate, glucose, and choline simulated using real pulses. Macromolecules were not modelled.*	*The basis set included spectra of 2HG, NAA, GABA, glutamate, glycine, creatine, myo-inositol, glutamine, lactate, alanine, acetate, aspartate, ethanolamine, glutathione, phosphorylethanolamine, scyllo-inositol, taurine, N-acetylaspartylglutamate, glucose, and choline simulated using real pulses. Macromolecules were not modelled.*

(Continues)

3. Data analysis methods and outputs		
4. Data quality		
a. Reported variables (SNR, linewidth (with reference peaks))	*SNR and linewidths not reported*	*SNR and linewidths not reported*
b. Data exclusion criteria	SNR < 5 or FWHM of creatine peak > 0.143 ppm	75th percentile 2HG/creatine values of the selected voxels
c. Quality measures of postprocessing model fitting (eg CRLB, goodness of fit, SD of residual)	2HG CRLB < 30%	2HG CRLB < 30%
d. Sample spectrum	Figures 1-3	Figures 1-3

The example above used the following paper: Zhou M, Zhou Y, Liao H, et al. Diagnostic accuracy of 2-hydroxyglutarate magnetic resonance spectroscopy in newly diagnosed brain mass and suspected recurrent gliomas. *Neuro-Oncol*. 2018;20(9):1262-1271. https://doi.org/10.1093/neuonc/noy022.
Items listed in italics are details that were not included in the paper that served as the source for this example.

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Commentary

Additional considerations for studying brain metabolite levels across pain conditions using proton magnetic resonance spectroscopy

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A B S T R A C T

Advances in proton magnetic resonance spectroscopy (MRS) allow for the non-invasive examination of neuroinhibitory and neuroexcitatory processes in humans. In particular, these methods have been used to understand changes across chronic pain conditions. While a recent meta-analysis supports the idea that underlying brain metabolite levels may be unique to different pain conditions and may serve as biomarkers for specific pain conditions, the lack of consideration of differential brain aging processes across heterogenous pain conditions introduces a significant source of bias. Future studies need to address the interactions between pain and brain aging across different MRS metabolite measures.

1. Commentary

Chronic pain is a growing public health problem that is highly prevalent in older individuals negatively impacting quality of life and overall well-being (Eggermont et al., 2014, van der Leeuw et al., 2016, Leveille et al., 2002, Leveille et al., 2002, Leveille et al., 2001, Patel et al., 2013, Patel et al., 2013). Effective pain treatments are currently lacking for this vulnerable population and the identification of potential neurobiological mechanisms underlying pain in older individuals may potentially aid development of effective therapeutic targets. Of particular relevance to the field of pain and aging neurobiology is the ability to measure the brain's major inhibitory and excitatory neurochemicals, gamma amino-butyric acid (GABA) and glutamate or the combined resonance of glutamate and glutamine (Glx) using proton magnetic resonance spectroscopy (1H-MRS). In this issue of *Neuroimage*, Peek and colleagues (Peek et al., 2020) significantly advance the use of 1H-MRS in the pain field by performing a systematic literature review and meta-analysis of 1H-MRS studies in relation to brain GABA, glutamate, and glutamine levels across various pain conditions. The authors also introduce the new Magnetic Resonance Spectroscopy quality assessment tool (MRS-Q), which is much needed to improve reporting of spectroscopy methods. In general, their results support the idea that underlying brain metabolite levels may be unique to different pain conditions and may serve as biomarkers for specific pain conditions. The authors are applauded for the examination of these pain studies including detailed information regarding MRS acquisition parameters as well as analyses considerations that will serve as important sources of variability and impact results. The study has a strong methodological approach, conducted in adherence with the Preferred Reporting Items for Systematic Reviews and Meta-analysis statement (PRISMA).

Beyond the various strengths of this outstanding work, we would like to offer additional considerations that currently limit the interpretation of the meta-analysis as it relates to brain glutamate-glutamine-GABA levels across pain conditions. Across and within the pain conditions examined, there was a wide variability in participant's age that may introduce a significant source of bias. This may be specifically problematic when examining glutamate-glutamine-GABA levels obtained from middle-to-older age individuals. Previous studies indicate age-related changes in cortical glutamate-glutamine-GABA levels, with lower glutamate-GABA and higher glutamine levels reported in older compared to younger adults (Gao et al., 2013, Grachev and Vania Apkarian, 2001, Hädel et al., 2013, Kaiser et al., 2005, Rowland et al., 2016, Sailasuta et al., 2008). Lower frontal GABA levels have also been associated with worse cognitive performance in older individuals even when bulk tissue changes are accounted for (Porges et al., 2017). However, this finding is somewhat contradictory to a study reporting that age-related differences in GABA levels are driven by bulk tissue changes (Maes et al., 2018). Given the vast literature documenting non-uniform structural brain changes with age including evidence of brain atrophy across widespread brain regions (Fjell et al., 2014, Fotenos et al., 2005, Horvath, 2013, Marner et al., 2003, Pelvig et al., 2008, Soreq et al., 2017, Taubert et al., 2020, Terry et al., 1987, Toescu, 2005), future MRS studies need to account for voxel placement location, age of participants and tissue corrections. It is plausible that conclusions regard-

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ing glutamate-glutamine-GABA levels are significantly confounded by biological brain aging processes that may be distinct and potentially independent of pathological pain processes.

To further complicate interpretations, age may have a differential effect by brain region and by pain condition where metabolite levels differ in younger versus older cohorts, as well as across pain conditions with different underlying mechanisms. Thus, grouping of clinical pain conditions must also be performed very carefully. While migraine is likely a distinct condition that occurs mainly in younger individuals, musculoskeletal pain is highly prevalent across the lifespan with certain pain types like osteoarthritis being most common in older adults. Similarly, pain conditions like pelvic pain, fibromyalgia, types of facial pain (e.g., temporomandibular pain) and irritable bowel syndrome (IBS) are functional pain syndromes, while low back pain and pains after spinal cord injury are very heterogeneous with both neuropathic and musculoskeletal pain components. While these issues are not explicitly stated in the article, the authors are commended for their detailed presentation of pain conditions in tables and figures, allowing the readers to make their own inferences.

Ultimately, the validity of inferences made from a meta-analysis depend on a number of factors such as: a) the degree to which the participants in the individual studies are representative of those in the population, (b) validity of inferences made from each of the independent studies included, (c) number of studies included, and d) sample size of the individual studies included. As an example, the small number of studies in musculoskeletal pain conditions that measure brain GABA levels suffer from small sample sizes across wide age ranges and different voxel locations, which likely lead to low statistical power and large standard errors. Thus, meta-analyses based on these studies can produce findings that are difficult to interpret (Bobko and Stone-Romero, 1998). Nonetheless, we are extremely enthusiastic about the foundation laid by Peek and colleagues (Peek et al. 2020), particularly the MRS-Q, which if followed, will allow for future H1-MRS studies focused on brain glutamate-glutamine-GABA and other metabolite levels to be more readily integrated across pain conditions. In particular, studies are urgently needed that specifically address the interactions between pain and aging brain processes to identify neurobiological mechanisms that may be targeted for treatment in our aging population.

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CHAPTER THREE

Increased GABA+ in people with Migraine, Headache and Pain Conditions- A Potential Marker of Pain

PREFACE

Chapter 3 presents a head to head comparison that investigates GABA levels in people with migraine, headache and pain conditions compared to controls.

Citation

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Publication metrics

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Dissemination

The work presented in this chapter has been presented at the following conferences:

- **Peek AL**; Leaver A and Rebbeck T. GABA+ a potential marker of pain- A step closer towards a better understanding of pain neurochemistry. [Poster presentation online with oral commentary], Australian Pain Society, 21st April 2021

- **Peek AL**; Leaver A; Galloway G and Rebbeck T. Brain Neurochemicals in Pain Conditions: A Cross-Sectional Study, JWCRR Forum, 30th July 2019

Impact

The art-work from this paper was featured on the front cover of the Journal of Pain, December 2021 (Forward to chapter). This paper received attention from Migraine Australia which attracted two interviews (Appendix 3). Migraine Australia is a charitable organisation and support group with over 10K follower. The Facebook video has received 500+ views, and the interviews have also been posted on YouTube for open access viewing.

AUTHORSHIP ATTRIBUTION STATEMENT

The co-authors of the paper '*Increased GABA+ in people with Migraine, Headache and Pain Conditions- A Potential Marker of Pain'* confirm that Aimie Laura Peek has made the following contributions:

- Design of the work
- Acquisition of the data
- Analysis and interpretation of the data
- Manuscript preparation, revision and critical appraisal for important intellectual content

As the primary supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

[redacted]

As. Prof. Trudy Rebbeck

Faculty of Medicine and Health, University of Sydney

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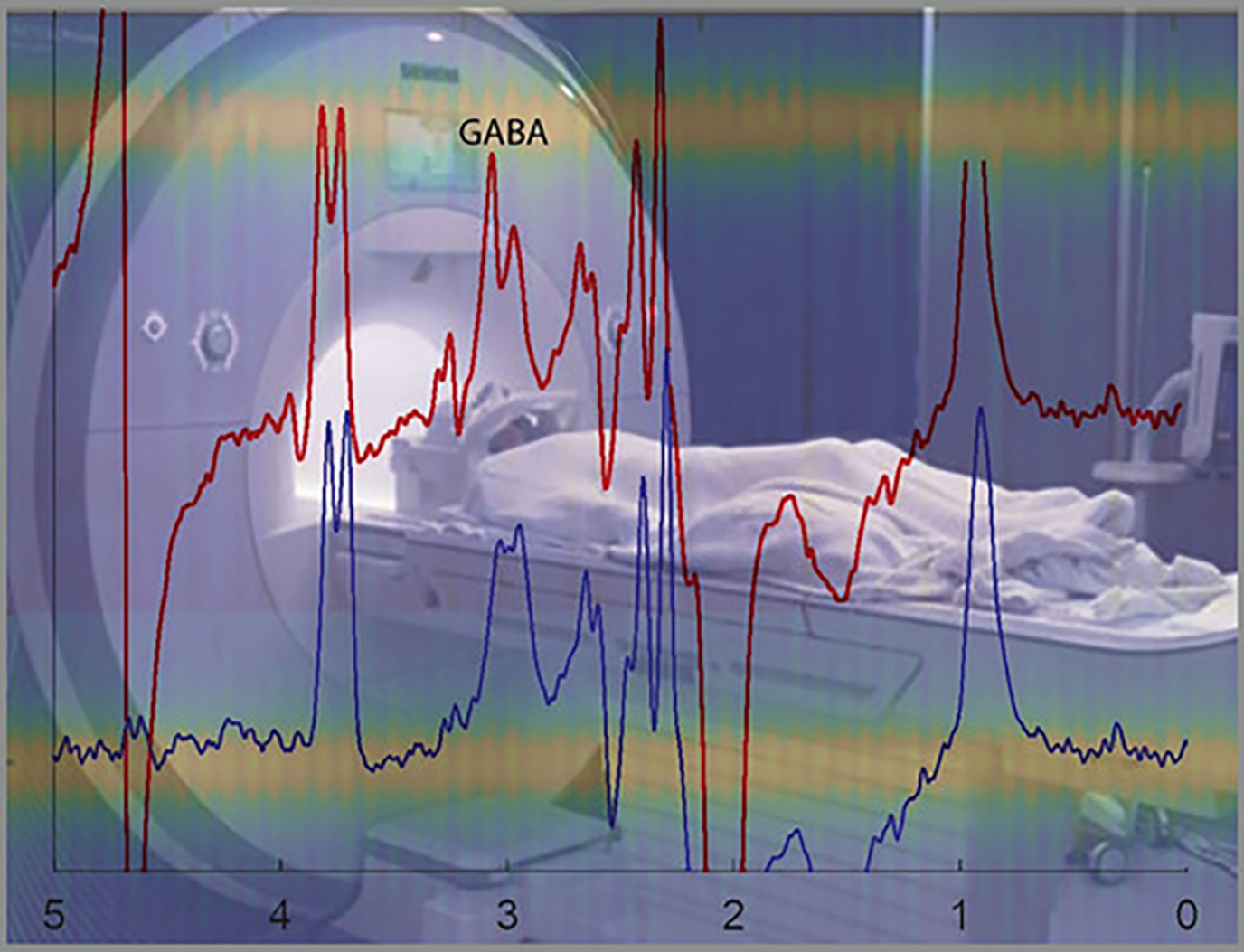

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Increased GABA+ in People With Migraine, Headache, and Pain Conditions- A Potential Marker of Pain

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Abstract: **Treatment outcomes for migraine and other chronic headache and pain conditions typically demonstrate modest results. A greater understanding of underlying pain mechanisms may better inform treatments and improve outcomes. Increased GABA+ has been identified in recent studies of migraine, however, it is unclear if this is present in other headache, and pain conditions. We primarily investigated GABA+ levels in the posterior cingulate gyrus (PCG) of people with migraine, whiplash-headache and low back pain compared to age- and sex-matched controls, GABA+ levels in the anterior cingulate cortex (ACC) and thalamus formed secondary aims. Using a cross-sectional design, we studied people with migraine, whiplash-headache or low back pain (n = 56) and compared them with a pool of age- and sex-matched controls (n = 22). We used spectral-edited magnetic resonance spectroscopy at 3T (MEGA-PRESS) to determine levels of GABA+ in the PCG, ACC and thalamus. PCG GABA+ levels were significantly higher in people with migraine and low back pain compared with controls (eg, migraine 4.89 IU ± 0.62 vs controls 4.62 IU ± 0.38; *P* = .02). Higher GABA+ levels in the PCG were not unique to migraine and could reflect a mechanism of chronic pain in general. A better understanding of pain at a neurochemical level informs the development of treatments that target aberrant brain neurochemistry to improve patient outcomes.**

Perspective: ***This study provides insights into the underlying mechanisms of chronic pain. Higher levels of GABA+ in the PCG may reflect an underlying mechanism of chronic headache and pain conditions. This knowledge may help improve patient outcomes through developing treatments that specifically address this aberrant brain neurochemistry.***

Keywords: *Pain, GABA+, Proton magnetic resonance spectroscopy, MRS, 1-HMRS.*

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Chronic pain is the leading cause of disability worldwide, when considering both migraine, and musculoskeletal pain conditions such as low back pain.[82] Randomized controlled trials in musculoskeletal pain[40,81] and headache conditions,[13,71,78] have investigated numerous approaches to reduce the burden of chronic pain. However, results remain modest with some patients failing to respond to interventions.[17,34,68,74] It is not fully understood why some people fail to recover from an initial onset of pain. However, gaining a better understanding of potential underlying neurochemical mechanisms may offer a further insight into the development and persistence of chronic pain.

One proposed mechanism of chronic pain is dysfunction in the metabolism of GABA, the main inhibitory neurometabolite of the central nervous system.[20] Chronic pain has been proposed to be associated with reduced levels of GABA+. Here, the term GABA+ is used rather than GABA to acknowledge the unwanted co-edited macromolecules and homocarnosine that are typically edited alongside GABA in GABA-edited magnetic resonance spectroscopy (MRS). This reduced GABA+ is considered reflective of a state of hyperexcitability.[23] The hyperexcitability, or loss of inhibition in combination with altered thalamocortical connectivity is said to give rise to a constant perception of pain.[16,33,39] However, the reduction in GABA+ has not been observed across all pain conditions. Two contrasting examples are migraine and low back pain. Increased GABA+ was found in adults and children with migraine compared to controls, using MRS sequences optimized to detect GABA+[2,5] and macromolecule suppressed GABA respectively[4]. It was hypothesized that increased GABA/GABA+ levels could reflect a migraine-specific mechanism such as cortical spreading depression, neurogenic inflammation or vasodilation.[2,43,60] In contrast, no differences in GABA+ levels were detected in people with low back pain compared to healthy controls. This may suggest that the dysfunction in hyperexcitability could be driven by an increase in excitatory neurometabolites such as glutamate rather than the inhibitory GABA.[31] Alternatively, it might reflect that the MRS parameters were not sufficiently optimized to detect GABA+ or GABA in these studies. Grachev *et al*[28] used a non-edited STEAM sequence- ie, not optimized to measure GABA or GABA+, and Janetzki *et al*[42] failed to report which parameters were used. Taken together, an increase in GABA+ may be a unique underlying mechanism of migraine, however, further studies optimized to detect GABA+ are required in other pain conditions.

To date a direct comparison of GABA+ levels in headache and pain groups has not been completed in the same GABA+ optimized MRS study. A recent meta-analysis of studies reporting GABA+ levels in pain conditions concluded that GABA+ levels appeared increased in migraine but variable in other pain conditions.[62] This suggests that GABA+ levels could possibly be specific to the pain condition and might reflect a migraine specific process such as cortical spreading depression, rather than more generic pain states such as central sensitization which can be seen across all chronic pain conditions. In addition to the limitation that pain groups were not compared within a single study, the quality of the methods of MRS acquisitions varied significantly.

In recent years, methods to quantify GABA+ have significantly evolved. High-quality studies use optimized sequences (MEscher GArwood point resolved spectroscopy; MEGA-PRESS[48]) and acquisition parameters specifically designed for detecting GABA+.[54] However, none of the 4 studies[29,42,67] of musculoskeletal pain included in the meta-analysis[62] reported utilizing these parameters. Furthermore, there was substantial variation in the brain region studied; a variable considered a potential confounder in the measurement of GABA+.[30,65,80,83] Consequently, it is unclear whether the variation in GABA+ levels seen across pain conditions are reflective of a true between-group difference or rather a reflection of the studies' methodological quality or brain region examined.

A number of brain regions have been proposed to be involved in the modulation of pain. The posterior cingulate gyrus formed our primary aim following our previous work which showed increased GABA+ in this region in people with migraine, which was associated with higher levels of central sensitisation.[1,2] The anterior cingulate cortex, and thalamus formed secondary aims due to their well-documented role in the pain experience.[16,39,84]

Therefore, the aim of this study was to establish whether the increased levels of GABA+ reported in high quality studies of migraine were present in other headache or pain conditions. The results of the study will provide further insight into mechanisms or consequences of chronic pain conditions, which in turn may direct future treatment options for those with persistent chronic pain.

Materials and Methods

Study Design

The study used a cross-sectional, case-control design to measure levels of GABA+ determined using MRS in people with migraine, other pain conditions and healthy control subjects. Ethical approval was granted from Western Sydney Local Health District (WSLHD) study number HREC/17/WMEAD/429. Written consent from participants was gained in line with the principles of the Declaration of Helsinki.

Participants

Inclusion/Exclusion Criteria

We aimed to recruit a total of sixty participants with a pain condition (n = 20 migraine, n = 20 whiplash-headache and n = 20 low back pain) to be age- and sex-matched to an individual from a pool of pain-free controls (n = 22) (Fig 1). Participants with migraine were included if they satisfied the conditions according to the International classification of headache disorders (ICHD)-3[41] criteria for migraine with or without

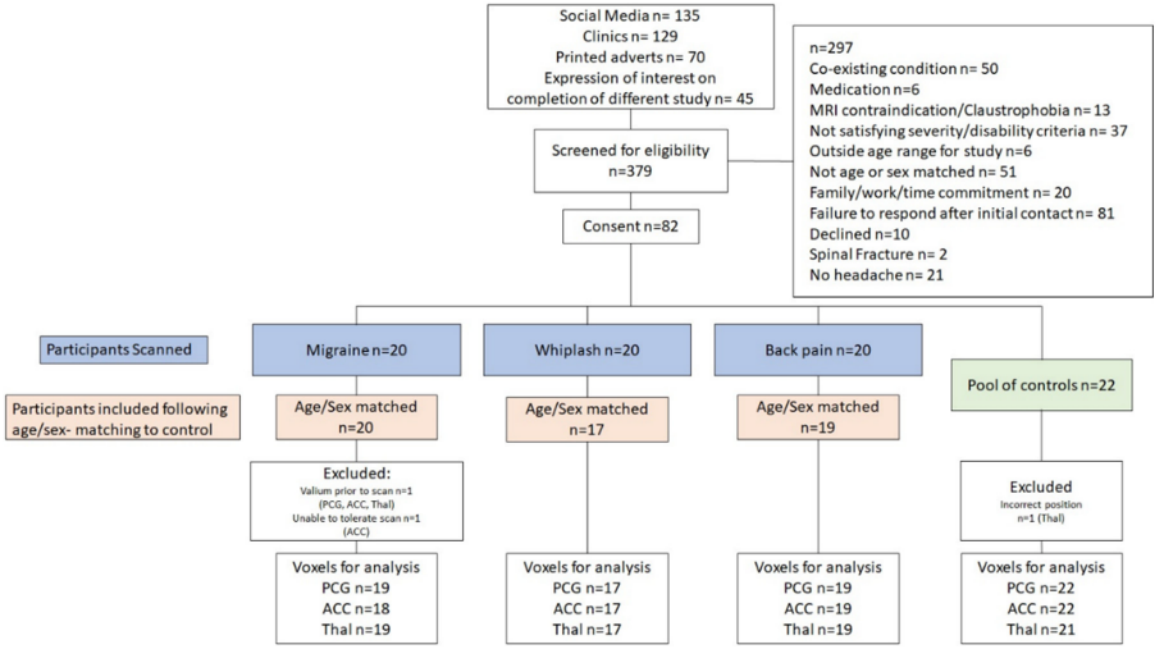

Figure 1. Flow of participants through the study.

aura (https://ichd-3.org/1-migraine/), had experienced migraines for at least 3 months, and had a Headache Impact Test (HIT-6) score over 50 (exceeding moderate headache-related disability). Participants with whiplash-headache were included if they satisfied ICHD-3 for 'whiplash with persistent headache', had symptoms for at least 3 months, and had moderate headache-related disability (HIT-6 >50). Participants with low back pain were included if they had pain in the region between T12 to S1 in accordance with the International Classification of Diseases (ICD) diagnosis for low back pain,[86] had experienced symptoms for at least 3 months, and had an Oswestry Disability Index (ODI) score over 20% (exceeding moderate low-back-pain-related disability). Pain-free controls were included if they had not experienced a previous migraine, regular headaches or a pain condition in the last 3 months or had not experienced a previous pain condition that had lasted for over 3 months.

Participants were excluded from the study if they had any contraindications for MRI or conditions that compromised MR spectroscopy such as pregnancy, metal implants, claustrophobia, or metal braces. Participants were also excluded if they experienced severe depression, had a diagnosed neurological or psychiatric condition or took medications which might influence GABA or glutamate levels in the brain such as gabapentin, pregabalin, topiramate, diazepam or glutamate. Furthermore, participants were not included in the study if they had symptoms in common with the other groups (eg, both migraine and low back pain). Additional exclusion criteria applying to all groups included not being independently ambulant, health complaints in the last 5 days and the inability to communicate in the English language. Further exclusion criteria were applied to the whiplash-headache group, including: any self-reported pre-existing headache condition, or if the road traffic crash was major (eg, involved spinal fracture or occupant fatality).

Age and Sex Matching

Both age and sex matching are important in the analysis of GABA+. There is a growing body of evidence suggesting GABA+ is lower in females than males[57,69] and decreases with age, although the rate of this has yet to be established.[25,63,73] To address the impact of age without age-restricting, a pair-wise sampling approach was adopted. Participants with pain were matched to a participant from a pool of twenty-two controls (within 5 years of age and the same sex). The pool of controls was established through age- and sex-matching to the migraine group with the addition of 2 participants matched to accommodate the whiplash-headache group.

Recruitment

Participants with headache and pain conditions were recruited from primary and secondary care settings. These included physiotherapy, general practitioner and neurology clinics as well as hospital outpatient departments. Potential participants either responded to an advertisement placed in a participating clinic, or clinicians working within the clinic gained consent from the potential participant to be contacted by the research team. Controls were recruited through advertisements placed on University, hospital and community noticeboards and on social media. Potential participants were first screened

by telephone and eligible participants were given detailed information about the study before providing written informed consent. Participants then completed self-reported measures of pain and disability online and underwent MRS examination.

Participant Characteristics

Participant demographic and clinical characteristics were collected using the online survey platform, REsearch Data Capture (REDCap)[37]. Demographic data included age, gender, height, weight, and educational level. Symptom data collected at baseline were pain duration and intensity and a self-reported description of symptoms. Participants also completed self-reported measures of pain, disability, and psychological distress at baseline (detailed below).

Self-report Measures of Pain and Disability

The Numeric Rating Scale (NRS) is the most widely used measure of pain and has been validated for use in multiple pain conditions. The score range is 0-100 with higher scores reflecting higher levels of pain.[22,85] The Central Sensitization Inventory (CSI) is a validated 100-point scale, where higher scores are associated with higher levels of hypersensitivity to both noxious and non-noxious stimuli.[47,56] Scores over 22.5 have been associated with migraine,[1] and scores over 40 have been associated with the presence of central sensitization syndromes such as fibromyalgia.[56] The Headache Impact Test (HIT-6) is a validated tool measuring headache-related disability. Scores range from 36 to 78 with higher scores demonstrating higher disability, and scores over 50/78 are considered high.[11,87,88] The World Health Organization Disability Assessment Schedule (WHODAS 2.0-12) is a validated measure of general disability. The score ranges from 12 to 60 with higher levels indicating greater disability.[4,14,64] The Depression, Anxiety and Stress Scale (DASS- 21) is a validated tool to measure depression, anxiety and stress, with sub-scores over 7/21, 6/21 and 10/21, respectively, indicating moderate levels of distress.[59] The Post Traumatic Stress Disorder (PTSD) Checklist identifies those who are experiencing PTSD; it is scored from 0 to 80, and with scores over 31 indicating the likely diagnosis of PTSD[8].

Magnetic Resonance Spectroscopy

Brain neurometabolite concentration was assessed using a J-difference-edited proton magnetic resonance spectroscopy (MRS) sequence, with the metabolite of interest in this study being GABA, the primary inhibitory neurotransmitter in the human brain. J-difference editing is a widely used method to study coupled signals from low-concentration molecules such as GABA that are overlaid by larger signals from other metabolites. The most commonly used method for GABA detection, MEGA-PRESS,[26,48] consists of 2 experiments: In the first ("ON") experiment, a frequency-selective radiofrequency pulse is applied to refocus the evolution of the scalar coupling in the GABA molecule; in the second ("OFF") experiment, this coupling is allowed to evolve freely. Subtracting the OFF from the ON spectrum yields the GABA-edited difference spectrum with a prominent GABA peak at 3.02 ppm, while the overlaying strong signals from creatine and phosphocreatine are cancelled out. Due to the finite selectivity of the editing pulses, the edited peak contains contaminations from co-edited macromolecules and homocarnosine, and is therefore termed "GABA+" in the literature.[15,54] The levels of GABA+ were reported in institutional units, ie, relative to the internal concentration reference of tissue water. GABA+ levels were corrected for magnetization relaxation effects of tissue water, and for differing concentration of GABA between grey and white matter.[36] Potential confounders of GABA+ concentration have been reported. In order to reduce the impact of these potential pharmacological confounders, participants were not allowed to consume pain medications, nicotine, alcohol or caffeine on the day of scanning.[6,10,53,58]

MRI and MRS Acquisitions

Structural imaging and spectroscopic data were acquired on a clinical Siemens PRISMA 3T scanner (Siemens Healthineers, Erlangen, Germany, software version VE11C) at a large teaching hospital in Sydney, Australia, using a 64-channel head/neck array coil for RF signal reception. Anatomical images were acquired as follows. Firstly, a high-resolution 3D T_1-weighted isotropic scan (MPRAGE) was acquired sagittally to guide voxel placement using all 3 reformatted planes for accurate positioning (repetition time (TR) = 2400 ms; echo time (TE) = 2.21 ms; inversion time (TI) = 1150ms; voxel size = 1. 0 × 1.0 × 1.0mm^3; iPAT = 3; FOV = 256mm; matrix = 256 × 256; acquisition time = 4min 35sec). Secondly, an axial 2D T_2-weighted series (TR = 7490ms; TE = 99ms; voxel size = 0.6 × 0.3mm^3; iPAT = 2; FOV = 220mm; matrix = 384 × 288; acquisition time = 2min 24sec) was also acquired to further aid voxel placement. Both the T_1-W and T_2-W series were sent for review and reporting by a consultant radiologist to allow for identification and follow up of any incidental findings.

Following the sequence Application Guide and Release Notes,[12] flip angle calibration was then performed for each voxel location (TR = 2000ms; TE= 30ms, 1 average). The RF power value identified to achieve the optimal flip angle resulting in maximal signal-to-noise ratio (SNR) and subsequent peak height was then used for the MEscher-Garwood Point RESolved Spectroscopy (MEGA-PRESS)[48] acquisition for GABA+ detection. MEGA-PRESS data were then acquired with the following parameters: TR = 2000ms; TE = 68 ms; 192 averages; 2048 data points; spectral width = 2000 Hz; editing pulse frequencies set to 1.9 ppm and 7.5 ppm for editing of GABA; editing pulse bandwidth = 70 Hz; center of slice selection frequency ("delta frequency") = -1.7 ppm relative to water for optimal co-localization of the 3.0 ppm GABA signal with the prescribed voxel. Finally, water-unsuppressed MEGA-PRESS data were acquired (1

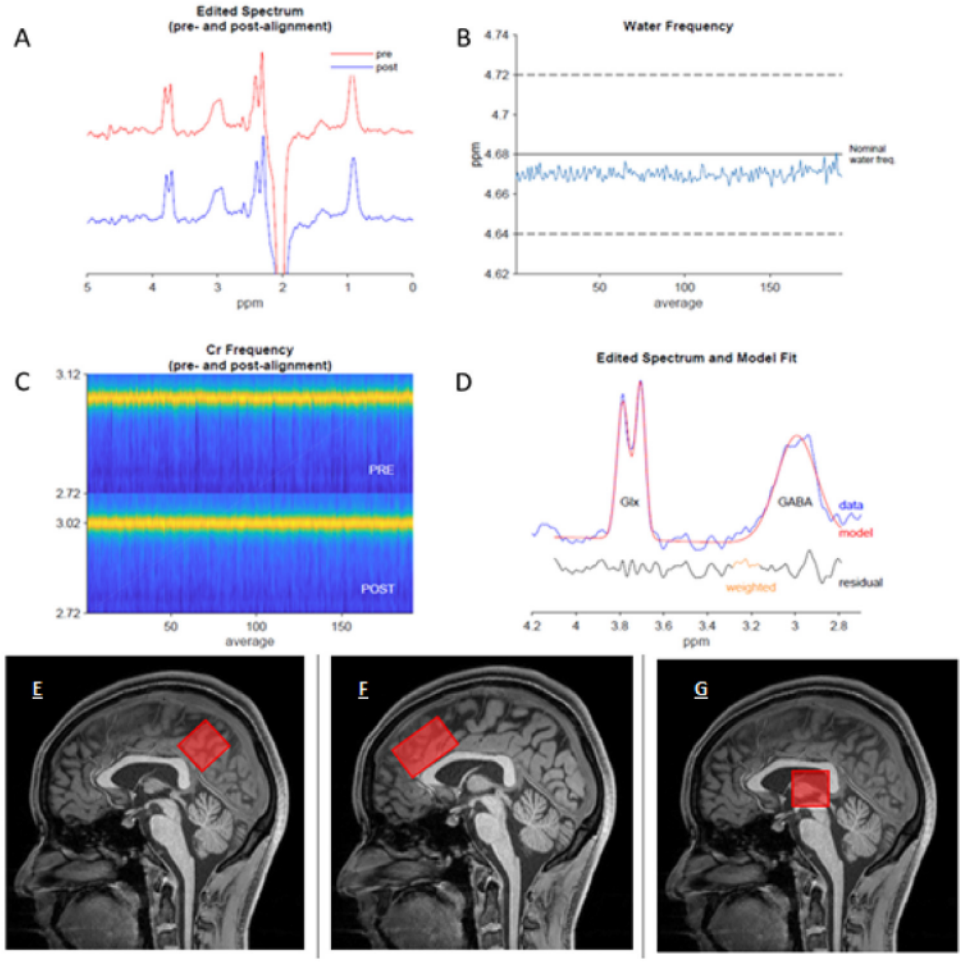

Figure 2. Example spectra from the PCG of a participant following fitting, and visual representation of voxel positioning. Example Gannet output taken from a migraine participant. (A) Demonstrates the processed GABA-edited difference spectrum before frequency and phase correction (red) and after (blue) (B) Shows the residual water signal plotted against time, demonstrating the stability of the experiment with respect to eg, field drift, or motion, (C) Shows the creatine signal (yellow) over the duration of the experiment, pre- refers to before frequency-and-phase correction and post refers to after. (D) Shows the output from the GannetFit module. The GABA-edited spectrum is shown in blue, the model in red, and the fit residual in black below. Schematic diagram to demonstrate the voxel placement for (E) PCG, (F) ACC and (G) thalamus (Color version of the figure is available online.).

average; delta frequency = 0.0 ppm for optimal co-localization of the unsuppressed water signal).

Brain Regions

The primary brain region of interest was the posterior cingulate gyrus (PCG) because it has been shown to be implicated in migraine[2] and associated with reports of increased central sensitization.[1] Further, posterior regions of the brain have demonstrated good reliability for GABA+ measurements.[65,72] Secondary brain regions of interest were the anterior cingulate cortex (ACC) and thalamus. The ACC was selected for its potential association with the affective and psychosocial aspects of pain.[84] The thalamus has been shown to be critically involved in central pain processing networks using fMRI, volumetric studies and MR spectroscopy.[16,39] Whilst the insula has also been identified as a potential contributor to pain mediation,[75,79] it was not deemed feasible to be included due to protocol time restrictions. Imaging was limited to 1 hour, in order to ensure participant compliance and reduce the chance of movement-related artifacts.

Voxel Placement

The PCG voxel measured 25(AP) × 40 (RL) × 25 (CC) mm^3 and was positioned closely aligned parallel to the superior posterior border of the splenium of the corpus callosum, inferior to the cingulate sulcus and antero-superior to the parieto-occipital sulcus, covering the PCG (and a portion of the inferior precuneus; Fig 2E). The ACC voxel measured 40 (AP) × 25 (RL) × 25 (CC) mm^3 and was aligned parallel to the superior anterior border of the genu of the corpus callosum and inferior to the supero-anterior portion of the cingulate sulcus (Fig 2F). The thalamic voxel measured 25(AP) × 40 (RL) × 25 (CC) mm^3 and was angled slightly over the thalamus on para-sagittal images to encompass maximal thalamic tissue. (Fig 2G). The long axis of the voxel was aligned anterior-to-posterior in the ACC and right-to-left in the PCG and thalamus.

MRS Processing

MEGA-PRESS data were processed using the MATLAB-based toolbox Gannet (version 3.1.3).[18] Gannet consists of several modules: *GannetLoad* applied zero-filling, 3-Hz exponential line broadening, and frequency-and-phase-correction of individual averages using the spectral registration algorithm.[55] A new, improved method of spectral alignment called "RobustSpecReg", has been found to reduce subtraction artefacts in a majority of datasets, and was therefore used alongside spectral registration in order to minimize fit error.[52] The aligned transients are then averaged to yield the edit-ON and edit-OFF spectra, which are subsequently subtracted to create the GABA-edited MEGA-PRESS difference spectrum. Each dataset was processed using both alignment algorithms, and a decision as to which result to use for further analysis was made based on visual inspection by a researcher with over 8 years of experience working on spectral editing (GO) who was blinded to group allocation. Spectral fitting was performed by the GannetFit module, which applies a single Gaussian model to fit the 3.02 ppm GABA+ signal and models the creatine peak in the "OFF" spectrum as well as the water signal in the water-unsuppressed data as Lorentzian peaks. The GannetCoRegister and GannetSegment modules call SPM12[24] to determine the tissue volume fractions of grey matter, white matter and cerebrospinal fluid. GannetQuantify then returns GABA levels in institutional units (IU), corrected for effects of tissue water content and relaxation effects as well as for GABA being present in grey matter at approximately twice the concentration of that in white matter.[36]

MRS Quality Assessment

The quality of the spectra was inspected by an independent investigator (GO) who was blinded to participant group. The spectra were analyzed visually for artifacts and insufficient water suppression. Where the fit error (standard deviation of the fit residual divided by the amplitude of the model) exceeded 10%, data were processed, and fitted using spectral registration rather than Robust spectral registration.[52] In cases where the fit error improved, this new analysis was retained. Should fit error exceed 12%; the cut off typically recommended in GABA spectroscopy papers,[18,65,66] the scan was excluded from the analysis.

Statistical Analysis

A sample size of 17 per group was calculated a priori. This was based on our previous study[2] and allows detection of a between-group GABA+ level difference of 0.2 IU with 80% power. Participants were age- and sex-matched within a maximum of 5 years in order to account for potential age-related GABA changes that have been observed.[25,45,46,63] (See above *section 2.2.2.* Age and Sex matching).

Data were tested for normality of distribution using the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Descriptive statistics (mean, standard deviation (SD) or median, interquartile range (IQR)) were used to report normally and non-normally distributed participant demographics and pain characteristics respectively. Between-group differences in participant characteristics were assessed using a Chi-square test for data presented as proportions (for example sex), and analysis of variance (ANOVA) or Kruskal-Wallis for normally and non-normally distributed data, respectively. Significance values were then adjusted by the Bonferroni correction for multiple tests.

In order to detect the difference in GABA+ levels between the pain groups and their paired age- and sex-matched controls, we used Wilcoxon signed rank tests. This test is recommended for pair-wise comparisons when data are non-parametric,[44] was used in our previous study[2] and allows for the close age- and sex-matching required for the analysis of GABA+ which is not possible when using a comparison of means such as an ANOVA.

In order to adjust for multiple comparisons, tests were split into 3 groups dependent on brain region and adjusted for using the Holm-Bonferroni procedure.[70]

To determine the difference in GABA+ levels between the different pain groups, the same analysis was used. We calculated pair-wise differences in 3 age- and sex-matched comparisons 1) migraine versus whiplash-headache, 2) whiplash-headache versus back pain, 3) back pain versus migraine. Whilst this method has the benefit of allowing for pair-wise comparison between similar individuals in terms of age and sex between the groups, it did limit the number of pairs that could be included in the analysis between pain groups.

To assess data quality between groups we also investigated group differences in fit error, SNR, frequency drift, and the full-width half-maximum (FWHM) of the modelled water peak using the Kruskal-Wallis non-parametric test.

A post-hoc analysis investigating the correlation between PCG GABA+ levels and pain duration was conducted by determining Pearson's r correlation coefficient.

Statistical analyses were conducted using SPSS version 26 (SPSS Inc, Chicago, IL). A *P*-value of $<.05$ was deemed significant across the analyses.

Data Availability

Gannet 3.1.3 code is available from: https://github.com/richardedden/Gannet3.1/releases/tag/v3.1.3. The data supporting the study's findings are available from the corresponding author, upon reasonable request following approval from the University of Sydney.

Results

Participants

Three hundred and seventy-nine participants were screened. Of these 297 were excluded, and 82 (migraine n = 20, whiplash-headache n = 20, low back pain n = 20 and controls n = 22) were enrolled. Following age- and

sex-matching, 56 participants with a pain condition (migraine n = 20, whiplash-headache n = 17, low back pain n = 19) and 22 controls were included in the final analysis. Recruitment was halted in April 2020 due to research restrictions related to the 2020 COVID-19 pandemic leaving 4 unmatched participants (whiplash-headache n = 3, low back pain n = 1). These participants were excluded from the analysis. One participant in the migraine group was excluded after scanning because they took diazepam prior to the scan. Data were missing from the ACC voxel of a single migraine participant and the thalamic voxel of a single control participant due to acquisition error (Fig 1).

Baseline Characteristics

All groups were similar with respect to age, sex, body-mass index (BMI), and educational status (Table 1). The *pain groups* were all similar with respect to pain intensity (eg, migraine mean ± SD 66.05 ± 22.9, whiplash-headache mean 58.8 ± 24.4 and low back pain mean 57.88 ± 20.26). The whiplash-headache group had significantly higher scores for DASS anxiety (median 30, interquartile range [IQR] 22 to 76) and depression (10, [4 to 24]) compared to the other migraine and back pain groups. The low back pain group had significantly lower CSI (mean ± SD 31.26 ± 14.74) and HIT-6 compared to both the whiplash and headache groups (Table 1). Participants were asked to rate their pain at time of scanning, pain levels were similar across the pain groups, all pain participants reported pain that related to their specified pain condition (eg, current headache levels in the migraine group and back pain in the back pain group), controls reported 4/100 pain on the NRS which related to inconsequential aches from everyday living (eg, post-exercise soreness).

Spectroscopy Quality

The fit error reflecting spectroscopy quality was under 10% for 209/212 (98%) of voxels with a mean fit error of 4.64% ± 1.08% for the PCG, 4.93% ± 0.96% for the ACC and 5.59% ± 1.8% for the thalamus (Fig 2). Where fit error exceeded 10% (3 voxels) the alternative alignment methods of robust spectral registration was applied (n = 1 control ACC, n = 1 control thalamus, n = 1 whiplash-headache thalamus). After this procedure, all spectra were modelled with a fit error below the recommended value,[18,66] and were in line with those reported in the largest MRS dataset available.[49] Therefore, all spectra were included in the analysis (Fig 1). Average frequency offset, representing frequency drift was -0.02 ppm ± 0.01 in the PCG, -0.01 ppm ± 0.02 in the ACC and -0.02 ppm ± 0.01 in the thalamus, which is also in line with the Big GABA dataset.[49] Spectral quality in terms of fit error, SNR, frequency drift, and full-width half maximum (FWHM) of modelled water signal were the same across all groups

Table 1. Characteristics of Participants.

	Migraine (n = 20)	*Whiplash (n = 17)*	*Back Pain (n = 19)*	*Controls (n = 22)*	P *value*	*Post-hoc- P < .05*
Age	39.65 ± 9.95	41.12 ± 12.1	38.68 ± 12.79	39.27 ± 11.49	0.78 b	-
Sex (female n, %)	16, 80	11, 65	14, 74	16, 73	0.78c	-
BMI	26.1 (22.2 – 32)	25.3 (20.8 – 33)	25 (22.8 – 27.6)	25.7 (23.4 – 26.5)	0.91 a	-
Educational level (University n, %)	12, 60	7, 41	8, 42.1	16, 72.7	0.13 c	-
Pain Characteristics						
Duration- ys	18 (10.5 – 25)	2.67 (1.5 – 4)	5 (2 – 12)	N/A	0.01a	^ Ⅱ
Average pain intensity in last wk (NRS 0 – 100)	66.05 ± 22.9	59.18 ± 21.29	54.58 ± 20.05	N/A	0.27b	-
Pain/Symp–ms						
Pain at time of scan (NRS 0-100)	40 (5 – 62)	31 (21 – 50)	30 (20 – 38)	0 (0 – 5)	0.01a	ǂ § †
CSI	48.65 ± 16.5	51.24 ± 16.77	31.26 ± 14.74	9 ± 9.1	0.01b	ǂ § † Ⅱ ≠
Disability						
WHODAS 2.0	26.9 ± 18.4	31.71± 18.84	19.74 ± 10.84	0.64 ± 1.56	0.01b	ǂ § †
HIT-6 (36 – 78)	68 (62 – 70)	65 (61 – 67)	40 (36 – 44)	36 (36 – 38)	0.01a	ǂ § Ⅱ ≠
Psychological Status						
DASS	20 (12 – 32)	30 (22 – 76)	16 (8 – 34)	2 (0 – 6)	0.01a	ǂ § †
Depression	2 (0 – 9)	10 (4 – 24)	4 (2 – 8)	0 (0 – 0)	0.01a	§ † ^
Anxiety	6 (2 – 12)	6 (4 – 16)	2 (0 – 6)	0 (0 – 2)	0.01a	ǂ § ≠
Stress	10 (8 – 17)	16 (10 – 24)	6 (4 – 20)	0 (0 – 4)	0.01a	ǂ § †
PTSD Checklist	9 (5 – 16)	21 (9 – 36)	6 (2 – 17)	0 (0 – 4)	0.01a	ǂ § †

Normally distributed data presented as Mean ± SD, and non-normally distributed data presented as median and (IQR). Between group analysis are denoted as follows: 1) Kruskal-Wallis test; 2) ANOVA; 3) Chi-Squared. Symbols represent significant post-hoc comparisons ($P < .05$) following Bonferroni correction as follows:
^Migraine versus Whiplash,
ⅡMigraine versus Low back pain,
ǂMigraine versus Control,
≠Whiplash versus Low back pain,
§Whiplash versus Control,
†Low back pain versus Control.

Table 2. Pair-wise Comparisons Between Pain Group and Control.

	N	Pain GABA+ IU (Mean± SD)	Control GABA+ IU (Mean± SD)	Mean difference± SD	Z score	P-value
PCG						
Migraine	n = 19	4.89 ± 0.62	4.62 ± 0.38	0.28 ± 0.63	-2.29	.02*
Whiplash	n = 17	4.78 ± 0.43	4.63 ± 0.44	0.16 ± 0.33	-1.82	.07
Back Pain	n = 19	4.88 ± 0.44	4.6 ± 0.32	0.31 ± 0.47	-2.67	.01*
ACC						
Migraine	n = 19	4.51 ± 0.38	4.59 ± 0.51	-0.06 ± 0.64	-0.28	.77
Whiplash	n = 17	4.47 ± 0.79	4.69 ± 0.99	-0.22 ± 1.33	-0.73	.46
Back Pain	n = 19	4.60 ± 0.47	4.47 ± 0.5	0.13 ± 0.73	-0.85	.40
Thalamus						
Migraine	n = 19	6.16 ± 1.23	5.75 ± 0.78	0.41 ± 1.05	-1.49	.14
Whiplash	n = 17	6.08 ± 0.79	5.61 ± 0.91	0.38 ± 1.47	-1.40	.16
Back Pain	n = 18	6.55 ± 1.78	5.53 ± 0.80	1.01 ± 1.81	-2.11	.04*

*denotes statistical significance (*P*-value <.05).

and in line with the largest collected GABA+ dataset[49,51] (Supplement 1).

Primary Analysis

Comparison Between Individual Pain Groups and Controls in the PCG

GABA+ levels were significantly higher in people with migraine (mean ± SD 4.89 ± 0.62 IU) compared with controls (4.62 ± 0.38 IU) in the PCG (mean difference ± SD 0.28 ± 0.63 IU, P = .02; Table 2, Fig 3B). Similarly, GABA+ levels were significantly higher in people with low back pain (mean ± SD 4.88 ± 0.46 IU) compared with controls (4.58 ± 0.34 IU) in the PCG (mean difference 0.32 ± 0.50 IU, P = .02). Both of these results maintained their significance when corrected for multiple family-wise comparisons (migraine P = .04, low back pain P = .03). In contrast, whilst there was a trend towards an increase in GABA+ levels in people with whiplash-headache (mean ± SD 4.78 ± 0.43) compared to controls (4.63 ± 0.44 IU), it did not reach statistical significance (mean difference 0.16 ± 0.33, P = .07). The majority of participants had higher GABA+ compared to controls irrespective of pain group (73.5% of the migraine group, 70.5% of the whiplash-headache group and 78.9% of the back pain group).

Secondary Analysis

Comparison Between Individual Pain Groups and Controls in the Thalamus and ACC

In the thalamus, GABA + levels were significantly higher in people with back pain (mean ± SD 6.55 ± 1.78 IU) compared with controls (5.53 ± 0.80 IU; mean difference ± SD 1.01 ± 1.81 IU, P = .04). However, when corrected for multiple family-wise comparisons significance was lost (P = .12). There were no other statistically significant differences between people with migraine or whiplash-headache compared with controls. (Table 2, Figure 3B)

In the ACC, GABA+ levels were not significantly different in any of the pain groups compared to controls. (Table 2, Figure 3B).

Comparison Between Pain Groups

Both age and sex matching were possible in 14 pairs of migraine-whiplash participants, 16 pairs of whiplash-back pain participants and 16 pairs of back pain-migraine participants. There were no significant differences in GABA+ levels between any of the pain groups in any of the brain regions (Table 3).

Discussion

This study found that GABA+ in the PCG was significantly increased in people with migraine and low back pain compared to controls. Whilst not statistically significant, the whiplash-headache group also showed a trend for increased GABA+ compared to controls. In addition, we found that the increase in GABA+ observed in the PCG was not present in the other brain regions examined, except for the thalamus in the low back pain group.

Increased GABA+ levels observed across all pain groups in this study suggest that the mechanism may be reflective of a chronic pain condition rather than being unique to migraine. Although not statistically significant in the whiplash-headache group, GABA+ levels were found to be higher in 70% of these participants compared to their controls. The secondary analysis revealed no significant difference between the pain groups, suggesting each pain group displays similar GABA+ levels. Together, these findings propose that the increase in GABA+ is not unique to migraine and may be more reflective of a mechanism seen across chronic pain conditions.

We can speculate that the increased level of GABA+ observed in the pain groups could potentially be explained by 2 proposed mechanisms. Firstly, increased GABA+ could be reflective of an adaptive response developed over time in response to pain, as proposed

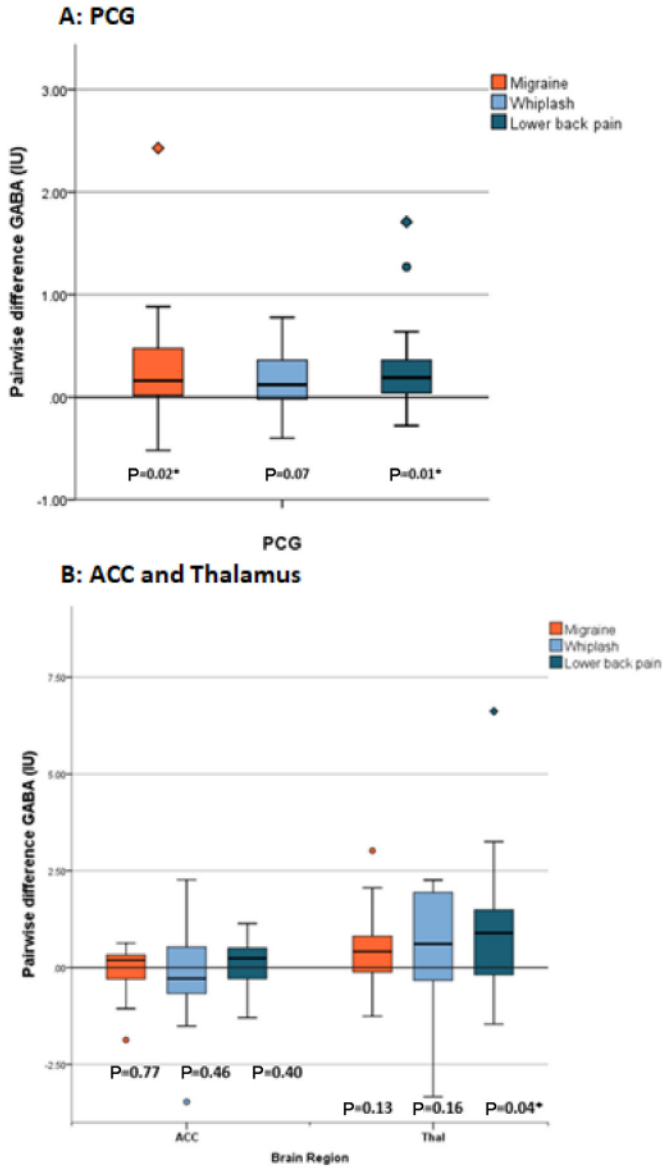

Figure 3. Pair-wise difference in GABA+ (IU) between pain groups and control. (A) Pairwise difference GABA+ (IU) in pain groups compared to control for the PCG. (B) Pairwise difference GABA+ (IU) in pain groups compared to control for the ACC and thalamus. Data are presented as box plots where the boxes represent the interquartile range (IQR), the line within the box demonstrate the median, and the outer whiskers represent the range of data. Outliers are plotted as circles where they reach >1.5 IQR below or above the 25th or 75th percentile and diamonds where they reach >3 IQR below or above the 25th and 75th percentile. *P* values with stars indicate the statistically significant difference in GABA+ levels between pain group and control.

Table 3. Pair-wise Comparisons Comparing Difference in GABA+ Levels Between Age- and Sex-matched Individuals From Different Pain Groups.

	Region	*Pain A Mean± SD*	*Pain B Mean ± SD*	*Pairwise Difference ± SD*	*Z score*	P *value*
Migraine (A) versus Whiplash (B)	PCG	4.83 ± 0.64	4.69 ± 0.40	0.14 ± 0.65	-0.47	.64
n = 14	ACC	4.43 ± 0.36	4.62 ± 0.78	-0.18 ± 0.78	-0.72	.47
	Thal	5.61 ± 1.02	6.27 ± 0.88	-0.66 ± 1.43	-1.48	.14
Whiplash (A) versus Back Pain (B)	PCG	4.66 ± 0.42	4.91 ± 0.47	-0.25 ± 0.54	-1.60	.11
n = 16	ACC	4.70 ± 0.75	4.63 ± 0.50	0.07 ± 1.01	-0.21	.84
	Thal	6.31 ± 0.78	6.56 ± 1.93	-0.24 ± 1.68	-0.21	.84
Back Pain (A) versus Migraine (B)	PCG	4.86 ± 0.67	4.89 ± 0.48	-0.03 ± 0.41	0.01	1.00
n = 16	ACC	4.5 ± 0.40	4.68 ± 0.44	-0.17 ± 0.62	-0.88	.38
	Thal	5.69 ± 1.01	6.56 ± 1.92	-0.87 ± 1.87	-1.76	.08

by Bigal *et al*[7] and supported by Bell *et al.*[5] All the included participants were experiencing chronic pain of over 3 months in duration: migraine (median [IQR], 18 years, [10.5 to 25 years]), whiplash-headache (2.7 years [1.5 to 4 years]) and low back pain (5 years [2 to 12 years]). It is therefore possible that the central nervous system has affected the metabolism of GABA, the main inhibitory neurometabolite of the central nervous system, in response to the ongoing levels of pain experienced by the participant. Whilst our post-hoc analysis shows a negligible correlation between PCG GABA+ and pain duration ($r = 0.05$, $P = .63$) it is hypothesized that GABA+ levels may be different in a different cohort experiencing acute pain.

Secondly, increased GABA+ may be reflective of a homeostatic mechanism perhaps to counteract increases in excitability that has been observed in some pain conditions.[21,27,38,61,89] Whilst the results from this cross-sectional study cannot determine cause or effect, they do challenge hypotheses from previous work that theorized that GABA+ levels could be associated with migraine specific processes such as cortical spreading depression, cortical vasodilation[43] or sensitization of the trigeminovascular system.[2,43] Therefore, a mechanism of chronic pain in general is more likely to underpin the higher GABA+ levels seen in this study.

Our findings suggest GABA+ is associated with chronic pain, however, these results differ somewhat with the conclusions of our recent meta-analysis.[62] To further elucidate proposed mechanisms, we need to review what is already known about neurometabolite behavior in each pain group. Firstly, the higher GABA+ levels observed in the migraine group compared with controls in this study were hypothesized *a priori* and are consistent with previous data. Previous data demonstrating this same increase in GABA/GABA+ in migraine includes pooled data from our meta-analysis, a recent high-quality study in a pediatric cohort[5] and our previous cohort study.[2] Conversely, decreased GABA+ has been reported in a single study of people with migraine[9] and related to headache severity[7] in another. It could be argued the differences might reflect a different subgroups of participants with a different nature of symptoms, or alternatively it may reflect that neither Bridge *et al,*[9] nor Bigal *et al*[7] used a sequence fully optimized for the detection of GABA+. The increase in GABA+ seen in this study and corroborated by other studies that were optimized to detect GABA/GABA+ gives us confidence that this may reflect a true biological finding.

In contrast to migraine and low back pain (LBP), there are no studies that have examined GABA+ levels in a whiplash-headache group, meaning our data cannot be interpreted with known data. We consciously included a musculoskeletal group who also experienced headaches (whiplash-headache) to explore whether the increased GABA+ levels observed in the migraine group were unique to migraine or also present in other headache types. We are confident that the whiplash-headache group are representative of the group in general, as the baseline profile includes higher levels of psychological distress and central sensitization symptoms, typically seen in people with whiplash.[76,77] Whilst the differences in GABA+ levels between the whiplash-headache group and controls did not reach statistical significance, the group appears to be clinically similar to the migraine and LBP groups. Firstly, the increased GABA+ concentration (Mean ± SD 4.78 ± 0.43 IU) in the headache-whiplash group and secondly, that similar percentage of people in all groups demonstrating increased GABA+ compared with controls (70.5% of the whiplash-headache group versus 73.5% and 78.9% in the migraine and back pain group respectively). If we accept that the whiplash-headache group appears to behave similarly to the other pain groups, it would strengthen the conclusion that GABA+ is reflective of a pain mechanism rather than a headache mechanism.

In considering our findings in relation to the reports of GABA+ in chronic pain, there is some variation. Our meta-analysis included all studies of GABA+ in chronic pain at the time of print,[62] results from the included studies showed mixed findings, with 4 of 21 results demonstrating a decrease in GABA.[23,33,35,39] However, these studies differed from ours in terms of type of pain conditions (non-musculoskeletal eg, neuropathic pain,[39] spinal cord injury,[32,33] pelvic pain[35] and fibromyaglia[23]), study methodology (smaller sample sizes, low signal to noise as a result of small voxel sizes: 8 mL vs 25 mL) and brain region studied (thalamus[32,39]). Overall, we are confident our data was derived from high quality spectra, using parameters optimized for GABA+.

Our data demonstrated higher quality spectra in the PCG and the ACC compared to the thalamus. The PCG was our primary region of interest due to previous studies demonstrating change within this region, whilst the ACC and thalamus were selected for their known involvement in chronic pain.[16,39,84] Since all spectra had an acceptable line width, the larger confidence interval demonstrated in the data from the thalamus, may reflect the region's small volume and deep location making it less suitable for high signal-to-noise (SNR) data acquisition (Fig 3B). The difficulties associated with reliably scanning the thalamus using MEGA-PRESS at 3T are also seen in other brain regions proposed to be involved in chronic pain such as the insula and the amygdala[3]. Whilst these regions along with others such as the pre-frontal cortex may demonstrate GABA+ changes, the sequence would require specific optimization to ensure adequate reliability. One method of improving reliability in these regions is to increase the number of averages acquired in these regions.[50] However, the resultant increase in scan time increases the chance of movement in regions that are already susceptible to motion artifact and was not feasible in a chronic pain cohort.

The nature of MR spectroscopy presents some further limitations namely the manual localization of the region of interest, the comparably large voxel size required for MEGA-PRESS acquisitions, and contamination of the modelled signal with co-edited macromolecules. Firstly, to address the limitation of manual localization, senior technologists who are

experts in both anatomy and MRS positioned the voxel following a strict protocol, guided by anatomical landmarks. Secondly, the reasonably largest voxel size maintaining regional specificity was chosen to enhance SNR. Finally, the decision to not use a macromolecule-suppressed sequence, was made in light of their vulnerability to frequency drift, reflected in better reliability of the observed signal in non-macromolecule suppressed studies.[19,49,54]

The presence of increased GABA+ in pain conditions provides an insight into a potential underlying mechanism of chronic pain; ongoing research is now required to elucidate the mechanism. Firstly, we need to understand how GABA+ correlates with clinical measures of pain sensitivity associated with chronic pain conditions such as pressure pain thresholds, and other quantitative measures of pain such as heat/cold pain thresholds. This will allow better understanding of the complexity of chronic pain and identify which domains are associated with neurometabolite changes, and further explore whether aberrant neurochemistry is present in everyone experiencing chronic pain condition. Secondly, longitudinal studies are required to investigate the cause or effect relationship between pain and higher GABA+ levels to determine whether increased GABA+ is a cause of chronic pain or an adaptive response to living with chronic pain. Finally, the addition of glutamate metabolism to future studies may allow to further pinpoint the underlying mechanisms. Answering these questions opens future avenues which can explore the matching of treatments to neurometabolite imbalances in chronic pain in an attempt to improve patient outcomes.

Conclusion

In conclusion, the results from this study suggest altered metabolism of GABA+ in migraine, and low back pain. GABA+ is more likely an underlying mechanism of chronic pain in general rather than a potential underlying neurometabolite marker of migraine. Studying the PCG using a method optimized for the detection of GABA+ provides a reliable method to quantify GABA+ levels in chronic pain populations.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jpain.2021.06.005.

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Supplementary Table 1: Quality metrics broken down by group

		Migraine	Whiplash	Back Pain	Control	p-value
PCG	**FWHM H_20**	8.73 (8.64 to 8.94)	8.67 (8.55 to 9.03)	8.67 (8.42 to 8.79)	8.52 (8.24 to 8.85)	0.48
	Fit error (%)	4.38 (3.62 to 5.14)	4.56 (4.12 to 5.43)	4.17 (3.81 to 4.80)	4.80 (4.23 to 5.26)	0.36
	Frequency drift (Hz)	-1.66 (-1.67 to -1.66)	-1.66 (-1.66 to -1.66)	-1.66 (-1.67 to -1.66)	-1.66 (-1.66 to -1.66)	0.52
	GABA SNR	13.79 (12.48 to 6.73)	13.97 (12.82 to 15.99)	14.42 (13.35 to 16.82)	13.75 (13.10 to 15.35)	0.51
ACC	**FWHM H_20**	8.73 (8.24 to 9.28)	8.42 (8.12 to 8.91)	8.42 (8.36 to 9.22)	8.45 (8.18 to 8.79)	0.22
	Fit error (%)	4.49 (4.05 to 5.00)	4.56 (4.12 to 5.43)	4.69 (4.38 to 5.24)	4.01 (4.45 to 6.28)	0.79
	Frequency drift (Hz)	-1.66 (-1.67 to -1.66)	-1.66 (-1.66 to -1.66)	-1.66 (-1.66 to -1.66)	-1.66 (-1.66 to -1.66)	0.93
	GABA SNR	15.73 (13.28 to 16.29)	16.03 (13.54 to 19.34)	16.68 (14.45 to 18.03)	16.03 (13.68 to 19.76)	0.61
Thal	**FWHM H_20**	10.68 (10.04 to 11.14)	10.68 (9.95 to 11.29)	10.74 (10.19 to 11.54)	10.99 (9.95 to 11.55)	0.59
	Fit error (%)	4.78 (4.05 to 5.62)	5.3 (4.29 to 5.76)	6.03 (4.96 to 6.86)	5.82 (4.79 to 6.38)	0.36
	Frequency drift (Hz)	-1.66 (-1.67 to -1.66)	-1.66 (-1.67 to -1.66)	-1.66 (-1.67 to -1.65)	-1.66 (-1.67 to -1.66)	0.48
	GABA SNR	11.29 (10.34 to 12.59)	11.73 (10.14 to 12.89)	11.76 (10.63 to 12.68)	11.05 (9.67 to 11.99)	0.69

Data presented as median and (IQR) to account for the non-normal distribution of some of the quality parameters. The non-parametric Kruskal-Wallis test was used to determine between group difference. Multiple comparisons were not conducted because the overall test did not identify any significant between group differences.

CHAPTER FOUR

Increase in ACC GABA+ levels correlate with decrease in migraine frequency, intensity and disability over time

PREFACE

Chapter 4 presents a longitudinal study investigating change in neurochemical level with change in clinical characteristics of people with migraine.

Citation

Peek AL; Leaver AM; Foster S; Puts NA; Oeltzschner G; Henderson L; Galloway G; Ng K; Refshauge K and Rebbeck T. Increase in ACC GABA+ levels correlate with decrease in migraine frequency, intensity and disability over time. *Journal of Headache and Pain,* 2021, 22 (150): 1-13

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Dissemination

The work presented in this chapter has been accepted for presentation at the following conferences:

- **Peek AL;** Leaver AM; Foster S; Puts NA; Oeltzschner G; Henderson L; Galloway G; Ng K; Refshauge K and Rebbeck T. GABA+ levels correlate with change in migraine

Impact

This paper is the first to explore the relationship between change in GABA levels and change in migraine status over time. The paper contributes new knowledge to the field suggesting that previously reported elevated GABA levels may not be a cause of migraine but actually a pain supressing mechanism. This finding could influence the direction of future development of migraine- and pain-specific medications.

AUTHORSHIP ATTRIBUTION STATEMENT

The co-authors of the paper *'Increase in ACC GABA+ levels correlate with decrease in migraine frequency, intensity and disability over time'* confirm that Aimie Laura Peek has made the following contributions:

- Design of the work
- Acquisition of the data
- Analysis and interpretation of the data
- Manuscript preparation, revision and critical appraisal for important intellectual content

As the primary supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

[redacted]

As. Prof. Trudy Rebbeck

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RESEARCH ARTICLE Open Access

Increase in ACC GABA+ levels correlate with decrease in migraine frequency, intensity and disability over time

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Abstract

Background: An imbalance between inhibitory and excitatory neurometabolites has been implicated in chronic pain. Prior work identified elevated levels of Gamma-aminobutyric acid + macromolecules ("GABA+") using magnetic resonance spectroscopy (MRS) in people with migraine. What is not understood is whether this increase in GABA+ is a cause, or consequence of living with, chronic migraine. Therefore, to further elucidate the nature of the elevated GABA+ levels reported in migraine, this study aimed to observe how GABA+ levels change in response to changes in the clinical characteristics of migraine over time.

Methods: We observed people with chronic migraine (ICHD-3) over 3-months as their treatment was escalated in line with the Australian Pharmaceutical Benefits Scheme (PBS). Participants underwent an MRS scan and completed questionnaires regarding migraine frequency, intensity (HIT-6) and disability (WHODAS) at baseline and following the routine 3 months treatment escalation to provide the potential for some participants to recover. We were therefore able to monitor changes in brain neurochemistry as clinical characteristics potentially changed over time.

Results: The results, from 18 participants who completed both baseline and follow-up measures, demonstrated that improvements in migraine frequency, intensity and disability were associated with an increase in GABA+ levels in the anterior cingulate cortex (ACC); migraine frequency ($r = -0.51$, $p = 0.03$), intensity ($r = -0.51$, $p = 0.03$) and disability ($r = -0.53$, $p = 0.02$). However, this was not seen in the posterior cingulate gyrus (PCG). An incidental observation found those who happened to have their treatment escalated with CGRP-monoclonal antibodies (CGRP-mAbs) ($n = 10$) had a greater increase in ACC GABA+ levels (mean difference 0.54 IU IQR [0.02 to 1.05], $p = 0.05$) and reduction in migraine frequency (mean difference 10.3 IQR [2.52 to 18.07], $p = 0.01$) compared to those who did not ($n = 8$).

Conclusion: The correlation between an increase in ACC GABA+ levels with improvement in clinical characteristics of migraine, suggest previously reported elevated GABA+ levels may not be a cause of migraine, but a protective mechanism attempting to suppress further migraine attacks.

Keywords: GABA, MRS, Migraine, Anti-CGRP, Pain, Longitudinal

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Background

Migraine is the leading neurological cause of disability worldwide [1], with an estimated global prevalence of 14.7% [2]. Chronic migraine is defined by the International Classification of Headache disorders (ICHD-3) as headaches that persist for more than or equal to 15 days a month, with at least 8 of those days having features of migraine and persisting for at least 3-months [3]. Despite chronic migraine only representing 7.7% of the entire migraine population [4], compared to episodic migraine it is associated with higher healthcare utilization, work disability, and reduction in quality of life [5–7] . Even though recent treatments are showing better effects, the responsiveness rate remains less than 50%, leaving 50% of people with ongoing symptoms of chronic migraine [8, 9]. Treatment development is hindered by the limited understanding of the pathophysiology of chronic migraine. Given this, there is a global call to understand the mechanism of chronic migraine to enable the development of more effective treatment strategies [10].

Chronic migraine has been attributed to several proposed mechanisms involving both peripheral and central mediators. One proposed mechanism of migraine is an imbalance between the main inhibitory and excitatory neurometabolites, gamma-aminobutyric acid (GABA) and glutamate. Studying these metabolites has previously proved challenging owing to spectral overlap of more abundant neurometabolites at clinical magnetic resonance imaging (MRI) field strength [11]. However, Advanced ^{1}H-Magnetic Resonance Spectroscopy (MRS) techniques such as MEGA-PRESS [12] address these limitations and allow for more reliable quantification of GABA or GABA+ co-edited macromolecules (GABA+) whilst also reporting the composite glutamate-glutamine-glutathione (Glx).

Several GABA/ GABA+ optimized cross-sectional MRS studies have investigated the imbalance of inhibition and excitation as a potential underlying cause of chronic migraine [13–16]. Our recent meta-analysis pooled results from 5 studies that reported levels of GABA and GABA+ in the anterior cingulate cortex (ACC), insula, occipital lobe and posterior cingulate cortex (PCG) and 6 studies that reported levels of Glx in the ACC, occipital lobe, PCG and thalamus of people with migraine [17]. We found GABA or GABA+ levels were significantly elevated in individuals with migraine compared to controls, yet there was no difference in Glx levels. These results challenge the concept of loss of inhibition leading to cortical hyperexcitability, where reduced levels of GABA compared to controls might be anticipated [18]. We might postulate that directional differences in GABA levels may be dependent on whether GABA is working within inhibitory or facilitatory circuits in the region being studied, which may result in either decreased or increased axonal firing respectively [19]. Nevertheless, this somewhat unexpected increase in GABA suggests a more complex relationship between the inhibitory and excitatory neurometabolites involved in migraine and warrants further investigation.

In investigating the role of neurometabolites in migraine, the region of brain to be examined must be considered. Studies of the PCG have demonstrated both elevated levels of GABA+ [14, 16] and an association between elevated GABA+ levels and central sensitization [20] in people with migraine. Another region, the ACC, has a well-established role in pain processing and modulation [21], and changes in ACC GABA+ levels have been reported in pain conditions such as fibromyalgia [22] and pelvic pain [23].

The elevated baseline levels of GABA+ observed in this cohort [24] and by others [14, 20, 25] have been proposed as a potential cause of migraine, due to being present in people with migraine but not in healthy controls. However, given the cross-sectional nature of the studies, the temporal and directional nature of these findings are unknown. Therefore, to further elucidate the nature of the elevated GABA+ levels in people with migraine, longitudinal studies, that examine the association between *change* in migraine characteristics (e.g. migraine frequency, pain intensity and disability) and *change* in GABA+ or Glx levels are required. Examining a cohort before and after treatment that is known to have a reasonable response rate (e.g. Onabotulinumtoxin A or calcitonin gene-related peptide monoclonal antibodies (CGRP-mAbs); response rate ~ 40% [8, 9], allows such an opportunity.

Aims

Our primary aim was to determine whether there is an association between *change* in GABA+ levels and *change* in migraine characteristics over time to further elucidate the nature of GABA's role in migraine. Secondary aims were to determine whether there is an association between baseline neurometabolite levels and change in clinical characteristics and / or change in neurometabolite levels. This would establish whether baseline levels of GABA+ could predict change either clinically or neurochemically.

Methods

Study design

This study was a longitudinal cohort study, observing a group of migraine participants who formed part of a larger cross-sectional multi-group study [16].

Participants

There were 20 participants with chronic migraine with or without aura as diagnosed by the ICHD-3 [3] recruited by a neurologist (KN) working in private practice (4 males, 16 females, mean age 39.7 ± 10 years). To be eligible to receive treatment (Botox® or CGRP- mAbs) under the Australian Pharmaceutical Benefits Scheme (PBS), participants were required to have experienced an average of at least 15 headache days a month, (8 days of which migrainous) for over 6-months and having failed three or more prophylactic migraine medications [26]. In addition, they were required to have at least moderate headache related disability measured as a HIT-6 score [27] exceeding 50 at the time of recruitment. All participants were recruited when they were due to start a new treatment regimen based on escalating care in line with the PBS guidelines [26]. Participants were included if they received any peripherally acting evidence-based medication to escalate their care (e.g. Onabotulinumtoxin A, CGRP-monoclonal antibodies (CGRP-mAbs). Treatment could be escalated with either Onabotulinumtoxin A injections 155 mg administered 12 weekly according to the PREEMPT protocol [8] or CGRP-mAbs as erenumab 70 to 140 mg self-administered by injection monthly.

Participants were excluded if they were taking medication known to affect GABA levels at baseline (e.g. diazepam, topiramate or gabapentin), had contraindications to MRI (e.g. claustrophobia, MR-unsafe devices/implants) or conditions that compromised MR spectroscopy (e.g. metal braces). In addition, participants were excluded if they experienced any acute health complaints in the 5 days prior to the scan, were diagnosed with a psychiatric or neurological condition, experienced pain in other regions of the body, or if they were unable to communicate in the English language.

To test the reliability of collecting longitudinal MRS, 5 healthy participants (2 males, 3 females, mean age 44.8 ± 10.0 years) were recruited for the study through advertisements placed on university and hospital noticeboards. These participants had no history of chronic pain, headache or health conditions and had no contraindications of MRI.

Procedure

Potential participants were identified by the treating neurologist, who provided study information, and gained consent to be contacted by the research team. The research team screened potential participants for eligibility by telephone, further explained the study and gained written informed consent. Participants immediately started an online pain diary, completed initial questionnaires of headache severity (Headache Impact Test-HIT-6), disability (World Health Organization Disability Assessment Schedule-WHODAS 2.0–12), pain sensitivity (Central Sensitisation Inventory-CSI) and psychological wellbeing (Depression Anxiety and Stress Scale- DASS-21). Questionnaires were completed using the online platform, REsearch Data Capture® (REDcap) [28]. Participants were scanned in their interictal phase and asked to refrain from taking pain medication, caffeine, nicotine or alcohol on the day of the MRI/MRS scan. Following the initial scan participants started their new treatment regimen. At 3-month follow-up participants repeated headache pain and disability questionnaires and attended for a follow-up MRI/MRS scan under the same conditions as their first scan.

Clinical outcome measures

Validated patient reported outcome measures were chosen to evaluate change in headache severity and disability over time. The HIT-6 was chosen due to being specifically designed and validated as a measure of adverse headache impact in both clinical practice and research [27, 29]. Scores range from 36 to 78 with higher scores corresponding to higher levels of disability. The WHODAS 2.0–12 was chosen as a global measure of disability due to its reliability and sensitivity to detecting change in disability over time [30]. Scores range from 12 to 60, with 60 indicating the highest level of disability.

Headache frequency was measured through an online weekly pain diary. Participants recorded the number of days they experienced migraine each week and rated the migraine severity using the Numerical Rating Scale (NRS) where 0 was no pain, and 100 the worst imaginable pain.

Neurometabolites of interest

The primary aim of the study was to understand the role of GABA. We therefore focused on GABA+ levels, as this currently reflects the most reliable method to report GABA when using a repeat measure design. The Glx composite signal was a secondary target, representing glutamate with additional contributions from glutamine (Gln) and glutathione (GSH). The composite signal is reported since Glu, Gln and GSH are heavily overlapped at 3 T field strength and therefore difficult to resolve from each other reliably [31].

MRI/MRS data acquisition

All participants were scanned on a Siemens 3 T Magnetom Prisma (Erlangen, Germany) with 64-channel head coil. High resolution 3D T_1-weighted structural images (repetition time (TR) = 2400 ms; echo time (TE) = 2.21 ms; inversion time (TI) = 1150 ms; voxel size = 1.0 × 1.0 × 1.0 mm^3; FOV = 256 mm; matrix = 256 × 256; acquisition time = 4 min 35 s) were acquired to inform voxel placement (previously described [24]) and for use in tissue segmentation. A 2D T_2-weighted series (TR = 7490

ms; TE = 99 ms; voxel size = 0.6 × 0.3 mm^3; FOV = 220 mm; matrix = 384 × 288; acquisition time = 2 min 24 s) was also acquired and sent to a consultant radiologist to review and report any incidental finding. MRS data were acquired using the MEGA-PRESS sequence from two regions shown in Fig. 1, the posterior cingulate gyrus (PCG, voxel size 25 (AP) × 40 (RL) × 25 (CC) mm^3) and the anterior cingulate cortex (ACC, 40 (AP) × 25 (RL) × 25 (CC) mm^3). Common parameters for both voxels were: TR = 2000 ms; TE = 68 ms; 192 averages (96 ON, 96 OFF); 2048 data points; spectral width = 2000 Hz; editing pulse frequencies set to 1.9 ppm and 7.5 ppm for editing of GABA+; editing pulse bandwidth = 70 Hz. Water-unsuppressed MEGA-PRESS data (with water suppression RF pulses deactivated) were also acquired from each voxel to perform eddy-current correction and water-scaled quantification.

MRS data processing

MRS data were processed using the open-source MATLAB-based analysis toolbox Gannet 3.1 [32], including data loading, coil combination, frequency-and-phase-correction of individual transients using the Spectral Registration algorithm [33], and averaging. The 3-ppm GABA+ and 3.75-ppm Glx signals in the difference spectrum were modelled with a single Gaussian and a dual-Lorentzian peak, respectively, including terms for the baseline slope between the two signals. The water signal in the water reference spectrum was modelled with a single Voigtian peak. The voxels were co-registered to the T_1-weighted structural acquisition, which was segmented using built-in SPM12 functions. GABA+ and Glx levels were quantified relative to the internal tissue water signal, accounting for tissue composition of the voxel, as well as different water content and relaxation times for grey matter, white matter, and cerebrospinal fluid. Alpha-corrected GABA concentration estimates were reported, accounting for the fact that GABA+ and Glx concentrations differ between grey and white matter at a ratio of ~ 2:1 [34].

Spectroscopy quality

Spectra were visually examined for artefacts by two investigators with eight and ten years' experience of spectral editing (GO, NP). Spectra were excluded if they demonstrated significant motion artefact or insufficient water suppression ($n = 1$, healthy participant, ACC voxel). All remaining spectra achieved a fit error below the recommended quality threshold of 10% [32]. The mean fit error was 4.61 ± 0.95% in the PCG and 4.57 ± 0.67% in the ACC.

MRS test-retest reliability

The test-retest reliability of the MEGA-PRESS acquisition over the same 3-month time period was determined in the 5 healthy control participants. One ACC acquisition was excluded from the analysis due to substantial motion artefact. Results demonstrated a test-retest coefficient of variance (CV) of 10% in PCG, and 12.6% in the ACC. This is in agreement with previous MEGA-PRESS studies of GABA+ [35–37] and provides evidence of the reliability of the MRS collection in this longitudinal investigation.

Statistical analysis

The power calculation was based on our previous work [14]. A sample size of $n = 17$ was required to detect a 0.2 IU change in GABA+ level with 80% power and

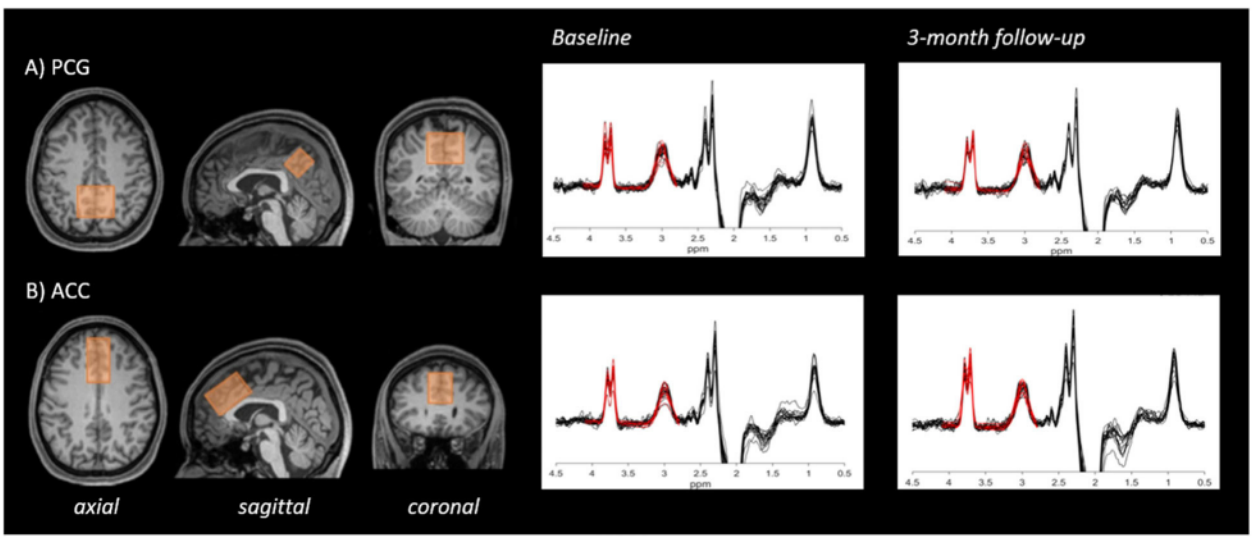

Fig. 1 Exemplary voxel placement and an overlay of all spectra from A) PCG and B) ACC at baseline and 3-month follow-up

therefore, 20 participants were recruited to allow for a 15% dropout rate.

Participants' baseline characteristics were reported using descriptive statistics, mean and standard deviation for normally distributed data, and median interquartile range for non-normally distributed data.

The outcomes of GABA+ levels, Glx levels, migraine days per month, HIT-6 scores and WHODAS scores were included in the statistical analysis. Normality for each variable was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests. Paired sample t-tests were used to examine change in clinical characteristics (migraine days, HIT-6, WHODAS) and change in neurometabolite levels (GABA+, Glx) from baseline to 3-month follow-up.

In addressing our primary aim to determine the degree of correlation between *change* in characteristics of migraine and *change* in neurometabolite levels, and to determine the correlation between *baseline* and *change* in neurometabolite levels, Pearson's (r) correlation was used- due to the normal distribution of data. Correlations $r > 0.07$ were considered strong, 0.4 to 0.69 moderate, 0.1–0.39 weak, < 0.1 negligible [38, 39].

Neurometabolite levels and clinical outcome measures were plotted as raincloud plots, which provide a transparent method of data visualisation [40]. Raincloud plots consist of a half-violin plot to visualize the distribution, a box plot to highlight the median and 95% confidence intervals, and scatter plots with lines connecting each individual participants score at baseline and 3-month follow-up.

Post-hoc testing was carried out to explore if *change* in GABA+ or Glx level was different in those who were escalated with CGRP-mAbs treatment compared to Onabotulinumtoxin A. Due to the normal distribution of data an independent sample t-test was used to determine any between-group differences. A point biserial correlation (Pearson's (r) correlation using one dichotomous variable and one continuous variable) was then used to determine correlations between group (CGRP-mAbs Yes/No) with headache frequency, intensity and disability.

An alpha level of 0.05 was used for all statistical tests. Statistical testing was carried out using SPSS version 26 [41] and data visualisation was performed in R version 4.0.2 [42].

Results

Of the 20 participants recruited, 18 were included in the final analysis. One was excluded following MRS because they had taken diazepam prior to being scanned, thus not meeting the study's inclusion criteria, and one did not complete their follow-up scan. Of the final 18 participants, 8 had been escalated to Onabotulinumtoxin A, and 10 with CGRP-mAbs. All participants had received two doses of medication between baseline and 3-month follow-up.

Participants

Baseline characteristics of study population

The mean ± SD duration of migraine was 21 ± 11.0 years in the participants included in the final analysis ($n = 18$). Participants experienced on average 16.7 ± 5.1 headache days in the month preceding the baseline scan, had an average pain intensity of 66.1% ± 22.9 in the week preceding the scan (Table 1). Participants were scanned in the interictal phase, however due to the chronic nature of the symptoms some still had residual head pain as reflected in the time since migraine (Table 1). Overall, the group's baseline psychological status indicated that on average, participants were mildly depressed and had moderate anxiety and stress levels [43].

Clinical changes from baseline to 3-month follow-up

In order to address our primary aim, we first report observed changes in migraine characteristics and brain neurometabolite levels from baseline to 3-month follow-up.

Change in migraine characteristics from baseline to 3-month follow-up

Overall the group experienced an improvement in clinical characteristics of migraine from baseline to 3-month follow-up. The mean ± SD headache frequency was 16.7 ± 5.1 days per month at baseline and 12.4 ± 10.0 days at 3-month follow-up (mean difference − 4.22 days, 95% CI [− 8.78 to 0.33] days, t (17) = − 1.96, $p = 0.07$). Headache intensity (HIT-6) decreased significantly from 65.7 ± 6.6 at baseline to 59.0 ± 8.4 at 3-months (mean difference − 6.72, 95% CI[− 9.15 to − 4.29], t (17) = − 5.84, $p = 0.01$) and disability (WHODAS) was 24.8 ± 16.1 at baseline and 22.0 ± 23.1 at 3-months (mean difference − 2.78 95% CI[− 12.38 to 6.82], t (17) = − 0.61, $p = 0.55$) (Fig. 2).

Change in neurometabolite levels from baseline to 3-month follow-up

Overall, mean GABA+ levels in the PCG significantly decreased between baseline and 3-month follow-up from 4.93 ± 0.62 IU to 4.48 ± 0.45 IU (mean difference − 0.45 IU, 95% CI [− 0.79 to − 0.10] IU, t (17) = − 2.72, $p = 0.02$). At an individual level, a decrease in PCG GABA level was observed in 12 participants, and 6 displayed an increase over the 3-month period. Prior to final analysis, the two outliers (baseline PCG GABA+ 6.5+ and baseline PCG Glx 18+) were reassessed for data quality and modelling and subsequently retained.

Table 1 Clinical characteristics of participants

	Migraine (n = 18)
Demographics (mean ± SD)	
Age	39.0 ± 10.0
Female (n, %)	15 (79%)
BMI	26.8 ± 6.2
Educational level (University educated n, %)	12 (63.1%)
Migraine Characteristics (mean ± SD)	
Duration (years)	21.0 ± 11.0
Aura (n, %)	12.0 (66.7%)
Pain intensity in last week (NRS %)	66.1 ± 22.9
Escalated with CGRP-mAbs (n, %)	10.0 (55.6%)
Psychological Status (mean ± SD)	
DASS total score (range: 0 to 126)	21.6.0 ± 14.0
- Depression (range: 0 to 42)	4.4 ± 5.7
- Anxiety (range: 0 to 42)	6.0 ± 5.4
- Stress (range: 0 to 42)	11.2 ± 6.1
Symptoms (median (IQR))	
Pain at time of 1st scan [NRS %]	40.0 (10.0 to 60.0)
Pain at time of 2nd scan [NRS %]	10.5 (0.0 to 50.0)
Time since migraine 1st scan [hours]	11.0 (0.0 to 48.0)
Time since migraine 2nd scan [hours]	36.0 (4.0 to 168.0)
Central Sensitisation Inventory^ (range: 0 to 100)	45.0 (36.0 to 50.0)
Baseline Clinical status (mean ± SD)	
Migraine frequency (days per month)	16.7 ± 5.1
WHODAS 2.0 (range: 12 to 60)	24.8 ± 16.1
HIT-6 ǂ (range: 36 to 78)	65.7 ± 6.6
GABA+ ACC [institutional units, IU]	4.51 ± 0.38
GABA+ PCG [IU]	4.93 ± 0.62
Glx ACC [IU]	13.51 ± 1.25
Glx PCG [IU]	12.90 ± 1.86

Clinical characteristics of participants included in final analysis ($n = 18$). Data reported as mean ± SD, except where stated non-normally distributed data is reported as Median (IQR). For DASS, CSI, WHODAS, HIT-6 a higher score indicates greater infliction. ^CSI > 40 indicates central sensitization, ǂHit-6 score > 60 indicate severe impact.

In contrast to the PCG, mean GABA+ levels in the ACC did not significantly change from baseline to 3-month follow-up. ACC GABA+ levels in participants with migraine at baseline were 4.51 ± 0.38 IU and 3-month follow up 4.40 ± 0.55 IU (mean difference − 0.12 IU, 95% CI [− 0.41 to 0.18] IU, t (17) = − 0.85, $p = 0.41$). At an individual level, a decrease in ACC GABA+ levels were observed in 11 participants and an increase in 7. Glx levels were not significantly different between baseline and 3-month follow-up in either the PCG or the ACC (Fig. 3).

Primary result

Correlation between change in brain neurometabolite levels and change in migraine characteristics

There were moderate inverse correlations between GABA+ levels in the ACC and all clinical outcomes. Specifically, we found a moderate inverse correlation between increase in GABA+ levels in the ACC and reduction in headache frequency at 3-month follow-up ($r = -0.51$, $p = 0.03$ (Table 2, Fig. 4). Similarly, moderate inverse correlations were found between increase in ACC GABA+ levels and both reduction in headache intensity ($r = -0.51$, $p = 0.03$) and reduction in disability ($r = -0.53$, $p = 0.03$). In contrast to the findings in the ACC, correlations between change in PCG GABA+ levels and changes in migraine frequency, intensity or disability were negligible and not significant (Table 2). In the case of Glx, there were only negligible correlations between change in Glx and change in clinical characteristics of migraine in both the PCG and the ACC (Table 2, Supplementary materials I).

Secondary results

Correlation between baseline brain neurometabolite levels and change in migraine characteristics

The baseline levels of both GABA+ and Glx in the ACC and PCG demonstrated a negligible correlation with change in migraine frequency, intensity and disability (Supplementary materials I).

Correlation between baseline brain neurometabolite levels and change in brain neurometabolite levels

In determining whether baseline neurometabolite levels predict the extent of change in neurometabolite level we found a moderate inverse correlation between baseline levels of GABA+ and change in GABA+ in the ACC ($r = -0.54$, $p = 0.01$) and strong inverse correlation in the PCG ($r = -0.72$, $p = 0.01$) respectively. This reflects that those with higher levels of GABA+ at baseline experienced greater reductions in GABA+ over time.

The baseline level of Glx and change in Glx level demonstrated a strong inverse correlation in the PCG ($r = -0.92$, $p = 0.01$), which was not present in the ACC ($r = -0.34$, $p = 0.16$).

Post-hoc analysis

Change in GABA+ levels and clinical characteristics in those receiving CGRP-mAbs

Post-hoc analysis demonstrated that those who received CGRP-mAbs ($n = 10/18$) had a significantly greater increase in ACC GABA+ levels and significantly greater improvement in migraine symptoms than those who did not (Supplementary materials II). There was also a moderate positive correlation between receiving CGRP-mAbs (No/Yes) and an increase in ACC GABA+ levels

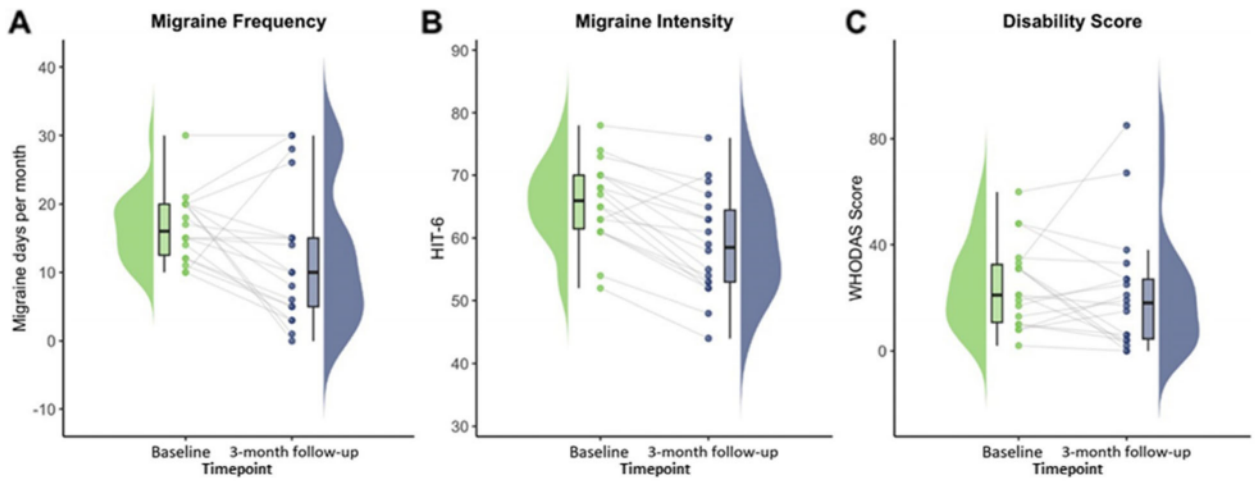

Fig. 2 Change in migraine characteristics from baseline to 3-month follow-up. Raincloud plots for change in A) migraine frequency (migraine days per month), B) migraine intensity (HIT-6 score) and C) disability (WHODAS score). Each individual plot represents change in clinical characteristics from baseline to 3-month follow-up in participants with migraine ($n = 18$). Green data points represent migraine characteristics at baseline and blue at 3-month follow-up. The grey lines represent change in individual participants scores over time

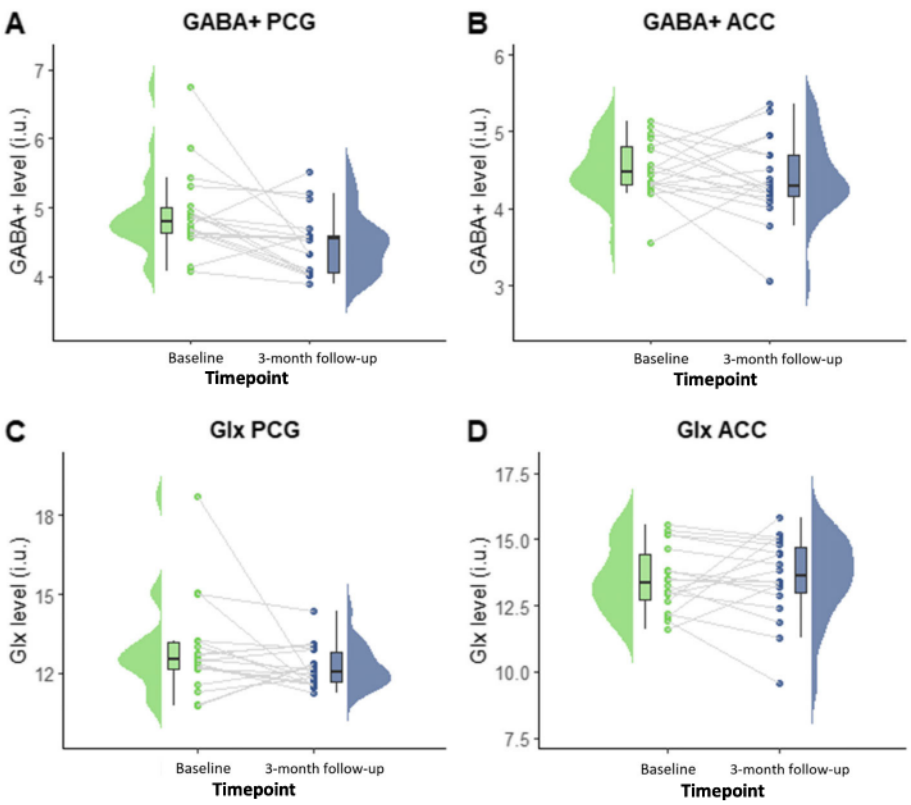

Fig. 3 Change in neurometabolite levels between timepoints for participants with migraine. GABA+ in A) PCG, B) ACC, and Glx in C) PCG and D) ACC. Each individual plot represents change in neurometabolite levels from baseline to 3-month follow-up in participants with migraine ($n = 18$). Green data points represent neurometabolite levels at baseline and blue at 3-month follow-up. The grey lines represent change in individual participants neurometabolite levels over time

Table 2 Correlation (Pearson's *r*) between change in GABA+ levels and change in measures of headache frequency, pain intensity and disability in people with migraine ($n = 18$)

Neurometabolite Levels		Change in frequency (days/month) *r* (*p*-value)	Change in intensity (HIT-6) *r* (p-value)	Change in disability (WHODAS) *r* (*p*-value)
Change in GABA+	**PCG**	−0.10 (0.69)	0.01 (0.96)	−0.23 (0.38)
	ACC	−0.51 (0.03)*	− 0.51 (0.03)*	− 0.53 (0.02)*
Change in Glx	**PCG**	0.08 (0.74)	−0.07 (0.8)	0.09 (0.74)
	ACC	−0.30 (0.23)	−0.32 (0.2)	−0.40 (0.10)

**statistically significant $p < 0.05$*

[r_{pb} (18) = 0.47, $p = 0.05$] which was not seen in the PCG. Furthermore, there was also a moderate inverse correlation between receiving CGRP-mAbs (No/Yes) and a reduction in migraine frequency [r_{pb} (18) = − 0.58, $p = 0.01$] and migraine intensity [r_{pb} (18) = − 0.49, $p = 0.04$].

Discussion

This study sought to measure any changes in GABA+ levels in a group of participants with chronic migraine as their care was escalated. We found that as GABA+ levels increase in the ACC, there was a corresponding moderate correlation with a decrease in migraine frequency, intensity and disability. A chance finding illustrated a greater increase in ACC GABA+ level in those taking CGRP-mAbs. These same correlations were not found in the PCG, despite group mean PCG GABA+ levels changing from baseline to 3-month follow-up. Results from this study suggest that GABA is a key neurometabolite of migraine. Proposed reasons for the differences observed between brain regions are discussed.

A major finding of this study was that an increase in ACC GABA+ levels were associated with an improvement in all three migraine outcomes; namely migraine frequency, intensity, and disability. Furthermore, the associations between change in neurometabolite levels and migraine characteristics were only seen for inhibitory GABA+ but not for excitatory Glx. Together this supports the hypothesis that the balance in cortical excitability in migraine is primarily mediated through inhibitory GABA, rather than excitatory Glx.

To date, correlations between GABA levels and clinical characteristics of migraine have only been measured in cross-sectional studies. Results from these studies have been mixed, with some showing an association with higher GABA/GABA+ levels and higher pain levels [15, 20] and others showing either the opposite [44] or

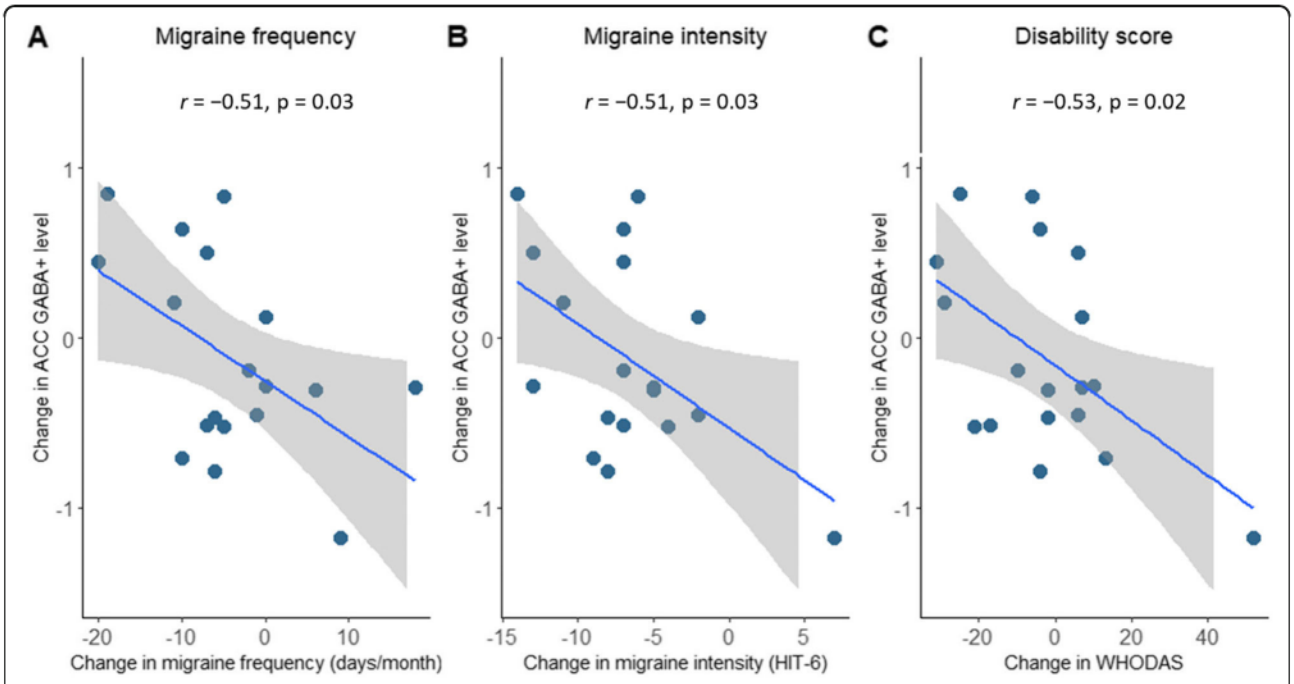

Fig. 4 Correlation between change in ACC GABA+ and change in clinical characteristics. Dots represent participants with migraine, the grey ribbon represents the 95% confidence interval, and the blue regression line represents the Pearson's correlation coefficient *(r)*

negligible associations [45]. Whilst this discrepancy may be related to methodological differences, the variance in these prior results questions the extent to which GABA+ is related to the clinical characteristics of migraine. A single longitudinal study reported a group mean reduction in PCG GABA+ level in 14 people with migraine following treatment with levetiracetam [46]. Although on average the group in that study improved in terms of both migraine frequency and intensity, the relationship between GABA and clinical characteristics was not explored. Our study examined these associations longitudinally and demonstrated that change in clinical characteristics were associated with change in ACC GABA+ levels. This consequently provides plausible evidence that ACC GABA+ levels are related to pain-related measures of migraine.

An observed correlation between the change in GABA+ levels and change in clinical characteristics of migraine in the ACC but not the PCG is consistent with our understanding of how different brain regions process pain. The role of the ACC in pain processing has been well documented in both pre-clinical and human studies. These studies have demonstrated decreased affective pain behaviour, such as reduced escape behaviour following ACC damage [47], and analgesic responses to direct ACC stimulation [48]. Furthermore, human studies have demonstrated ACC activity during both observing and receiving a painful stimulus [49]. These findings combined with altered ACC GABA+ levels in other pain conditions [22, 23], mean the observed correlation between change in ACC GABA+ and change in clinical characteristics of pain are consistent with the role of the ACC in pain processing.

In contrast, the role of the PCG in pain is less clear. As part of the default mode network, *deactivation* of the PCG has been associated with higher levels of catastrophising in people with migraine [50] and attention to pain in people without a pain condition [51, 52]. Several cross-sectional studies have also demonstrated higher levels of GABA+ in the PCG/visual cortex of people with migraine compared to controls [13–16]. Taken together with the findings of this study, we can posit that GABA+ levels in the PCG might not directly reflect a measure of pain, rather they reflect another aspect of the migraine experience not captured within this study. Consequently, it could be proposed that the ACC provides a more relevant region to explore when investigating the association between GABA+ levels and pain in people with chronic migraine.

Our findings raise the possibility that GABA+ has a pain suppressing role in migraine. Whilst previous cross-sectional reports have identified elevated baseline GABA+ levels in people with migraine compared to pain-free controls, it was not clear if this difference reflected the underlying cause of migraine or an adaptive response to having migraine. Our data support the latter hypothesis suggesting the role of GABA+ is suppressive given that ACC GABA+ further increased as all three clinical measures of migraine reduced. i.e. where ACC GABA+ increased over time, migraine symptoms improved. Further, the reduction in migraine *frequency* may suggest that GABA+ has a role in suppressing cortical sensitivity in migraine, thus increasing the threshold required to trigger a migraine, rather than just modulating the migraine's severity. This hypothesis suggesting a suppressive role of GABA does not support the proposal that future treatments are required to reduce the elevated GABA+ levels to that observed in healthy participants to better treat migraine [14, 17].

The hypothesised suppressive mechanism of GABA+ in the ACC is further supported by our post-hoc analysis. Although this study is not a drug trial and was not designed to evaluate drug interventions, the use of CGRP-mAbs in 10/18 participants provides a subgroup of people with migraine who experienced greater recovery, e.g. decreased migraine frequency (CGRP-mAbs mean ± SD − 8.8 ± 7.4 days versus Onabotulinumtoxin A 1.5 ± 8.2 days, mean difference 10.3 days, 95% CI [2.52 to 18.07], $p = 0.01$). Accompanying the greater improvement in the CGRP-mAbs group there was also a greater mean increase in ACC GABA+ levels (Supplementary materials II, III). Further, an increase in ACC GABA+ level was observed in 60% ($n = 6/10$) of the CGRP-mAbs group compared to just 12.5% ($n = 1/8$) of the Onabotulinumtoxin A group. This supports the hypothesis that those who improve are more likely to experience an increase in ACC GABA+ levels, providing further evidence that ACC GABA+ levels have a pertinent role in the recovery of people with chronic migraine.

Since both CGRP-mAbs and Onabotulinumtoxin A medications are thought to have a peripheral mode of action and do not cross the blood-brain barrier [53, 54], it is likely that they do not directly influence brain GABA levels. We might speculate that CGRP-mAbs or Onabotulinumtoxin A block the activation of trigeminal afferents by blocking peripheral receptors or inhibiting neuropeptide release [54, 55]. Consequently, tonic or phasic trigeminal afferent drive is inhibited, reducing the activity of neurons in the spinal trigeminal nucleus, thalamus and cingulate cortex pathway [56]. This reduction in afferent drive may ultimately underpin the alteration in excitatory and inhibitory balance observed here in the ACC. In addition, altered descending drive from the cingulate cortex to brainstem pain modulatory circuits, [57, 58] may suppress the ability of trigeminal inputs to evoke a migraine attack. Therefore, it is likely that the correlation between change in ACC GABA+ levels and change in pain levels, reflects the central effects of

altered trigeminal afferent drive rather than a direct effect of the medication itself.

Future directions

This longitudinal study provides the next stage of exploratory research aimed at understanding the role of the neurometabolites GABA and glutamate in migraine. This study reported the composite measures, GABA+ (GABA + macromolecules) and Glx (glutamate-glutamine-glutathione) as they currently represent the most reliable method when using a repeated-measures design [11, 34, 59]. Therefore, some attention should be paid to the macromolecule content of the signal. As technology advances and the specificity and reliability of GABA and glutamate acquisition improve, future studies may wish to use methods that attempt to separate GABA from macromolecules and report glutamate specifically rather than the composite Glx. Further Glx was obtained from the difference spectra. The reliability of this method has been discussed in several studies which suggest although Glx and glutamate can be measured using MEGA-PRESS the measurement of Glx and glutamate may be more reliable if measured using a PRESS sequence [31, 60, 61]. Therefore, our results for Glx would benefit from further investigation using an experiment specifically optimised for Glx.

Further investigation of the temporal nature of GABA+ levels in chronic migraine would aid our understanding. It is hypothesised that the change in GABA+ levels reported in this study might reflect a chronic shift in GABA levels. However, fluctuation of GABA levels in a person with migraine in the short term or throughout the migraine cycle remains unknown. A study of time-resolved measurements, yet to be conducted in a migraine population, may further elucidate the nature of GABA+ changes reported in this study.

The exploratory nature of the study inevitably meant that we were not adequately powered to fully investigate (beyond exploratory testing) subgroups of participants in terms of treatments received or responsiveness. Future research aimed at investigating neurometabolite profiles of people who respond to particular treatments would significantly benefit the migraine community, providing the next step in delivering targeted treatment for migraine. Treatment strategies based on those most likely to respond would not only reduce the unnecessary prescription of medication, but improve patient outcomes, reduce the risk of side-effects, and reduce unnecessary health care costs.

Conclusion

In conclusion, we found that an increase in ACC GABA+ levels over time was associated with a decrease in migraine frequency, intensity and disability. Suggesting previously reported elevated GABA+ levels may not be a cause of migraine, but a protective mechanism attempting to suppress further migraine attacks. The findings of this study support that ACC GABA may have a pertinent role in the recovery of people with chronic migraine.

Abbreviations

GABA: Gamma-aminobutyric acid; GABA+: GABA + macromolecules; MRS: Magnetic resonance spectroscopy; ICHD: International classification of headache disorders; PBS: Pharmaceutical benefits scheme; HIT-6: Headache intensity scale; WHODAS: World health organization disability assessment schedule; ACC: Anterior cingulate cortex; PCG: Posterior cingulate gyrus; MRI: Magnetic resonance imaging; Glx: Glutamate-gluatmaine-glutathione; CGRP-mAbs: Calcitonin gene-related peptide monoclonal antibodies

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s10194-021-01352-1.

Additional file 1.

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Authors' contributions

TR, KR, AL, GG, AP and KN supported the conception and design of the project. SF and AP acquired the data. AP analysed the data, NP, GO, LH, TR contributed to the interpretation of the data. AP, AL, TR, KR produce the first draft. All authors contributed intellectual content to revised manuscripts and read and approved the final manuscript.

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Availability of data and materials

The open-source software code of Gannet 3.1.3 that was used to process and analyze the MRS data is available from https://github.com/richardedden/Gannet3.1/releases/tag/v3.1.3. The scripts for the batch analysis and the R code for the visualization is available through the OSF repository https://osf.io/y8gps/?view_only=0a35454b80dc456f93e84a99b86fbfb4. Datasets generated during the current study are available from the corresponding authors upon reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval was granted through Western Sydney Local Health District reference number HREC/17/WMEAD/429. Written informed consent was gained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

9. SUPPLEMENTARY MATERIALS

9.1 Supplementary Materials I

Table 1: Correlation between baseline GABA+ and clinical characteristics of migraine

	Change in frequency (days/month) *r* (p-value)	**Change in intensity (HIT-6)** *r* (p-value)	**Change in disability (WHODAS)** *r* (p-value)
Baseline GABA+			
PCG	−0.07 (0.80)	−0.17 (0.50)	0.05 (0.85)
ACC	−0.15 (0.57)	−0.23 (0.37)	0.01 (0.10)

**statistically significant p <0.05, all negligible correlations*

9.2 Supplementary Materials II

Table 2: Post-hoc between group comparison of primary outcomes

	CGRP-mAbs (n = 10)	**Botox** (n = 8)	**Mean difference** [95% CI]	**Independent t-test** *t* (df), p-value
Change in frequency (days/month)	−8.8 ± 7.38	1.5 ± 8.16	10.3 [2.52 to 18.07]	*t* (16) = 2.81, p = 0.01*
Change in intensity (HIT-6)	−8.8 ± 2.97	−4.13 ± 5.72	4.68 [0.26 to 9.09]	*t* (16) = 2.25, p = 0.04*
Change in disability (WHODAS)	−8.4 ± 14.92	4.25 ± 22.75	12.65 [−6.21 to 31.5]	*t* (16) = 1.42, p = 0.17
Change in ACC GABA+	0.12 ± 0.63	−0.41 ± 0.37	0.54 [0.02 to 1.05]	*t* (16) = 2.10, p = 0.05*
Change in PCG GABA+	−0.59 ± 0.82	−0.27 ± 0.51	0.31 [−0.39 to 1.02]	*t* (16) = 0.95, p = 0.36

*Reported as mean ± SD unless stated, *statistically significant p <0.05;*
Botox: OnabotulinumtoxinA

9.3 Supplementary Materials III

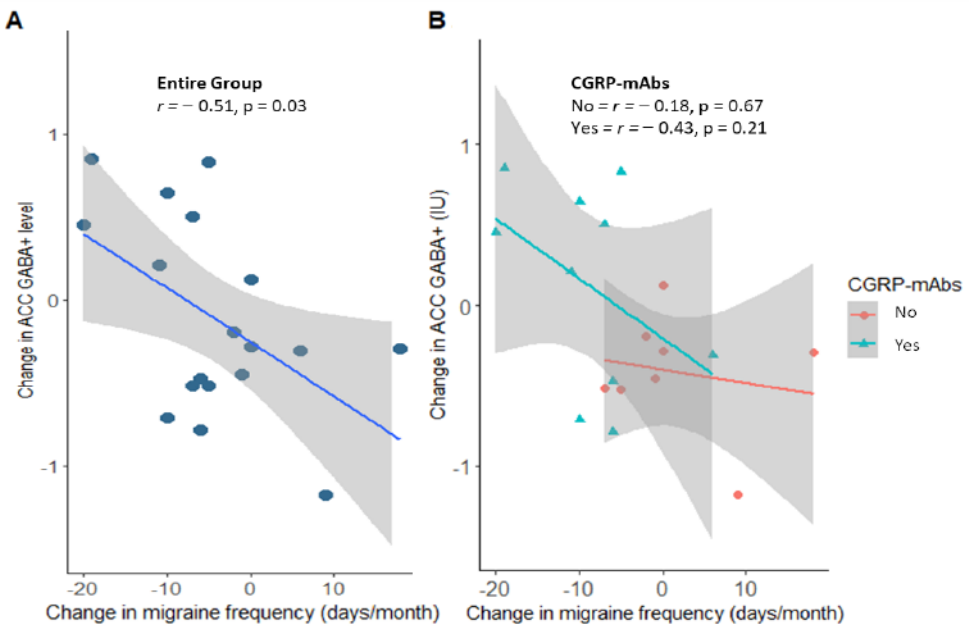

Figure 1: Correlation between change in GABA+ levels and change in migraine frequency in ACC. A) entire group B) divided by group: treatment escalated with CGRP-mAbs: Yes /No.

Figure 1A demonstrates the correlation of the whole group of people with migraine reported in this study, Figure 1B demonstrates the correlation divided by group and shows that the group escalated with CGRP-mAbs had a stronger correlation with an increase in ACC GABA+ than those who did not, although neither correlation reached statistical significance. Further, the people escalated with CGRP-mAbs generally had a greater reduction in migraine days a month compared to those who were not escalated with CGRP-mAbs.

CHAPTER FIVE

A Comprehensive Guide to MEGA-PRESS for GABA Measurement

PREFACE

Chapter 5 presents an evidence based guideline for measuring GABA using a MEGA-PRESS experiment. The chapter consists of the manuscript with three supplements, the extended guideline and the infographic.

Citation

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Publication metrics

The work presented in this chapter has been submitted to *NeuroImage* which has an impact factor of 6.556, and is Q1 for Cognitive Neuroscience and Neurology. The Scimago Journal and Country Rank (2020) is 3.26.

Dissemination

The Guideline will be submitted for presentations and workshops at International Conferences in 2022 the International Society for Magnetic Resonance in Medicine (ISMRM), Society for MR Radiographers & Technologists (SMRT) and Organization for

Human Brain Mapping (OHBM), 7th MRS International symposium on Advanced MRS and GABA editing school,

Impact

Chapter 5 saw the collaboration of 23 International MRS experts representing 15 Universities from 8 Countries, to reach consensus on best practice in MRS. The work further engaged experts in guideline development and translation with end users such as radiographers, PhD students and pain researchers to form a multi-disciplinary working group.

AUTHORSHIP ATTRIBUTION STATEMENT

The co-authors of the paper *'A Comprehensive Guide to MEGA-PRESS for GABA Measurement'* confirm that Aimie Laura Peek has made the following contributions:

- Design of the work
- Collection and extraction of the data
- Analysis and interpretation of the data
- Manuscript preparation, revision and critical appraisal for important intellectual content

As the primary supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

[redacted]

As. Prof. Trudy Rebbeck

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14th December 2021

A COMPREHENSIVE GUIDE TO MEGA-PRESS FOR GABA MEASUREMENT

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Data availability and code: No neuroimaging data analysis was performed in this guideline

ABSTRACT

Background: The aim of this guideline is to provide a series of evidence-based recommendations that allow those new to the field of MEGA-PRESS to produce high-quality data for the measurement of GABA levels using edited magnetic resonance spectroscopy with the MEGA-PRESS sequence at 3T. GABA is the main inhibitory neurotransmitter of the central nervous system and has been increasingly studied due to its relevance in many clinical disorders of the central nervous system. MEGA-PRESS is the most widely used method for quantification of GABA at 3T, but is technically challenging and operates at a low signal-to-noise ratio. Therefore, the acquisition of high-quality MRS data relies on avoiding numerous pitfalls and observing important caveats.

Methods: The guideline was developed by a working party that consisted of experts in MRS and experts in guideline development and implementation, together with key stakeholders. Strictly following a translational framework, we first identified evidence using a systematically conducted scoping literature review, then synthesised and graded the quality of evidence that formed recommendations. These recommendations were then sent to a panel of 21 world leaders in MRS for feedback and approval using a modified-Delphi process across two rounds.

Results: The final guideline consists of 23 recommendations across six domains essential for GABA MRS acquisition (Parameters, Practicalities, Data acquisition, Confounders, Quality/reporting, Post-processing). Overall, 78% of recommendations were formed from high-quality evidence, and 91% received agreement from over 80% of the expert panel.

Conclusion: These 23 expert-reviewed recommendations and accompanying extended documentation form a readily usable guideline to allow those new to the field of MEGA-PRESS to design appropriate MEGA-PRESS study protocols and generate high-quality data.

KEYWORDS: GABA, MEGA-PRESS, 1H-MRS, MRS, edited-MRS, guideline

1. INTRODUCTION

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter of the central nervous system (CNS) and plays an important role in regulating healthy brain function. For example, GABA is implicated in sensory processing (Puts *et al.*, 2017; Wood *et al.*, 2021), learning (Kolasinski *et al.*, 2019; Zacharopoulos *et al.*, 2021), memory (Gasbarri and Pompili, 2014) and motor function (Kolasinski *et al.*, 2019; Zacharopoulos *et al.*, 2021).

GABA is of particular interest in clinical conditions of the CNS and altered GABAergic function has been associated with chronic pain (Peek *et al.*, 2020), psychological disorders e.g. stress and depression (Schür *et al.*, 2016; Godfrey *et al.*, 2018), substance addiction (Vengeliene *et al.*, 2008) and neurodevelopmental disorders, e.g. autism spectrum disorder (Marotta *et al.*, 2020). Evidence for altered GABA function comes through multiple lines of enquiry including animal models (Enna and McCarson, 2006; Sun *et al.*, 2018), genetics (Baulac *et al.*, 2001; Coghlan *et al.*, 2012), post-mortem studies (de Jonge *et al.*, 2017), blood plasma (Petty, 1994; Bhandage *et al.*, 2019) and *in-vivo* Magnetic Resonance Spectroscopy (MRS) (Schur *et al.*, 2016; Peek *et al.*, 2020). Given the wealth of evidence, targeting the GABAergic system with therapeutic interventions may therefore prove fundamental to improving patient outcomes in these conditions. However, this requires a better understanding of the role of GABA in humans, which requires the reliable measurement of GABA in the human brain. The only currently available approach to measure GABA *in-vivo* in humans is through tailored MRS.

MRS is a non-invasive brain imaging technique which enables the *in-vivo* quantification of endogenous brain neurometabolites based upon their chemical structure. Conventional proton MRS has been successfully used to quantify numerous neurometabolites, such as glutamate, N-acetylaspartate (NAA) and choline-containing compounds. GABA is also present in the MR spectrum, however, due to its lower concentration and complicated peak pattern, its signal is difficult to reliably separate from more abundant neurometabolites such as creatine (Mullins *et al.*, 2014)- particularly at field strengths typical for current clinical MRI scanners. The most widely used technique for measuring GABA levels at 3T is J-difference editing, most famously implemented in the MEscher–GArwood Point RESolved Spectroscopy (MEGA-PRESS) experiment (Mescher *et al.*, 1998). MEGA-PRESS consists of two sub-experiments (usually acquired in an interleaved fashion), one applying editing pulses at a frequency of 1.9 ppm to selectively refocus the coupling evolution of the GABA signal at 3 ppm ('Edit-ON'), while the other allows the free evolution of the spin system throughout the echo time ('Edit-OFF'). Subtracting the Edit-OFF from the Edit-ON spectrum reveals a difference-edited GABA signal while removing the stronger overlapping signals from creatine-containing compounds. (see de Graaf 2019 for review). The edited signal at 3 ppm is contaminated by co-edited macromolecular signals (estimated to account for about 50% of the edited signal area) and is commonly referred to as GABA+. While the macromolecular contamination can be reduced by adding a second editing pulse at 1.5 ppm (Henry *et al.*,

2001), the increased specificity comes at the expense of a much greater sensitivity to experimental instability, particularly thermal drift of the magnetic field strength (Edden *et al.*, 2016).

The separability of the GABA signal is significantly improved using MEGA-PRESS, but accurate detection and quantification still require high-quality data. Data quality is determined to a great extent by the choice of acquisition parameters, however, few studies provide sufficient detail of these. For example, in a recent meta-analysis (Peek *et al.*, 2020) investigating the use of MRS to measure GABA levels in pain conditions, only two out of fourteen studies reported using parameters that were deemed adequate for quantification of GABA levels. The remaining studies either documented using inadequate parameters or sequences, or altogether failed to fully report the parameters used, a finding resonated in other reviews such as Schur *et al*. 2016. The heterogeneity in MRS acquisition parameters used within the field has been acknowledged as a significant barrier to the reproducibility and comparability of quantitative MRS outcome measures (Mullins *et al.*, 2014; Peek *et al.*, 2020). In response, multiple expert panels have recently formed to establish consensus guidelines for minimal best practice in acquisition and analysis of MRS data (Mullins *et al.*, 2014; Öz *et al.*, 2020; Choi *et al.*, 2021; Kreis *et al.*, 2021). While some aspects covered in these consensus guidelines might apply to GABA measurement using MEGA-PRESS, the specific requirements for its successful application are not addressed in detail.

A further barrier to implementing these consensus documents is that they are typically written by experts with a high level of technical knowledge, leading to some recommendations being difficult for those new to the field to interpret and adequately implement. The growing field of translational research has increasingly seen those from fields outside of magnetic imaging physics wishing to use advanced MRS methods in both clinical and research populations. Examples include clinician-researchers and higher degree research students in areas such as pain medicine, physiotherapy and psychology. Typically these researchers do not have a background in magnetic resonance physics, and often do not have direct access to the resources or expertise required to interpret and implement technical consensus documents. We have therefore identified a need for an easily accessible and translatable guideline to the adequate use of MEGA-PRESS for the measurement of GABA. However, the substantial heterogeneity in preferred acquisition parameters, even among leading MRS experts, is a challenge for creating widely applicable methodological guidelines.

We therefore used an established translational framework widely used for developing clinical guidelines in order to maximize the objectivity of our recommendations. The National Health and Medical Research Council (NHMRC), the leading governmental authority on medical research in Australia, recommends a multi-stage process for guideline development (NHMRC, 2021). Four key aspects to ensure robustness include: 1) engaging subject, and methodological expertise alongside end-users, 2) evidence synthesis, 3) establishing quality and strength of evidence using the Grading of recommendations, Assessment, Development and Evaluation (GRADE) (NHMRC, 2009), and 4) independent expert review of the recommendations (NHMRC, 1999, 2021). These steps ensure guidelines are credible, useable and ready for implementation into practice.

The result of this study is a robust, translatable, evidence-based, and expert-reviewed guideline that will enable those new to the field to use MEGA-PRESS to acquire high-quality data for the reliable quantification of brain GABA levels. The adherence to a translational framework ensures that the guidelines are evidence-based, rather than a narrative of personal opinions and experiences. Whilst the guideline has been written specifically for the reliable measurement of GABA using MEGA-PRESS at 3T, many of the recommendations will, with certain modifications, also be applicable to similar techniques employing different signal localization (e.g. MEGA-sLASER, MEGA-SPECIAL (Edden *et al.*, 2012)), editing schemes (HERMES) (Saleh *et al.*, 2016b), and target metabolites (Harris *et al.*, 2017).

2. METHODS

We followed the NHMRC framework Guidelines for Guidelines (NHMRC, 2021) and utilized the ADAPTE toolkit (The ADAPTE Collaboration, 2009) to develop this guideline. This framework divides the evidence synthesis and recommendation formation workflow into three stages: set up, adaptation and finalisation (The ADAPTE Collaboration, 2009). The stages are summarised in Figure 1.

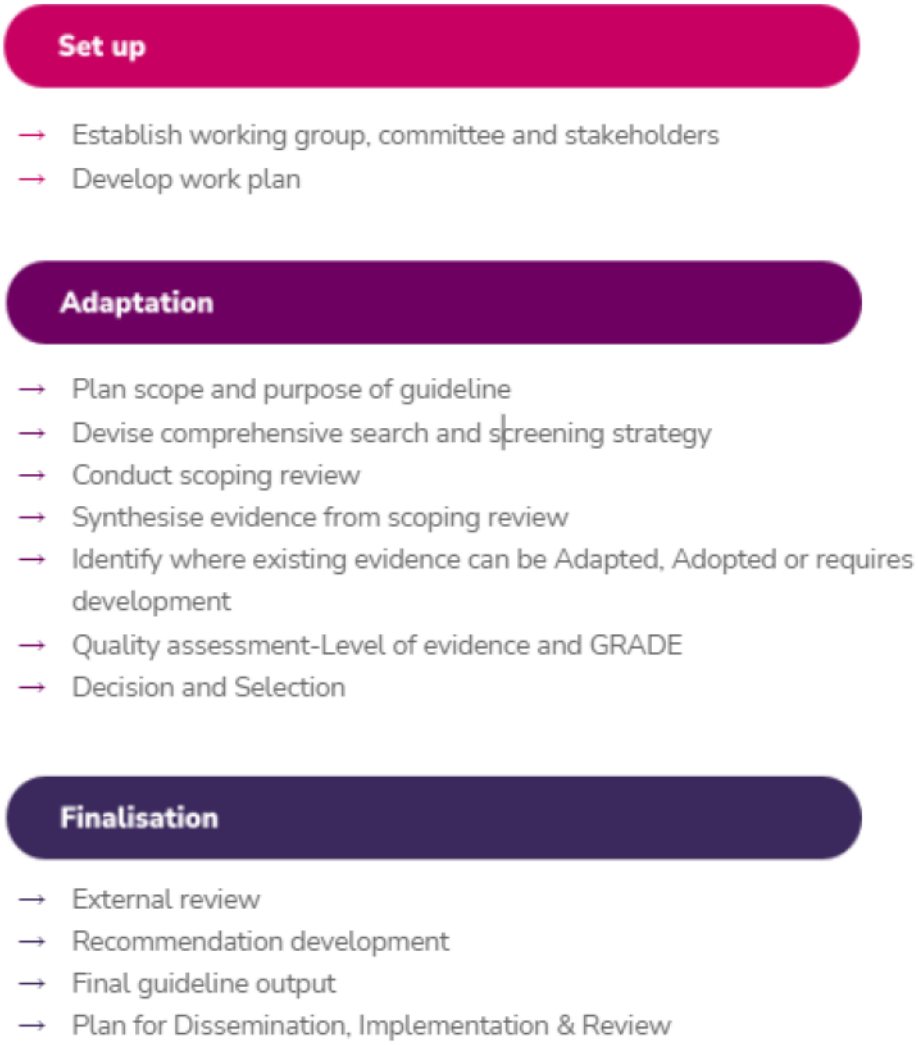

Figure 1: Demonstrating the process followed to develop the guideline based on the ADAPTE process (The ADAPTE Collaboration, 2009)

The purpose of the set up stage was to establish the guideline working party and sub-committees, identify key stakeholders and formulate a work plan (The ADAPTE Collaboration, 2009).

2.1.1 Committee establishment and stakeholder engagement

The guideline working party included a core team of six co-authors. The working party consisted of two sub-committees; i) Guideline development/implementation sub-committee

(four members with a total of over 40 years of experience in forming clinical/therapeutic guidelines) and ii) MRS sub-committee (three members with a total of over 23 years experience in MRS of GABA). One author was included in both sub-committees to ensure consistency, communication and continuity across meetings. Key stakeholders reflect proposed end-users and those with an interest in the final guideline. Stakeholders were identified and engaged by the working party to be involved in the development process. The key stakeholders were a research radiographer, a PhD student studying MRS, three clinician-researchers who were investigating GABA levels in multiple pain conditions, and two MRS experts who provide training to new MRS users.

2.1.2 Work plan

A work plan identifying and recruiting all expertise required for project completion through large international collaborative networks was developed. Stages were identified through NHMRC Guidelines for Guidelines (NHMRC, 2021) and a time-line established. Details of the Adaptation and Finalisation stages are described as follows.

2.2 Adaptation

The adaptation stage was the largest of stages and included several steps from systematically identifying literature through a scoping review, through to the formulation of guideline recommendations.

2.2.1 Scope and purpose

The working party met with key stakeholders on two occasions through an iterative discussion process to arrive at the scope and purpose of the Comprehensive Guide to MEGA-PRESS for GABA measurement. The result of the discussions led to the identification of six key domains critical for high-quality data: Parameters, Practicalities, Data Acquisition, Confounders, Quality/Reporting, Post-processing. The working party and stakeholders agreed the following were not within scope: i) providing in-depth review of all differences between vendor-specific user interfaces, hardware, and implementations of the MEGA-PRESS sequence; ii) details regarding post-processing, modelling and quantification methods, except for those aspects with direct implications for the acquisition protocol design for example, the necessity of acquiring a water-reference signal (see 4. *Discussion*). Further it was agreed that the focus would be set on recommendations for measuring GABA at 3T in clinical and

research populations using MEGA-PRESS, although some recommendations would translate to other metabolites, field strengths and sequences.

2.2.2 Search and Screening

Evidence to inform the guideline was identified through a systematically conducted scoping review. A search strategy was developed using terms for GABA editing (e.g. MEGA-PRESS, spectral editing, GABA) AND magnetic resonance spectroscopy (e.g. MRS, magnetic resonance spect*) AND terms specific to GABA MRS acquisition stages (e.g. gradients, shim). Three databases were searched (Ovid MEDLINE, Embase and PubMed) and reference lists of included studies were screened by the MRS expert sub-committee for any missing publications. A two-stage approach was used to screen studies for inclusion against the pre-specified inclusion criteria regarding study methods and study design (For further details of review methodology see Supplement 1). Studies were included if they used methods involving single-voxel MRS data acquired in humans, phantoms or using computer simulations. Study designs were included if they were consensus documents, systematic reviews, randomised controlled trials or methodological investigations. Studies were excluded if the methods included animals, used multi-voxel or spectroscopic imaging techniques (beyond the scope of these guidelines), or used designs that were narrative (non-evidence-based) reviews, commentaries or conference proceedings. In the first stage of screening, two reviewers independently screened titles and abstracts to identify studies appropriate for full text review (AP, GO). In the second stage, full texts were screened for inclusion. Data were then extracted independently by two authors using a standardised form for each of the six pre-identified domains. Inconsistencies in screening and disagreement on exclusion/inclusion were discussed and resolved with a third reviewer (NP). The MRS sub-committee reviewed the results of the search and identified any key missing papers.

2.2.3 Results of the scoping review

The initial search retrieved 2664 studies, 21 additional publications were identified following the reference list search of included publications, the MRS-subcommittee review, and following the release of a special issue of *NMR in Biomedicine.* The special issue *"Advanced methodology for in vivo magnetic resonance imaging"* (Choi *et al.*, 2021) contained a series of expert consensus guidelines in MRS published after the commencement of the search. Following removal of duplicates, 1460 studies were screened against the inclusion and exclusion criteria, resulting in exclusion of a further 1283 records, leaving 176 records for

full-text screening. Following the exclusion of 87 studies (32 due to study design, 39 due to content e.g. not MEGA-PRESS, or not relevant to 3T, and 16 for both content and design reasons), 90 publications were used to inform the guidelines (For PRISMA Flowchart see Supplement 2). Nine of the 90 publications were consensus documents, one randomized control trial, one seminal textbook describing the theory of *in-vivo* MR spectroscopy, one seminal paper documenting MEGA-PRESS practices, four systematic reviews, three multi-site trials, and seventy-one methodological publications. The publications used to inform each recommendation are listed in Supplement 3.

2.2.4 Evidence Synthesis

The MRS sub-committee summarised evidence from the studies identified by the scoping review under the six pre-identified domains. The MRS sub-committee used an iterative process to establish where recommendations currently existed in consensus documents, and could be later considered for adoption or adaptation or where recommendations would require development. Following the ADAPTE framework for guideline adaptation (The ADAPTE Collaboration, 2009), a recommendation is considered suitable for *Adoption-* when it can be lifted directly from an existing guideline or for *Adaption-* when the recommendation needs to be adjusted to suit the audience or context. Where no evidence exists the recommendations require development *DeNovo* ('from scratch') (NHMRC, 2021). This first scoping draft (Draft 1) included 20 recommendations under the six domains. Furthermore, the MRS sub-committee identified five areas that required recommendation development.

2.2.5 Evidence Level Assessment and GRADING the certainty of evidence

An NHMRC *Level of Evidence* was assigned to each study included in the evidence synthesis for each of the recommendations. The Level of Evidence describes the suitability of a study design to address a research question (ranging from Level 1 indicating the most robust design to Level 4 indicating the least robust design) (NHMRC, 2009). Studies involving confounders of GABA levels were assessed using the traditional hierarchy of evidence (NHMRC, 2009) given that such research questions are best answered through systematic reviews of randomised controlled trials (Level 1). For studies reporting MRS principles and acquisition parameters, the MRS sub-committee considered consensus documents the highest level of evidence (Level 1). Hence, to appraise these publications, the traditional NHMRC evidence hierarchy was adapted following the recommendations for hierarchy modification by the

NHMRC (NHMRC, 2009). Details of the traditional and modified evidence hierarchy are detailed in Table 1.

The modified Grading of recommendations, Assessment, Development and Evaluation (GRADE) (NHMRC, 2009) was then utilized to determine the degree of certainty in the body of evidence used to inform each of the recommendations. The GRADE process considers the Level of Evidence and direction of findings to determine the level of confidence that can be placed in the recommendation (NHMRC, 2009). The modified-GRADE ranges from GRADE A where a recommendation is informed by a number of Level 1 studies providing consistent recommendations through to GRADE I where there is insufficient evidence to provide a recommendation (Table 2). The GRADE process was carried out independently by four blinded member of the development/ implementation sub-committee. Disagreements were resolved through discussion.

2.2.6 Decision and Selection

The first draft (Draft 1) consisting of 20 recommendations was circulated to the key stakeholders prior to an in-person consensus meeting held at the 5th International GABA Symposium (19th-21st November 2019, Park City, UT, United States). The aims of the consensus meeting were: 1) to discuss key information required by those new to the field of MRS, and identify any gaps not addressed through the draft recommendations; 2) to identify and reach agreement where recommendations could be adopted or adapted from existing evidence; 3) to determine the process to develop recommendations for areas currently not supported by evidence.

As a result of the stakeholder meeting, the 20 recommendations were revised and augmented to 26 recommendations. Agreement was reached that 12 recommendations were suitable for direct adoption, nine for adaptation, and five required development (note: four of these five were later adapted from recommendations in newly released consensus documents). It was agreed that the process for development would be led by the MRS sub-committee. The MRS sub-committee would use and customise evidence from other fields or sequences for GABA MEGA-PRESS. Discussion regarding the key information required for those new to the field was agreed upon.

These decisions were then forwarded to the development/implementation sub-committee. This sub-committee wrote recommendations in easily understandable language suitable for those new to the field of MRS. The revised draft was then circulated back to the stakeholders

and a finalised draft (Draft 2) was prepared to be circulated for review by an external expert panel.

2.3 Finalisation

The finalisation stage included; external expert review, production of this peer review publication, one-page infographic and extended guideline, and agreeing upon the implementation and dissemination plan and schedule for review and update (The ADAPTE Collaboration, 2009).

2.3.1 External review

The finalised draft (Draft 2) was sent for agreement and review by a panel of experts using a modified-Delphi process. The modified-Delphi process is a group consensus strategy, designed to transform opinions into group consensus using an iterative multi-stage process (Hasson *et al.*, 2000; Miller *et al.*, 2020). The expert panel was established through invitation by the MRS sub-committee. Experts were identified based on their contribution to recent MRS consensus documents, and their contribution to the field of MRS. The panel consisted of 21 expert MRS researchers from 15 universities in eight different countries. In Round 1, experts rated a) their agreement with the content of the recommendation, and b) the suitability of the recommendation for use in a beginner's guide. Ratings were on a Likert scale of -5 to +5 (where -5 to -1 indicated disagreement, 0 represented a neutral agreement, and 1 to 5 indicated agreement). Experts were also given the opportunity to comment on each of the recommendations and submit suggestions for modifications. The results from Round 1 and 2 expert panel agreement were analysed using percentages.

Recommendations were classified as having 'expert panel endorsement' and accepted into the final guideline where at least 80% of the expert panel had agreed to the recommendation. In cases where recommendations did not reach the 80% threshold, they were revised, taking into account the written feedback from the expert panel. These revised recommendations were then re-sent to the expert panel for a second rating (Round 2). The Round 2 expert panel consisted of 20 experts, as one expert was unavailable to review the revised recommendations. Any recommendation not achieving agreement of at least 80% of the expert panel in Round 2 was not given the 'expert panel endorsement' label. In these instances, evidence was reviewed by the working party, and the significance of removing the recommendation from the guideline was deliberated until a final verdict on the recommendation was reached.

2.3.2 Recommendation development

The finalised Comprehensive Guide to MEGA-PRESS for GABA Measurement consists of 23 recommendations across the six key domains. Nineteen of the 26 recommendations sent for expert panel review (Draft 2) received expert panel endorsement (over 80% agreement) in Round 1 (Figure 2). Sixteen of these were immediately accepted into the guideline. Three of the nineteen required further refinement (1 due to new evidence being published, one due to not being deemed suitable for those new to the field by the expert panel, one due to being deemed too long by the expert panel). Following expert feedback from Round 1, the recommendations were consolidated and re-grouped from 26 to 23 recommendations. Overall, eight recommendations were revised and submitted to the expert panel for Round 2 assessment. Following Round 2, a further six recommendations received expert panel endorsement and were accepted into the guideline (Figure 3). Two recommendations did not receive expert endorsement ('gradient order' - 75% and 'water reference' - 55%). In these cases, the MRS sub-committee revisited the evidence for these recommendations and debated the inclusion of these recommendations in the guideline. In both cases the result of the debate was to include the recommendation, without expert panel endorsement, with the addition of further explanatory notes in the consideration section of the extended document.

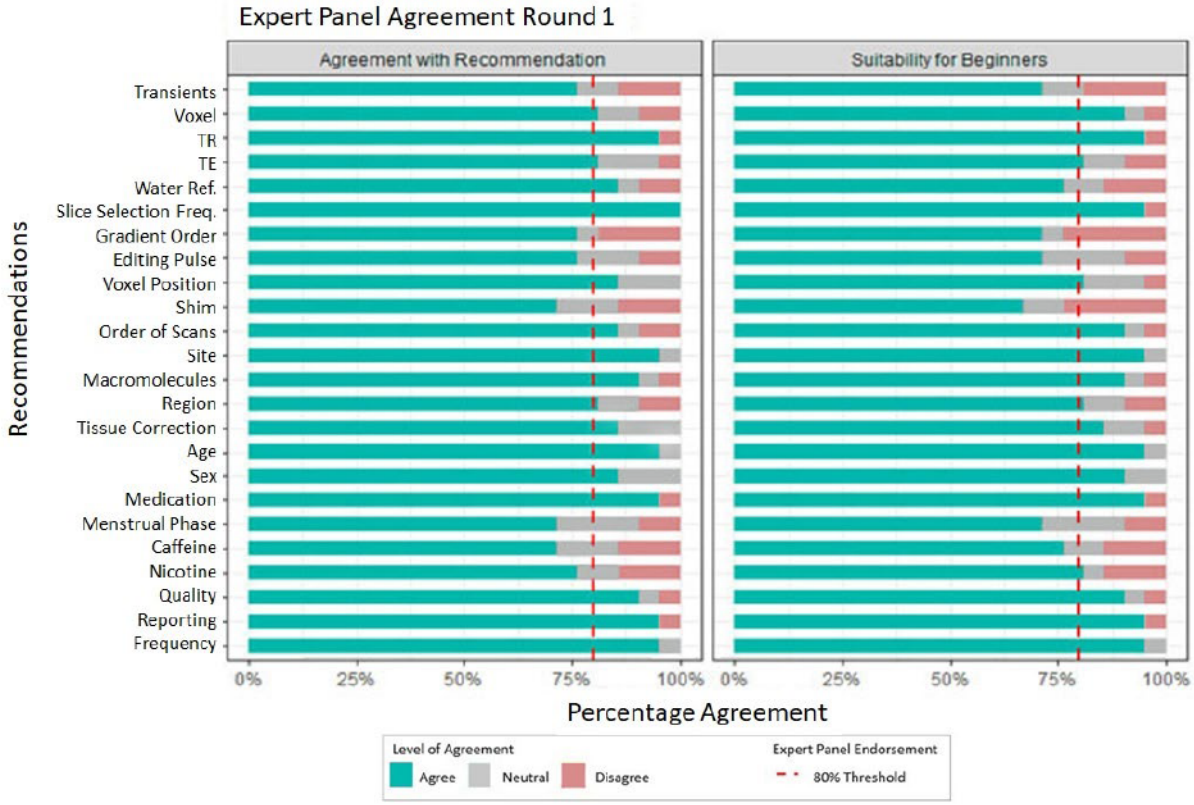

Figure 2 Results from Round 1 of the Expert panel review

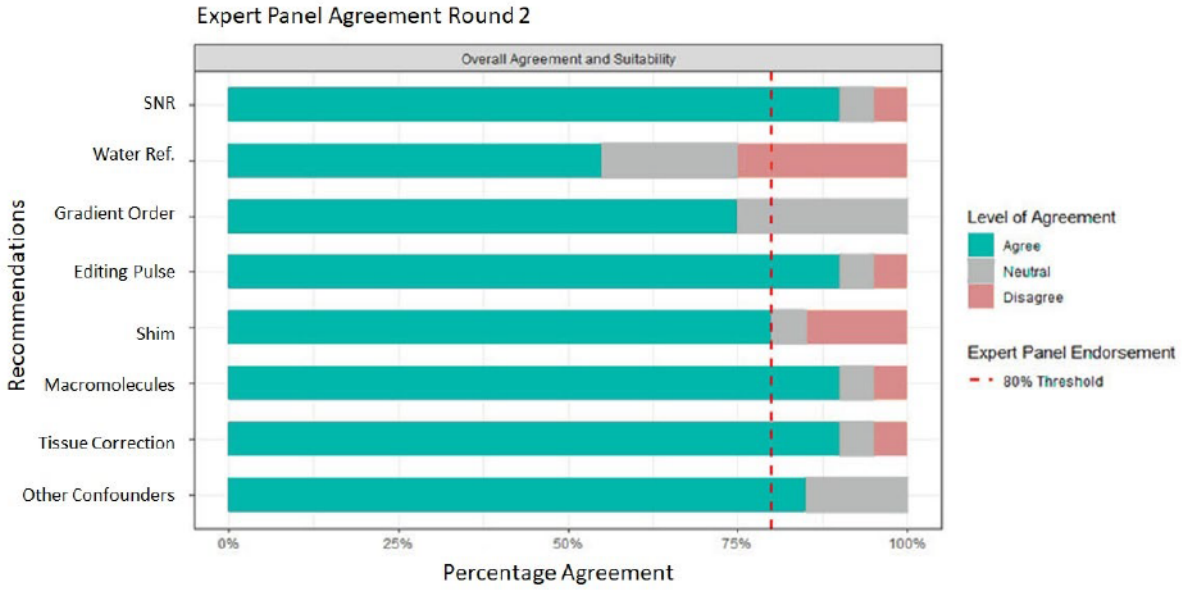

Figure 3 Results from Round 2 of the Expert panel review

2.3.3 Final Guideline outputs

The three outputs from this work include a full guideline (Supplement 4), this peer reviewed publication summarising the recommendations and a one-page infographic summary (Supplement 5).

1) A Comprehensive Guide to MEGA-PRESS for GABA measurement (Full guideline)

The full guideline (Supplement 4) is a detailed document providing background information on the subject of each recommendation. The full-length guideline is recommended for consultation when upskilling in the field of MEGA-PRESS, particularly during the study protocol design phase. Each final recommendation included in the guideline is the result of the evidence synthesis and the expert panel feedback. Therefore this guideline consists of the full evidence summary that informed the recommendation, and includes the key considerations added by the expert panel that resulted in the final recommendation.

2) The peer reviewed publication (This manuscript)

This peer reviewed publication first outlines the rigor of the methodological process of recommendation development and then provides a summary of the recommendations. This manuscript provides GRADE of evidence, percentage of expert panel agreement and a shortened summary of the evidence synthesis and expert panel feedback that informed the recommendation. This manuscript can be used instead of the full-length guideline when a brief overview of parameters that determine data quality is sufficient.

3) One-page infographic summary

The infographic (Supplement 5) provides a quick visual reference guide, summarizes the key messages of the Comprehensive Guide and provides a memory aid to users who have previously read the full guideline. Its purpose is to improve the translation of the guideline into standard practice.

2.3.4 Dissemination, Implementation and Review

The working party designed the dissemination and implementation plan. Dissemination will occur at key annual meetings and conferences where target markets, such as junior researchers, applications-oriented scientists, and educators will be in attendance. This includes the International Society for Magnetic Resonance in Medicine (ISMRM), Society for MR Radiographers & Technologists (SMRT) and Organization for Human Brain

Mapping (OHBM). In addition, the guideline will be presented in GABA-MRS-focused workshops and educationals such as the International Symposium on GABA and Advanced MRS and EDITINGSCHOOL, where attendees have a specific interest in GABA MRS.

Pilot implementation will commence at all of the working parties' collaborative sites (over 25 sites worldwide), where the guideline will be integrated in current operating procedures, and the infographic will be distributed. In addition, members of the guideline working party will integrate it into their supervision and teaching procedures to students (graduate and undergraduate), residents and researchers. The guideline will be reviewed for currency by the working party in 2026 and updated should further high-quality evidence provide recommendations differing to those presented in this guideline.

Table 1: Level of evidence modified from NHMRC (2009) (NHMRC, 1999)

	MODIFIED EVIDENCE HIERARCHY		ORIGINAL EVIDENCE HIERARCHY	
Level	**Design**	**Justification**	**Design**	**Justification**
Level 1	Consensus Document	Traditionally a systematic review of the most appropriate study design is considered Level 1 evidence. In this case we consider expert consensus documents as Level 1 because akin to systematic reviews in other fields, these consensus documents draw on the most appropriate study designs to inform the parameters required to run a MEGA-PRESS study. All consensus documents included within this review had a panel of authors from multiple institutions across multiple countries. They also benefit from recency, with 7/9 included consensus being published in 2020/2021.	Systematic review	In line with the NHMRC recommendations (NHMRC, 2009) a systematic review of Level 2 studies will be considered Level 1. In this case meta-analysis of the studies will likely improve precision of the results. In cases where systematic reviews are of lower levels of evidence, they will be considered the same level as the studies they include, as they may increase the chance of bias (NHMRC, 2009).
	Seminal texts	Where core principles of physics are required to inform the guideline, seminal text of these fundamental physical properties are also considered highest level of text.		
Level 2	Systematic Review	Systematic reviews are considered Level 2 evidence as they pool together results from methodological publications which have been specifically designed to test parameters required to run a MEGA-PRESS study. However, the methodological publications typically have small sample sizes, and limitations and suffer from publication bias.	Randomised Control Trial	In order to investigate the impact of a confounder a randomized control trial would be considered the best design to address the research question.
	Large multi-site studies	Large multi-site studies provide the most information on applying parameters in a clinical context; however, the purpose of such trials is rarely to investigate a single parameter required to run a MEGA-PRESS study.		
Level 3	Methodological publications	For the purpose of this study, methodological publications were considered as any study that had a specific aim to investigate a parameter required to run a MEGA-PRESS study. These might include studies on humans, phantoms, or simulations. These did not include animal studies. These methodological publications will often test a specific parameter required to run a MEGA-PRESS study and directly inform this guideline.	i) Comparative study with concurrent controls	Studies designs that investigate a condition compared to a control group, or situation are considered Level 3 evidence as they have the potential for bias.

		However, these studies are typically performed using small samples, and are often tested on healthy subjects in non-clinical environments.	ii) Comparative study without concurrent controls Consensus document	Consensus documents are considered Level 3 when investigating confounders, as these research questions are better answered using a scientifically rigorous design, and therefore a consensus document is potentially biased.
Level 4	Narrative Reviews	Narrative reviews are commonly published in the field of ^{1}H-MRS spectroscopy but must be interpreted with caution due to the high risk of bias and personal opinion.	Case series	Case series are considered Level 4 due to being underpowered to answer these research questions, with no control for comparison.

Table 2 GRADE of Recommendation

GRADE	Criteria	Description
A	• Good evidence (One or more Level 1 study or studies with consistent findings)	Body of evidence can be trusted to guide recommendation
B	• Fair evidence (One or more Level 2 or 3 study or studies with consistent findings)	Body of evidence can be trusted to guide recommendation in most situations
C	• Conflicting evidence (One or more Level 1 to 3 study or studies with inconsistent findings) OR • Low level evidence (More than one Level 4 study)	Body of evidence provides some support for recommendation, but care should be taken in its application
D	• Insufficient evidence (no studies) OR • Poor evidence (Level 4-5 studies with inconsistent findings)	Body of evidence is weak, and recommendation must be applied with caution

Adapted from Guyatt *et al.* (2008) and Wright *et al.* (2006)

3. RESULTS

The final guideline consisted of 23 recommendations, under six domains essential for GABA MRS acquisition; Parameters, Practicalities, Data acquisition, Confounders, Quality/reporting, Post-processing. Overall 78.3% of recommendations were formed from high quality evidence (Level A or B) and 91.3% received agreement from over 80% of the expert panel (Table 3).

Table 3 SUMMARY OF RECOMMENDATIONS

			Evidence GRADE	Experts: R1 (%) Agreement	Experts: R2 (%) Agreement
Acquisition	SNR	-Number of Transients -Voxel Size	A	76.2 81	90
	TR		A	95.2	-
	TE		A	81	-
	Water Reference		A	85.7	55
	Slice selection for water ref		A	100	-
	Gradient		D	76.2	75
	Editing Pulse		A	76.2	90
Practicalities	Voxel Position		A	85.7	-
	Shimming		A	71.5	80
	Order of Scans		A	85.7	-
Confounders	Scanner Site		B	95.2	-
	Macromolecules		A	90.5	100
	Region		C	81	-
	Tissue Composition		A	85.7	90
	Age		A	95.2	-
	Sex		C	85.7	-
	Medications		B	95.2	-
	Other	Caffeine	D	71.4	
		Nicotine		76.2	85
		Menstrual Phase		71.4	
Data Acquisition	Quality Assessment		A	90.5	-
	Export		I	90.5	-
Quality and Reporting	Quality Metrics		A	90.5	-
	Reporting		A	95.2	
Post-Processing	Frequency and Phase Correction		A	95.2	

3.1 *PARAMETERS*

3.1.1 Signal-to-Noise Ratio Considerations (Number of transients and Voxel volume)

ADAPT: Start with at least 192 transients (i.e. 96 Edit-ON + 96 Edit-OFF) and a voxel volume of 27 ml (e.g 3 ´ 3 ´ 3 cm^3) to quantify GABA when scanning a favourable brain region.

- Consider increasing the total number of transients when scanning smaller or more challenging brain regions (see 3.3.3 *Region*).

Evidence GRADE A. *Round 1 Expert Panel Agreement number of transients 76.2%, voxel size 81%, Round 2 Expert Panel Agreement 90%*

There were eight studies (Level 1 to Level 4) (Bhattacharyya *et al.*, 2007; Harris *et al.*, 2014; Mullins *et al.*, 2014; Brix *et al.*, 2017; Mikkelsen *et al.*, 2017; Mikkelsen *et al.*, 2018; Sanaei Nezhad *et al.*, 2018; Mikkelsen *et al.*, 2019) with recommendation about the number of transients, and seven studies (Level 1 to Level 4) (Mullins *et al.*, 2014; Bai *et al.*, 2015; Bergmann *et al.*, 2016; Chen *et al.*, 2017; Mikkelsen *et al.*, 2017; de Graaf, 2019; Mikkelsen *et al.*, 2019) with recommendation about voxel volume. The studies recommended using a range of transients from 126 (Sanaei Nezhad *et al.*, 2018) to 320 (Mikkelsen *et al.*, 2017; Mikkelsen *et al.*, 2019) transients, with the majority recommending a minimum of 192 transients when using a voxel volume of e.g 3 ´ 3 ´ 3 cm^3. A further two studies (Level 1)(Peek *et al.*, 2020; Lin *et al.*, 2021) highlight the importance of reporting the number of transients used and whether they refer to the total number of transients or separate (as Edit-ON and Edit-OFF). Failure to achieve adequate signal to noise has a significant effect on quality of the spectra as demonstrated in Figure 4. Round 1 agreement for this recommendation was 76.2% for number of transients and 81% for voxel size. Two key considerations were made: first, experts recommended combining the two separate recommendations to highlight the interdependence of the number of transients and voxel size. Second, the number of transients are best selected in multiples of 16 to allow for full phase cycles to be included. Round 2 agreement increased to 90%. Therefore, the revised recommendation was accepted.

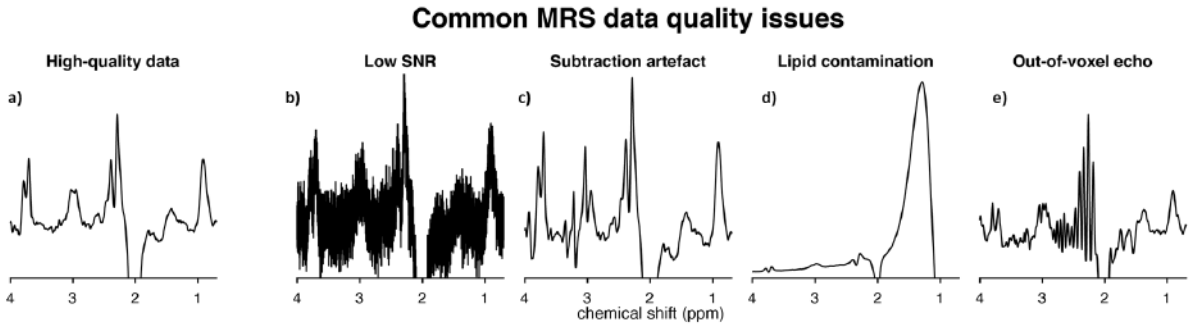

Figure 4: Common MEGA-PRESS data quality issues. a) High-quality data with sufficient SNR, narrow linewidths, a well-defined edited signal at 3 ppm, and no substantial artefacts; b) very high noise levels due to low number of transients or small voxel volume; c) severe subtraction artefacts due to scanner frequency drift; d) lipid contamination due to participant motion or voxel positioning too close to the skull; e) out-of-voxel echo ("ghost signal").

3.1.2 Repetition Time (TR):

ADOPT: Use a TR of around 2000 ms at 3T.

Evidence GRADE A; *Round 1 Expert Panel Agreement 95.2%*

There were five studies (Level 1 to Level 3) (Puts *et al.*, 2013; Mikkelsen *et al.*, 2017; Deelchand *et al.*, 2019; Mikkelsen *et al.*, 2019; Wilson *et al.*, 2019) that provided recommendations on TR. The studies all concur that a TR of ~2000ms is suitable for the measurement of GABA with edited MRS. Round 1 agreement was 95.2%. Therefore, this recommendation was adopted.

3.1.3 Echo Time (TE):

ADOPT: TE should be 68 ms (GABA+); 80 ms (macromolecule-suppressed GABA)

Evidence GRADE A; *Round 1 Expert Panel Agreement 81%*

There were ten studies (Level 1 to Level 3) (Mescher *et al.*, 1998; Edden *et al.*, 2012; Mullins *et al.*, 2014; Harris *et al.*, 2015a; Edden *et al.*, 2016; Mikkelsen *et al.*, 2017; Deelchand *et al.*, 2019; Mikkelsen *et al.*, 2019; Wilson *et al.*, 2019; Cudalbu, 2020) that provided recommendation on TE. The consensus across studies was to keep TE as close to 68 ms as possible when estimating GABA+, and 80 ms for macromolecule-suppressed measurements. Round 1 agreement was 81%. Therefore, this recommendation was accepted.

ADAPT: Water reference scans (required for eddy-current correction and water-scaled quantification): acquire two water reference scans for each volume of interest: one

using the same parameters as MEGA-PRESS, but deactivated water suppression for eddy-current correction, and one short-TE (TE ~ 30 ms) for quantification.

Evidence GRADE A; *Round 1 Expert Panel Agreement 85.7%, Round 2 Expert Panel Agreement 55%*

There were seven studies (Level 1 to Level 3) (Hall *et al.*, 2014; Mullins *et al.*, 2014; Oeltzschner *et al.*, 2016; de Graaf, 2019; Wilson *et al.*, 2019; Near *et al.*, 2020; Öz *et al.*, 2020) that provided recommendation on water reference scans. There was consensus across studies recommending that water reference scans are acquired from the same volume of interest using the same parameters and gradients in order to facilitate eddy-current correction. Round 1 agreement was 85.7%, but only 70% felt the recommendation was suitable for a beginner's guide. Experts reasoned that those new to the field might not be aware that using long-TE water data for quantification purposes may introduce T_2-weighting, which inadvertently has implications for quantification (Gasparovic *et al.*, 2018). In line with the feedback and the publication of a new consensus document (Near *et al.*, 2020), the recommendation was revised to recommend acquiring a separate short-TE water reference scan to be used for quantification. However, Round 2 agreement reduced to 55% due to several experts (n=8/20, 40%) not considering a short=TE scan necessary for quantification. The inclusion of this guideline was discussed by the working party. The decision was made to retain the revised recommendation due to it reflecting the most up-to-date recommendation in the literature. It was decided to further develop the preface and consideration section for educational purposes to help the translation of this new recommendation, given the feedback from the experts.

3.1.4 Slice-selection centre frequency of water reference scan:

ADOPT: Set the water reference to be acquired from the same volume as the GABA signal.

Evidence GRADE B; *Round 1 Expert Panel Agreement 100%*

There were three studies (Level 2 to Level 3) (Mikkelsen *et al.*, 2017; Deelchand *et al.*, 2019; Mikkelsen *et al.*, 2019) that provided recommendations on slice-selection centre frequency of the water reference scan. The consensus across studies was that the frequency should be set to 0 ppm offset, i.e. localizing the 4.7 ppm water signal. Round 1 agreement was 100%. Therefore, this recommendation was accepted.

3.1.5 Order of slice-selective gradients:

ADAPT: When artefacts appear in pilot data, consider changing the order of the slice-selective gradients for each volume of interest.

Evidence GRADE D; *Round 1 Expert Panel Agreement 76.2%; Round 2 Expert Panel Agreement 75%*

There was one paper (Level 3) (Ernst and Chang, 1996) that provided recommendations on the order of slice-selective gradients. The paper highlighted how changing the order of gradients can remove artefacts from data. Round 1 agreement was 76.2% due the recommendations suggesting that trial acquisitions with different orders should be conducted. In line with feedback from expert consensus, the recommendation was revised to suggest this as a troubleshooting option only when artefacts are consistently present in data. Round 2 agreement reduced to 75% due to concerns that those new to the field would not know which artefacts could be helped by changing gradient order (n=3/20, 15%) and that some systems do not allow for simple adjustment of gradient order. The decision to maintain the recommendation was made by the MRS sub-committee who felt this troubleshooting advice might be helpful to those new to the field with the addition of Figure 4, which demonstrates the artefacts that can be addressed through this method. This recommendation therefore was included, but not given expert approval.

3.1.6 Editing pulse specifications

ADOPT: Editing pulses can be applied as follows (Table 4):

Table 4 editing pulse specifications

	GABA+	Macromolecule-suppressed
Frequency (ppm)		
Edit-ON	1.9 ppm	1.9 ppm
Edit-OFF	7.46 ppm	1.5 ppm
Bandwidth	60 Hz	Usually 80 Hz (60 Hz on some implementations)
Spacing	0.5 TE apart (this parameter is usually not accessible to the user)	

Evidence GRADE A; *Round 1 Expert Panel Agreement 76.2%; Round 2 Expert Panel Agreement 90%*

There were nine studies (Level 1 to Level 3) (Keltner *et al.*, 1996; Mescher *et al.*, 1998; Henry *et al.*, 2001; Mullins *et al.*, 2014; Edden *et al.*, 2016; Mikkelsen *et al.*, 2017; Deelchand *et al.*, 2019; Mikkelsen *et al.*, 2019; Saleh *et al.*, 2019) that provided recommendation on editing pulse parameters. Recommendations were dependent on whether GABA+ or macromolecule-suppressed GABA was being acquired (see Supplement 4 full-length guideline for explanation). Round 1 agreement was 76.2%, the recommendation was therefore revised. Key points from the expert panel were that some sequence implementations do not allow for the adjustment of these parameters. The panel had many suggestions of variations that they use when applying editing pulses (n=8/21, 38.1%) which highlight the methodological heterogeneity even among experts in the MRS field. The revised recommendation removed recommendations for pulse duration and highlighted that editing pulses could be applied using these parameters as a starting point for those new to the field. Round 2 expert panel agreement was 90%. Therefore, this revised recommendation was accepted.

3.2 *PRACTICALITIES*

3.2.1 Voxel position

ADAPT: Use automated voxel positioning tools where available. If manually positioning the voxel, use a screenshot and clear instructions regarding positioning relative to anatomical landmarks and degree of rotation.

Evidence GRADE A; *Round 1 Expert Panel Agreement 85.7%*

There were five studies (Level 1 to Level 4) (Kreis, 2004; Bai *et al.*, 2017; Chen *et al.*, 2017; Park *et al.*, 2018; Öz *et al.*, 2020) that provided recommendations on positioning of the voxel. The studies recommended use of an automated voxel positioning tool. Although the expert panel agreed with this recommendation, 28.6% of experts highlighted that fully automated voxel positioning is not currently available as standard. Round 1 agreement was 85.7%. Therefore, this recommendation was accepted.

3.2.2 Shimming

ADAPT: A beginner should use a readily available automated field-map-based shim and minimize the use of manual adjustments.

Evidence GRADE A; *Round 1 Expert Panel Agreement 71.5%; Round 2 Expert Panel Agreement 80%*

There were eight studies (Level 1 to Level 3) (Gruetter and Tkáč, 2000; Saleh *et al.*, 2016a; Juchem and de Graaf, 2017; Deelchand *et al.*, 2018; Sanaei Nezhad *et al.*, 2018; Wilson *et al.*, 2019; Öz *et al.*, 2020; Juchem *et al.*) that provided recommendation on shimming to maximize the homogeneity of the static magnetic field (B_0). The studies demonstrated that projection-based shim optimisation or second-order pencil beam methods could provide narrower linewidths than the default 3D field map-based methods. These specific techniques may not be readily available on all systems, therefore the expert panel recommends that any readily-available automated field map-based methods are used with minimal manual adjustments where possible (9/21, 43%). Round 1 agreement was 71.5 %, subsequent adjustments were therefore made to highlight that linewidths are calculated differently by different vendors (see considerations in extended document). Despite evidence suggesting projection-based shim optimisation might achieve narrower linewidths, the recommendation states the beginner should use readily available field-map based shim methods. Round 2 expert agreement was 80%. Therefore, the revised recommendation was accepted.

3.2.3 Order of scans and field drift

ADOPT: Where possible, MRS should be conducted prior to gradient-heavy acquisitions or in small blocks of 2-5 minutes with frequency adjustments between adjustment blocks. Consider using real-time frequency correction if available.

Evidence GRADE A; Round 1 Expert Panel Agreement 85.7%

There were seven studies (Level 1 to Level 3) (Harris *et al.*, 2014; Edden *et al.*, 2016; Mikkelsen *et al.*, 2017; Andronesi *et al.*, 2020; Cudalbu, 2020; Öz *et al.*, 2020; Choi *et al.*, 2021) that provided recommendation on the order of scans and the effect it has on field drift. The studies highlighted the negative impact gradient-heavy scanning (e.g. diffusion tensor imaging) has on frequency drift during subsequent MRS scans. Previous recommendations were to avoid scanning after gradient-heavy acquisitions, however owing to this not being practical due to scan scheduling problems, a recent consensus document made a new proposal. The recommendation was to acquire MRS data in small blocks with frequency adjustment after each block whilst monitoring the residual water signal on the inline display during the scan acquisition in order to detect drift. Round 1 agreement was 85.7%. Therefore, this recommendation was accepted.

3.3 *CONFOUNDERS*

3.3.1 Scanner site and vendor

ADOPT: In multi-site studies, standardised protocols should be used, and the degree of systematic differences between site/scanner should be reported.

Evidence GRADE B; *Round 1 Expert Panel Agreement 95.2%*

There were three multi-site studies (Level 2) (Mikkelsen *et al.*, 2017; Mikkelsen *et al.*, 2019; Saleh *et al.*, 2019) that provided recommendations on managing scanner site and different vendors as a confounder of GABA. The studies reported a coefficient of variation across all data sets of around 12% for GABA+/Cr and 17% for water-scaled GABA+. Macromolecule-suppressed MEGA-PRESS had larger CVs of 28%-29% for both GABA/Cr and water-scaled GABA (Mikkelsen *et al.*, 2017; Mikkelsen *et al.*, 2019). Round 1 expert panel agreement was 95.2%. Therefore, this recommendation was accepted.

3.3.2 Macromolecules

ADAPT: A beginner should use conventional MEGA-PRESS reporting GABA+. Macromolecule contamination should be acknowledged as a limitation, and consideration paid to whether macromolecules could be responsible for between-group differences.

Evidence GRADE A; *Round 1 Expert Panel Agreement 90.5%; Round 2 Expert Panel Agreement 100%*

There were twelve studies (ranging from Level 1 to Level 3) (Mullins *et al.*, 2014; Cudalbu, 2020; Choi *et al.*, 2021) (Henry *et al.*, 2001; Harris *et al.*, 2015a; Edden *et al.*, 2016; Mikkelsen *et al.*, 2016b; Shungu *et al.*, 2016; Gu *et al.*, 2018; Oeltzschner *et al.*, 2018a; Duncan *et al.*, 2019) that provided recommendation on macromolecule contamination as a confounder of GABA. Contrary to the original consensus document for MEGA-PRESS (Level 1) (Mullins *et al.*, 2014), the latest consensus documents recommend the use of macromolecule-suppressed editing where possible (Level 1)(Cudalbu, 2020). However, both consensus documents acknowledge this approach has a number of limitations including its susceptibility to frequency drift. The expert panel agreed that a macromolecule-suppressed study is more difficult to control and run as a beginner and therefore endorsed the recommendation that a beginner should acquire GABA+ data. Both consensus documents

agree that in cases where GABA+ is acquired, results must be reported as GABA+ macromolecules, with macromolecule contaminations explicitly acknowledged as a limitation. Round 1 expert panel agreement was 90.5%. This recommendation was revised following publication of a new consensus document and therefore sent out for Round 2 grading despite achieving over 80% expert panel agreement on Round 1. Round 2 agreement was 100%. Therefore, the revised recommendation was accepted.

3.3.3 Region

ADAPT: Select brain regions relevant to the research question, however, acknowledge that brain regions have differing reliability with respect to data acquisition.

Evidence GRADE C; *Round 1 Expert Panel Agreement 81%*

There were fourteen studies (Level 1^T to Level 4^T) (Gruetter and Tkáč, 2000; Harada *et al.*, 2011; Waddell *et al.*, 2011; Puts and Edden, 2012; Gao *et al.*, 2013; van der Veen and Shen, 2013; Harris *et al.*, 2015c; Long *et al.*, 2015; Greenhouse *et al.*, 2016; Brix *et al.*, 2017; Chen *et al.*, 2017; Porges *et al.*, 2017; Puts *et al.*, 2018; Dhamala *et al.*, 2019) that provided recommendation on brain region as a confounder of GABA levels. The studies demonstrated that GABA levels appear to be region-specific rather than reflective of a global GABAergic tone as once proposed (Puts and Edden, 2012). Therefore, it is important to consider the suitability of the brain region for ^{1}H-MRS acquisition and recognize that different brain regions have different reliability with respect to signal-to-noise ratio and the likelihood of artefacts. Round 1 agreement was 81%. Therefore, this recommendation was accepted.

3.3.4 Tissue composition

ADAPT: Water-scaled quantification methods should consider the impact of partial volume effects on GABA estimation.

-Segmented structural images should be used along with a tissue-correction method to account for grey matter, white matter and cerebrospinal fluid composition of the voxel. Grey-matter only correction should be avoided.

Evidence GRADE A; *Round 1 Expert Panel Agreement 85.7%; Round 2 Expert Panel Agreement 90%*

There were nine studies (Level 1 to Level 3) (Bhattacharyya *et al.*, 2011; Geramita *et al.*, 2011; Mullins *et al.*, 2014; Harris *et al.*, 2015b; Mikkelsen *et al.*, 2016a; Porges *et al.*, 2017;

Gasparovic *et al.*, 2018; Puts *et al.*, 2018; Choi *et al.*, 2021) that provided recommendation on tissue composition as a confounder of GABA estimation. The studies agreed that GABA levels were higher in grey matter than white matter, and therefore needed to be accounted for when quantifying GABA. The additional considerations from the expert panel were that tissue composition should be considered as a covariate in order to clarify whether between-group differences were being driven by GABA levels rather than tissue composition (n= 3/21, 14.3%). Round 1 agreement was 85.7%. However, the original recommendation included a significant number of caveats. Therefore, to improve clarity the recommendation was revised, where the caveats were removed from the recommendation and placed in the considerations section of the full document. Round 2 agreement was 90%. Therefore the revised recommendation was accepted.

3.3.5 Age

ADOPT: Age is likely to affect GABA levels, therefore age should be accounted for in study design or statistical analysis.

Evidence GRADE A; *Round 1 Expert Panel Agreement 95.2%*

There were seven studies (Level 1^T to Level 3^T) (Aufhaus *et al.*, 2013; Gao *et al.*, 2013; Porges *et al.*, 2017; Maes *et al.*, 2018; Marenco *et al.*, 2018; Simmonite *et al.*, 2019; Porges *et al.*, 2020) that provided recommendations on age as a confounder of GABA levels. The studies all suggest that GABA+ decreases with age in adulthood. The recent meta-analysis (Porges *et al.*, 2020) describes an early period of increase in frontal GABA levels, which stabilized throughout adulthood, and then decreased with aging. Round 1 agreement was 95.2%. Therefore, this recommendation was accepted.

3.3.6 Sex

ADOPT: Sex is likely to impact on GABA levels, therefore sex should be accounted for in study design or statistical analysis.

Evidence GRADE C; *Round 1 Expert Panel Agreement 85.7*

There were four studies (Level 3^T to Level 4^T) (O'Gorman *et al.*, 2011; Aufhaus *et al.*, 2013; Gao *et al.*, 2013; Saleh *et al.*, 2017) that provided recommendation on sex as a confounder for GABA levels. The variation in outcome across the studies suggest that differences in GABA

levels between males and females may be region-specific. Round 1 agreement was 85.7%. Therefore, this recommendation was accepted.

3.3.7 Medications

ADAPT: Medications may impact GABA levels, as minimum best practice all medications should be recorded.

-Consider excluding participants taking medications likely to affect the GABAergic system.

Evidence GRADE B; *Round 1 Expert Panel Agreement 95.2%*

There were eight studies (Level 1^{T} to Level 4^{T}) (Rothman *et al.*, 1993; Petroff *et al.*, 1996a; Petroff *et al.*, 1996b; Bhagwagar *et al.*, 2004; Licata *et al.*, 2009; Cai *et al.*, 2012; Puts and Edden, 2012; Myers *et al.*, 2014) that provided recommendations on medications that may confound GABA. The studies reported that medications that alter GABA concentration directly and those that affect GABA receptor agonists and antagonists may both influence brain GABA levels. Round 1 agreement was 95.2%. Therefore, this recommendation was accepted.

3.3.8 Other potential confounders: Nicotine, Caffeine, Phase of menstrual cycle

ADAPT: Potential confounders such as caffeine and nicotine intake and phase of menstrual cycle may affect GABA levels, as minimum best practice potential confounders should be recorded.

Evidence GRADE D; *Round 1 Expert Panel Agreement Caffeine 71.4%, Nicotine 76.2%, Phase of Menstrual Cycle 71.4%, Round 2 Expert Panel Agreement 85%*

There were six studies (Level 3^{T} to 4^{T}) (Epperson *et al.*, 2002; Epperson *et al.*, 2005; Harada *et al.*, 2011; De Bondt *et al.*, 2015; Schulte *et al.*, 2017; Oeltzschner *et al.*, 2018b) that provided recommendation on other potential confounders of GABA levels which included caffeine, nicotine, and phase of menstrual cycle. The studies were inconclusive to the degree of effect these potential confounders may have on GABA levels. Round 1 agreement was caffeine 71.4%, nicotine 76.2%, phase of menstrual cycle 71.4%. Expert panel feedback was that there was not sufficient high-quality evidence confirming these factors as confounders of GABA levels, and therefore the expert panel did not feel it was essential to control for all in

study design. The recommendation was adjusted to reflect this. Round 2 agreement was 85%. Therefore, the revised recommendation was accepted.

3.4 *DATA ACQUISITION*

3.4.1 Quality assessment during the scan

ADOPT: It is recommended to monitor the quality of the acquisition using the inline data display at time of scanning.

-Scans should be cancelled, and voxel position adjusted if evidence of weak water suppression, strong lipid contamination or other artefacts.

Evidence GRADE A; *Round 1 Expert Panel Agreement 90.5%*

There were two studies (Level 1) (Öz *et al.*, 2020; Choi *et al.*, 2021) that provided recommendations on quality assessment during the scan. Both recommended that the MR operator should evaluate and monitor water suppression efficiency, spectral linewidth and signal-to-noise ratio at the beginning and during the MRS acquisition. Round 1 expert panel agreement was 90.5%. Therefore, this recommendation was accepted.

3.4.2 Data export

DEVELOP: Export data in a format that saves individual transients to allow adequate post-processing.

Evidence GRADE I; *Round 1 Expert Panel Agreement 90.5%*

There were no studies discussing file format export for MEGA-PRESS acquisitions. The recommendation was therefore developed based on a consensus document that made recommendations on the file format to export for ^{1}H-MRS studies which also can be applied to MEGA-PRESS acquisitions (Near *et al.*, 2020). Round 1 expert panel agreement was 90.5%. Therefore, the developed recommendation was accepted.

3.5 *QUALITY AND REPORTING*

3.5.1 Quality Metrics

ADOPT: Report spectral quality in terms of the signal-to-noise ratio, linewidth, water suppression efficiency, fit quality, and the presence of unwanted spectral features

Evidence GRADE A; *Round 1 Expert Panel agreement 90.5%*

There were seven studies (Level 1 to Level 3) (Bolliger *et al.*, 2013; Mullins *et al.*, 2014; Kreis, 2016; Chen *et al.*, 2017; Deelchand *et al.*, 2018; Wilson *et al.*, 2019; Öz *et al.*, 2020) that provided recommendations on which variables should be used to assess data quality. The studies agree that spectral quality should be assessed using a number of aspects including signal-to-noise ratio, linewidth, water suppression efficiency, modelling quality, and presence of unwanted spectral features. Round 1 agreement was 90.5%. Therefore, this recommendation was accepted.

3.5.2 Reporting

ADOPT: When reporting results use one of these two checklists (MRS in MRS, Lin *et al.* 2020 or MRS-Q, Peek *et al.* 2020) using the appropriate terminology (Kreis *et al.* 2020). Include detailed reporting of hardware, MEGA-PRESS-specific acquisition parameters, quantification details, quality metrics, and analysis methods.

Evidence GRADE A; *Round 1 Expert Panel Agreement 95.2%*

There were three studies (Level 1 to Level 3) (Deelchand *et al.*, 2019; Peek *et al.*, 2020; Lin *et al.*, 2021) that provided recommendations on reporting in MEGA-PRESS GABA studies. Two studies provided checklists that could be utilized to improve reporting in these studies. Round 1 agreement was 95.2%. Therefore, this recommendation was accepted.

3.6 *POST-PROCESSING*

3.6.1 Frequency-and-Phase Correction (Post-processing)

ADOPT: Frequency-and-phase alignment of individual transients should be performed during post-processing.

Evidence GRADE A; *Round 1 Expert Panel Agreement 95.2%*

There were ten studies (Level 1 to Level 3) (Edden and Barker, 2007; Edden *et al.*, 2014; Harris *et al.*, 2014; Near *et al.*, 2015; Cleve *et al.*, 2017; van der Veen *et al.*, 2017; Wiegers *et al.*, 2017; Tapper *et al.*, 2019; Near *et al.*, 2020; Choi *et al.*, 2021) that provided recommendation on frequency-and-phase correction. The studies found that using frequency-and-phase correction was able to significantly improve editing efficiency. Round 1 agreement was 95.2%. Therefore, this recommendation was accepted.

4 DISCUSSION

The Comprehensive Guide to MEGA-PRESS for GABA Measurement presented in this manuscript, was developed following a translational framework to produce robust, user-friendly guidelines for those new to the field of MEGA-PRESS. The key strengths of this approach were conducting a systematically delivered scoping review to inform the evidence synthesis and the involvement of multiple stakeholders with diverse experience and expertise. Further, we performed blinded GRADEing of the quality of evidence for each recommendation, and then finally incorporated expert peer review through the modified-Delphi process. The result was a guideline with 23 recommendations; 73.9% of these recommendations had a high GRADE of evidence and high expert panel agreement, 4.4% had a high GRADE of evidence but low expert panel agreement, 17.4% had a low GRADE of evidence and high expert panel agreement, and 4.4% had a low GRADE of evidence and low expert panel agreement. Reasons for the differences between GRADE of evidence and the degree of expert panel agreement plus the decisions to retain the recommendations that did not gain expert panel approval are discussed below.

Both 3.1 ‘Parameters’ and 3.2 ‘Practicalities’ sections contain recommendations with a high GRADE of evidence and a high percentage of expert panel agreement, with the exception of just two recommendations (Table 3). This high level of evidence supported by the expert panel encourages confidence in our recommendations, as expert evaluation reflects practice in experienced MRS groups worldwide. The two recommendations in the guideline that were not sufficiently endorsed by the experts were both in the 3.2 ‘Practicalities’ section; *Order of slice-selective gradients* (Evidence GRADE I, Experts: 75% agreement, 25% neutral) and *Water reference scans for eddy current correction and water-scaled quantification* (Evidence GRADE A, Experts 55% agreement, 20% neutral, 25% disagreement). This lower level of expert panel agreement suggests that these recommendations are less reflective of current

standard practice. The MRS sub-committee saw an opportunity to encourage the translation of evidence to practice and decided to keep the recommendations in the guideline without expert panel endorsement but with further discussion: Firstly, while experts were concerned that *Order of slice selective gradients* is not applicable to all systems, the MRS sub-committee found it a valuable troubleshooting option worth adopting as regular practice on systems where it is available. Secondly, *Water reference scans for eddy-current correction and quantification* failed to gain expert panel endorsement following the addition of a separate short-TE scan for quantification, although this practice is recommended in the latest consensus document (Near *et al.*, 2020). The MRS sub-committee maintained this recommendation to further facilitate the implementation of this new recommendation into practice.

The 3.3 'Confounders' section contained recommendations with generally lower levels of evidence but achieved high expert panel agreement. Firstly, five of the eight recommendations in this domain were assessed using a traditional hierarchy of evidence, where Level 1 evidence represents a systematic literature review of randomised controlled trials, a study design that has not been frequently adopted in the field of MRS to date. Secondly, many of the recommendations in this section reflect principles and practices historically adopted from expert opinion and practical experience rather than from clear and systematic evidence collection. This explains the high level of expert agreement, but also shows further high quality research is required to establish the degree of confounding these factors present.

The 3.4 'Data Acquisition', 3.5 'Quality and Reporting' and 3.6 'Post-processing' sections generally had high levels of evidence and high expert panel agreement. The high levels of evidence adapted from consensus documents and high expert panel agreement reflect that areas included in these domains are topical, relevant and are considered important in the acquisition of GABA using MEGA-PRESS. The one recommendation that had no evidence (Level I), and therefore required active development instead of adoption or adaption was *File export*. Previous consensus documents may not have included this explicit recommendation as it might be considered 'assumed knowledge', but the MRS-subcommittee and stakeholders valued its inclusion given the intended audience. This is especially relevant since failure to save the correct file type at time of scanning prevents appropriate post-processing and compromises data quality considerably; an easily-avoidable mistake that has been commonly observed by the MRS sub-committee.

The results of the evidence synthesis did not always provide recommendations suitable for a those new to the field. In two instances (*Shimming* and *Macromolecules*), the MRS sub-committee and expert panel agreed that a beginner would likely achieve a better result using a different approach to that recommended in the most recent consensus documents (Cudalbu, 2020; Juchem *et al.*, 2021). An example was *shimming*: a recent consensus document (Juchem *et al.*, 2021) recommends use of a tool that is not readily available on all systems, has limited technical support, requires approved distribution from its developers, and is more technically challenging to operate than system based shim methods (FASTMAP). Whilst proof-of-concept studies (Grewal *et al.*, 2016; Saleh *et al.*, 2016b; Deelchand *et al.*, 2018) have demonstrated that narrower linewidths can be achieved using this approach compared to readily available automated field map-based methods, it requires specific expertise to be set up and used. Therefore, the MRS sub-committee and expert panel recommend that a beginner use a readily-available automated field-map-based shim method. Doing so is well-established in the field and should produce sufficient B_0 homogeneity to generate high-quality spectra (Mullins *et al.*, 2014). In summary, while this recommendation is not consistent with the recent consensus document, it is directly aligned with our aim of enabling a beginner to produce high-quality MEGA-PRESS spectra for the reliable quantification of GABA.

The second recommendation adapted for the beginner in our guideline was *Macromolecules* and how they should be handled. Feedback from 90.5% (20/21) experts was that beginners should choose sequence parameters to acquire GABA plus macromolecule (GABA+) data, despite the latest consensus document recommending the acquisition of macromolecule-suppressed data. This is supported by a previous consensus document (Mullins *et al.*, 2014) and methodological publications (Mullins *et al.*, 2014; Harris *et al.*, 2015a; Mikkelsen *et al.*, 2017) that all agree that symmetric macromolecule suppression is an order of magnitude more susceptible to frequency drift and that other methods of macromolecule signal removal all have substantial technical and practical limitations (Mullins *et al.*, 2014; Harris *et al.*, 2015a; Mikkelsen *et al.*, 2017). The MRS sub-committee reviewed the evidence once more, and decided that despite our recommendation differing from the latest consensus document (Cudalbu, 2020), acquiring GABA+ currently offers the most robust, reliable and widely used method to measure GABA levels for a beginner user. Further the likelihood of failure acquiring GABA+ is substantially lower than if they were to use the delicate macromolecule suppression. Therefore, the recommendation to acquire GABA+ data and acknowledge the

macromolecule contamination as a limitation (or discuss as a potential source of observed effects) was deemed most suitable for inclusion in the Comprehensive Guide.

The scope of the Comprehensive Guide was to largely focus on study design and data acquisition. We considered it to be beyond the scope to discuss further details of post-processing (beyond frequency-and-phase correction and the file format export it requires), modelling, or quantification of MEGA-PRESS data. We therefore direct the reader to comprehensive efforts on best practices in MEGA-PRESS (Mullins *et al.*, 2014) and two recent consensus papers on pre-processing, modelling and quantification (Near *et al.*, 2020) and spectral editing in general (Choi *et al.*, 2021). Further, the beginner is advised to liaise with representatives from their vendor and sequence developers with regard to system-specific functions that may or may not be available, as highlighted throughout this Comprehensive Guide. Finally, the MRSHub (https://www.mrshub.org) provides an online resource hosting processing and analysis software, normative example data, and a discussion forum frequented by beginners and experts alike where questions about study design and protocol can be posed.

In conclusion, this Comprehensive Guide combines a robust evidence synthesis on the measurement of GABA levels with edited MRS and expert panel review. The result is an evidence-based, peer-reviewed guideline for those new to using MEGA-PRESS including higher degree research students, clinician-researchers, MRI technicians or anyone new to the field of MEGA-PRESS. The guideline helps to ensure sufficient quality of acquisition and reporting is achieved. The high level of agreement between evidence and expert assessment instils confidence in the validity, longevity, and applicability of these recommendations. The full accompanying documentation is freely available online here: https://osf.io/5v9cp/?view_only=b6c70761abdd4d6e9e38d2a7b9944f9f

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SUPPLEMENT 1:

Review Methods:

Database:

Search Ovid MEDLINE, Embase and PubMed

Search Strategy

((mega press OR gaba OR gamma-amino but* OR spectral editing)) AND (MRS OR magnetic resonance spectro* OR "magnetic resonance methods") AND (white paper OR consensus OR acquisition parameters OR gradients OR water suppression OR editing pulses OR voxel OR methods OR cramer lower bounds OR CRLB OR full width half maximum OR FWHM OR shim OR frequency drift OR phase drift OR quality OR reporting)

Inclusion/ Exclusion

P: Include: humans, phantom or computer simulations

Exclude: animals

I: Magnetic resonance spectroscopy, single voxel, spectral-edited, brain

Exclude: 2D techniques, MRSI

O: Contribute to the six identified domains

Exclude: studies reporting outcomes of clinical cohorts, except those related to medications

S: White papers, consensus, methods papers, systematic or quasi-systematic reviews, peer reviewed journal

Exclude conference proceedings, clinical commentaries, editorials, narrative reviews

Study selection

Studies were screened using a two-stage approach. Firstly, two reviewers (AP, GO) independently screened the titles and abstracts to identify papers potentially suitable for inclusion. Secondly, the same two reviewers independently reviewed the full text to determine the final eligible papers for each of the six domains. Disagreements were discussed and resolved through consultation of a third reviewer (NP). Rationale for exclusion was documented and duplicates were removed. The MRS sub-committee reviewed the results of the search and identified any missing papers.

Data Extraction

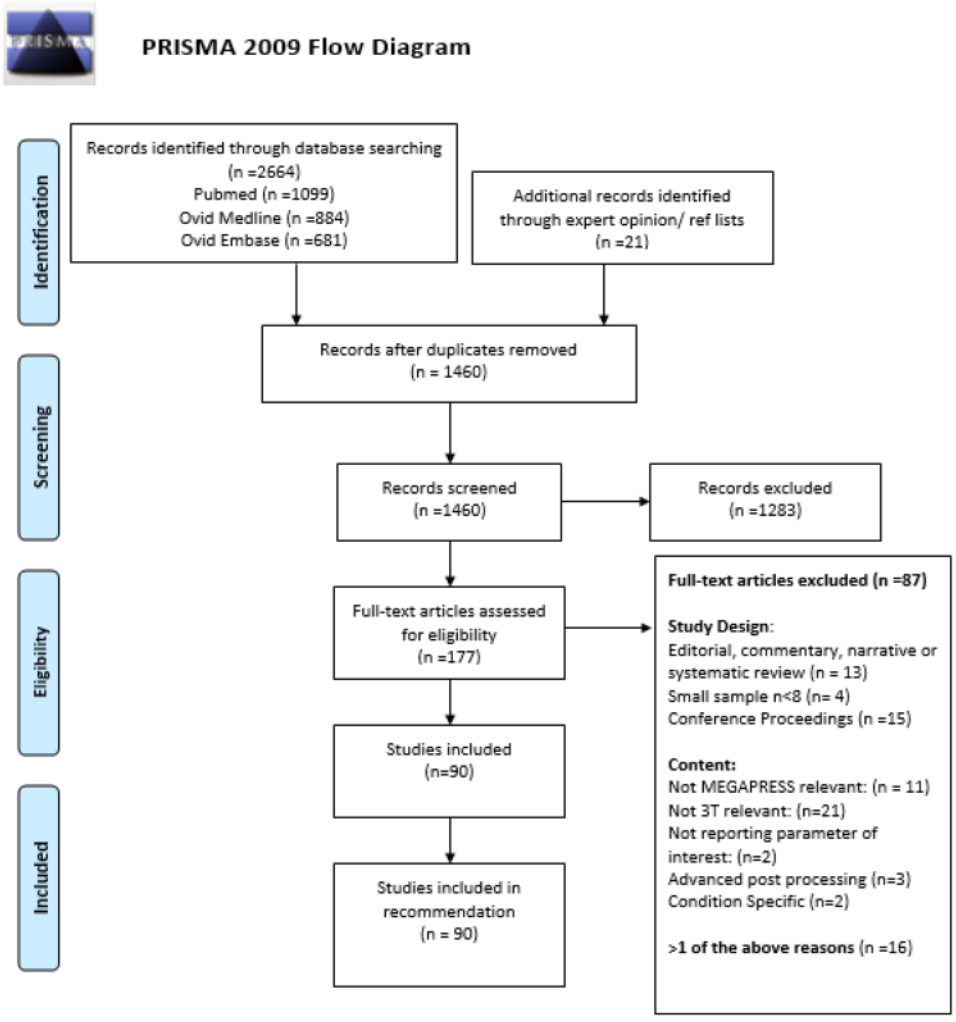
Data was independently extracted by two reviewers with a special interest and experience in GABA MRS into a predesigned data extraction sheet. Inconsistencies or disagreements were discussed for resolution. Data was then synthesised using qualitative analysis.

Parameters not addressed in publications specifically concerned with spectral editing (grey literature search)

In cases where parameters were not addressed in publications specifically concerned with spectral editing, literature concerning conventional (un-edited) single voxel spectroscopy, e.g. guidelines, white papers, consensus documents and other publications were drawn upon.

SUPPLEMENT 2

PRISMA Flow Diagram



SUPPLEMENT 3

Table 1: Summary of papers used to inform the guideline

		Papers with Recommendations	Study Design	Level of Evidence
Parameters	**Signal to noise**			
	- Transients	Mullins *et al.*, 2014	Cons	1
		Mikkelsen *et al.*, 2017	MSS	2
		Mikkelsen *et al.*, 2019	MSS	2
		Bhattacharyya *et al.*, 2007	M	3
		Harris *et al.*, 2014	M	3
		Brix *et al.*, 2017	M	3
		Mikkelsen *et al.*, 2018	M	3
		Sanaei Nezhad *et al.*, 2018	M	3
	Reporting of	Lin *et al.*, 2020	Cons	1
		Peek *et al.*, 2020	SR	2
	- Voxel Volume	Mullins *et al.*, 2014	Cons	1
		de Graaf, 2019	Sem	1
		Mikkelsen *et al.*, 2017	MSS	2
		Mikkelsen *et al.*, 2019	MSS	2
		Bai *et al.*, 2015	M	3
		Bergmann *et al.*, 2016	M	3
		Chen *et al.*, 2017	M	3
	TR	Wilson *et al.*, 2019	Cons	1
		Mikkelsen *et al.*, 2017	MSS	2
		Mikkelsen *et al.*, 2019	MSS	2
		Puts *et al.*, 2013;	M	3
		Deelchand *et al.*, 2019	M	3
	TE	Cudalbu *et al.*, 2020	Cons	1
		Mullins *et al.*, 2014;	Cons	1
		Wilson *et al.*, 2019	Cons	1
		Mescher *et al.*, 1998	SemP	1
		Puts *et al.*, 2012	SR	2
		Mikkelsen *et al.*, 2017	MSS	2
		Mikkelsen *et al.*, 2019	MSS	3
		Harris *et al.*, 2015a;	M	3
		Edden *et al.*, 2016;	M	3
		Deelchand *et al.*, 2019	M	1
	Water Reference	Mullins *et al.*, 2014;	Cons	1
		Wilson *et al.*, 2019;	Cons	1
		Öz *et al.*, 2020	Cons	1
		Near et al., *2021*	Cons	1
		de Graaf, 2019	Sem T	1
		Hall *et al.*, 2014;	M	3

		Oeltzschner *et al.*, 2016	M	3
	Slice-selection frequency of water reference	Mikkelsen *et al.*, 2017	MSS	2
		Mikkelsen *et al.*, 2019	MSS	2
		Deelchand *et al.*, 2019	M	3
	Slice-selective gradients	Ernst and Chang, 1996	M	3
	Editing pulse specifications	Mullins *et al.*, 2014	Cons	1
		Mikkelsen *et al.*, 2017	MSS	2
		Mikkelsen *et al.*, 2019	MSS	2
		Saleh *et al., 2019*	MSS	2
		Keltner *et al.*, 1996	M	3
		Mescher *et al.*, 1998	M	3
		Henry *et al.*, 2001	M	3
		Edden *et al.*, 2016	M	3
		Deelchand *et al., 2019*	M	3
Practicalities	Voxel Position	Öz *et al.*, 2020	Cons	1
		Bai *et al.*, 2017	M	3
		Chen *et al.*, 2017	M	3
		Park *et al.,* 2018	M	3
		Kreis, 2004	Nar	4
	Shimming	Juchem *et al. 2020*	Cons	1
		Wilson *et al.*, 2019	Cons	1
		Öz *et al.*, 2020	Cons	1
		Juchem et al 2017	SR	2
		Grewal et al 2016	M	3
		Saleh *et al.*, 2016	M	3
		Deelchand *et al.*, 2018;	M	3
		Sanaei Nezhad *et al.*, 2018	M	3
	Order of scans and field drift	Öz *et al.*, 2020	Cons	1
		Andronesi *et al.*, 2020	Cons	1
		Choi *et al.,* 2021	Cons	1
		Cudalbu *et al.,* 2020	Cons	1
		Mikkelsen *et al.*, 2017;	MSS	2
		Harris *et al.*, 2014a	M	3
		Edden *et al.*, 2016	M	3
Confounders	Scanner site and vendor	Mikkelsen *et al.*, 2017;	MSS	2
		Mikkelsen *et al.*, 2019;	MSS	2
		Saleh *et al.*, 2019	MSS	2
	Macromolecules	Mullins *et al.,* 2014	Cons	1
		Cudalbu *et al.,* 2020	Cons	1
		Choi *et al.,* 2021	Cons	1
		Henry *et al.*, 2001;	M	3
		Harris *et al.*, 2015a;	M	3
		Edden *et al.*, 2016;	M	3

	Mikkelsen *et al.*, 2016b;	M	3
	Shungu *et al.*, 2016;	M	3
	Gu *et al.*, 2018;	M	3
	Oeltzschner *et al.*, 2018a;	M	3
	Duncan *et al.*, 2019	M	3
Region	Puts and Edden, 2012	SR	1^{T}
	Harada *et al.*, 2011;	M	4^{T}
	Waddell *et al.*, 2011;	M	4^{T}
	Gao *et al.*, 2013;	M	4^{T}
	van der Veen 2013;	M	4^{T}
	Harris *et al.*, 2015c;	M	4^{T}
	Long *et al.*, 2015;	M	4^{T}
	Greenhouse *et al.*, 2016;	M	4^{T}
	Grewal *et al.*, 2016a;	M	4^{T}
	Brix *et al.*, 2017;	M	4^{T}
	Chen *et al.*, 2017b;	M	4^{T}
	Porges *et al.*, 2017a;	M	4^{T}
	Puts *et al.*, 2018;	M	4^{T}
	Dhamala *et al.*, 2019	M	4^{T}
Tissue Composition	Mullins *et al.*, 2014	Cons	1
	Choi *et al.*, 2006;	M	3
	Bhattacharyya *et al.*, 2011;	M	3
	Geramita *et al., 2011;*	M	3
	Harris *et al.*, 2015b;	M	3
	Mikkelsen *et al.*, 2016a;	M	3
	Porges *et al.*, 2017b;	M	3
	Gasparovic *et al.,* 2018		
Age	Porges *et al.*, 2020;	SR	1^{T}
	Aufhaus *et al.*, 2013a;	M	3^{T}
	Gao *et al.*, 2013;	M	3^{T}
	Porges *et al.*, 2017a;	M	3^{T}
	Maes *et al.*, 2018;	M	3^{T}
	Marenco *et al.*, 2018;	M	3^{T}
	Simmonite *et al.*, 2019	M	3^{T}
Sex	Aufhaus *et al.*, 2013a;	M	3^{T}
	Gao *et al.*, 2013;	M	3^{T}
	Saleh *et al.*, 2017;	M	3^{T}
	O'Gorman *et al.*, 2011a	M	4^{T}
Medication	Puts and Edden, 2012;	SR	1^{T}
	Bhagwagar *et al.*, 2004;	RCT	2^{T}
	Rothman *et al.*, 1993;	M	4^{T}
	Petroff *et al.*, 1996a;	M	3^{T}

		Petroff *et al.*, 1996b;	M	3 [T]
		Licata *et al.*, 2009;	M	4 [T]
		Cai *et al.*, 2012;	M	4 [T]
		Myers *et al.*, 2014	M	4 [T]
	Potential Confounders			
	- Caffeine	Oeltzschner *et al.*, 2018b;	M	4 [T]
	- Nicotine	Epperson *et al.*, 2005;	M	3 [T]
		Schulte *et al.*, 2017;	M	3 [T]
	- Menstrual Cycle	Epperson *et al.*, 2005;	M	3 [T]
		De Bondt *et al.*, 2015a	M	3 [T]
		Epperson *et al.*, 2002;	M	4 [T]
		Harada *et al.*, 2011;	M	4 [T]
Data Acquisition	Quality assessment during the scan	Öz *et al.*, 2020;	Cons	1
		Choi *et al.*, 2021	Cons	1
	Data Export	-		
Quality and Reporting	Quality Metrics	Mullins *et al.*, 2014;	Cons	1
		Wilson *et al.*, 2019;	Cons	1
		Öz *et al.*, 2020;	Cons	1
		Bolliger *et al.*, 2013;	M	3
		Kreis, 2016;	M	3
		Chen *et al.*, 2017b;	M	3
		Deelchand *et al.*, 2018	M	3
	Reporting	Lin *et al.*, 2020;	Cons	1
		Peek *et al.*, 2020;	SR	2
		Deelchand *et al.*, 2019	M	3
Post-processing	Frequency and Phase Correction	Near *et al.*, 2020;	Cons	1
		Choi *et al.*, 2021;	Cons	1
		Edden and Barker, 2007;	M	3
		Edden *et al.*, 2014b;	M	3
		Harris *et al.*, 2014c;	M	3
		Cleve *et al.*, 2015;	M	3
		Near *et al.*, 2015;	M	3
		van der Veen *et al.*, 2017;	M	3
		Wiegers *et al.*, 2017;	M	3
		Tapper *et al.*, 2019a		

Cons= consensus document; MSS= multi-site study SemT/P= seminal textbook/paper; SR= systematic review; M: methodological publication, Nar: Narrative. Note levels of evidence documented with 'Level X[T]' are assessed using a traditional hierarchy of evidence (NHMRC, 2009) rather than the modified-hierarchy of evidence (Table 1).

A Comprehensive Guide to MEGA-PRESS for GABA Measurement

Last updated 12th November 2021

Table of Contents

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Preface

These guidelines aim to enable those new to the field of MEGA-PRESS to acquire high-quality data for the reliable quantification of GABA

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter of the central nervous system (CNS) and plays an important role in regulating healthy brain function[1]. Altered GABAergic function has been identified in a number of pathological conditions that affect the central nervous system such as pain[2], psychological[3,4] and neurodevelopmental disorders[5].

Magnetic Resonance Spectroscopy (MRS) is currently the only non-invasive brain imaging technique which enables the *in-vivo* measurement of GABA. The measurement of GABA using conventional MRS is challenging given its relatively low concentration in the human brain, the spectral overlap by more abundant neurometabolites and its complicated peak pattern[6]. Therefore, an edited sequence such as MEGA-PRESS (MEscher–GArwood Point RESolved Spectroscopy[7]) is required.

MEGA-PRESS is the most widely used technique for measuring GABA levels at 3T. The sequence uses J-difference editing which consists of two sub-experiments, usually acquired in an interleaved fashion. One sub-experiment applies editing pulses at a frequency of 1.9 ppm to selectively refocus the coupling evolution of the GABA signal at 3 ppm ('Edit-ON'), while the other allows the free evolution of the spin system throughout the echo time ('Edit-OFF'). Subtracting the Edit-OFF from the Edit-ON spectrum reveals a difference-edited GABA signal while removing the stronger overlapping signals from creatine-containing compounds[7]. The composite edited signal at 3ppm contains up to 50% co-edited macromolecules and is therefore commonly referred to as GABA+ (GABA+ macromolecules). Macromolecule signals can be suppressed by adding a second editing pulse at 1.5 ppm, however this experiment is significantly less stable[8].

MEGA-PRESS significantly improves the accurate detection of GABA, however, it is a technically challenging process that relies on the observation of a number of caveats and avoiding numerous pitfalls. At present the large heterogeneity of sequences and parameters used to study GABA demonstrates the lack of standardisation within the field, resulting in variable reliability of the data[2]. The study of GABA has been met with growing interest from those with clinical backgrounds but without a background in magnetic resonance physics. This has motivated an easily translatable guideline to assist the avoidance of pitfalls and ensure the accurate detection of GABA in clinical and research populations.

Purpose

These guidelines are intended to assist those new to the field of MEGA-PRESS to plan and implement a study to reliably measure brain GABA levels in clinical and research populations.

Specifically these guidelines will assist those new to the field to:

- → Select appropriate **'acquisition parameters'** depending on the brain region of interest and specific study population
- → Be aware of the impact the choice of **'acquisition parameters'** is likely to have on the spectral output
- → Be aware of specific '**practicalities'** to be considered when running a MEGA-PRESS experiment.
- → Be aware of identified and potential '**confounders'** of GABA and methods to handle these confounders.
- → Be aware of the implications associated with the methods for handling **'confounders**'.
- → Have knowledge of key aspects during **'data acquisition'**
- → Conduct appropriate **'quality assessment and reporting'** of the experiment
- → Understand the importance of frequency-and-phase correction for **'post-processing'**

Scope

These guidelines largely focus on study design and data acquisition to ensure steps are followed to collect high-quality data. It was not within the scope of the guideline to discuss further details of post-processing (beyond frequency-and-phase correction and the requirements for file export at the time of acquisition), modelling, or quantification of MEGA-PRESS data. Resources to assist the next stages of post-processing and quantification have been outlined in the accompanying manuscript[9]. Further it is advised that the beginner liaise with MRS experts and representatives from their scanner vendor to provide further information on vendor-specific variances highlighted in the Comprehensive Guide.

Development

The guideline was developed using a translation framework widely used for the development of clinical guidelines, the NHMRC framework Guidelines for Guidelines[10] and the ADAPTE toolkit[11]. Full details of the development process including search strategy of the scoping review are outlined in the accompanying manuscript[9]. In brief, this framework divides the evidence synthesis and recommendation formation into three stages: set up, adaptation and finalisation[11]. The stages are summarized in Figure 1. The key strengths of this approach include the involvement of multiple stakeholders with diverse experience and expertise, conducting a systematically delivered scoping review, the blinded quality assessment of each recommendation, and the modified-Delphi approach[12,13] used to integrate external expert peer review.

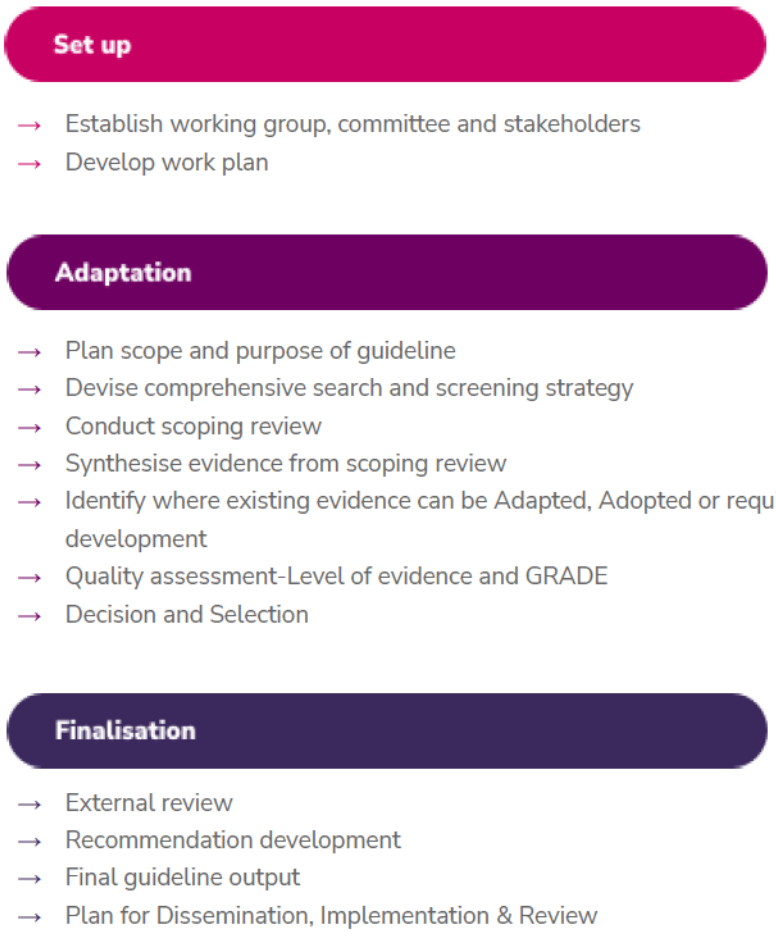

Figure 1: A summary of the process followed to develop the guideline based on the ADAPTE framework[11]

SET UP

A working party consisting of MRS experts, translation/implementation experts and key end-users including higher-degree research students, research radiographers and MRS mentors was established.

ADAPTATION

Evidence Synthesis

Evidence to inform the guideline was identified through a systematically conducted scoping review (see manuscript for full search strategy[9]). Evidence was summarized, and the ADAPTE framework for guideline adaptation[11] was used in an iterative process to establish where evidence currently exists for each recommendation. The process considers if recommendations are suitable for *Adoption*- when it can be lifted directly from an existing guideline or for *Adaptation*- when the recommendation needs to be adjusted to suit the audience or context. Where no evidence exists, the recommendations require development *DeNovo* ('from scratch')[10].

Quality Assessment

The quality of evidence was established in a two-stage process. First, the NHMRC Level of Evidence was established using the traditional and a modified NHMRC hierarchy of evidence framework (Supplement 1). The Level of Evidence describes the suitability of a study design to address a research question (ranging from Level 1 indicating the most robust design to Level 4 indicating the least robust design)[14].In this guideline, studies best answered through a systematic review of randomised controlled trials (e.g. 'Medications') were assessed using a traditional hierarchy of evidence, whilst those examining MRS principles and acquisition parameters, best answered through expert consensus, were assessed using a modified hierarchy (Supplement 1). Second, the modified Grading of recommendations, Assessment, Development and Evaluation (GRADE)[14] determined the degree of certainty in the body of evidence used to inform each of the recommendations (Table 1).

Table 1: GRADE matrix

GRADE	Criteria	Description
A	→ Good evidence (One or more Level 1 study or studies with consistent findings)	Body of evidence can be trusted to guide recommendation
B	→ Fair evidence (One or more Level 2 or 3 study or studies with consistent findings)	Body of evidence can be trusted to guide recommendation in most situations
C	→ Conflicting evidence (One or more Level 1 to 3 study or studies with inconsistent findings) OR → Low level evidence (More than one Level 4 study)	Body of evidence provides some support for recommendation, but care should be taken in its application
I	→ Insufficient evidence (no studies) OR → Poor evidence (Level 4-5 studies with inconsistent findings)	Body of evidence is weak, and recommendation must be applied with caution

FINALISATION

Expert Panel Agreement

The expert panel consisted of 21 expert MRS researchers from 15 universities in eight countries. A modified-Delphi process[12,13] was used to determine expert agreement on the content and suitability of the recommendation for the Comprehensive Guide (See manuscript[9] for further detail). In brief, recommendations were classified as having 'expert panel endorsement' and accepted into the final guideline if at least 80% of the expert panel had agreed to the recommendation. In cases where recommendations did not reach the 80% threshold in Round 1 or new evidence had become available, recommendations were revised and sent out for re-assessment in Round 2. Recommendations that did not reach the 80%-threshold in Round 2 were not given an 'expert panel endorsement' label.

Final Guideline Outputs

The three outputs from this work include this full guideline, a peer-reviewed publication and a one-page infographic summary.

A Comprehensive Guide to MEGA-PRESS for GABA measurement (This extended guideline)

This guideline briefly provides an overview of the background and the development process of the guideline, and then provides a detailed document which gives context for each recommendation, an evidence synthesis, and considerations from the expert panel. The full-length guideline is recommended for consultation when upskilling in the field of MEGA-PRESS, particularly during the study protocol design phase.

The peer-reviewed publication

The peer-reviewed publication[9] is an accompanying manuscript that first outlines the rigor of the methodological process of recommendation development and then provides a summary of the recommendations. The manuscript provides the GRADE of evidence, percentage of expert panel agreement and a shortened summary of the evidence synthesis and expert panel feedback that informed the recommendation. The manuscript can be used instead of the full-length guideline when a brief overview of parameters that determine data quality is sufficient.

One-page infographic summary

The infographic provides a quick visual reference guide, summarises the key messages of the Comprehensive Guide and provides a memory aid to users who have previously read the full guideline. Its purpose is to improve the translation of the guideline into standard practice.

Table 2: Summary of Recommendations

			Evidence GRADE	Experts: R1 (%) Agreement	Experts: R2 (%) Agreement
Acquisition	SNR	-Number of Transients -Voxel Size	A	76.2 81	90
	TR		A	95.2	-
	TE		A	81	-
	Water reference		A	85.7	55
	Slice selection for water reference		A	100	-
	Gradient		I	76.2	75
	Editing pulse		A	76.2	90
Practicalities	Voxel position		A	85.7	-
	Shimming		A	71.5	80
	Order of scans		A	85.7	-
Confounders	Scanner site		B	95.2	-
	Macromolecules		A	90.5	100
	Region		C	81	-
	Tissue composition		A	85.7	90
	Age		A	95.2	-
	Sex		C	85.7	-
	Medications		B	95.2	-
	Other	-Caffeine -Nicotine -Menstrual phase	I	71.4 76.2 71.4	85
Data Acquisition	Quality assessment		A	90.5	-
	Export		I	90.5	-
Quality and Reporting	Quality metrics		A	90.5	-
	Reporting		A	95.2	
Post-Processing	Frequency and phase correction		A	95.2	

1. Parameters

1.1. Signal-to-Noise Ratio Considerations (Number of transients and Voxel volume)

Recommendation	**ADAPT: Start with at least 192 transients (i.e. 96 Edit-ON + 96 Edit-OFF) and a voxel volume of 27ml (e.g. 3 x 3 x 3cm^3) to quantify GABA when scanning a favourable brain region.** Consider increasing the total number of transients when scanning smaller or more challenging brain regions (See Region)	GRADE A Agreement

1.1.1. Number of transients

Background

MRS measurements suffer from low signal-to-noise ratio (SNR) and require the acquisition of multiple repetitions of the experiment, which are averaged at the end of the scan. The terms transients, averages, excitations or acquisitions have all been used in the literature. Notably, the SNR of the averaged spectrum increases with the square root of the number of transients[15], but linearly with the voxel volume. In order to achieve sufficient SNR, the number of transients and the voxel volume must therefore be considered as a whole. Our recommendation serves to provide a starting point from which further optimisation can be performed, but does not take into account specific adjustments for particular brain regions, conditions or populations.

Evidence summary

Eight studies provided recommendations for the number of transients required to acquire data for a MEGA-PRESS experiment (one consensus document; Level 1[6], two large multi-site trials; Level 2[16,17] and five methodological publications; Level 3[8,18-21]). The number of transients recommended for MEGA-PRESS acquisitions ranged from the lowest recommending 126 [20] to the highest recommending 320 [16,17], equating to 4-13 minutes of scan time (at typical repetition time (TR) = 2s). The consensus document suggested 10 minutes scan-time will typically suffice (Level 1). Three of the five methodological publications (Level 3) directly investigated the number of averages required for a MEGA-PRESS dataset and found little improvement in variation when increasing the number of transients from 128 to 296[20], or 200 to 300[18], respectively, with only modest gains demonstrated beyond 218 transients[19]. One methodological publication (Level 3) noted a decrease in stability in the ACC beyond 262 transients[3]. Reasons for reduced stability over longer scans could either be due to increased frequency drift[8,21] or motion artefact[18]. Although a single Level 3 study has demonstrated 126 transients are sufficient, the overall recommendation is to use at least 196 transients (96 Edit-ON and 96 Edit-OFF) to ensure adequate SNR.

A consensus document (Level 1)[22] and a quality assessment tool within a systematic review (Level 2)[2] highlight the importance of *reporting* the number of transients used in the study. Reporting should specify whether the number of acquisitions are separate (as Edit-ON and Edit-OFF) or total number of transients.

Considerations

The expert panel commented that the number of transients and voxel volume must be considered together (n=11/21, 52.4%). Choice of brain region and study population will also impact the number of transients required (6/21, 28.6%). Firstly, less favourable brain regions such as the thalamus or dorsolateral prefrontal cortex (DLPFC) may require a greater number of transients to maintain adequate SNR, or alternatively, a larger sample size[18,23], whereas regions such as the occipital and parietal lobe are more favourable to MRS. Secondly, certain study populations, such as paediatric or clinical cohorts, may be more likely to move in longer acquisitions compared to healthy control participants. Therefore, a balance between gaining sufficient SNR and length of scan time needs to be achieved. In addition, it is recommended to choose multiples of 16 to allow for full phase cycles to be included.

Furthermore, it should be noted that the reference to number of transients (total vs. number of ON/OFF) has not been standardized across implementations of MEGA-PRESS and therefore must be checked at time of setup.

1.1.2. Voxel volume

Background

Voxel volume refers to the volume of the area the spectroscopic signal originates from, i.e. the product of the voxel dimensions. There is variation in how voxel volume is reported in the literature, and may be reported as mm^3, cm^3 or ml, although consensus documents recommend all three dimensions are reported in the format 30 × 30 × 30 mm^3 [22].

Evidence Summary

Seven studies provided recommendations for voxel volume (one consensus document; Level 1[6], one seminal text; Level 1[24], two large multi-site trials; Level 2[16,17] and three methodological publications; Level 3[25-27]). All seven studies recommended the use of a ~27ml voxel (e.g. 3 × 3 × 3 cm^3, although the voxel does not have to have equal dimensions) for MEGA-PRESS acquisitions (Level 1 to 4). The rationale for this large voxel volume is to compensate for the low SNR of MRS methods, particularly for low-concentration compounds like GABA. One consensus document[6] supports a reduction in voxel volume if the number of averages is increased to adequately compensate for the loss in SNR. It should also be noted that studies using smaller voxel volumes may result in lower GABA estimates (Level 3)[27].

Considerations

The relationship between the number of transients and SNR provides 'diminishing returns'. SNR increases only with the square root of the number of transients, while the relationship between voxel volume and the number of transients is linear (Level 1)[24]. For example, an 8-ml volume (e.g. a 2 × 2 × 2 cm^3 voxel) only has approximately 30% of the SNR of a 27-ml volume when the number of transients is the same[15]. In this scenario (using an 8-ml vs. 27-ml voxel), the scan time would need to be increased nine-fold to obtain comparable SNR, which is not feasible in most studies. A larger voxel volume will increase SNR; however, it also reduces regional specificity, and further increases partial volume effects (See section 3.4 Tissue composition). To improve regional specificity, the dimensions can be adjusted to make the voxel more rectangular-cuboid-shaped. Quantification is not discussed in detail here, however, using a larger voxel volume increases the importance of including partial volume correction during data analysis. The expert panel commented that high quality data could be obtained with a slightly smaller voxel volume, e.g. 25 ml, (n=6, 28.6%) but it was agreed that 27 ml is an appropriate volume for the beginner to start with when using 192 transients (96 Edit-ON + 96 Edit-OFF).

1.2. Repetition Time

Recommendation	Use a TR of around 2000 ms at 3T.	GRADE A Agreement

Background

Repetition time (TR) refers to the amount of time from the application of an excitation pulse to the application of the next pulse. TR determines the degree of recovery of the longitudinal magnetization between each repetition of the experiment. This section reports the most commonly used TR across 3T MEGA-PRESS in milliseconds (ms)

Evidence Summary

Five studies provided recommendations on TR (one consensus document; Level 1[28], two large multi-site trials; Level 2[16,17], and two methodological publications; Level 3[29,30]). All five papers recommended a TR of 2000 ms for edited MRS of GABA. One methodological publication (Level 3)[29] investigated the T_1 of GABA and determined it as 1310 ms. This T_1 is the same order of magnitude as other commonly measured metabolites, and therefore does not require the TR to be adjusted beyond 2000 ms for GABA acquisitions.

Considerations

This recommendation reached 95.2 % consensus in the first round. However, 3/21 14.3 % of the expert panel commented that TR was unlikely to have a large effect on SNR. Therefore, an appropriate TR could be considered as anywhere between 1500 and 3000 ms. The effect of changing the TR has not been specifically investigated in the literature, however, 2000 ms is most commonly used for single-voxel MRS.

1.3. Echo Time (TE)

Recommendation	ADOPT: TE should be 68 ms (GABA+); 80 ms (macromolecule-suppressed GABA).	GRADE A Agreement

Background

Echo time (TE) is defined as the time between the application of the excitation pulse and the time where optimal refocusing of the signal occurs[13]. For in-vivo proton MRS, TE is usually between 30 and 200 ms. Longer TE results in decreased SNR due to T_2 relaxation losses. However, multiplet signals from coupled resonances change with increasing TE, and facilitate the detection of certain compounds with higher sensitivity at longer echo times using experiments such as MEGA-PRESS.

Evidence Summary

Ten studies provided recommendations on TE (three consensus documents; Level 1[6,28,31], a systematic review; Level 2[32] two large multi-site trials; Level 2[16,17], three methodological publications; Level 3[30,33,34] and a seminal paper; Level 1[7]). A TE of 68 ms for measuring GABA was first adopted in 1998 when the first seminal MEGA-PRESS study was published. The rationale for using 68 ms is to allow for complete evolution of the GABA multiplet in the edit-OFF acquisition, thus allowing for maximum editing efficiency [7]. The ten studies (Level 1 to 4) [6,7,16,17,28,30-34] have discussed the length of TE. As a result, the consensus remains to keep the TE as close to 68 ms as possible when estimating GABA+. When using a method to suppress macromolecule (MM) contamination, four studies (Level 1 to 4) [17,31-33] agree that the TE should be lengthened to 80 ms to allow for longer, more selective editing pulses, without substantial signal loss due to T_2 relaxation (Level 1-4)[7,16,30,31].

Considerations

The longer TE used to measure MM-suppressed GABA may not be required for some Siemens scanners as they can apply MM-suppression at 68 ms due to their higher maximum B_1 (Level 3)[17].

1.4. Water reference scan required for eddy-current correction and water-scaled quantification:

Recommendation	ADAPT: Acquire two water reference scans for each volume of interest: One using the same parameters as MEGA-PRESS but deactivated water suppression for eddy-current correction and one short-TE (~30 ms) for quantification.	GRADE A Agreement

Background

A water reference scan is an additional acquisition from the same volume, but without water suppression. A water reference scan using the same timing as the water-suppressed MEGA-PRESS experiment is required to perform eddy-current correction. To clarify, the water reference scan should have identical localization, TR, TE and water suppression gradients, but water suppression radiofrequency pulses deactivated. While this acquisition can also be used to perform metabolite quantification relative to tissue water, it will be heavily T_2-weighted due to the longer TE. For quantification purposes, an additional water reference scan with the shortest possible TE is therefore suggested. Due to the high concentration of water in the brain, water reference scans require only a few transients, so the time penalty of acquiring two separate water reference scans is negligible[24].

Evidence Summary

Seven studies provided recommendations for water reference scans without water suppression for eddy-current correction and water-scaled quantification (five consensus documents; Level 1[6,28,35-37], one seminal text; Level 1[24] and two methodological publications; Level 3[38,39]). One study recommends acquiring a separate short-TE scan to account for the difference in T_2 weighting (one consensus document[37]; Level 1). There is consensus across the studies recommending that the water reference scan is acquired from the same volume of interest, using the same gradients in order to facilitate eddy-current correction, water-scaled quantification, and receiver-coil combination.

Considerations

Some sequences automatically acquire a water reference during the MEGA-PRESS acquisition, whereas others require a separate scan. While not explicitly stated in the literature, it is necessary to ensure the water suppression gradients are active, while the water suppression pulses are deactivated. The strong signal requires only a few transients for sufficient SNR; the expert panel recommends that typically between 4-8 transients will suffice (4/21, 19%). Round 2 saw a separate short-TE scan for quantification added to the recommendation following expert panel feedback, and a recent consensus document[37]. This significantly reduced expert panel agreement from 85.7 to 55%, suggesting this has yet to be widely accepted in the field.

1.5. Slice-selection center frequency of water reference scan

Recommendation	ADOPT: Set the water reference to be acquired from the same volume as the GABA signal	GRADE B Agreement

Background

The slice-selection frequency is the carrier frequency of the slice-selective RF pulses for the water reference scan. Due to the chemical shift displacement effect, the slice-selection frequency needs to be adjusted appropriately to ensure that the metabolite signals and the water signals originate from the same volume.

Evidence Summary

Three studies provided recommendations on the slice-selection centre frequency for the localization of the water reference (two large multi-site trials; Level 2[16,17] and a methodological publication; Level 3[30]). All three studies agree that it should be set to 0 ppm offset, i.e. localizing the 4.7-ppm water signal[16,17,30]. The water-suppressed MEGA-PRESS data is commonly collected with –1.7 ppm offset relative to water, i.e. localizing the 3-ppm GABA signal. This ensures that the water reference scan is co-localized with the same volume as the GABA signal from the MEGA-PRESS scan.

Considerations

Implementing the slice-selection centre frequency (also referred to as the delta frequency) may vary between different vendors. Some need to be set explicitly, some can be selected via a drop-down menu and others are fully automated.

1.6. Order of slice-selective gradients

Recommendation	ADAPT: When artefacts appear in pilot data, consider changing the order of the slice-selective gradients for each volume of interest.	GRADE I Agreement

Background

For PRESS localization, the MRS signal is generated from a cuboid volume by applying three consecutive slice-selective radiofrequency pulses and slice-selective gradients in three orthogonal planes, anterior to posterior, head to foot, and left to right[24]. MRS experiments are sensitive to the appearance of spectral artefacts. These artefacts are often caused by incomplete dephasing of unwanted signal from outside the volume of interest, resulting in out-of-voxel echos (Figure 2). Artefacts are common in regions subjected to abrupt changes of magnetic susceptibility, for example the sinuses or the mouth[24,40]. The order in which the three slice-selective gradients are applied can usually be modified to minimize the appearance of these artefacts.

Evidence Summary

One study provided recommendations on the order of slice-selective gradient application (methodological publication; Level 3[40]). The study recommended that the axial gradient should be applied last for frontal voxels, in order to minimize out-of-voxel water ("ghost") artefacts in the data[40]. No other studies have discussed the order of slice-selective gradients, however this was discussed at an international expert workshop in 2018[41]. The consensus from this workshop was to conduct 2-minute pilot scans with varied gradient order prior to commencing data collection. Beginners should then observe the impact of gradient order on spectra with respect to reducing artefacts (e.g. lipid contamination or out-of-voxel echoes) and improving spectral quality. This recommendation is supported by experiments conducted yet unpublished (Level 5)[15], where substantially less lipid in a motor region was demonstrated when gradient order was optimized.

Considerations

Not all vendors allow for the easy adjustment of gradient order. In instances where MEGA-PRESS has not been run on a scanner before, a series of pilot acquisitions (as detailed above) should be completed to select the optimal gradient order. The expert panel suggest if artefacts exist and gradient order cannot be adjusted, the VOI can be rotated slightly (2/21, 9.5%). Note that rotations beyond 45 degrees may automatically flip the direction of the gradients, which can make the directions of the chemical shift displacement difficult to predict, particularly when rotating the voxel in more than one plane. Experts also suggested using slice-selective pulses with large bandwidth (e.g. adiabatic localization) to reduce effects of static magnetic field (B_0) inhomogeneity, although these may not be available in every implementation (2/21, 9.5%). Some possible artefacts are demonstrated in Figure 2, but for further information it is recommended to refer to Kreis *et al.* (2004)[42] for visual examples of other common spectral artefacts (2/21, 9.5%).

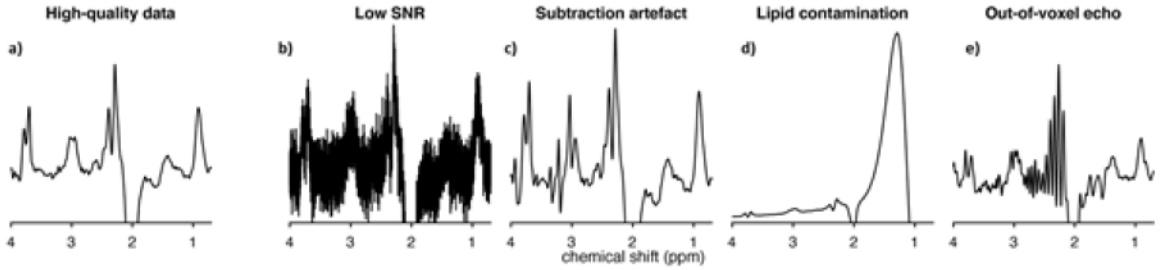

Figure 2: Common MEGA-PRESS data quality issues. a) High-quality data with sufficient SNR, narrow linewidths, a well-defined edited signal at 3 ppm, and no substantial artefacts; b) very high noise levels due to low number of transients or small voxel volume; c) severe subtraction artefacts due to scanner frequency drift; d) lipid contamination due to participant motion or voxel positioning too close to the skull; e) out-of-voxel echo ("ghost signal").

1.7. Editing pulse specifications

Recommendation

ADOPT: Editing pulses can be applied as follows:

	GABA+	Macromolecule -suppressed
Frequency (ppm) Edit-ON Edit-OFF	 1.9 ppm 7.46 ppm	 1.9 ppm 1.5 ppm
Bandwidth	60 Hz	Usually 80Hz (60 Hz on some implementations)
Spacing	0.5 TE apart (this parameter is usually not accessible to the user)	

GRADE

A

Agreement

Background

Editing pulse frequency is defined as the frequency at which the frequency-selective editing pulses are applied. The frequencies of the editing pulses are different dependent on whether GABA+ or MM-suppressed GABA is being acquired. In GABA+ data, the edited signal at 3 ppm is contaminated by co-edited MM signal (estimated to account for about 50% of the edited signal area[43,44]). To reduce the MM contamination, a second editing pulse can be applied at 1.5 ppm. However, the increase in specificity comes at the expense of a much greater sensitivity to experimental instability, particularly thermal drift of the magnetic field. Editing pulse bandwidth refers to the full-width half-maximum (FWHM) bandwidth of these pulses, which is a measure for their selectivity, and inversely related to their duration. Editing pulse spacing refers to the time between the two editing pulses. Not all these settings can be adjusted by the user, depending on the sequence.

Evidence Summary

Nine studies provided recommendations on the frequency, bandwidth, and spacing of the editing pulses. Five discussed the frequency of the editing pulse for GABA+ (one consensus document; Level 1[6], two large multi-site trials; Level 2[16,17] and four methodological publications; Level 3[7,30,45,46]) and four discussed the position of the editing pulse for MM-suppressed GABA (Level 3) [17,34,46,47]. The consensus for GABA+ was that editing pulses should be placed at 1.9 ppm and 7.46 ppm (Level 3 to 4) [7,16,30,45,46]. For MM-suppressed GABA, the editing pulses need to be positioned symmetrically around the MM resonance at 1.7 ppm (i.e. 1.9 ppm / 1.5 ppm)[17,34,47]. Five of the nine studies specifically recommend that the editing pulses are spaced TE/2 apart (Level 1 to 3)[6,16,17,30,46] . However, certain implementations on Siemens and Canon platforms do not comply with the TE/2 requirement, therefore reducing editing efficiency if deviating from TE = 68 ms (Level 3)[30,46]. The editing pulse bandwidth should be kept as narrow as possible (FWHM = 60–80 Hz). The minimum achievable bandwidth may depend on vendor, sequence implementation,

and available hardware (Level 1 and 3). Given the duration of the editing pulse is inversely proportional to the bandwidth, the duration should therefore be as long as the TE permits- usually around 15 ms for GABA+ and 20 ms for MM-suppressed GABA (Level 2 to 3)[16,17,30].

Considerations

The definition and specification of the editing pulse bandwidth differs between sequence implementations. Some implementations require the editing pulse duration as the input, while others require the FWHM of the bandwidth. Notably, the FWHM entered on the exam card may differ from the actual FWHM of the editing pulse. For example, Siemen's implementations that apply a smoothing filter to the pulse, result in an actual FWHM considerably larger than the nominal one. Therefore, specifications for bandwidth duration were not added to this recommendation. The expert panel had numerous suggestions for variations on these parameters (n=8, 38.1%), which highlight that there is variation on what can be applied at an expert level.

2. Practicalities

2.1. Voxel position

Recommendation	ADAPT: Use automated voxel positioning tools where available. If manually positioning the voxel use a screenshot and clear instructions regarding positioning relative to anatomical landmarks and degree of rotation.	GRADE A Agreement

Background

Voxels are generally positioned according to the research interest of the investigator. However, several factors limit the freedom to position voxels. In this section, practical approaches to voxel placement are discussed.

Evidence Summary

Five studies provided recommendations on the practicalities of voxel positioning (one consensus document; Level 1[35], three methodological publications; Level 3[27,48,49] and one narrative review; Level 4[42]). One of the 5 studies (Level 4)[42] demonstrates the implications of positioning the voxel. The positioning of voxels has the potential to cause significant variation in data: Two of the five studies (Level 3) examined reproducibility of manual voxel placement and found that the overlap in repositioning the voxel within a scan ranged between 75% and 85%, 75%, corresponding to a 2-3 mm displacement along three axes[48,38]. Therefore, to aid manual voxel placement it is recommended that a screen-shot is used with detailed written instructions including reference to anatomical landmarks. A consensus document (Level 1) and a methodological publication (Level 3) recommend the use of an automated voxel positioning tool . This has been found to improve reliability of voxel repositioning both within and between scans[35,49].

Considerations

When manually positioning a voxel, care needs to be taken to not position the voxel too closely to the skull, since dural fat signals may contribute strong lipid signals that significantly distort the spectrum and hamper subsequent quantification (Figure 2). Significant lipid signals may still occur even if the voxel shown on the inline display did not contact the dura, this is due to the chemical shift displacement effect. Some vendors allow the display of a second voxel box to visualize the origin of a shifted metabolite signal (e.g. the lipid resonance), which can help guide voxel placement.

Brain regions such as the occipital lobe or parietal lobe are considered favourable for scanning. It is therefore recommended that when first utilizing MEGA-PRESS

sequences, the beginner pilots their methodology in these regions prior to scanning more challenging regions. More challenging regions involve positioning voxels deeper in the brain (i.e. further away from receiver coils), close to ventricles or iron deposits (e.g. subcortical regions). These may suffer from decreased signal-to-noise ratio (SNR) or spectral quality compared to cortical voxels (Figure 2).

Fully automated voxel positioning software is not currently integrated into standard scanner operating software (6/21, 28.6%). The expert panel note that freely available AutoAlign (Siemens), ReadyBrain (GE), SmartExam (Philips) or NeuroLine (Canon) software all can improve alignment reproducibility by referencing the anatomical images on which a volume of interest (VOI) is planned. The accuracy of these tools relies on the quality of the anatomical images and consistency of voxel placement should be carefully reviewed during acquisition (2/21, 9.5%).

2.2. Shimming

Recommendation	A beginner should use a readily available automated field-map-based shim and minimize the use of manual adjustments.	GRADE A Agreement

Background

Shimming is the process of maximizing the homogeneity of the static magnetic field (B_0) over the measurement volume of interest. Since high homogeneity results in narrow linewidths and increased SNR, the quality of the shim is considered one of the most important parameters for determining spectral quality[24]. Shim is typically reported in terms of the full-width at half-maximum (FWHM) of the water linewidth and is reported in Hz, although this may vary between vendors. Linewidth values largely depend on brain region and the surrounding interfaces between air/tissue and tissue/bone. These will influence the magnetic field causing field distortions[28]. Most vendors offer automated field map-based shim routines and/or projection-based shim routines and may also offer manual adjustment of the shim currents. Dynamic shim updates are the subject of ongoing research, and not readily available across all systems (refer to Juchem *et al.* 2020[50] for visual representation of the impact of shim quality on the spectra.)

Evidence Summary

Eight studies provided recommendations on the practicalities of shimming (three consensus documents; Level 1[28,35,50], one systematic review; Level 2[51] and four methodological publications; Level 3[20,52-54]). The consensus across the eight studies was that FASTMAP, FASTESTMAP (projection-based shim optimization) or second-order pencil beam methods could provide narrower linewidths than the default 3D-field-map-based methods. However, these systems are not openly available and are technically more challenging to operate. It is further recommended that manual shimming should only be implemented to optimize suboptimal automated shims to minimise user intervention (Level 1) [35]. Three consensus documents (Level 1)[28,35,50] and one methodological publication (Level 3) [52] recommend that the attainable values of B_0 shim quality are expressed as linewidths of the water peak. The three consensus papers (Level 1)[28,35,50] report that; at 3T a FWHM of 5-7 Hz is considered excellent, 8-10 Hz is considered good and 11-13 Hz acceptable for the brain. However, these values are significantly lower than those reported in the methodological publication (Level 3)[52]. These values are likely to increase in brain regions that are more difficult to shim, such as frontal, temporal or subcortical regions. An example is the frontal region where a shim of <16 Hz is considered acceptable[52].

Considerations

Evidence suggests that projection-based shim optimization (e.g. FASTMAP, FASTESTMAP) methods or pencil-beam methods can potentially achieve a better shim than default 3D-field-map-based methods. However, experts highlight these are not readily available and can be more challenging for a beginner to use (3/21, 14.3%). Therefore, the expert panel recommend that readily available automated field-map-based methods are used by the beginner and that manual adjustments are avoided wherever possible (9/21, 43%).

The expert panel commented that different vendors calculate spectral linewidths differently (8/21, 38.1%). Therefore, the inline display values of linewidth may not correspond to the above mentioned quality criteria. It is therefore useful to measure the actual FWHM from processed spectra to determine how it relates to the inline-displayed value for that system.

2.3. Order of scans and field drift

Recommendation	ADOPT: Where possible MRS should be conducted prior to gradient-heavy acquisitions or in small blocks of 2-5 minutes with frequency adjustments between adjustment blocks. Consider using real-time frequency correction if available.	GRADE A Agreement

Background

The order of scans refers to the position of the MRS acquisitions within the study protocol. Several critical effects relating to the order of scans need to be considered during protocol design. This section focuses on issues associated with field drift resulting from imaging sequences widely used in neuroscientific and clinical studies.

Evidence Summary

Eight studies provided recommendations with regard to field drift. Five specifically with regard to the order of scanning (three consensus documents; Level 1[31,35,36] and two methodological publications; Level 3[8,34]). Three discussed the use of real-time frequency correction (three consensus documents; Level 1[31,36,55]) and two investigated the impact of field drift on reported GABA level (large multi-site trials; Level 2)[16,17]. Five of the eight studies (Level 1-4)[8,31,34,36,55] highlighted the negative impact gradient-heavy scanning such as diffusion-tensor imaging (DTI) has on frequency drift during subsequent MRS scans. Frequency drift was observed for as long as 30 minutes following a fMRI scan[8,36]. The degree of frequency drift can vary between scanners. The range of frequency drift reported in the two methodological publications following fMRI/DTI (Level 3) were; –2 Hz/min[8] and 4.6 Hz/min respectively on a MM-suppressed acquisition[34]. While all MRS studies are susceptible to drift, MM-suppressed MEGA-PRESS is an order of magnitude more susceptible[34]. The impact of frequency drift is that it reduces editing efficiency, changes signal and increases subtraction artefacts (Figure 2) [8,34]. For conventional MEGA-PRESS, drifts of 10 Hz will result in a moderate signal change of 4-6%, while the MM-suppressed GABA signal may change by approximately 30%[8,34]. Given the potential impact of frequency drift on editing efficiency, it is recommended that MRS be conducted before any fMRI or DTI with application of real-time (prospective) frequency correction if available[8,34,35]. A recent consensus document (Level 1) proposes that data be acquired in small blocks of 2-5 minutes to monitor frequency drift or subject motion with interleaved scanner frequency adjustments between acquisition blocks [36].

An additional recommendation regarding the order of scanning is that the water reference scan is acquired first (Level 1)[26]. This will ensure that the water reference is acquired from the same VOI in case the metabolite acquisition needs to be stopped and/or repeated due to participant motion.

Considerations

It is acknowledged that conducting MRS prior to high-gradient imaging is not always possible. The expert panel recommended establishing the drift behaviour of an individual scanner by piloting MRS before and after a functional MRI (fMRI) or diffusion tensor imaging (DTI) sequence in order to gauge the potential effects on subsequent MRS (2/21, 9.5%). Alternatively, experts endorse the new recommendation to acquire MRS data in small blocks with frequency adjustment after each block, whilst monitoring the water residual during the scan acquisition in order to detect drift (3/21, 14.3%).

Although one consensus document (Level 1) [35] recommends acquiring the water reference scan prior to the metabolite spectrum, this might not be possible for all vendors e.g. GE. An alternative might be to consider interleaving the water reference.

3. Confounders

This section covers known and potential confounders to MRS studies of GABA. This list is not exhaustive due to the specific nature of our search strategy and the wide range of known and unknown potential confounders. This section focuses on major confounders including:

3.1 Scanner site and vendor

3.2 Macromolecules

3.3 Regional difference

3.4 Tissue composition

3.5 Age

3.6 Sex

3.7 Medication

3.8 Other potential confounders which include caffeine, nicotine and phase of menstrual cycle.

(Note levels of evidence documented with 'Level X^{T}' are assessed using a traditional hierarchy of evidence[14] rather than the modified-hierarchy of evidence (Supplement 1).

3.1. Scanner site and vendor

Recommendation	In multi-site studies standardized protocols should be used, and the degree of systematic differences between site/scanner should be reported.	GRADE B Agreement

Evidence Synthesis

Three studies discussed site and vendor as confounders for GABA estimates (three multi-site trials; Level 2 [16,17,46]). Two of these studies (Level 2)[16,17] analysed a dataset from 272 participants across 24 sites, using vendor-specific MEGA-PRESS implementations. The studies reported a coefficient of variation across all data sets of around 12% for GABA+/Cr and 17% for water-scaled GABA+. MM-suppressed MEGA-PRESS had larger CVs of 28%-29% for both GABA/Cr and water-scaled GABA [16,17].

Linear-mixed effects analysis of variance showed that only 20% of the overall variance of GABA+/Cr measures was accounted for by site-level differences, while 8% was accounted for by differences between scanner vendors. In contrast, water-scaled GABA+ data variance was mainly accounted for by between-vendor difference (53% of total variance) with just 11% being accounted for by site-level differences. The third study (Level 2)[46] showed that using a 'universal' MEGA-PRESS sequence that was implemented for all major vendors (with identical timing, RF pulses, and gradients) improved the within-subject agreement of GABA+/Cr estimates acquired on different systems compared to the vendor-specific implementations. However, this study only included eight participants.

Considerations

There is considerable difference between individual scanners, especially with different vendors. To establish the difference between sites scanning the same phantoms or control participants on all the scanners might help to quantify the between scanner differences. When designing a multi-site study always use a balanced design where the same number of controls and participants are scanned on each of the scanners (2/21, 9.5%).

3.2. Macromolecules

Recommendation	ADAPT: A beginner should use conventional MEGA-PRESS reporting GABA+. Macromolecule contamination should be acknowledged as a limitation, and consideration paid to whether macromolecules could be responsible for between-group differences.	GRADE A Agreement

Evidence Synthesis

Eleven studies discussed MM-contamination as a confounder of GABA (three consensus documents; Level 1 [6,31,36] and eight methodological publications; Level 3 [33,34,43,44,47,56-58]). A limitation of conventional MEGA-PRESS is the co-editing of MM that underlie the 3-ppm GABA signal. The degree of this contamination has been reported to be within 41%[43] and 60% of the total GABA+ signal area (Level 3)[44]. Three main approaches to account for MM contamination have been proposed, however all three approaches have significant limitations as agreed in two consensus documents[6,31]. The most widely used approach is symmetric MM-suppressed editing. This technique, however, is highly susceptible to frequency drift, thus reducing the reliability of MM-suppressed GABA measurements compared to conventional editing for GABA+2. Only one of seven methodological publications (Level 3)[44] found comparable repeatability of symmetric MM-suppressed editing compared to conventional GABA+ MEGA-PRESS. Recent consensus documents (Level 1) on MM in MRS[31] and edited MRS[36] recommend using MM-suppressed editing where possible. However, they also acknowledge the limitations of this approach and that it might not be practical in a clinical environment.

When interpreting data it should be noted that, while there was a moderate correlation between GABA and GABA+ levels pooled across brain regions in two methodological publications (Level 3)[33,56], there was only a weak correlation in a region-specific analysis[33]. Therefore, care needs to be taken when comparing or pooling results from conventional GABA+ and MM-suppressed GABA studies.

Considerations

The expert panel recommended that beginners should use conventional MEGA-PRESS at present despite consensus documents recommending the use of MM- suppressed sequences (19/21, 90.5%). A MM-suppressed sequence is more challenging for beginners to acquire because it is more susceptible to experimental instabilities such as frequency drift. Therefore, the expert panel recommends that in line with a previous consensus document (Level 1)[6] beginners adopt the most widely utilized approach of conventional GABA+ acquisition. They should report this as GABA+, acknowledging MM-contamination of the edited signal as a limitation. The MM baseline could be measured in a group of control participants, if differences in MM might explain between group-differences in the study population or between the time points[31].

3.3. Region

Recommendation	ADAPT: Select brain regions relevant to research question, however, acknowledge that brain regions have differing reliability with respect to data acquisition.	GRADE A Agreement

Evidence Synthesis

Fourteen studies discussed brain region as a confounder of GABA (1 review; Level 1^{T} [59], 13 methodological publications; Level 4^{T} [19,27,52,60-69]). Historically, it was hypothesised that brain GABA Levels may be universal across all brain regions, reflecting a "global GABAergic tone"[59]. However, there is growing evidence that this is not the case (Level 4^{T}) [27,52,60-62,65,68,69]. Several methodological publications have demonstrated that GABA levels are different between anterior and posterior brain regions (Level 4^{T})[52,61,68-70], but less so between hemispheres (Level 4^{T})[52,66].

Considerations

It is important to consider that certain brain regions may be less suitable for stable and reliable data acquisition than others depending on size, depth, tissue composition of the voxel and whether signals are obtained from cortical or subcortical regions. The occipital lobe, and posterior cingulate gyrus, for example, are associated with high quality spectra whereas regions such as the amygdala are more challenging. (See sections 1.1 SNR and 2.1 voxel position).

3.4. Tissue composition

Recommendation		GRADE
	ADAPT: Water-scaled quantification methods should consider the impact of partial volume effects on GABA estimation. Segmented structural images should be used along with a tissue correction method to account for grey matter, white matter and cerebrospinal fluid. Grey-matter only correction should be avoided.	A Agreement

Evidence Synthesis

Nine studies discussed the relative volumes of grey and white matter within the MRS volume, as a confounder for GABA estimates (one consensus document; Level 1 [6], eight methodological publications; Level 3 [66,71-77]). There was agreement across all nine studies that GABA levels are higher in grey matter than white. This is substantiated by data using brain tissue extracted during surgery[78] and chemical shift imaging studies[79] (not included in this review). The studies that used MEGA-PRESS and optimised parameters found that GABA levels are approximately twice as high in grey compared to white matter[74-77]. The three other studies reported a range of ratios from 2:1[72] to 8:1[71,73]. All of these studies investigated a healthy population or simulation, the ratio may be altered in the presence of pathology.

The recommended approach for handling grey and white matter differences within the MRS volume is debated. One methodological publication (Level 3)[76] demonstrated that choice of tissue correction method significantly impacts the water-scaled quantification of GABA+ and therefore needs to be considered with care. All studies agree that correction for grey matter alone is insufficient and leads to over estimation of GABA, especially in voxels containing less than 50% grey matter. All methodological publications recommend a degree of tissue correction which allow for the different voxel composition of grey matter, white matter and CSF when using water-scaled quantification. However, this approach alone does not take into account tissue specific relaxation times. One methodological publication (Level 3)[74] investigated tissue relaxation times and recommends the use of pulse sequence parameters that minimize the effect of signal relaxation, owing to not knowing the composition of the voxel *a priori*. The consensus document (Level 1)[6] recommends that the most appropriate approach is to use grey matter to white matter ratios as a covariate in any statistical analysis rather than to attempt to correct measures based on the reported differences in concentration between tissue types.

Considerations

There are a number of tissue correction algorithms available, however there are limitations to each approach. Beginners should be aware of the limitations of their chosen approach (e.g. unaccounted difference in relaxation times). Using tissue composition as a covariate helps to clarify that between-group differences in GABA are driven by differences in GABA levels rather than by differences in tissue composition (3/21, 14.3%). However, including tissue composition as an additional covariate may reduce power in study with a small sample size (1/21, 4.8%).

3.5. Age

Recommendation	ADOPT: Age is likely to affect GABA levels, therefore age should be accounted for in study design or statistical analysis.	GRADE A Agreement

Evidence Synthesis

Seven studies investigated age as a confounder for GABA (one systematic literature review and meta-analysis[80]; Level 1^T and six methodological publications; Level 3^T [68,69,81-84]). All seven papers report that GABA+ decreases with age, however, one found no relationship between MM-suppressed GABA and age. One methodological publication (Level 3^T)[68] proposed that the observed decrease in GABA levels is a result of grey matter atrophy, and further supports the recommendation to correct for tissue composition. One of the six methodological publications (Level 3^T)[69] reported a 5% decrease in GABA/Cr and 4% decrease for GABA/NAA per decade, however, this was not calculated for GABA to water ratios or investigated in any other study. The meta-analysis[80] (Level 1) that extracted single-subject data found an increase in GABA in early development, plateauing in adolescence and early adulthood, followed by a steady decline with age.

Considerations

Age should be considered as a covariate due to a substantial age trajectory, however, including age as an additional covariate will reduce the power of a study with a small sample size. Use of an age-matched design, i.e. matching the age of participants across all groups may avoid the need to include age as an additional covariate.

3.6. Sex

Recommendation	ADOPT: Sex is likely to affect GABA levels, therefore sex should be accounted for in study design or statistical analysis.	GRADE C Agreement

Evidence Synthesis

Four studies investigated sex as a confounder for GABA (four methodological publications; three Level 3^{T} [69,81,85] and one Level 4^{T}[86]. The sample size ranged from 14[86] to 100[69] participants. The study with the largest number of participants found no difference in GABA+ levels between males and females in the anterior cingulate cortex (ACC). These results were reproduced by two other studies investigating the ACC[81,85]. Conversely, two studies of the parietal and dorsolateral prefrontal cortex (DLPFC) found statistically significantly higher levels of GABA in males compared to females[85,86]. Taken together, these results suggest that sex differences in GABA may be region-specific.

Considerations

When designing a study, consider recruiting equal numbers of female and male participants unless the study has an important sex component, or the condition being studied is more prevalent in a particular sex. A study design with sex-matching between groups can also be used to account for sex differences.

3.7. Medication

Recommendation	ADAPT: Medications may affect GABA levels, as minimum best practice all medications should be recorded.	GRADE B Agreement

Evidence Synthesis

Eight studies discussed medications that may confound GABA (one systematic review; Level 1^{T} [59]one RCT; Level 2^{T} [87], six methodological publications; two Level 3^{T} [88,89] and four Level 4^{T} [90-93]). In the seven clinical studies returned by our search, five drugs were investigated: vigabatrin, citalopram, zolpidem, gabapentin, tiagabine. Level 4 evidence suggests vigabatrin, citalopram, zolpidem, gabapentin may confound GABA measurements, while the data were inconclusive regarding tiagabine. The systematic review (Level 1^{T}) [59] further concluded GABA levels might increase following administration of levetiracetam or topiramate but not valproate, carbamazepine and phenytoin, and lamotrigine. Taken together, brain GABA levels may be influenced by a variety of medications regardless of whether their primary mechanism of action is on the concentration of GABA itself for GABA receptor agonists or antagonists and therefore medication should be recorded and considered as a potential confounder of GABA (Level 1^{T})[59].

Considerations

The aim of this section was to highlight that medications may confound measures of GABA. Given the broad aim of our scoping review, our evidence synthesis is not a full systematic review of this question. As a result, studies investigating the confounding effects of specific medications on GABA levels may have been missed. It is recommended that a medical specialist is consulted to discuss the mechanism of action of any pertinent drugs in the planned studies population. It is important that a considered decision is made with regard to handling patients who are medicated. It is likely that exclusion of these participants will considerably bias the study population.

3.8. Other potential confounders: Nicotine, Caffeine, Phase of menstrual cycle

Recommendation	ADAPT: Potential confounders such as caffeine and nicotine intake and phase of menstrual cycle may affect GABA levels, as minimum best practice potential confounders should be recorded.	GRADE I Agreement

3.8.1. Nicotine

Evidence Synthesis

Two studies investigated nicotine as a confounder for GABA levels (two methodological publications; Level 3[T 94,95]). One study found no difference in GABA Levels between 48 heavy smokers (n=48) and healthy controls[94]. Another study found no difference in GABA Levels in 36 smokers between baseline measures and following 48 hours abstinence[95].

3.8.2. Caffeine

Evidence Synthesis

One study discussed caffeine as a confounder of GABA Levels (methodological publication; Level 4[T 96]). A study of 15 healthy participants found no significant difference in GABA levels before and after acute administration of 200 mg of caffeine.

3.8.3. Menstrual Cycle

Evidence Synthesis

Four studies discussed the menstrual cycle as a confounder of GABA (four methodological publications; two Level 3[T 97,98] two Level 4[T 60,95]). One[95] of the four studies investigated phase of menstrual cycle as a secondary aim looking at a subgroup of six participants and therefore did not provide sufficient data to determine the effect of phase of menstrual cycle. The three remaining studies had sample sizes ranging from seven[60] to 75[98] participants. The study with the largest sample size found higher GABA levels during ovulation compared to the rest of the cycle in women with a natural cycle[98]. There was no difference between the follicular and luteal phases. In contrast, the two remaining studies found higher levels of GABA in the follicular phase compared to the luteal phase, but did not investigate GABA during ovulation[60,97]. Furthermore, one paper investigated women taking the hormonal contraceptive pill and found no difference between the active or inactive pill[98]. Current evidence suggests menstrual cycle may affect GABA levels, although there are some methodological limitations to the included studies.

Considerations

The effect of caffeine, nicotine and phase of menstrual cycle on GABA cannot be fully established from current evidence. Therefore, it is suggested that the impact of these potential confounders are considered in the design of the study, especially when conducting longitudinal or repeat measure studies.

4. Data Acquisition

4.1. Quality assessment during the scan

Recommendation	**ADOPT: It is recommended to monitor the quality of the acquisition using the inline data display at time of scanning.** Scans should be cancelled, and voxel position adjusted if evidence of weak water suppression, strong lipid contamination or other artefacts.	GRADE A Agreement

Preface

Most modern MRI scanners offer inline displays showing the last acquired spectral transient. This display can be used to determine spectral quality during the acquisition (water suppression, potential lipid contaminations and other artefacts), and make time-saving decisions whether a scan should be cancelled (and potentially repeated), or the voxel should be repositioned.

Evidence summary

Two studies provided recommendations to monitor quality of data acquisition during the scan (two consensus documents; Level 1[35,36]). Both consensus recommended that the MR operator should evaluate and monitor water suppression efficiency, spectral linewidth and signal-to-noise ratio at the beginning and during the MRS acquisition. A change in linewidth, frequency or spectral pattern, or worsening water suppression, suggests the participant has moved. It is recommended that the participant is visually checked, and the acquisition repeated if necessary (potentially including the localizer/scout image to account for the new participant position).

Considerations

Experts highlight that not all vendors provide the option to monitor the scan using an inline display at time of scanning e.g. GE. One expert noted that running an inline display can affect the TR on certain systems (prolonging TR up to 200 ms), this has important implications for relaxation correction or functional MRS experiments.

4.2. Data export

Recommendation	DEVELOP: Export data in a format that saves individual transients to allow adequate post-processing.	GRADE I Agreement

Preface

Spectroscopic data is often saved in vendor-specific file formats with varying degrees of processing. To ensure that all necessary post-processing steps can be performed, export MEGA-PRESS data in a file format that stores all individual transients separately.

Evidence summary

There is currently no discussion concerning which files to export specifically for MEGA-PRESS acquisitions, however, one study generally discusses the file format to export for MRS studies which could also be applied to MEGA-PRESS studies (consensus document; Level 1[37]). This consensus document (Level 1)[37] recommends that data be saved as single transients to allow for post-acquisition frequency-and-phase aligment[37]. Based on MEGA-PRESS-applicable recommendations extracted from the consensus document, we have developed the following recommendation:

Scanner	Format	Description	Comment
Philips	SDAT/SPAR	Two files for each acquisition, SDAT contains acquired signal data, SPAR contains header info.	Use SDAT/SPAR only when individual transients are exported. If this is not the case, also export DATA/LIST (which does not contain voxel location information, so both formats are then required).
	DATA/LIST	As above, DATA, LIST respectively.	
GE	GE-P (.7)	Default combines RF coil channels and groups in a phase encoded step. Fully customizable to preserve or combine any/all dimensions.	

Scanner	Format	Description	Comment
Siemens	TWIX (.dat),	All dimensions (RF channels, transients) preserved without modification.	Older sequence implementations may not allow the export of single-average RDA files.
	single-average RDA	All dimensions (except time/spectral dimensions) are pre-combined. Can be customized to preserve or combine any/all dimensions.	
All Vendors	Single-average DICOM	Default setting: dimensions are collapsed. Depending on settings, individual transients can be exported in separate DICOM files.	

Considerations

Experts noted that specific customized options have to be set on the exam cards to enable the export of individual transients (2/21, 9.5%), however these options may not be available on all scanners or implementations.

5. Quality & Reporting

5.1. Quality Metrics

Recommendation	ADOPT: Report spectral quality in terms of the signal-to-noise ratio, linewidth, water suppression efficiency, fit quality and the presence of unwanted spectral features	GRADE A Agreement

Preface

Due to the inherently low SNR, MRS acquisitions require a high degree of stability from the participant and the equipment. Spectra of low quality will result in less reliable (or wrong) quantification of the metabolite of interest. Judging the quality of an MRS spectrum by visual inspection requires experience. Several quantitative metrics of data quality allow more objective judgement whether the acquisition has been successful in terms of shim quality, water suppression, presence of artefacts, and quality of the data modelling. Another commonly used expression of uncertainty is the Cramér-Rao lower bounds (CRLB). CRLB can be considered as the "maximum trust that can be associated with an area (and thus concentration) estimated in model fitting"[99]. While they can be a useful indicator of quality for quantitative MRS, if used as a percentage of the estimated value (relative CRLB) results can be significantly biased due to the exclusion of potentially clinically meaningful data.[28,99]. An alternative is to use absolute CRLBs [99]. However, no single quality measure alone is sufficient to demonstrate the overall quality of data.

Evidence summary

Seven studies provide recommendations on quality metrics (three consensus documents; Level 1[6,28,35] and four methodological publications; Level 3[27,54,99,100]). One consensus document (Level 1)[35] made 7 recommendations on the variables to assess in order to determine spectroscopy quality: (1) SNR, (2) metabolite and unsuppressed water resonance linewidths, (3), residual water signal, (4) line shape, (5) CRLBs of the data fit, (6) fit quality (relative size of residuals versus the standard deviation of noise), and (7) presence of artefacts (spurious signals, baseline distortions, contamination from subcutaneous lipids). Of the four methodological publications (Level 3), 2 discussed CRLBs[99,100], 1 discussed bootstrapping[27], and 1 reported expected values for water linewidth cutoffs[54]. Two studies recommend the use of absolute CRLBs but not relative CRLBs based on the risk of introducing selection bias[35,99] (Level 1 and Level 5). Relative-CRLB cut-off recommendations of between 20-50% have been shown to bias exclusion and potentially obscure clinically meaningful differences in clinical populations[28,99].

Considerations

In cases where MRS analysis software packages do not report CRLBs, an alternative metric of fit error is the standard deviation of the fit residual[6].

5.2. Reporting

Recommendation	ADOPT: When reporting results use one of these two checklists (MRS in MRS- Lin et al. 2020[22]) or the MRS-Q (Peek et al. 2020[2]) using the appropriate terminology (Kreis et al. 2020). Include detailed reporting of hardware, MEGA-PRESS specific acquisition parameters including quantification details, quality and analysis methods.	GRADE A Agreement

Preface

Reporting of methods needs to contain sufficient information for readers to replicate the study and the data analysis. The quantitative results of MRS measurements depend strongly on the acquisition parameters, data quality, and the choice of analysis methodology. It is therefore required to report every step of data acquisition, processing, and analysis in as much detail as possible.

Evidence summary

Three studies provide recommendations on what should be reported in a MEGA-PRESS GABA study (one consensus document; Level 1[22], one systematic review; Level 2[2] and one methodological publication; Level 3[30]). The consensus document and systematic review were in agreement. The consensus document (Level 1) [22] reported five areas that require reporting; 1) hardware, 2) acquisition, 3) data analysis, 4) methods and 5) outputs and data quality. The systematic review (Level 2)[2] produced an 11-point checklist (MRS-Q) under the broader domains; 1) scanner, sequence parameters, 2) quality measures, 3) sample size calculation, 4) partial volume correction, and 5) analysis. The methodological publication (Level 3)[54] highlighted five aspects of optimization often not reported in edited MRS studies: 1) procedure to calculate and set the frequency of editing pulse; 2) time when editing pulse frequency is set and whether it is updated during acquisition; 3) length and bandwidth of localization pulses; 4) GABA relaxation times used for quantification; 5) homocarnosine co-editing often not mentioned (while MM is).

Considerations

When reporting MRS studies, refer to the consensus document on terminology and concepts for characterization[101]

6. Post-Processing

6.1. Frequency-and-Phase Correction (Post-processing)

Recommendation	ADOPT: Frequency-and-phase alignment of individual transients should be performed during post-processing.	GRADE A Agreement

Preface

Frequency-and-phase correction (FPC) is the post-processing step of aligning individual transients of a MEGA-PRESS acquisition and aligning the averaged edit-ON and edit-OFF spectra to each other. FPC techniques have been developed to address the strong susceptibility of MEGA-PRESS to subtraction artefacts, i.e. unwanted artefacts arising from spectral misalignment during the calculation of the GABA-edited difference spectrum. These artefacts can commonly occur in clinical populations due to head position, and significantly reduce the precision of data modelling and quantification.

Evidence summary

Ten studies provide recommendations on frequency and phase correction (two consensus documents; Level 1[36,37] and eight methodological publications; Level 3 [8,102-108]). The two consensus documents [36,37] recommended that spectral alignment routines be used during post-processing to improve the quality of the final spectrum for both unedited and edited MRS data. Two of the methodological publications (Level 3)[104,105] found that using the spectral registration algorithm for FPC of individual averages improves the linewidth and SNR of MRS data, and reduces subtraction artefacts in MEGA-PRESS data. One methodological publication (Level 3)[8] concurs that subtraction artefact can be improved in scans showing significant drift, however, editing efficiency and the GABA-to-MM signal ratio cannot be improved with this step alone. Three papers[106-108] demonstrated that appropriate alignment of edit-ON and edit-OFF spectra reduces subtraction artefacts in MEGA-PRESS data, and improved quantification. One methodological publication (Level 3)[103] found that determining the individual frequency history of an acquisition and calculating individual basis sets for linear-combination modelling based on this history, improves modelling accuracy. However, this method is not implemented in any currently available analysis software package.

Considerations

Experts highlighted that post-processing cannot compensate retrospectively for the impact of acquiring data with incorrect editing pulse frequency, e.g. as a result of frequency drift (2/21, 9.52%). Therefore, frequency drift needs to be monitored at time of acquisition (see Quality assessment during the scan).

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Supplementary Material

Supplement 1: Level of evidence modified from NHMRC (1999, 2009)

	MODIFIED EVIDENCE HIERARCHY		ORIGINAL EVIDENCE HIERARCHY	
Level	**Design**	**Justification**	**Design**	**Justification**
1	Consensus Document	Traditionally a systematic review of the most appropriate study design is considered Level 1 evidence. In this case we consider expert consensus documents as Level 1 because akin to systematic reviews in other fields, these consensus documents draw on the most appropriate study designs to inform the parameters required to run a MEGA-PRESS study. All consensus documents included within this review had a panel of authors from multiple institutions across multiple countries. They also benefit from recency, with 7/9 included consensus being published in 2020/2021.	Systematic review	In line with the NHMRC recommendations (NHMRC, 2009) a systematic review of Level 2 studies will be considered Level 1. In this case meta-analysis of the studies will likely improve precision of the results. In cases where systematic reviews are of lower levels of evidence, they will be considered the same level as the studies they include, as they may increase the chance of bias (NHMRC, 2009).
	Seminal texts	Where core principles of physics are required to inform the guideline, seminal text of these fundamental physical properties are also considered the highest level of text.		
2	Systematic Review	Systematic reviews are considered Level 2 evidence as they pool together results from methodological publications which have been specifically designed to test parameters required to run a MEGA-PRESS study. However, the methodological publications typically have small sample sizes, and limitations and suffer from publication bias.	Randomised Control Trial	In order to investigate the impact of a confounder a randomized control trial would be considered the best design to address the research question.
	Large multi-site studies	Large multi-site studies provide the most information on applying parameters in a clinical context; however, the purpose of such trials is rarely to investigate a single parameter required to run a MEGA-PRESS study.		
3	Methodological publications	For the purpose of this study, methodological publications were considered as any study that had a specific aim to investigate a parameter required to run a MEGA-PRESS study. These might include studies on humans, phantoms, or simulations. These did not include animal studies. These methodological publications will often test a specific parameter required to run a MEGA-PRESS study and directly inform this guideline. However, these studies are typically performed using small samples, and are often tested on healthy subjects in non-clinical environments.	i) Comparative study with concurrent controls ii) Comparative study without concurrent controls	Studies designs that investigate a condition compared to a control group, or situation are considered Level 3 evidence, as they have the potential for bias.
			Consensus document	Consensus documents are considered Level 3 when investigating confounders, as these research questions are better answered using a scientifically rigorous design, and therefore a consensus document is potentially biased.
4	Narrative Reviews	Narrative reviews are commonly published in the field of 1H-MRS spectroscopy but must be interpreted with caution due to the high risk of bias and personal opinion.	Case series	Case series are considered Level 4 due to being underpowered to answer these research questions, with no control for comparison.

A Comprehensive Guide to MEGA-PRESS for GABA Measurement

1. Starting Parameters

- At least 192 transients (96 Edit-ON + 96 Edit-OFF) and a 27 ml voxel (i.e 3 x 3 x 3 cm^3 in favourable regions)
- TR around 2000 ms, TE 68 ms (GABA+)
- Two water reference scans: One using same parameters as MEGA-PRESS but deactivated water suppression and one short TE (~30 ms)
- Set water reference to be acquired from the same volume as GABA
- Consider changing order of gradients if artefacts appear
- Editing pulse frequency 1.9 ppm (edit-ON), 7.46 ppm (edit-OFF) and editing pulse bandwidth 60-80 Hz, with pulses 0.5 TE apart

2. Practicalities

- ✔ Use automated voxel positioning or manually position using a screenshot
- ✔ Use a readily-available automated field-map-based shim and minimise manual adjustments
- ✔ Acquire data in small blocks with frequency adjustments between blocks

3. Confounders

- ⚠ In multi-site studies use standardised protocols and evaluate between scanner differences.
- ⚠ Beginners should use conventional MEGA-PRESS and acknowledge macromolecule contamination
- ⚠ Select a clinically relevant brain region that is also favourable to MRS
- ⚠ Consider partial volume effects when using water scaled quantification
- ⚠ Account for age and sex in study design or statistical analysis
- ⚠ Record all medications and other potential confounders

4. Data Acquisition

Monitor the quality of aquisition using the inline data display at time of scanning

Export data as individual transients

5. Quality & Reporting

Report signal-to-noise ratio, linewidth, water suppression efficiency, fit quality, and presence of unwanted spectral features

Use checklists to aid reporting such as MRS in MRS[1] MRS-Q[2]

6. Post-Processing

Perform frequency-and-phase alignment of individual transients

1. Lin et al (2021) Minimum Reporting Standards for in vivo Magnetic Resonance Spectroscopy (MRSinMRS): Experts' consensus recommendations. NMR Biomed 34(5) e4484
2. Peek et al (2020) Brain GABA and glutamate levels across pain conditions: A systematic literature review and meta-analysis of 1H-MRS studies using the MRS-Q quality assessment tool. Neuroimage 210, 116532

CHAPTER SIX

Discussion

DISCUSSION

The findings of the studies reported in this thesis achieved both of the main aims 1) to advance the understanding of the role of GABA in migraine (and pain conditions), and 2) to improve the standardisation of methods for quality assessment, accurate measurement and the reporting of GABA in studies using MEGA-PRESS. In this Discussion the term GABA will be used when referring to the neurotransmitter Gamma-aminobutyric acid, or concept involving the neurotransmitter. The MRS measurement of GABA will be referred to as GABA if a macromolecule-suppressed technique has been used, otherwise the term GABA+ (GABA+ macromolecules) will be used to acknowledge that the GABA signal from a conventional MEGA-PRESS sequence also contains signal from macromolecules. Where evidence is from studies using both macromolecule suppressed and conventional sequences the term GABA/GABA+ will be referred to.

The body of work contained in this thesis has progressed the understanding of the involvement of GABA in migraine in several key ways. The initial finding was that levels of GABA/GABA+ in the brain of people with migraine potentially differ from other pain conditions and thus could represent a unique biomarker or mechanism of migraine (Chapter 2). This finding suggested that GABA levels should be investigated by directly comparing different pain populations in a single study to confirm whether this observation was unique to migraine or present in other headache or pain types. By conducting a cross-sectional study, levels of GABA+ (GABA+ macromolecules) were found to be increased not only in migraine, but also in other headache (whiplash-headache) and pain conditions (low back pain). This finding suggested that elevated GABA+ levels were not a unique biomarker or

mechanism of migraine but more reflective of pain in general (Chapter 3). Finally by following people with migraine longitudinally, an increase in levels of GABA+ over time was found to be associated with a decrease in clinical measurement of pain and disability demonstrating GABA+ levels were sensitive to change in pain status (Chapter 4).

The lack of standardisation for GABA measurement in combination with the rapidly developing nature of the field of MRS has resulted in a large variety of sequences and parameters being used to measure GABA. Prior to this thesis there was no formal method for assessing the quality of MRS parameters utilised, despite the significant impact on reliability of GABA measurement. This observation prompted the development and application of a quality assessment tool (the MRS-Q in Chapter 2). The lack of standardisation in the field was further addressed through the development of an evidence-based, clinically-orientated guideline that built on the domains included in the MRS-Q. The guideline was robustly developed through both evidence synthesis, and the independent assessment of an external expert panel consisting of 21 internationally renowned MRS experts. The result was a guideline consisting of 23 user-friendly recommendations. The guideline will also assist those new to the field of MEGA-PRESS to accurately measure GABA in clinical and research populations (Chapter 5).

Advancing understanding of the role of GABA in migraine and pain

Elevated levels of GABA+ have been reported in people with migraine compared to controls using a GABA optimised sequence, but elevated GABA+ levels have not been observed in people with other pain conditions. This initial evidence suggested that elevated GABA+ levels may represent a unique biomarker or mechanism involved in migraine (Chapter 2). However, the studies included in the systematic review in Chapter 2 generally compared pain populations with control populations, rather than directly comparing GABA/GABA+ levels

between people with pain conditions. This prompted a direct comparison of GABA+ levels across headache and pain conditions in a cross-sectional study (Chapter 3). The finding that GABA+ levels were elevated in all pain conditions compared with controls, challenged the initial hypothesis that elevated GABA+ levels were unique to migraine (Chapter 3 vs 2). The elevated GABA+ levels observed across pain conditions therefore appear to more likely reflect an aspect of pain common across chronic headache and pain conditions rather than being a migraine-specific phenomenon. To further test this hypothesis, the change in GABA+ levels over time was measured in one pain group (migraine), and correlated with changes in clinical presentation (Chapter 4). Here, an increase in GABA+ levels over time was associated with a decrease in pain and disability over time, supporting the notion that GABA+ levels are likely to reflect a change in pain status. Finally, these changes in GABA+ levels over time were observed in the anterior cingulate cortex (ACC), a brain region understood to have a role in pain modulation[1-3]. The findings, considered together, support a hypothesis that elevated GABA+ levels are reflective of a chronic pain state, specifically increasing as pain decreases, rather than reflecting a migraine-specific mechanism.

The findings of this thesis challenge prior hypotheses that proposed elevated GABA levels were associated with the migraine-specific mechanism of cortical spreading depression. Prior authors proposed that elevated GABA levels could be responsible for the inhibition that occurs following the wave of cortical excitation during the process of cortical spreading depression[4-6]. However, if elevated GABA/GABA+ levels were associated specifically with cortical spreading depression, then increased GABA+ levels would not have been expected in other pain conditions such as whiplash-headache or low back pain as found in Chapter 2.

The elevated GABA+ levels observed across pain conditions in this thesis have yet to be reproduced in other studies. While recent work has corroborated the finding of elevated GABA/GABA+ levels in people with migraine[7,8], there have yet to be any further

GABA/GABA+ optimised studies in people with low back pain or whiplash-headache. Further, there have been no addition head to head comparisons of GABA/GABA+ levels across pain conditions. Therefore, additional work to assess the reproducibility of the elevated GABA/GABA+ levels across pain conditions in now required.

The recent study that reproduced elevated GABA levels in people with migraine did so using a macromolecule-suppressed sequence[7]. Findings from this study interpreted together with evidence from Chapter 3 suggest that GABA and GABA+ measurements are likely to reflect the same construct. The use of macromolecule-supressed sequences is an area of debate within the field of MRS[9]. While it is considered that a macromolecule-suppressed sequence is more specific for the measurement of GABA, it is also more sensitive to scanner instabilities and therefore less reliable[10]. A more stable method, which was used in this thesis, is to acquire GABA+ macromolecules and report it as GABA+[9]. Use of either approach has been accepted within the field, however, confusion remains where studies use conventional MEGA-PRESS, which does not separate the macromolecule signal from the GABA signal, yet report it as GABA. In this case caution needs to be applied in the interpretation of the results of the study, particularly if macromolecular changes could be driving between-group, or time-point differences.

The elevated levels of GABA+ observed across pain conditions (Chapter 3) were not anticipated and challenge the long-standing hypothesis that chronic pain is the result of '*loss of inhibition*'. Findings from this thesis are the first to use a GABA+ optimised sequence to study posterior cingulate gyrus (PCG) GABA+ levels in people with low back pain, and whiplash-headache. The results from Chapter 3 appear to refute those of previous studies, and one recent study. These studies observed a reduced level of GABA+ (reported as GABA) in the thalamus and medial prefrontal cortex of people with neuropathic pain[11-13], the insula of people with fibromyalgia[14], and the ACC of people with chronic pelvic pain[15]. Collectively,

the findings of these studies suggested that the lower GABA level in the pain groups reflected a *'loss of inhibition'*, a theory that has fundamentally underpinned the rationale for studying the GABAergic system in chronic pain. Historically the theory suggests that a lower level of inhibitory GABA would lead to an imbalance between inhibition and excitation resulting in a state of cortical hyperexcitability[12,14,16] and thus chronic pain.

The elevated GABA+ levels observed across pain conditions in this thesis (Chapter 3), appear to challenge the theory of *'loss of inhibition'*. However, evidence suggests the resulting effect from a change in GABA levels can be overall inhibitory or excitatory dependent on the brain circuit within which it operates. While GABAergic neurons in themselves have an inhibitory effect, the cascading effect of axonal firing within a circuit might be increased or decreased. Therefore, the resulting output will be dependent on whether GABA is working in inhibitory or faciliatory circuits within or between regions being investigated[17]. The complex relationships between brain regions, circuits and neural pathways cannot be explored using MRS alone. However, gaining an understanding of the relationship between change in GABA levels and change in clinical characteristics of pain over time (Chapter 4) can further elucidate the role of GABA in pain conditions.

Finding an association between increasing GABA+ levels and decreasing pain over time (Chapter 4) provides a new insight into the interpretation of previous studies. Traditionally elevated GABA+ levels observed in people with migraine compared to controls were considered aberrant, and a likely cause of migraine[4,7,18,19]. As a result it was proposed that future attempts to improve outcomes in people with migraine should be aimed at reducing the elevated GABA+ levels to the level observed in healthy controls. However, given the cross-sectional nature of these studies, vital information regarding the nature of this relationship was missing. The longitudinal design elucidated this relationship by observing change in both brain neurochemistry and migraine status over time. The finding of an inverse relationship

challenged the interpretation of previous studies that suggested elevated GABA+ levels were a cause of migraine. Instead, this finding suggests that the role of elevated GABA+ levels is an adaptive mechanism that attempts to suppress pain. Consequently, reducing elevated GABA+ levels through targeted treatment in people with migraine or chronic pain conditions may not reduce pain and disability as formerly anticipated.

The PCG and ACC may be key regions to scan based on their role in modulating pain and their favourability to MRS acquisition. Findings from this thesis provide new evidence that GABA+ levels in the PCG are elevated in people with pain compared to controls. The posterior brain regions, such as the PCG and occipital lobe, have been scanned more frequently in migraine than in other pain conditions. The rationale is that high-quality spectra can be produced from these regions, given their large and homogenous nature, and the localisation of the visual cortex and other retinopic areas proposed to be associated with migraine with aura[20-22]. However, given that the PCG has not been fundamentally associated with pain modularity pathways, GABA levels in the PCG of those with other non-headache chronic pain conditions have yet to be established. This thesis, therefore, provides new evidence to suggest that elevated GABA+ levels are present in the PCG across pain conditions and confirms that high-quality MRS data can be obtained from this region in chronic pain populations. Therefore, the PCG may present a region of interest in future studies of chronic pain.

The observed association between increased GABA+ and decreased symptoms in migraine, and high-quality data produced from the ACC suggests the ACC is also a favourable region for further investigation in future studies of migraine and pain. GABA/GABA+ levels in the ACC of people with pain conditions have been widely investigated, unlike in the PCG. The rationale for studying GABA levels in the ACC has been based on the large body of evidence indicating that the ACC has a role in pain modulatory circuits of the brain[1-3]. The ACC is

technically a more challenging region than the PCG from which to acquire MEGA-PRESS data, given the region's close proximity to sinus, bone and dura. The proximity of these structures risks unwanted artefacts, which in turn could challenge the reliability of GABA measurement in this region[23,24]. However, evidence from this thesis demonstrates that high-quality MEGA-PRESS data can be acquired from the ACC in people with chronic pain using an angulated rectangular voxel aligned parallel to the superior anterior border of the genu of the corpus callosum (Figure 1). Taken together, the findings from this thesis demonstrate the possible involvement of the GABAergic system in both the ACC and PCG of people with pain. Furthermore, it appears that we can confidently accept these findings, given the high-quality of spectra obtained from these regions (Chapter 3, Supplementary Table 1).

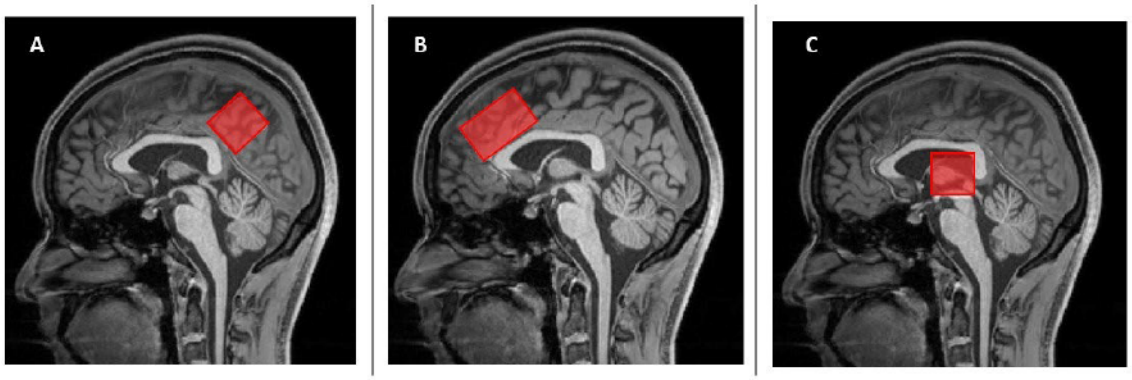

Figure 1: Demonstrating the positioning of the voxel used to acquire data in this thesis for A) the PCG, B) the ACC and C) the thalamus. *Reprinted from The Journal of Pain, 22 (12), Peek et al. Increased GABA+ in people with migraine, headache and pain conditions- a potential marker of pain, 2021[25], through open source licence.*

Other regions with a proposed role in chronic pain are the thalamus, insula and amygdala, however these regions are not naturally favourable to MRS acquisition. In this thesis the thalamus was investigated because of its proposed role in migraine[26,27] and pain[12,28,29]. However, the quality of the data acquired from the thalamus was not comparable to that from

the ACC and PCG despite using the same parameters (Chapter 3, Supplementary Table 1). Results from this thesis corroborated those of other studies that demonstrated regions such as the occipital lobe, and the ACC were significantly more favourable for data acquisition using MRS than other more challenging brain regions, such as the thalamus[30,31]. The risk of comparing data from the thalamus to other regions more favourable to MRS is that GABA levels are more likely to be underestimated in regions that return poor signal-to-noise ratio. Therefore, consideration should be given to the quality of data produced from each brain region when interpreting and comparing studies of GABA[32]. In some instances high-quality data can be obtained from more challenging regions using specific shim optimisation and acquisition parameter adjustments. However, these adjustments have yet to become standard practice and have not been applied in pain studies to date[33-36].

In summary, elevated GABA levels appear to be present across pain conditions and associated with changes in pain status. High-quality data was produced from both the ACC and the PCG, however, MRS data using the same acquisition parameters in the thalamus produced less reliable data. The difference in quality highlights the importance of selecting the appropriate acquisition parameters for the region of interest to produce reliable data. Therefore, the reliability of conclusions drawn from meta-analyses and individual MRS studies in the field are largely underpinned by the quality of methodology used for data acquisition. The development of a quality assessment tool and standardisation of methods to accurately acquire and report GABA data therefore form a research priority.

Development of methods for quality appraisal and improving the quality of GABA measurement using MEGA-PRESS

The two new tools reported within this thesis were developed to fill a gap, given there was no formal method to assess the quality of MRS methods, and no standardised methods for

GABA measurement or reporting. The MRS-Q presented in Chapter 2 allows critical appraisal of the body of evidence to date, allowing accurate conclusions to be drawn with regard to what is known about brain neurochemistry in pain conditions. The Comprehensive Guide to MEGA-PRESS for GABA measurement presented in Chapter 5 built upon this quality assessment tool to provide an evidence based guideline to facilitate the planning of future studies, through providing standardisation for the measurement of GABA using MEGA-PRESS. Here we discuss the application of the tools presented in this thesis to facilitate improvement in: i) quality assessment; ii) planning and design; and iii) study reporting. The translational framework used to develop the guidelines is also discussed, including the engagement of end users and experts ensuring credibility and facilitation of direct implementation into practice.

The development of a quality assessment tool to critically appraise MRS studies

The MRS-Q provides the first quality assessment tool for studies of MRS. The MRS-Q is a 12-point evidence based checklist that establishes the quality of MRS studies across three domains considered critical for determining the quality of GABA acquisition. The domains are: i) sequence / acquisition parameters; ii) the reporting of quality metrics; and iii) the reporting of study design / analysis procedures. Evaluation of studies based on these criteria determines data quality and consequently the accuracy of the data.

The prior absence of a tool to critically appraise MRS methods of data acquisition has substantially limited the confidence with which conclusions of work to date can be interpreted. The absence of quality assessment is particularly apparent when appraising the literature and synthesising evidence. Before the MRS-Q was developed, reviews of MRS studies did not include a quality appraisal stage in their methodology, instead quality

appraisal was *ad-hoc* without a systematic process, and usually was opinion-based and confined to the discussion section of a manuscript [37-39]. As a result the conclusions of earlier work, including meta-analyses of MRS studies should be interpreted with caution[40].

The importance of quality appraisal in MRS studies is critical, given the substantial impact that study design and methods have on the quantification of GABA[41]. For example, previous studies that used sequences or parameters now deemed sub-optimal to detect GABA, reported that GABA levels were either reduced or did not differ between people with migraine and control participants in the occipital lobe and ACC[42,43]. Conversely, four recent studies that used methods optimised to detect GABA/GABA+ reported an elevated level of GABA/GABA+ in the PCG in people with migraine compared to controls [4,7,8,44]. While these differences might be explained by the different brain regions studied, it is also likely that the quality of the data acquired affected the quantification of GABA[30,31]. Specifically, lower GABA levels have been reported in instances where insufficient signal has been obtained due to small voxel size, or insufficient transients, compared to levels recorded under optimal conditions[31,41,45]. Therefore, ensuring that meta-analyses of MRS studies formally acknowledge the quality of the data acquisition methods is a priority that will enable appropriate interpretation of the evidence, thereby improving the certainty of the conclusions drawn.

The MRS-Q addressed the gap in the field by providing a tool to assess the quality of MRS studies across parameters considered critical for attaining high-quality data. The MRS-Q has already been implemented in a meta-analysis of brain neurochemicals in alcohol use disorder (Appendix 4) and has been well received within the field (Commentary in NeuroImage, 2021[46] Chapter 2 supplementary material; and GABA editing school, 2019). It appears, therefore, that the MRS-Q is well positioned for adoption into future practice as a quality assessment tool.

A Comprehensive Guide to MEGA-PRESS to assist planning of studies measuring GABA

The Comprehensive Guide provides recommendations to facilitate the planning of studies that use MEGA-PRESS to accurately measure GABA in clinical populations. The Comprehensive guide consists of 23 evidence-based recommendations across six domains considered vital for acquiring high-quality MRS data. The development of the guideline was in response to the lack of standardisation within the field, which has led to the varying quality of research into GABA. The guideline was developed using a translational framework to ensure credibility and facilitate translation.

The lack of standardisation in GABA measurement, is a recognised issue within the field of MRS, which has led to a wide variety of methodology and acquisition parameters being used to study GABA. One problem with the lack of agreed standards is that it provides a substantial barrier to learning best practice methods for those new to the field or clinician-researchers without direct access to MRS expertise[9]. The lack of standardisation is particularly relevant when investigating clinical populations, where the production of high-quality data relies on the balance between what is pragmatically possible and the laws of physics. The wide variety of acquisition parameters used in MRS studies, observed in Chapter 2, demonstrates that suitable data acquisition parameters for GABA have yet to be standardised. The lack of standardisation is further compounded by poor standards of reporting, and failure to report the rationale underpinning choice of acquisition parameters. Together, these practices provide challenges to those new to the field of MEGA-PRESS, including the need to determine which parameter adjustments can be made without threatening data quality. This thesis therefore provides those new to the field with the rationale to underpin vital decisions required for planning an MRS study in a clinical population.

The Comprehensive Guide assists those new to the field of MEGA-PRESS to consider factors beyond the data acquisition parameters highlighted by the MRS-Q, which might also impede data quality. It is well recognised in recent consensus documents[47] and methodological publications[41,48] that the quality of MRS data is highly dependent on the acquisition parameters selected. However, critical MEGA-PRESS idiosyncrasies, beyond acquisition parameters, had yet to be comprehensively documented.

The importance of planning an experiment using MEGA-PRESS, beyond the use of suitable *acquisition parameters,* is supported by individual methodological studies. Idiosyncrasies specific to MEGA-PRESS that can impact data quantification have been documented to include conducting a MEGA-PRESS study following gradient heavy sequences [10,47,49-53] and imaging a brain region not favourable to MRS[31,54-66]. Furthermore, failure to account for potential confounders of GABA levels may also threaten the reliability of results[54,67-71]. While each of these caveats can have significant effects on the results of a study, the absence of a comprehensive guide documenting these caveats in a single resource has led to the potential for overlooking them at the planning stages.

To develop the first beginner-friendly comprehensive guide to MEGA-PRESS for the measurement of GABA, we therefore collated evidence, including consensus documents and methodological publications, to provide recommendations across six domains that experts considered essential for producing high-quality data: *Acquisition parameters, practicalities, confounders, data acquisition, quality and reporting, and post-processing*.

The guideline development process resulted in 17 of the 23 (74 %) recommendations having both a high GRADE of evidence, and a high level of expert panel agreement. Of the remaining recommendations 2 recommendations (4 %) had a high GRADE of evidence but low expert panel agreement, 4 recommendations (17 %) had a low GRADE of evidence and

high expert panel agreement, and 2 recommendations (4 %) had a low GRADE of evidence and low expert panel agreement. Overall we can have confidence that a high proportion of our recommendations were formed from high-quality evidence, and were supported by experts.

The recommendations formed from low-quality evidence were mainly related to controlling for confounders of GABA. Therefore only conservative recommendations could be made until further high-quality research has been conducted. The two recommendations that did not gain a high level of expert panel agreement were *order of slice selective gradients*, and *water reference scans for eddy-current correction and quantification*. These two recommendations highlight aspects of MRS that have yet to become standard practice in the MRS community. The inclusion of such aspects in the guideline further the educational value of the resource in translating new knowledge into practice.

In summary, the evidence-based approach taken to develop the guideline resulted in a comprehensive resource that provides recommendations on all aspects of planning and conducting a study using MEGA-PRESS to accurately measure GABA. Further, the extended document provides a clear rationale to inform those new to the field of the impact that parameter adjustments and study design modifications may have on data quality (Chapter 5, Supplement 4).

The improvement in reporting of MRS

Together the MRS-Q and the Comprehensive guide can also improve the quality of reporting in studies of MRS. The MRS-Q was initially designed to establish the quality of MRS acquisition through use of a 12-point checklist. In conducting the appraisal of MRS studies, it also became apparent where vital elements of the study had not been sufficiently reported. The application of the MRS-Q in Chapter 2 exposed the poor level of reporting within the

field, a problem also recognised by other groups[72]. Whilst the poor level of reporting might not reflect the methods used in each study it precludes the reproducibility and critical appraisal of the quality of the results [73]. Improvement in reporting in MRS is, therefore, a priority[72].

The use of the MRS-Q as a reporting checklist was identified by an external group and was adopted into the consensus guideline: *Minimal Required Reporting Standards in MRS (MRS in MRS)*[72] (Chapter 2-Supplementary materials). The benefits of using this checklist include assisting authors to report vital information, and enabling journal reviewers and editors to easily confirm sufficient reporting in submitted manuscripts. Prior to the MRS-Q the only reporting checklist developed in magnetic resonance physics was for the use of functional MRI[74] , however, that checklist has yet to be widely implemented. In contrast, there are two well established reporting checklists for clinical research: the PRISMA[75] checklist for systematic reviews, and the CONSORT statement[76] for randomised controlled trials. Use of these two checklists is standard practice, primarily because they are now generally required for publication.

Preliminary evidence suggests that the MRS-Q is suitable for implementation as standard practice and appears to have wide acceptability within the field. Firstly, the MRS-Q was adopted by an expert consensus guideline to improve reporting of MRS studies[72]. Secondly, more than 95% of the international expert panel (reported in Chapter 5) agreed to the recommendation to use the MRS-Q or *MRS in MRS*[72] (which recommends the use of the MRS-Q) when reporting MRS studies.

The use of a translational framework to improve implementation

The use of a translational framework to develop the Comprehensive guide to MEGA-PRESS was unique for the field of MRS. To date, guidelines in MRS are typically developed through

expert consensus. The use of a translational framework to develop the guideline ensured credibility and facilitated translation. The credibility of the guideline was achieved through conducting a robust evidence synthesis and quality assessment of all current knowledge of MEGA-PRESS methodology. To encourage translation and implementation, the framework recommends the engagement of a wide range of end-users from the initial stages of guideline development.

The Comprehensive Guide to MEGA-PRESS engaged end-users including a PhD candidate studying MRS, two MRS tutors, three clinician researchers and a research radiographer. The engagement of end-users ensures that the guidelines are fit for purpose and user-friendly, a key step that has been shown in translational research to improve the uptake of guidelines in clinical practice[77-79]. In addition, engaging researchers with expertise in guideline development and translation, and MRS expertise in the working group, ensures both rigor of the guideline development process, and credibility of the final guideline. Engaging an independent expert panel through a modified-Delphi process provided a rigorous method to gauge the agreement and acceptability of the guideline without introducing personal bias, a stage also shown to facilitate translation into standard practice[80].

The expert panel consisted of 21 renowned MRS experts from 15 universities across eight countries. The expert panel, alone, oversee research in more than 45 research sites in Australia, America, Europe and the UK, and therefore provide a substantial international network to facilitate widespread implementation of the guideline into practice. The use of the robust translational framework to develop the guideline, therefore, increases the likelihood that the guidelines will be translated to practice with the potential to become a seminal resource for the accurate measurement of GABA.

Future Directions

The future study of the involvement of GABA in pain

The findings of this thesis significantly expand what is known about the involvement of GABA in migraine and pain. Together, the findings reported in this thesis allow for advances to reliably identify neurochemical profiles such as patterns of neurochemical alterations across brain regions to: inform diagnosis, develop mechanism-specific individualised treatment, and identify those likely to respond to specific treatments.

The next steps in elucidating the role of GABA in pain would be to i) determine if the elevated GABA levels observed across pain conditions in this thesis are reproducible; ii) to determine if GABA levels change in response to change in pain status over time in people with chronic pain conditions; and iii) to establish if GABA levels individually or as part of a neuroimaging profile can predict individuals likely to respond to treatment.

The first step to determine whether the elevated GABA levels observed across pain conditions are a biomarker or part of a pain mechanism, is to reproduce the findings. The findings need to be independently reproduced in a larger cohort of people with varying chronic pain conditions. If elevated GABA levels were a reliable marker of pain it would be expected that the results would be consistently reproduced across pain conditions in various settings. However, studies using a GABA optimised sequence have yet to be conducted by independent researchers investigating participants with low back pain, whiplash or other pain conditions.

Secondly, to determine whether *change* in GABA level reflects *change* in pain status in chronic pain conditions. The longitudinal study presented in Chapter 4 needs to be conducted in people with other chronic pain conditions. Should elevated GABA levels be a biomarker or

part of a mechanism of pain it would be anticipated that as observed in migraine (Chapter 4), GABA levels should also change to reflect a change in pain status across other chronic pain conditions.

Finally, to determine if GABA levels form part of a neuroimaging profile that predicts treatment response, a multimodal controlled trial would be required. The study of GABA, using MRS, provides information on neurochemical levels in specific brain regions. However, the complexity of neural networks and pain pathways in the central and peripheral nervous system requires a more comprehensive and multimodal approach to better understand the context of the neurochemical observations. Therefore, a study that uses multiple imaging methods covering central and peripheral mechanism is required.

In addition to using multiple imaging techniques the study would need to be suitably powered for multiple group comparisons, longitudinal in design and use a control group. The post-hoc analysis (Chapter 4) provided preliminary evidence that GABA levels may change depending on the treatment provided or response to treatment. We observed that GABA levels increased more in those who received CGRP-mAbs than those who received OnabotulinumtoxinA (Chapter 4). However, given that those treated with CGRP-mAbs also experienced better recovery than those who were treated with OnabotulinumtoxinA it remains unclear as to whether the treatment or treatment response drove the increase in GABA levels. Therefore, a study sufficiently powered to compare the neuroimaging profile of those who respond to treatment with those who do not is now required. Results from such a study would further clarify the potential role of GABA levels as i) a therapeutic target for treatment; and ii) a part of a responder profile. The systematic progression of research to better understand neuroimaging profiles of pain and treatment responsiveness consequently provide steps towards the provision of individually targeted treatment to improve outcomes of those with chronic migraine and pain.

In addition to exploring neuroimaging and neurochemical profiles, my post-doctoral studies will also advance the line of enquiry through investigating correlations between neurochemical profiles and clinical measures of pain sensitivity and salivary measures of GABA. Work underway builds on work completed as part of this thesis, with the intention of identifying a battery of clinical tests that can be used as a surrogate marker of potentially detrimental brain neurochemical changes. The aim of these studies is to design tests amenable to use within the clinic environment without relying on expensive and time consuming MRI procedures. These studies will provide further steps toward the better understanding of GABA levels in pain and in the future might aid the provision of personalised, targeted treatments to improve outcomes for people with migraine and other chronic pain conditions.

Monitoring the uptake of the MRS-Q and Comprehensive Guide to MEGA-PRESS

To gauge the impact of the MRS-Q and The Comprehensive Guide to MEGA-PRESS, uptake needs to be monitored. The gold standard for the implementation of the MRS-Q as a reporting checklist would be it becoming a requirement for publication. *NMR Biomedicine* has recently delivered a special edition that features the consensus document, and recommends the use of the MRS-Q when reporting MRS studies (Chapter 2, Supplement 1). Therefore, approaching such a journal to discuss a trial implementation would be a logical next step in implementation of the MRS-Q.

The early phase of the implementation plan for the Comprehensive Guide (which also recommends the use of the MRS-Q as a reporting checklist) is to encourage its use in the 45 research sites where the expert panel members provide input. In addition, the guideline will be disseminated using both passive and active implementation strategies such as presentations at International conferences and interactive workshops delivered at educational

institutions by opinion leaders, methods shown to improve uptake of guidelines in other fields[81,82]. One method to evaluate uptake would be to send a questionnaire to the expert panel (21 international experts) and their students to evaluate the uptake of the guideline and any barriers to implementation at 1 year post dissemination.

Future development of the Comprehensive Guide to MEGA-PRESS

When designing the Comprehensive Guide, several gaps in the field were identified. The main gap informing future research required in the field, was the lack of high-quality evidence for the investigation of potential confounders of GABA measurement. To date, only small proof of concept studies exist, providing weak evidence that factors such as caffeine or nicotine intake and phase of menstrual cycle may impact GABA levels. Due to the paucity of research in these areas the guideline could only provide the weak recommendation to consider recording potential confounders of GABA levels until further high-quality evidence is available. Therefore, additional robust investigation of the effect of potential confounders on GABA levels is now required to increase the strength of the recommendation.

The focus of the Comprehensive Guideline was on acquiring high-quality data, however, it does not detail the next stages of data processing and analysis. Similar to data acquisition the data analysis stage lacks standardisation within the field. The MRS-Q highlights the need to record analysis procedures due to the impact these can have on data interpretation. Therefore, future research using a translational framework to develop guidelines for the analysis of GABA data would be beneficial in bringing standardisation to the field.

Concluding Statement

The findings of this thesis significantly progress the field in terms of the understanding of GABA in migraine and pain. Findings suggest that elevated GABA+ levels were not a unique

biomarker or mechanism of migraine but more reflective of pain in general. This was further supported by the relationship between an increase in GABA+ level and improvement in pain status. Research is now required to determine the relationship between *change* in GABA levels and *change* in pain status in other pain conditions, and explore GABA as part of a neurochemical profile which may further elucidate the role of GABA in pain.

The MRS-Q and Comprehensive Guide to MEGA-PRESS has the potential to shape future research into GABA, through providing methods to assess quality and guide reporting in MRS studies. In addition to presenting guidelines which present agreement on what constitutes best practice for the accurate measurement of GABA using MEGA-PRESS. Together, evidence from this thesis allows for the progression of research using accurate methodology to further elucidate the role of GABA as a potential biomarker, therapeutic target or as part of a responder neurochemical profile. Enhancing our understanding of GABA levels in pain could provide the essential next steps towards the development of mechanism-specific treatment to improve outcomes in those with chronic migraine and other chronic pain conditions.

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APPENDICES

Appendix ONE: Study Protocols

Appendix TWO: Ethics approval letters

Appendix THREE: Media Engagement

Appendix FOUR: Related project not forming part of the thesis

APPENDIX ONE

Study Protocols

Appendix one includes

1 Recruitment posters for Chapters 3-4
Patient information and consent forms
3 Study recruitment screening forms
Self-reported history of pain form
Baseline questionnaires
6 Follow-up questionnaire
7 MRI screening form (Westmead Hospital)
8 Day of scanning questionnaire
9 MRS run sheet

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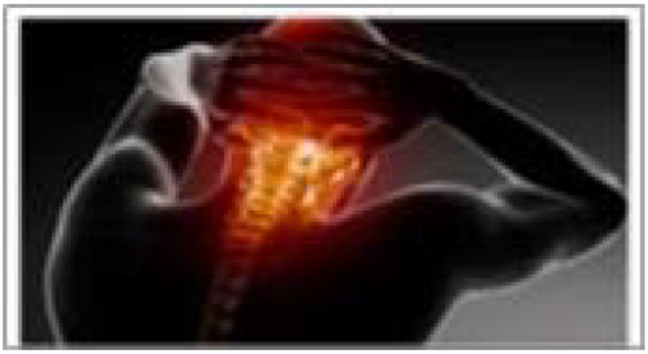
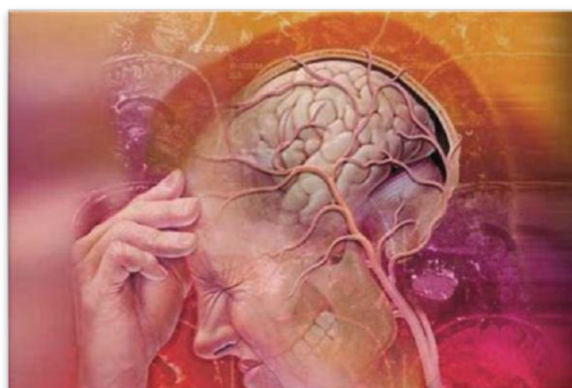


Headache after Whiplash?
Lasting over 3 months
18-65yrs old?

Find out more about your pain

Free brain MRI
Free clinical assessment
Full clinical report



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18-65yrs old?

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Healthy and Happy?
18-65yrs old?

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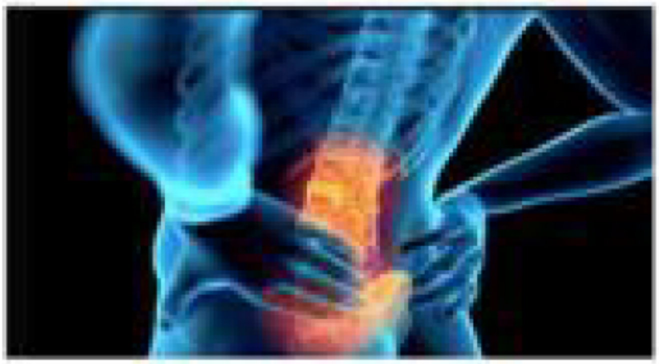

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Lasting over 3 months?
18-65yrs old?

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Discipline of Physiotherapy
Faculty of Health Sciences

Brain Neurochemicals in Headache and other Pain conditions

Participants wanted

We are using brain scans (MRI) to measure changes in the brain related to pain or headache. We are looking for differences between people who have migraine, headache due to whiplash, low back pain and those who do not have headaches or pain.

Your involvement will help us better understand these painful conditions and to design effective treatments for people with them! MRI is not suitable for everyone. We are seeking participants who have not had metal implants, surgery to the neck, or suffer claustrophobia.

Participants will answer some questionnaires (online or paper; 20 minutes), attend a clinical assessment session (1.5 hours) and undergo MRI scan of the brain (1 hour). The assessment comprises of an interview to determine the features of the painful condition and a series of clinical tests to be conducted by a physiotherapist to measure the mobility and muscle function of the neck and back, including ultrasound imaging. Clinical testing and MRI scanning will be conducted at Westmead Physiotherapy Department, Westmead Hospital on Hawkesbury Road in Westmead. You may be asked to return for a repeat scan in 3-6 months.

If you are interested in taking part in the study, whether you have headaches or low back pain or if you are pain-free, or if you seek further information, please contact:

Aimie Peek (apee6909@uni.sydney.edu.au / telephone 9351 9534 (Faculty of Health Sciences)
Trudy Rebbeck (trudy.rebbeck@sydney.edu.au / telephone 9351 9534 (Faculty of Health Sciences)

This research project has been approved by the Western Sydney Local Health District Human Research Ethics Committee.

Participant Information Sheet/Consent Form

Health/Social Science Research - *Adult providing own consent*

(University of Sydney/Westmead Hospital)

Title	An investigation of brain neurochemicals in migraine, whiplash associated disorder with persistent headache and low back pain
Short Title	Brain neurochemicals in headaches and other pain conditions
Protocol Number	HREC 5354
Coordinating Principal Investigator	Dr Trudy Rebbeck
Associate Investigators	Dr Andrew Leaver; Mrs Maria Eliza Aguila; Mrs Aimie Peek; A/Prof Karl Ng; Ms Sheryl Foster; Dr Sushil Bandodkar; Prof Kathryn Refshauge; Prof Graham Galloway; Prof Michele Sterling
Location	Westmead Hospital and The University of Sydney, NSW

Part 1 What does my participation involve?

1 Introduction

You are invited to participate in this research project called *An investigation of brain neurochemicals in migraine, whiplash associated disorder with persistent headache and low back pain*. You have been invited because you have been experiencing migraine, headache due to whiplash, or low back pain, or you are pain-free.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the processes involved with taking part. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or local health worker.

Participation in this research is voluntary. If you don't wish to take part, you don't have to.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- Understand what you have read
- Consent to take part in the research project
- Consent to be involved in the research described
- Consent to the use of your personal and health information as described.

This Participant Information Statement is yours to keep.

2 What is the purpose of this research?

Headache and persistent musculoskeletal pain (due to whiplash or low back pain) are health problems in Australia. This is partly because scientists and clinicians do not always know the causes of pain or headache. One of the proposed causes is that brain chemicals may be different in people with these headache or pain conditions, but we have yet to demonstrate high quality evidence for this. The purpose of this research is to measure these brain chemicals using a magnetic resonance imaging (MRI) scanner and a saliva sample to see if they differ between headache and pain conditions. This research will help us understand the underlying causes of headache and pain, potentially leading to better treatment.

3 What does participation in the research involve?

You will be asked to answer some questionnaires (online or paper; 30 minutes), attend a clinical assessment session (1.5 hours) and then, at a later time, undergo MRI scan of the brain and provide a saliva sample in a specially designed, discrete collection pot (45 mins).

Firstly, we will ask a few questions over the phone to make sure you are eligible and it is safe for you to enter the study. Then you will need to complete questionnaires online or on paper about your headache, experiences about pain, general and psychological health. At the clinical assessment, a trained physiotherapist will perform tests to assess your neck muscle performance, spinal mobility and pain sensitivity. Muscle performance assessment involves a video of your performance of exercises and real-time ultrasound imaging. Pain sensitivity tests involve applying a sensor to your neck, arm and leg and reducing the sensor's temperature from 30 degrees to 5 degrees to determine if this is painful. Other tests involve gently pressing a thin wire against your neck or arm then asking you about pain. Finally, we will apply a pressure cuff to your arm and measure your pressure tolerance using a laboratory pressure sensor. The tests are looking for the first moment you experience pain and therefore will be stopped the moment you say it is painful. There should be no lasting pain from any of the pain sensitivity tests.

You will then be required to attend Westmead Hospital on Hawkesbury Road in Westmead to undertake a series of brain scans using an MRI scanner. The scans will measure the concentration of chemicals in your brain, as well as the structure of your brain. Some people may experience some mild anxiety when placed in the MRI scanner. However, the imaging staff involved with this study are trained to deal with these issues and will be available for immediate support. We will also collect a saliva sample at the same time, analysing the same chemicals as those measured by the MRI Scanner.

You will be asked to keep a weekly pain diary (online or paper; 10 minutes) over the next 6-12 months and may be contacted between 3 to 12 months following the first scan to take a repeat saliva sample and MRI scan.

This research has been designed to make sure the researchers interpret the results in a fair and appropriate way and avoids researchers or participants jumping to conclusions.

There are no costs to you associated with participating in this research, nor will you be paid. However, we will reimburse your for your travel and parking associated with the research project visit (up to $50 per visit).

4 Other relevant information about the research project

The research will be conducted in three sites in two states (New South Wales and Queensland). Researchers from these sites are collaborating to undertake this research. A total of 80 subjects with migraine, headache due to whiplash, low back pain and people who are not experiencing any pain will be enrolled from these sites.

5 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine care, your relationship with professional staff or your relationship with the University of Sydney or Westmead Hospital.

6 What are the possible benefits of taking part?

We cannot and do not guarantee or promise that you will receive any benefits from the study. However, the physiotherapist will explain the results of your clinical tests. At your request information can be forwarded to your treating clinicians, which may help with your treatment.

If anything of clinical or health significance is suspected on your MRI scan by the radiologist, the neurologist member of the research team (A/Prof Karl Ng) will review the scans and discuss these with you and your general practitioner.

7 What are the possible risks and disadvantages of taking part?

There are minimal risks or potential discomfort associated with the clinical tests and MRI scanning in this research.

Some of the clinical tests may produce mild discomfort or exacerbation of pain. The physiotherapists are highly trained and they will perform tests in a way to minimise discomfort. They will also constantly ask you for feedback during the test procedures to ensure discomfort is avoided or minimised. They will also communicate with your general practitioner if any adverse reaction occurs.

A risk associated with the MRI scanning is the ability of the high magnetic field of the scanner to attract metals. To minimise this risk, we will make sure you have no metals

in your body and will make sure there are no other reasons you should not be scanned (such as claustrophobia). You may also feel anxious in the scanner. You can communicate with the radiologist at any time during MRI scanning, and the procedure can be stopped if needed. MRI scanners are noisy, so you will be provided with ear protection such as disposable earplugs to minimise the noise. Repeating the scan puts you at no known additional risk.

There is a small chance that the MRI scan will detect something of clinical or health significance. If this is the case, the research team neurologist will discuss this finding with you and your nominated primary health care practitioner.

There are no risks to yourself involved with the saliva collection. The researcher will follow health and safety guidelines throughout the collection process.

8 What if I withdraw from this research project?

If you do consent to participate, you may withdraw at any time. If you decide to withdraw from the project, please notify a member of the research team before you withdraw. A member of the research team will inform you if there are any special requirements linked to withdrawing. If you do withdraw, you will be asked to complete and sign a 'Withdrawal of Consent' form; this will be provided to you by the research team.

If you decide to leave the research project, the researchers will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected up to the time you withdraw will form part of the research project results. If you do not want your data to be included, you must tell the researchers when you withdraw from the research project.

9 Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons, although this is unlikely. Potential reasons are unforeseen such as loss of funding or inability of lead researchers to continue participation.

10 What happens when the research project ends?

We will be analysing the results as pooled participant groups. If you wish to be informed about the overall research findings, please contact Dr Trudy Rebbeck, The University of Sydney Faculty of Health Sciences [telephone: (02) 9351 9534]

A report of the research may be submitted for publication, but individual participants will not be identifiable in such a report.

Part 2 How is the research project being conducted?

11 What will happen to information about me?

By signing this consent form, you consent to the research team collecting and using personal information about you for the research project. We may also use your information in a future related research. When your data will be used for a future related research, the future research will be approved by an appropriate ethics committee.

By signing the separate consent form for Tissue storage, you consent to the research team collecting a sample of your saliva, and storing it for further analysis.

Any information obtained in connection with this research project and for any future research that can identify you will remain confidential and will only be used for the purpose of this study. It will only be disclosed with your permission, or as required by law.

The research information that is collected about you during the study will be accessible by the research team. To protect your privacy, these records will be identified not with your name, but with a code, and stored in locked offices in the Faculty of Health Sciences, The University of Sydney. Data that are stored electronically will be protected with a firewall-protected on a password-protected computer.

Your name and other identifying information that can link you to these records will be known only to the principal researcher and to the research team. In accordance with regulatory guidelines, the information collected will be stored for 20 years, at which point it will be destroyed. with exception of the saliva sample which will be destroyed after two years.
It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your express permission.

In accordance with relevant Australian and/or State privacy and other relevant laws, you have the right to access the information collected and stored by the researchers about you. You also have the right to request that any information, with which you disagree, can be corrected. Please contact one of the researchers named at the end of this document if you would like access to your information.

12 Complaints and compensation

If you suffer any distress or psychological injury as a result of this research project, you should contact the Coordinating Principal Investigator, Dr Trudy Rebbeck [(02) 93519534; trudy.rebbeck@sydney.edu.au as soon as possible. You will be assisted with arranging appropriate treatment and support.

13 Who is organising and funding the research?

This research project is being conducted in two states. The principal investigator in NSW is: Dr Trudy Rebbeck at The University of Sydney. The principal investigators in QLD are Prof Michele Sterling and Prof Graham Galloway at University of Queensland. Other members of the research team are Mrs Maria Eliza Aguila, Prof Kathryn Refshauge, Mrs Aimie Peek, and A/Prof Karl Ng at The University of Sydney; Dr Sushil Bandodkar at Children's Hospital at Westmead and Ms Sheryl Foster at Westmead Hospital.

This research has been funded by the Centre of Research Excellence in Recovery Following Road Traffic Injury (CRERTI) and The University of Sydney.

14 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of the Western Sydney Local Health District.

This project will be carried out according to the National Statement on Ethical Conduct in Human Research (2007). This statement has been developed to protect the interests of people who agree to participate in human research studies.

15 Further information and who to contact

The person you may need to contact will depend on the nature of your query. If you want any further information concerning this project or if you have any problems which may be related to your involvement in the project, you can contact any of the following members of the researcher team:

Research contact persons:

Name	Dr Trudy Rebbeck
Role	Coordinating Principal Investigator
Telephone	(02) 9351 9534
Email	trudy.rebbeck@sydney.edu.au

Name	Mrs Aimie Peek
Role	Associate Researcher
Telephone	(02) 9351 9534
Email	Apee6909@uni.sydney.edu.au

For matters relating to research at the site at which you are participating, the details of the local site complaints person are:

Complaints contact person

Name	Patient Advice and Liaison Service (PALS)
Position	Westmead Hospital Patient Representative
Telephone	(02) 9845 7014
Email	wslhd-pals-mail@health.nsw.gov.au

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	WSLHD Human Research Ethics Committee
HREC Executive Officer	Kellie Hansen
Telephone	8890 9007
Email	Wslhd-researchoffice@health.nsw.gov.au

Local HREC Office contact (Single Site -Research Governance Officer)

Name:	Lani Attwood
Position	Research Governance Manager
Telephone	8890 9007
Email	Wslhd-rgo@health.nsw.gov.au

Consent Form - *Adult providing own consent*

Title	An investigation of brain neurochemicals in migraine, whiplash associated disorder with persistent headache and low back pain
Short Title	Brain neurochemicals in headaches and other pain conditions
Protocol Number	HREC 5354
Coordinating Principal Investigator	Dr Trudy Rebbeck
Associate Investigators	Mrs Maria Eliza Aguila; Mrs Aimie Peek; A/Prof Karl Ng; Ms Sheryl Foster; Dr Sushil Bandodkar; Prof Kathryn Refshauge; Prof Graham Galloway; Prof Michele Sterling
Location	Westmead Hospital and the University of Sydney, NSW

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the project without affecting my future care.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signature _____ Date _____

Declaration by Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Researcher† (please print) Aimie Peek

Signature _____ Date _____

† An appropriately qualified member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

Form for Withdrawal of Participation - *Adult providing own consent*

Title	An investigation of brain neurochemicals in migraine, whiplash associated disorder with persistent headache and low back pain
Short Title	Brain neurochemicals in headaches and other pain conditions
Protocol Number	HREC 5354
Coordinating Principal Investigator	Dr Trudy Rebbeck
Associate Investigators	Mrs Maria Eliza Aguila; Mrs Aimie Peek; A/Prof Karl Ng; Ms Sheryl Foster; Dr Sushil Bandodkar; Prof Kathryn Refshauge; Prof Graham Galloway; Prof Michele Sterling
Location	Westmead Hospital and the University of Sydney, NSW

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine care, or my relationships with the researchers or The University of Sydney or Westmead Hospital.

Name of Participant (please print) _____

Signature _____ Date _____

In the event that the participant's decision to withdraw is communicated verbally, the Senior Researcher must provide a description of the circumstances below.

Name of Researcher† (please print) _____

Signature _____ Date _____

Declaration by Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Researcher† (please print) Aimie Peek

Signature _____ Date _____

† An appropriately qualified member of the research team must provide information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

PARTICIPANT INFORMATION AND CONSENT FORM

Study Title: Brain neurochemicals in headaches and other pain conditions

Chief Investigator: Dr Sushil Bandodkar
Department of Biochemistry Department Children's Hospital at Westmead

Request
We ask that you consider giving your permission for storage of a sample of your saliva at Children's Hospital at Westmead for possible use in future research approved by a Human Research Ethics Committee (HREC). You will have previously needed to give consent for collection of the saliva and for it to be used for research purposes.

This form provides you with information to help you decide whether you will allow this. Please take the time to read the following information carefully and discuss it with others if you wish.

What kind of sample will be taken, and how?
A single saliva sample will be taken from your mouth. You will be asked to chew a cotton wad then spit the wad into a specially designed tube which is then then sealed and frozen. This will collect roughly 2ml of saliva.

Possible future use of stored saliva, please pick one of the choices below –

☐ **My saliva may be kept and used in research to learn about, prevent, or treat Migraines and other forms of headaches**

☐ **My saliva may be kept and used in research to learn about, prevent or treat (Migraines and other forms of headaches) or other health problems eg heart disease, mental illness, chronic pain.**

☐ **My saliva may <u>not</u> be used in future research unless researchers contact me to tell me about the study and ask my permission**

☐ **My saliva may <u>not</u> be used in future research. I do not want researchers to contact me about future studies.**

Will the saliva sample be identifiable as mine after it is stored?
The stored saliva sample will not be identifiable as yours.

PARTICIPANT INFORMATION AND CONSENT FORM

Study Title: Brain neurochemicals in headaches and other pain conditions

What will happen to my saliva sample?
Your sample will be stored for up to 2 years at Children's Hospital at Westmead, Biochemistry Department.
We wish to store (or 'bank') the sample for potential, and as yet unspecified, HREC approved research in the future. Not all potentially beneficial future research can be known at any one time, as the need for future research is determined by ongoing developments in the field. If you agree to your sample being stored, you will be asked to sign a specific consent form to store your sample in this way.

How will I know if my samples are being used in the future?
If you agree to your saliva sample being stored for future research, it may only be used for research projects in the future with the approval of a HREC. The HREC will determine whether, or not, your consent should be obtained at that time for a particular research project. However, notifying you and obtaining your consent for specific research may not be possible if the stored sample is not linked to your identifying information.

It will not be possible to provide you with feedback about the findings of potential future research.

Who will have access to my saliva sample once it has been stored?
The custodians charged with ensuring appropriate standards are met in storing and managing the tissue bank will have access to your sample. Researchers involved in research approved by a HREC may also have access to your sample.

Will drug or biotechnology companies be able to use my sample for profit in the future?'
There is the possibility that research involving your saliva sample may result in commercially viable technology or treatments. You will not however be able to claim financial benefit from any discoveries arising from the use of your saliva sample.

How long will my saliva sample be stored?'
Your saliva sample will be stored for 2 years. The sample will be destroyed at the end of this period.

Will I be able to get my sample back if I change my mind once it has been stored in the 'tissue bank'?'

PARTICIPANT INFORMATION AND CONSENT FORM

Study Title: Brain neurochemicals in headaches and other pain conditions

It may not be possible to return your sample, therefore if you change your mind and no longer wish for the sample to be stored in the tissue bank it will be destroyed.

Complaints
This study has been approved by Western Sydney Local Health District Human Research Ethics Committee.

If you have any concerns about the conduct of the study, or your rights as a study participant, you may contact:

The Secretary, WSLHD Human Research Ethics Committee
Telephone No 8890 8183 or email: wslhd-researchoffice@health.nsw.gov.au

Contact details
When you have read this information, the researcher Aimie Peek will discuss it with you and any queries you may have. If you would like to know more at any stage, please do not hesitate to contact her on *apee6909@uni.sydney.edu.au*. If you have any problems while on the study, please contact

Dr Sushil Bandodkar
Working hours Telephone No – (02) 9845 3289
After hours Telephone No- 0436 655 669

Thank you for taking the time to consider this study.

PARTICIPANT INFORMATION AND CONSENT FORM

Study Title: Brain neurochemicals in headaches and other pain conditions

CONSENT TO PARTICIPATE IN RESEARCH

Name of Chief Investigator : Dr Sushil Bandodkar

1. I understand that the researcher will conduct this study in a manner conforming to ethical and scientific principles set out by the National Health and Medical Research Council of Australia and the Good Clinical Research Practice Guidelines of the Therapeutic Goods Administration.

2. I acknowledge that I have read, or have had read to me the Participant Information Sheet relating to this study. I acknowledge that I understand the Participant Information Sheet. I acknowledge that the general purposes, methods, demands and possible risks and inconveniences which may occur to me during the study have been explained to me by Aimie Peek ("the researcher") and I, being over the age of 18 acknowledge that I understand the general purposes, methods, demands and possible risks and inconveniences which may occur during the study.

3. I acknowledge that I have been given time to consider the information and to seek other advice.

4. I acknowledge that refusal to take part in this study will not affect the usual treatment of my condition.

5. I acknowledge that I am volunteering to take part in this study and I may withdraw at any time.

6. I acknowledge that this research has been approved by the Western Sydney Local Health District Human Research Ethics Committee.

7. I acknowledge that I have received a copy of this form and the Participant Information Sheet, which I have signed.

8. I acknowledge that regulatory authorities may have access to my medical records relevant to this study to monitor the research in which I am agreeing to participate. However, I understand my identity will not be disclosed to anyone else or in publications or presentations.

Before signing, please read 'IMPORTANT NOTE' following.

IMPORTANT NOTE:

This consent should only be signed as follows:

1. Where a participant is over the age of 18 years, then by the participant personally.

Name of participant _____ Date of Birth _____
Address of participant _____
Signature of participant _____Date: _____
Signature of researcher _____ Date: _____
Signature of witness _____Date: _____

Baseline Questionnaires

PERSONAL DETAILS

1. Participant ID: _____
2. Medicare No _____
3. Sex ☐ Male ☐ Female
4. Height (cm) _____
5. Weight (kg) _____
6. Marital status ☐ Single ☐ Married / Defacto
   ☐ Divorced / Widowed / Separated
7. Country of birth _____
8. Highest education level
   ☐ Primary
   ☐ Secondary
   ☐ Certificate
   ☐ Diploma or advanced diploma
   ☐ Graduate diploma or graduate certificate
   ☐ Bachelor degree
   ☐ Postgraduate degree
9. Occupation _____
10. Medications:

Drug name	Dose	Frequency	Length of time taken	Time/ date last taken

QUESTIONS ON PAIN (HEADACHE OR LOW BACK PAIN)

11. How long have you been experiencing headaches / low back pain?
    _____ months/ years

12. Have you been seen by a health professional for your headaches / low back pain?

    □ Yes □ No

13. Have you been given a headache/back pain diagnosis?

    □ Yes □ No

    If yes,

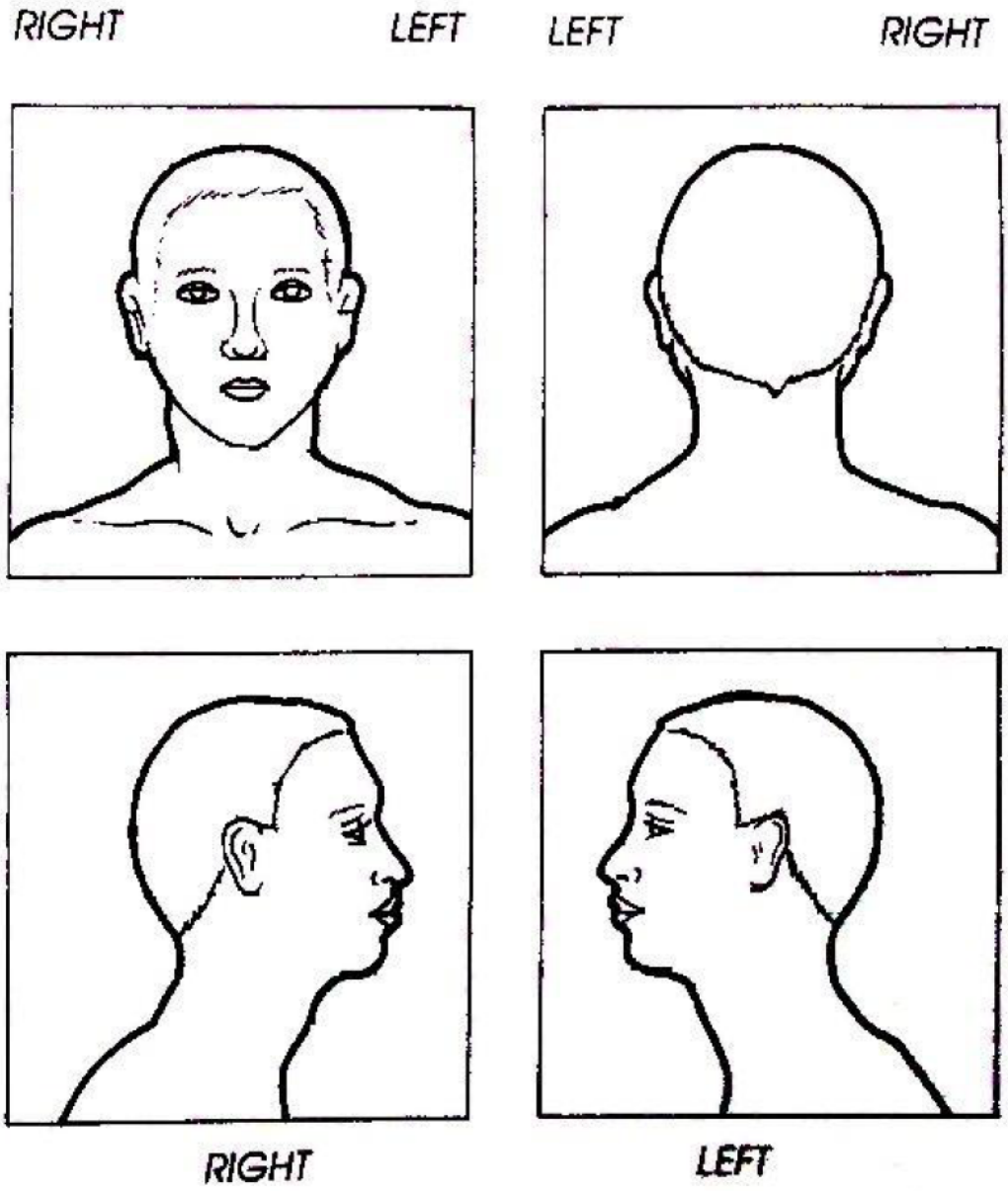
    a. What is your diagnosis?

        □ Migraine, please specify _____
        □ Persistent headache attributed to whiplash
        □ Non-specific low back pain
        □ Others, please specify _____

    b. Who diagnosed your headache or back pain?

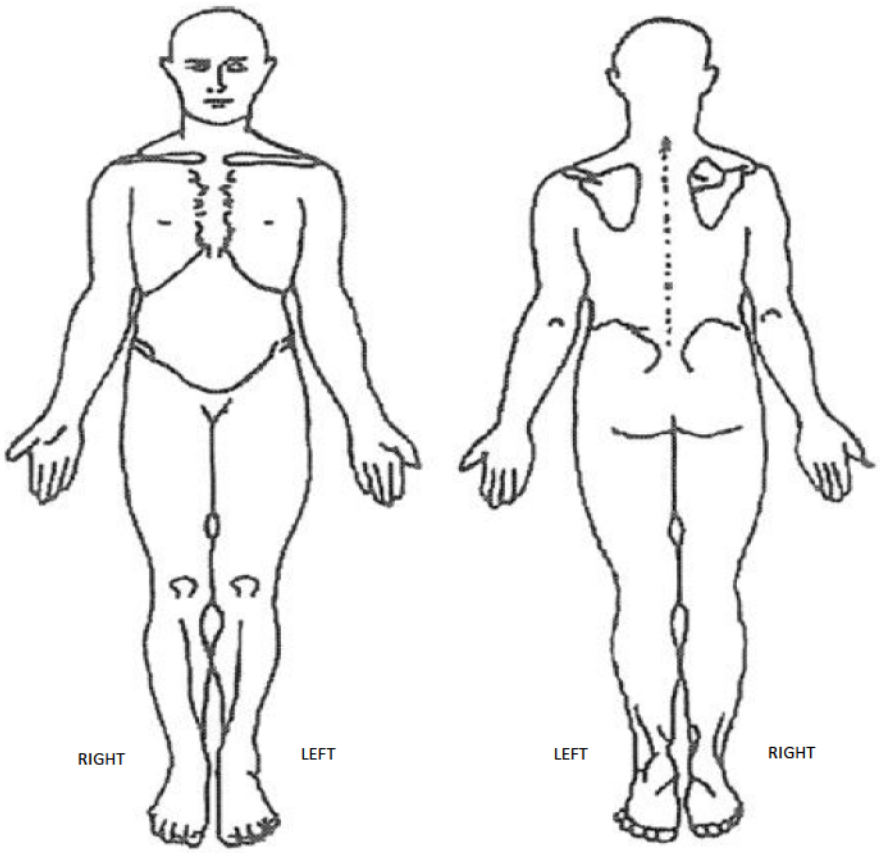
        □ GP □ Neurologist □ Other, please specify _____

14. Please shade areas on the head and body charts below to indicate where you are currently experiencing pain including headaches and low back pain. If you have pain in more than one location, indicate those additional locations also.



Please proceed to question 15, next page.

RESEARCH TEAM USE ONLY *(Other subjective information about headache / back pain):*
Frequency:
Duration:
Intensity:
Associated symptoms:
Triggers:
History:
Previous treatment:



You should then rate the **headache or back pain intensity** using the following three 0 – 10 scales:

15. Average pain intensity over the **last month** (Circle the most appropriate)

0	1	2	3	4	5	6	7	8	9	10
No pain										Worst possible pain

16. Average pain intensity over the **last week** (Circle the most appropriate)

0	1	2	3	4	5	6	7	8	9	10
No pain										Worst possible pain

17. Average pain intensity over the **last 24 hours** (Circle the most appropriate)

0	1	2	3	4	5	6	7	8	9	10
No pain										Worst possible pain

18. How many times in a **day** do you experience headaches or back pain?
_____times per day

19. How many times in a **week** do you experience headaches or back pain? _____ times per week

20. How many times in a **month** do you experience headaches or back pain? _____ times per month

Please continue to the following pages where there are 4 questionnaires measuring symptoms associated with pain.

Central Sensitization Inventory1: Part A

Please circle the best response to the right of each statement

1	I feel unrefreshed when I wake up in the morning	Never	Rarely	Sometimes	Often	Always
2	My muscles feel stiff and achy	Never	Rarely	Sometimes	Often	Always
3	I have anxiety attacks	Never	Rarely	Sometimes	Often	Always
4	I grind or clench my teeth	Never	Rarely	Sometimes	Often	Always
5	I have problems with diarrhea and/or constipation	Never	Rarely	Sometimes	Often	Always
6	I need help in performing my daily activities	Never	Rarely	Sometimes	Often	Always
7	I am sensitive to bright lights	Never	Rarely	Sometimes	Often	Always
8	I get tired very easily when I am physically active	Never	Rarely	Sometimes	Often	Always
9	I feel pain all over my body	Never	Rarely	Sometimes	Often	Always
10	I have headaches	Never	Rarely	Sometimes	Often	Always
11	I feel discomfort in my bladder and/or burning when I urinate	Never	Rarely	Sometimes	Often	Always
12	I do not sleep well	Never	Rarely	Sometimes	Often	Always
13	I have difficulty concentrating	Never	Rarely	Sometimes	Often	Always
14	I have skin problems such as dryness, itchiness, or rashes	Never	Rarely	Sometimes	Often	Always
15	Stress makes my physical symptoms get worse	Never	Rarely	Sometimes	Often	Always
16	I feel sad or depressed	Never	Rarely	Sometimes	Often	Always
17	I have low energy	Never	Rarely	Sometimes	Often	Always
18	I have muscle tension in my neck and shoulders	Never	Rarely	Sometimes	Often	Always
19	I have pain in my jaw	Never	Rarely	Sometimes	Often	Always
20	Certain smells, such as perfumes, make me feel dizzy and nauseated	Never	Rarely	Sometimes	Often	Always
21	I have to urinate frequently	Never	Rarely	Sometimes	Often	Always
22	My legs feel uncomfortable and restless when I am trying to go to sleep at night	Never	Rarely	Sometimes	Often	Always
23	I have difficulty remembering things	Never	Rarely	Sometimes	Often	Always
24	I suffered trauma as a child	Never	Rarely	Sometimes	Often	Always
25	I have pain in my pelvic area	Never	Rarely	Sometimes	Often	Always

Total =

[1] From Mayer, T.G., Neblett, R., Cohen, H., Howard, K.J., Choi, Y.H., Williams, M.J., Perez, Y., & Gatchel, R.J. (2012). The development and psychometric validation of the Central Sensitization Inventory. *Pain Practice,* **12**(4), 276-285.

CENTRAL SENSITIZATION INVENTORY: PART B

Have you been diagnosed by a doctor with any of the following disorders?

Please check the box to the right for each diagnosis and write the year of the diagnosis

		No	Yes	Year Diagnosed
1	Restless leg syndrome			
2	Chronic fatigue syndrome			
3	Fibromyalgia			
4	Temporomandibular joint disorder (TMJ)			
5	Migraine or tension headaches			
6	Irritable bowel syndrome			
7	Multiple chemical sensitivities			
8	Neck injury (including whiplash)			
9	Anxiety or panic attacks			
10	Depression			

Self-Completed Leeds Assessment of Neuropathic Symptoms and Signs Pain Score

Think about how your pain(s) that you have felt over the last week. Put a tick against the descriptions that best match your pain(s). These descriptions may, or may not, match your pain(s) no matter how severe it feels. Only circle responses that describe any of your pain(s).

1. In the area where you have pain, do you also have 'pins and needles', tingling or prickling sensations?

a) NO - I don't get these sensations (0)

b) YES - I get these sensations often (5)

2. Does the painful area change colour (perhaps looks mottled or more red) when the pain is particularly bad?

a) NO - The pain does not affect the colour of my skin (0)

b) YES - I have noticed that the pain does make my skin look different from normal (5)

3. Does your pain make the affected skin abnormally sensitive to touch? Getting unpleasant sensations or pain when lightly stroking the skin might describe this.

a) NO - The pain does not make my skin in that area abnormally sensitive to touch (0)

b) YES - My skin in that area is particularly sensitive to touch (3)

4. Does your pain come on suddenly and in bursts for no apparent reason when you are completely still? Words like 'electric shocks', jumping and bursting might describe this.

a) NO - My pain doesn't really feel like this (0)

b) YES - I get these sensations often (2)

5. In the area where you have pain, does your skin feel unusually hot like a burning pain?

a) NO - I don't have burning pain (0)

b) YES - I get burning pain often (1)

6. Gently rub the painful area with your index finger and then rub a non-painful area (for example, an area of skin further away or on the opposite side from the painful area). How does this rubbing feel in the painful area?

a) The painful area feels no different from the non-painful area (0)

b) I feel discomfort, like pins and needles, tingling or burning in the painful area that is different from the non-painful area (5)

7. Gently press on the painful area with your finger tip then gently press in the same way onto a non- painful area (the same non-painful area that you chose in the last question). How does this feel in the painful area?

a) The painful area does not feel different from the non-painful area (0)

b) I feel numbness or tenderness in the painful area that is different from the non-painful area (3)

Short-Form McGill Pain Questionnaire-2

This questionnaire provides you with a list of words that describe some of the different qualities of pain and related symptoms. Please put an **X** through the numbers that best describe the intensity of each of the pain and related symptoms you felt during a typical headache episode. Use 0 if the word does not describe your pain or related symptoms.

1. Throbbing pain	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
2. Shooting pain	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
3. Stabbing pain	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
4. Sharp pain	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
5. Cramping pain	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
6. Gnawing pain	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
7. Hot-burning pain	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
8. Aching pain	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
9. Heavy pain	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
10. Tender	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
11. Splitting pain	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
12. Tiring-exhausting	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
13. Sickening	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
14. Fearful	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
15. Punishing-cruel	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
16. Electric-shock pain	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
17. Cold-freezing pain	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
18. Piercing	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
19. Pain caused by light touch	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*Worst*

20. Itching	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
21. Tingling or 'pins and needles'	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*
22. Numbness	*none*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*worst*

Pain Vigilance and Awareness Questionnaire

INSTRUCTIONS: Consider your behavior over the last 2 weeks. Indicate how frequently, on a scale from 0 (never) to 5 (always), each item was a true description for you. Please answer each item by circling one number on the scale from0 (never) to 5(always).

1 I am very sensitive to pain.	0	1	2	3	4	5
2. I am aware of sudden or temporary changes in pain.	0	1	2	3	4	5
3. I am quick to notice changes in pain intensity.	0	1	2	3	4	5
4. I am quick to notice effects of medication on pain.	0	1	2	3	4	5
5. I am quick to notice changes in location or extent of pain.	0	1	2	3	4	5
6. I focus on sensations of pain.	0	1	2	3	4	5
7. I notice pain even if I am busy with another activity.	0	1	2	3	4	5
8. I find it easy to ignore pain,	0	1	2	3	4	5
9. I know immediately when pain starts or increases.	0	1	2	3	4	5
10. When I do something that increases pain, the first thing I do is check to see how much pain was increased,	0	1	2	3	4	5
11. I know immediately when pain decreases.	0	1	2	3	4	5
12. I seem to be more conscious of pain than others.	0	1	2	3	4	5
13. I pay close attention to pain. 14. I keep track of my pain level.	0	1	2	3	4	5
15. I become preoccupied with pain.	0	1	2	3	4	5
16. I do not dwell on pain.	0	1	2	3	4	5

WHO Disability Assessment Schedule 2.0

This questionnaire asks about difficulties due to health conditions. Health conditions include diseases or illnesses, other health problems that may be short or long lasting, injuries, mental or emotional problems, and problems with alcohol or drugs.

Think back over the past 30 days and answer these questions, thinking about how much difficulty you had doing the following activities. For each question, please circle only one response.

.

In the past 30 days, how much difficulty did you have in:						
S1	Standing for long periods such as 30 minutes?	None	Mild	Moderate	Severe	Extreme or cannot do
S2	Taking care of your household responsibilities?	None	Mild	Moderate	Severe	Extreme or cannot do
S3	Learning a new task, for example, learning how to get to a new place?	None	Mild	Moderate	Severe	Extreme or cannot do
S4	How much of a problem did you have joining in community activities (for example, festivities, religious or other activities) in the same way as anyone else can?	None	Mild	Moderate	Severe	Extreme or cannot do
S5	How much have you been emotionally affected by your health problems?	None	Mild	Moderate	Severe	Extreme or cannot do

Please continue to next page...

In the past 30 days, how much difficulty did you have in:						
S6	Concentrating on doing something for ten minutes?	None	Mild	Moderate	Severe	Extreme or cannot do\
S7	Walking a long distance such as a kilometre [or equivalent]?	None	Mild	Moderate	Severe	Extreme or cannot do
S8	Will behing your whole body?	None	Mild	Moderate	Severe	Extreme or cannot do
S9	Getting dressed?	None	Mild	Moderate	Severe	Extreme or cannot do
S10	Dealing with people you do not know?	None	Mild	Moderate	Severe	Extreme or cannot do
S11	Maintaining a friendship?	None	Mild	Moderate	Severe	Extreme or cannot do
S12	Your day-to-day work?	None	Mild	Moderate	Severe	Extreme or cannot do

H1	Overall, in the past 30 days, how many days will be these difficulties present?	*Record number of days* _____
H2	In the past 30 days, for how many days will be you totally unable to carry out your usual activities or work because of any health condition?	*Record number of days* _____
H3	In the past 30 days, not counting the days that you will be totally unable, for how many days did you cut back or reduce your usual activities or work because of any health condition?	*Record number of days* _____

This completes this questionnaire. Thank you.

Headache Impact Test -6 (HIT-6)TM

This questionnaire was designed to help you describe and communicate the way you feel and what you cannot do because of headaches.

To complete, please check one box for each question.

1. When you have headaches, how often is the pain severe?

☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always

2. How often do headaches limit your ability to do usual daily activities including household work, work, school, or social activities?

☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always

3. When you have a headache, how often do you wish you could lie down?

☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always

4. In the past 4 weeks, how often have you felt too tired to do work or daily activities because of your headaches?

☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always

5. In the past 4 weeks, how often have you felt fed up or irritated because of your headaches?

☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always

6. In the past 4 weeks, how often did headaches limit your ability to concentrate on work or daily activities?

☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always

COLUMN 1	*COLUMN 2*	*COLUMN 3*	*COLUMN 4*	*COLUMN 5*
(6 points each)	*(8 points each)*	*(10 points each)*	*(11 points each)*	*(13 points each)*

To score, add points for answers in each column

Please share your HIT-6 results with your doctor.

Total Score: _____

Higher scores indicate greater impact on your life. Score range is 36-78.

HIT-6™ US Original (English) Version 1.0

Headache Disability Questionnaire

Name:..... Date:...../...../..... Score / 90

Please read each question and circle the response that best applies to you

1. How would you rate the usual pain of your headache on a scale from 0 to 10?

0 NO PAIN	1	2	3	4	5	6	7	8	9	10	WORST PAIN

2. When you have headaches, how often is the pain severe?

NEVER	1-9%	10-19%	20-29%	30-39%	40-49%	50-59%	60-69%	70-79%	80-89%	90-100%	ALWAYS
0	1	2	3	4	5	6	7	8	9	10	

3. On how many days in the last month did you actually lie down for an hour or more because of your headaches?

NONE	1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24	25-27	28-31	EVERY DAY
0	1	2	3	4	5	6	7	8	9	10	

4. When you have a headache, how often do you miss work or school for all or part of the day?

NEVER	1-9%	10-19%	20-29%	30-39%	40-49%	50-59%	60-69%	70-79%	80-89%	90-100%	ALWAYS
0	1	2	3	4	5	6	7	8	9	10	

5. When you have a headache while you work (or school), how much is your ability to work reduced?

NOT	1-9%	10-19%	20-29%	30-39%	40-49%	50-59%	60-69%	70-79%	80-89%	90-100%	UNABLE TO
0 REDUCED	1	2	3	4	5	6	7	8	9	10	WORK

6. How many days in the last month have you been kept from performing housework or chores for at least half of the day because of your headaches?

NONE	1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24	25-27	28-31	EVERY DAY
0	1	2	3	4	5	6	7	8	9	10	

7. When you have a headache, how much is your ability to perform housework or chores reduced?

NOT	1-9%	10-19%	20-29%	30-39%	40-49%	50-59%	60-69%	70-79%	80-89%	90-100%	UNABLE
0 REDUCED	1	2	3	4	5	6	7	8	9	10	TO PERFORM

8. How many days in the last month have you been kept from non-work activities (family, social or recreational) because of your headaches?

NONE	1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24	25-27	28-31	EVERY DAY
0	1	2	3	4	5	6	7	8	9	10	

9. When you have a headache, how much is your ability to engage in non-work activities (family, social or recreational) reduced?

NOT	1-9%	10-19%	20-29%	30-39%	40-49%	50-59%	60-69%	70-79%	80-89%	90-100%	UNABLE
0 REDUCED	1	2	3	4	5	6	7	8	9	10	TO PERFORM

Neck Disability Index (NDI)

INSTRUCTIONS: This questionnaire is designed to enable us to understand how much your neck pain has affected your ability to manage everyday activities. Please answer each question by ticking (✓) ONE CHOICE that most applies to you. We realise that you may feel that more than one statement may relate to you, but PLEASE JUST TICK (✓) THE ONE CHOICE, WHICH CLOSELY DESCRIBES YOUR PROBLEM RIGHT NOW.

1. PAIN INTENSITY

❑ I have no pain at the moment.
❑ The pain is mild at the moment.
❑ The pain comes and goes and is moderate.
❑ The pain is moderate and does not vary much.
❑ The pain is severe but comes and goes.
❑ The pain is severe and does not vary much.

2. PERSONAL CARE

❑ I can look after myself without causing extra pain.
❑ I can look after myself normally but it causes extra pain.
❑ It is painful to look after myself and I am slow and careful.
❑ I need some help, but manage most of my personal care.
❑ I need help every day in most aspects of self-care.
❑ I do not get dressed, I wash with difficulty and stay in bed.

3. LIFTING

❑ I can lift heavy objects without extra pain
❑ I can lift heavy objects but it causes extra pain
❑ Pain prevents me from lifting heavy objects off the floor, but I can if they are conveniently positioned, for example on a table.
❑ Pain prevents me from lifting heavy objects, but I can manage light to medium weights if they are conveniently positioned.
❑ I can lift very light weights.
❑ I cannot lift or carry anything at all.

4. READING

❑ I can read as much as I want to with no pain in my neck.
❑ I can read as much as I want with slight pain in my neck.
❑ I can read as much as I want with moderate pain in my neck.
❑ I cannot read as much as I want because of moderate pain in my neck.
❑ I cannot read as much as I want because of severe pain in my neck.
❑ I cannot read at all.

5. HEADACHE

❑ I have no headaches at all.
❑ I have slight headaches which come infrequently.
❑ I have moderate headaches which come infrequently.
❑ I have moderate headaches which come frequently.
❑ I have severe headaches which come frequently.
❑ I have headaches almost all the time.

6. CONCENTRATION

❑ I can concentrate fully when I want to with no difficulty.
❑ I can concentrate fully when I want to with slight difficulty.
❑ I have a fair degree of difficulty concentrating when I want to.
❑ I have a lot of difficulty concentrating when I want to.
❑ I have a great deal of difficulty concentrating when I want to.
❑ I cannot concentrate at all.

8. DRIVING (omit this question if you never drive a car when in good health)

❑ I can drive my car without neck pain.
❑ I can drive my car as long as I want with slight pain in my neck.
❑ I can drive my car as long as I want with moderate pain in my neck.
❑ I cannot drive my car as long as I want because of moderate pain in my neck.
❑ I can hardly drive my car at all because of severe pain in my neck.
❑ I cannot drive my car at all.

9. NECK PAIN AND SLEEPING

❑ I have no trouble sleeping.
❑ My sleep is slightly disturbed (less than 1 hour sleepless).
❑ My sleep is mildly disturbed (1-2 hours sleepless).
❑ My sleep is moderately disturbed (2-3 hours sleepless).
❑ My sleep is greatly disturbed (3-5 hours sleepless).
❑ My sleep is completely disturbed (5-7 hours sleepless).

10. RECREATION

❑ I am able to engage in all recreational activities with no pain in my neck at all.
❑ I am able to engage in all recreational activities with some pain in my neck.
❑ I am able to engage in most, but not all recreational activities because of pain in my neck.
❑ I am able to engage in a few of my usual recreational activities because of pain in my neck.
❑ I can hardly do any recreational activities because of pain in my neck.
❑ I cannot do any recreational activities at all.

Oswestry Low Back Pain Disability Questionnaire

Instructions
This questionnaire has been designed to give us information as to how your back or leg pain is affecting your ability to manage in everyday life. Please answer by checking ONE box in each section for the statement which best applies to you. We realise you may consider that two or more statements in any one section apply but please just shade out the spot that indicates the statement which most clearly describes your problem.

Section 1 – Pain intensity

☐ I have no pain at the moment
☐ The pain is very mild at the moment
☐ The pain is moderate at the moment
☐ The pain is fairly severe at the moment
☐ The pain is very severe at the moment
☐ The pain is the worst imaginable at the moment

Section 2 – Personal care (washing, dressing etc)

☐ I can look after myself normally without causing extra pain
☐ I can look after myself normally but it causes extra pain
☐ It is painful to look after myself and I am slow and careful
☐ I need some help but manage most of my personal care
☐ I need help every day in most aspects of self-care
☐ I do not get dressed, I wash with difficulty and stay in bed

*Note: Distances of 1 mile, ½ mile and 100 yards have been replaced by metric distances in the Walking section

Section 3 – Lifting

☐ I can lift heavy weights without extra pain
☐ I can lift heavy weights but it gives extra pain
☐ Pain prevents me from lifting heavy weights off the floor, but I can manage if they are conveniently placed eg. on a table
☐ Pain prevents me from lifting heavy weights, but I can manage light to medium weights if they are conveniently positioned
☐ I can lift very light weights
☐ I cannot lift or carry anything at all

Section 4 – Walking*

☐ Pain does not prevent me walking any distance
☐ Pain prevents me from walking more than 2 kilometres
☐ Pain prevents me from walking more than 1 kilometre
☐ Pain prevents me from walking more than 500 metres
☐ I can only walk using a stick or crutches
☐ I am in bed most of the time

Section 5 – Sitting

☐ I can sit in any chair as long as I like
☐ I can only sit in my favourite chair as long as I like
☐ Pain prevents me sitting more than one hour
☐ Pain prevents me from sitting more than 30 minutes
☐ Pain prevents me from sitting more than 10 minutes
☐ Pain prevents me from sitting at all

Section 6 – Standing

☐ I can stand as long as I want without extra pain
☐ I can stand as long as I want but it gives me extra pain
☐ Pain prevents me from standing for more than 1 hour
☐ Pain prevents me from standing for more than 3 minutes
☐ Pain prevents me from standing for more than 10 minutes
☐ Pain prevents me from standing at all

Section 7 – Sleeping

☐ My sleep is never disturbed by pain
☐ My sleep is occasionally disturbed by pain
☐ Because of pain I have less than 6 hours sleep
☐ Because of pain I have less than 4 hours sleep
☐ Because of pain I have less than 2 hours sleep
☐ Pain prevents me from sleeping at all

Section 8 – Sex life (if applicable)

☐ My sex life is normal and causes no extra pain
☐ My sex life is normal but causes some extra pain
☐ My sex life is nearly normal but is very painful
☐ My sex life is severely restricted by pain
☐ My sex life is nearly absent because of pain
☐ Pain prevents any sex life at all

Section 9 – Social life

☐ My social life is normal and gives me no extra pain
☐ My social life is normal but increases the degree of pain
☐ Pain has no significant effect on my social life apart from limiting my more energetic interests eg, sport
☐ Pain has restricted my social life and I do not go out as often
☐ Pain has restricted my social life to my home
☐ I have no social life because of pain

Section 10 – Travelling

☐ I can travel anywhere without pain
☐ I can travel anywhere but it gives me extra pain
☐ Pain is bad but I manage journeys over two hours
☐ Pain restricts me to journeys of less than one hour
☐ Pain restricts me to short necessary journeys under 30 minutes
☐ Pain prevents me from travelling except to receive treatment

Orebro Musculoskeletal Pain Questionnaire Short-Form

1. How long have you had your current pain problem?
Tick (√) one.

0-1 weeks [1]	1-2 weeks [2]	3-4 weeks [3]	4-5 weeks [4]	6-8 weeks [5]
9-11 weeks [6]	3-6 months [7]	6-9 months [8]	9-12 months [9]	over 1 year [10]

2. How would you rate the pain that you have had during the past week?
Circle one.

0 1 2 3 4 5 6 7 8 9 10 [ ]
No pain Pain as bad as it could be

For items 3 and 4, please circle the one number that best describes your current ability to participate in each of these activities.

3. I can do light work (or home duties) for an hour.

0 1 2 3 4 5 6 7 8 9 10 [ ]
No pain Without any difficulty

4. I can sleep at night.

0 1 2 3 4 5 6 7 8 9 10 [ ]
No pain Without any difficulty

5. How tense or anxious have you felt in the past week?
Circle one.

0 1 2 3 4 5 6 7 8 9 10 [ ]
Absolutely calm and relaxed As tense and anxious as I've ever felt

6. How much have you been bothered by feeling depressed in the past week?
Circle one.

0 1 2 3 4 5 6 7 8 9 10 [ ]
Not at all Extremely

7. In your view, how large is the risk that your current pain may become persistent?

0 1 2 3 4 5 6 7 8 9 10 [ ]
No risk Very large risk

8. In your estimation, what are the chances you will be working your normal duties (at home or work) in 3 months

0 1 2 3 4 5 6 7 8 9 10 [ ]
No chance Very Large Chance

9. An increase in pain is an indication that I should stop what I'm doing until the pain decreases.

0 1 2 3 4 5 6 7 8 9 10 [ ]
Completely disagree Completely agree

10. I should not do my normal work (at work or home duties) with my present pain.

0 1 2 3 4 5 6 7 8 9 10 [ ]
Completely disagree Completely agree

SUM:_____

Depression Anxiety Stress Scales – 21 items (DASS)

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

1	I found it hard to wind down	0	1	2	3
2	I will be aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (e.g. excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I found it difficult to work up the initiative to do things	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I experienced trembling (e.g. in the hands)	0	1	2	3
8	I felt that I will be using a lot of nervous energy	0	1	2	3
9	I will be worried about situations in which I might panic and make a fool of myself	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting agitated	0	1	2	3
12	I found it difficult to relax	0	1	2	3
13	I felt down-hearted and blue	0	1	2	3
14	I will be intolerant of anything that kept me from getting on with what I will be doing	0	1	2	3
15	I felt I will be close to panic	0	1	2	3
16	I will be unable to become enthusiastic about anything	0	1	2	3
17	I felt I will be not worth much as a person	0	1	2	3
18	I felt that I will be rather touchy	0	1	2	3
19	I will be aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life will be meaningless	0	1	2	3

PTSD Checklist for DSM-5 (PCL-5)2

Instructions: Below is a list of problems that people sometimes have in response to a very stressful experience. Please read each problem carefully and then circle one of the numbers to the right to indicate how much you have been bothered by that problem in the past month.

In the past month, how much were you bothered by:	Not at all	A little bit	Moderately	Quite a bit	Extremely
1. Repeated, disturbing, and unwanted memories of the stressful experience?	0	1	2	3	4
2. Repeated, disturbing dreams of the stressful experience?	0	1	2	3	4
3. Suddenly feeling or acting as if the stressful experience were actually happening again (as if you were actually back there reliving it)?	0	1	2	3	4
4. Feeling very upset when something reminded you of the stressful experience?	0	1	2	3	4
5. Having strong physical reactions when something reminded you of the stressful experience (for example, heart pounding, trouble breathing, sweating)?	0	1	2	3	4
6. Avoiding memories, thoughts, or feelings related to the stressful experience?	0	1	2	3	4
7. Avoiding external reminders of the stressful experience (for example, people, places, conversations, activities, objects, or situations)?	0	1	2	3	4
8. Trouble remembering important parts of the stressful experience?	0	1	2	3	4
9. Having strong negative beliefs about yourself, other people, or the world (for example, having thoughts such as: I am bad, there is something seriously wrong with me, no one can be trusted, the world is completely dangerous)?	0	1	2	3	4

[2] Weathers, F. W., Litz, B. T., Keane, T. M., Palmieri, P. A., Marx, B. P., & Schnurr, P. P. (2013). The PTSD Checklist for DSM-5 (PCL-5) – Standard [Measurement instrument]. Available from http://www.ptsd.va.gov/

In the past month, how much were you bothered by:	Not at all	A little bit	Moderately	Quite a bit	Extremely
10. Blaming yourself or someone else for the stressful experience or what happened after it?	0	1	2	3	4
11. Having strong negative feelings such as fear, horror, anger, guilt, or shame?	0	1	2	3	4
12. Loss of interest in activities that you used to enjoy?	0	1	2	3	4
13. Feeling distant or cut off from other people?	0	1	2	3	4
14. Trouble experiencing positive feelings (for example, being unable to feel happiness or have loving feelings for people close to you)?	0	1	2	3	4
15. Irritable behavior, angry outbursts, or acting aggressively?	0	1	2	3	4
16. Taking too many risks or doing things that could cause you harm?	0	1	2	3	4
17. Being "superalert" or watchful or on guard?	0	1	2	3	4
18. Feeling jumpy or easily startled?	0	1	2	3	4
19. Having difficulty concentrating?	0	1	2	3	4
20. Trouble falling or staying asleep?	0	1	2	3	4

12-item medical outcomes study short form health survey version 2.0 (SF-12v2) SF12 included SF6D

1. On a scale of 1 to 5, where:

1 Excellent

2 Very good

3 Good

4 Fair

5 Poor

In general, how would you rate your health after the motor vehicle accident? _____

The following questions are about activities you might do during a typical day. Does **your health** *now* limit you in these activities? If so, were the activities limited a lot or a little?

Circle the most appropriate option.

	Yes, Limited a Lot	Yes, Limited a Little	No, Not limited at All
2. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
3. Climbing **several** flights of stairs	1	2	3
3a Vigorous activities (eg running, lifting heavy objects)	1	2	3
3b Bathing or dressing yourself	1	2	3

During the *past week*, have you had any of the following problems with your work or other regular daily activities as a result of your *physical health*?

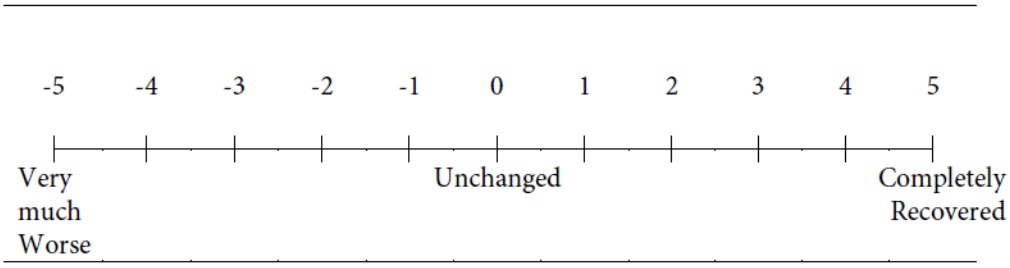
	Yes	No
4. Accomplished less than you would like	1	2
5. Were limited in the **kind** of work or other activities	1	2

During the *past week*, have you had any of the following problems with your work or other regular daily activities *as a result of any emotional problems* (such as feeling depressed or anxious)?		
	Yes	No
6. Accomplished less than you would like	1	2
7. Didn't do work or other activities as **carefully** as usual	1	2

On a scale of 1 to 5 where: 1 All of the time 2 Most of the time 3 Some of the time 4 A little of the time 5 None of the time	All of the Time	Most of the Time	Some of the Time	A little of the Time	None of the Time
8. During the *past week*, how much did pain interfere with your normal work (including both work outside the home and housework)?	1	2	3	4	5
9. Have you felt calm and peaceful?	1	2	3	4	5
10. Did you have a lot of energy?	1	2	3	4	5
11. Have you felt downhearted and depressed?	1	2	3	4	5
11a Have you been nervous?	1	2	3	4	5
12. During the *past week*, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?	1	2	3	4	5

Follow up Questionnaires

Participant ID:
Date:
The Global Rating of Change Scale



The Visual Analogue Scale
Please rate the migraine, headache or back pain intensity using the following 0-10 scales:

2. Average Pain intensity over the **last month** (Circle the most appropriate)

0 1 2 3 4 5 6 7 8 9 10
No Pain **Worst Possible Pain**

3. Average pain intensity over the **last week** (Circle the most appropriate)

0 1 2 3 4 5 6 7 8 9 10
No Pain **Worst Possible Pain**

4. Average pain intensity over the last **24 hours** (Circle the most appropriate)

0 1 2 3 4 5 6 7 8 9 10
No Pain **Worst Possible Pain**

5. How many days since your last pain episode over 4/10.....

6. How many times a **day** do you experience your migraines, headaches, or back pain?

..... times a day

7. How many times a **week** do you experience your migraines, headaches or back pain?

..... times a week

8. How many times in a **month** do you experience your migraines, headaches or back pain?

..... times a month

WHO Disability Assessment Schedule 2.0

This questionnaire asks about difficulties due to health conditions. Health conditions include diseases or illnesses, other health problems that may be short or long lasting, injuries, mental or emotional problems, and problems with alcohol or drugs.

Think back over the past 30 days and answer these questions, thinking about how much difficulty you had doing the following activities. For each question, please circle only one response.

.

In the past 30 days, how much difficulty did you have in:						
S1	Standing for long periods such as 30 minutes?	None	Mild	Moderate	Severe	Extreme or cannot do
S2	Taking care of your household responsibilities?	None	Mild	Moderate	Severe	Extreme or cannot do
S3	Learning a new task, for example, learning how to get to a new place?	None	Mild	Moderate	Severe	Extreme or cannot do
S4	How much of a problem did you have joining in community activities (for example, festivities, religious or other activities) in the same way as anyone else can?	None	Mild	Moderate	Severe	Extreme or cannot do
S5	How much have you been emotionally affected by your health problems?	None	Mild	Moderate	Severe	Extreme or cannot do

Please continue to next page...

In the past 30 days, how much difficulty did you have in:						
S6	Concentrating on doing something for ten minutes?	None	Mild	Moderate	Severe	Extreme or cannot do\
S7	Walking a long distance such as a kilometre [or equivalent]?	None	Mild	Moderate	Severe	Extreme or cannot do
S8	Will behing your whole body?	None	Mild	Moderate	Severe	Extreme or cannot do
S9	Getting dressed?	None	Mild	Moderate	Severe	Extreme or cannot do
S10	Dealing with people you do not know?	None	Mild	Moderate	Severe	Extreme or cannot do
S11	Maintaining a friendship?	None	Mild	Moderate	Severe	Extreme or cannot do
S12	Your day-to-day work?	None	Mild	Moderate	Severe	Extreme or cannot do

H1	Overall, in the past 30 days, how many days will be these difficulties present?	*Record number of days*
H2	In the past 30 days, for how many days will be you totally unable to carry out your usual activities or work because of any health condition?	*Record number of days* _____
H3	In the past 30 days, not counting the days that you will be totally unable, for how many days did you cut back or reduce your usual activities or work because of any health condition?	*Record number of days* _____

This completes this questionnaire. Thank you.

Headache Impact Test -6 (HIT-6)TM

This questionnaire was designed to help you describe and communicate the way you feel and what you cannot do because of headaches.

To complete, please check one box for each question.

1. When you have headaches, how often is the pain severe?

☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always

2. How often do headaches limit your ability to do usual daily activities including household work, work, school, or social activities?

☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always

3. When you have a headache, how often do you wish you could lie down?

☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always

4. In the past 4 weeks, how often have you felt too tired to do work or daily activities because of your headaches?

☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always

5. In the past 4 weeks, how often have you felt fed up or irritated because of your headaches?

☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always

6. In the past 4 weeks, how often did headaches limit your ability to concentrate on work or daily activities?

☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always

COLUMN 1	*COLUMN 2*	*COLUMN 3*	*COLUMN 4*	*COLUMN 5*
(6 points each)	*(8 points each)*	*(10 points each)*	*(11 points each)*	*(13 points each)*

To score, add points for answers in each column

Please share your HIT-6 results with your doctor.

Total Score: _____

Higher scores indicate greater impact on your life.
Score range is 36-78.

HIT-6™ US Original (English) Version 1.0

Headache Disability Questionnaire

Name:..... Date:...../...../..... Score / 90

Please read each question and circle the response that best applies to you

1. **How would you rate the usual pain of your headache on a scale from 0 to 10?**

0 NO PAIN	1	2	3	4	5	6	7	8	9	10 WORST PAIN

2. **When you have headaches, how often is the pain severe?**

NEVER 0	1-9% 1	10-19% 2	20-29% 3	30-39% 4	40-49% 5	50-59% 6	60-69% 7	70-79% 8	80-89% 9	90-100% 10 ALWAYS

3. **On how many days in the last month did you actually lie down for an hour or more because of your headaches?**

NONE 0	1-3 1	4-6 2	7-9 3	10-12 4	13-15 5	16-18 6	19-21 7	22-24 8	25-27 9	28-31 10 EVERY DAY

4. **When you have a headache, how often do you miss work or school for all or part of the day?**

NEVER 0	1-9% 1	10-19% 2	20-29% 3	30-39% 4	40-49% 5	50-59% 6	60-69% 7	70-79% 8	80-89% 9	90-100% 10 ALWAYS

5. **When you have a headache while you work (or school), how much is your ability to work reduced?**

NOT 0 REDUCED	1-9% 1	10-19% 2	20-29% 3	30-39% 4	40-49% 5	50-59% 6	60-69% 7	70-79% 8	80-89% 9	90-100% 10 UNABLE TO WORK

6. **How many days in the last month have you been kept from performing housework or chores for at least half of the day because of your headaches?**

NONE 0	1-3 1	4-6 2	7-9 3	10-12 4	13-15 5	16-18 6	19-21 7	22-24 8	25-27 9	28-31 10 EVERY DAY

7. **When you have a headache, how much is your ability to perform housework or chores reduced?**

NOT 0 REDUCED	1-9% 1	10-19% 2	20-29% 3	30-39% 4	40-49% 5	50-59% 6	60-69% 7	70-79% 8	80-89% 9	90-100% 10 UNABLE TO PERFORM

8. **How many days in the last month have you been kept from non-work activities (family, social or recreational) because of your headaches?**

NONE 0	1-3 1	4-6 2	7-9 3	10-12 4	13-15 5	16-18 6	19-21 7	22-24 8	25-27 9	28-31 10 EVERY DAY

9. **When you have a headache, how much is your ability to engage in non-work activities (family, social or recreational) reduced?**

NOT 0 REDUCED	1-9% 1	10-19% 2	20-29% 3	30-39% 4	40-49% 5	50-59% 6	60-69% 7	70-79% 8	80-89% 9	90-100% 10 UNABLE TO PERFORM

Neck Disability Index (NDI)

INSTRUCTIONS: This questionnaire is designed to enable us to understand how much your neck pain has affected your ability to manage everyday activities. Please answer each question by ticking (✓) ONE CHOICE that most applies to you. We realise that you may feel that more than one statement may relate to you, but PLEASE JUST TICK (✓) THE ONE CHOICE, WHICH CLOSELY DESCRIBES YOUR PROBLEM RIGHT NOW.

1. PAIN INTENSITY

☒ I have no pain at the moment.

☒ The pain is mild at the moment.

☒ The pain comes and goes and is moderate.

☒ The pain is moderate and does not vary much.

☒ The pain is severe but comes and goes.

☒ The pain is severe and does not vary much.

2. PERSONAL CARE

☒ I can look after myself without causing extra pain.

☒ I can look after myself normally but it causes extra pain.

☒ It is painful to look after myself and I am slow and careful.

☒ I need some help, but manage most of my personal care.

☒ I need help every day in most aspects of self-care.

☒ I do not get dressed, I wash with difficulty and stay in bed.

3. LIFTING

☒ I can lift heavy objects without extra pain

☒ I can lift heavy objects but it causes extra pain

☒ Pain prevents me from lifting heavy objects off the floor, but I can if they are conveniently positioned, for example on a table.

☒ Pain prevents me from lifting heavy objects, but I can manage light to medium weights if they are conveniently positioned.

☒ I can lift very light weights.

☒ I cannot lift or carry anything at all.

4. READING

☒ I can read as much as I want to with no pain in my neck.

☒ I can read as much as I want with slight pain in my neck.

☒ I can read as much as I want with moderate pain in my neck.

☒ I cannot read as much as I want because of moderate pain in my neck.

☒ I cannot read as much as I want because of severe pain in my neck.

☒ I cannot read at all.

5. HEADACHE

☒ I have no headaches at all.

☒ I have slight headaches which come infrequently.

☒ I have moderate headaches which come infrequently.

☒ I have moderate headaches which come frequently.

☒ I have severe headaches which come frequently.

☒ I have headaches almost all the time.

6. CONCENTRATION

☒ I can concentrate fully when I want to with no difficulty.

☒ I can concentrate fully when I want to with slight difficulty.

☒ I have a fair degree of difficulty concentrating when I want to.

☒ I have a lot of difficulty concentrating when I want to.

☒ I have a great deal of difficulty concentrating when I want to.

☒ I cannot concentrate at all.

8. DRIVING (omit this question if you never drive a car when in good health)

☒ I can drive my car without neck pain.

☒ I can drive my car as long as I want with slight pain in my neck.

☒ I can drive my car as long as I want with moderate pain in my neck.

☒ I cannot drive my car as long as I want because of moderate pain in my neck.

☒ I can hardly drive my car at all because of severe pain in my neck.

☒ I cannot drive my car at all.

9. NECK PAIN AND SLEEPING

☒ I have no trouble sleeping.

☒ My sleep is slightly disturbed (less than 1 hour sleepless).

☒ My sleep is mildly disturbed (1-2 hours sleepless).

☒ My sleep is moderately disturbed (2-3 hours sleepless).

☒ My sleep is greatly disturbed (3-5 hours sleepless).

☒ My sleep is completely disturbed (5-7 hours sleepless).

10. RECREATION

☒ I am able to engage in all recreational activities with no pain in my neck at all.

☒ I am able to engage in all recreational activities with some pain in my neck.

☒ I am able to engage in most, but not all recreational activities because of pain in my neck.

☒ I am able to engage in a few of my usual recreational activities because of pain in my neck.

☒ I can hardly do any recreational activities because of pain in my neck.

☒ I cannot do any recreational activities at all.

Oswestry Low Back Pain Disability Questionnaire

Instructions
This questionnaire has been designed to give us information as to how your back or leg pain is affecting your ability to manage in everyday life. Please answer by checking ONE box in each section for the statement which best applies to you. We realise you may consider that two or more statements in any one section apply but please just shade out the spot that indicates the statement which most clearly describes your problem.

Section 1 – Pain intensity

☐ **I have no pain at the moment**
☐ **The pain is very mild at the moment**
☐ **The pain is moderate at the moment**
☐ **The pain is fairly severe at the moment**
☐ **The pain is very severe at the moment**
☐ **The pain is the worst imaginable at the moment**

Section 2 – Personal care (washing, dressing etc)

☐ **I can look after myself normally without causing extra pain**
☐ **I can look after myself normally but it causes extra pain**
☐ **It is painful to look after myself and I am slow and careful**
☐ **I need some help but manage most of my personal care**
☐ **I need help every day in most aspects of self-care**
☐ **I do not get dressed, I wash with difficulty and stay in bed**

*Note: Distances of 1 mile, ½ mile and 100 yards have been replaced by metric distances in the Walking section

Section 3 – Lifting

☐ **I can lift heavy weights without extra pain**
☐ **I can lift heavy weights but it gives extra pain**
☐ **Pain prevents me from lifting heavy weights off the floor, but I can manage if they are conveniently placed eg. on a table**
☐ **Pain prevents me from lifting heavy weights, but I can manage light to medium weights if they are conveniently positioned**
☐ **I can lift very light weights**
☐ **I cannot lift or carry anything at all**

Section 4 – Walking*

☐ **Pain does not prevent me walking any distance**
☐ **Pain prevents me from walking more than 2 kilometres**
☐ **Pain prevents me from walking more than 1 kilometre**
☐ **Pain prevents me from walking more than 500 metres**
☐ **I can only walk using a stick or crutches**
☐ **I am in bed most of the time**

Section 5 – Sitting

☐ I can sit in any chair as long as I like
☐ I can only sit in my favourite chair as long as I like
☐ Pain prevents me sitting more than one hour
☐ Pain prevents me from sitting more than 30 minutes
☐ Pain prevents me from sitting more than 10 minutes
☐ Pain prevents me from sitting at all

Section 6 – Standing

☐ I can stand as long as I want without extra pain
☐ I can stand as long as I want but it gives me extra pain
☐ Pain prevents me from standing for more than 1 hour
☐ Pain prevents me from standing for more than 3 minutes
☐ Pain prevents me from standing for more than 10 minutes
☐ Pain prevents me from standing at all

Section 7 – Sleeping

☐ My sleep is never disturbed by pain
☐ My sleep is occasionally disturbed by pain
☐ Because of pain I have less than 6 hours sleep
☐ Because of pain I have less than 4 hours sleep
☐ Because of pain I have less than 2 hours sleep
☐ Pain prevents me from sleeping at all

Section 8 – Sex life (if applicable)

☐ My sex life is normal and causes no extra pain
☐ My sex life is normal but causes some extra pain
☐ My sex life is nearly normal but is very painful
☐ My sex life is severely restricted by pain
☐ My sex life is nearly absent because of pain
☐ Pain prevents any sex life at all

Section 9 – Social life

☐ My social life is normal and gives me no extra pain
☐ My social life is normal but increases the degree of pain
☐ Pain has no significant effect on my social life apart from limiting my more energetic interests eg, sport
☐ Pain has restricted my social life and I do not go out as often
☐ Pain has restricted my social life to my home
☐ I have no social life because of pain

Section 10 – Travelling

☐ I can travel anywhere without pain
☐ I can travel anywhere but it gives me extra pain
☐ Pain is bad but I manage journeys over two hours
☐ Pain restricts me to journeys of less than one hour
☐ Pain restricts me to short necessary journeys under 30 minutes
☐ Pain prevents me from travelling except to receive treatment

Orebro Musculoskeletal Pain Questionnaire Short-Form

1. How long have you had your current pain problem?
Tick (√) one.

0-1 weeks [1]	1-2 weeks [2]	3-4 weeks [3]	4-5 weeks [4]	6-8 weeks [5]
9-11 weeks [6]	3-6 months [7]	6-9 months [8]	9-12 months [9]	over 1 year [10]

2. How would you rate the pain that you have had during the past week?
Circle one.

0 1 2 3 4 5 6 7 8 9 10 [ ]
No pain — Pain as bad as it could be

For items 3 and 4, please circle the one number that best describes your current ability to participate in each of these activities.

3. I can do light work (or home duties) for an hour.

0 1 2 3 4 5 6 7 8 9 10 [ ]
No pain — Without any difficulty

4. I can sleep at night.

0 1 2 3 4 5 6 7 8 9 10 [ ]
No pain — Without any difficulty

5. How tense or anxious have you felt in the past week?
Circle one.

0 1 2 3 4 5 6 7 8 9 10 [ ]
Absolutely calm and relaxed — As tense and anxious as I've ever felt

6. How much have you been bothered by feeling depressed in the past week?
Circle one.

0 1 2 3 4 5 6 7 8 9 10 [ ]
Not at all — Extremely

7. In your view, how large is the risk that your current pain may become persistent?

0 1 2 3 4 5 6 7 8 9 10 [ ]
No risk — Very large risk

8. In your estimation, what are the chances you will be working your normal duties (at home or work) in 3 months

0 1 2 3 4 5 6 7 8 9 10 [ ]
No chance Very Large Chance

9. An increase in pain is an indication that I should stop what I'm doing until the pain decreases.

0 1 2 3 4 5 6 7 8 9 10 [ ]
Completely disagree Completely agree

10. I should not do my normal work (at work or home duties) with my present pain.

0 1 2 3 4 5 6 7 8 9 10 [ ]
Completely disagree Completely agree

SUM:_____

SYDNEY WEST AREA HEALTH SERVICE | NSW HEALTH

SWHR-2091w

Hospital/Community Health Centre: Westmead Hospital	M.R.N.
Title / Family name	
Given names	
DOB / Sex / Consultant: Aimie Peek / Ward/Unit: Outpatient Radiology	
Address:	Postcode
Patient Contact Number: (home)	(work)

Westmead Hospital
MAGNETIC RESONANCE IMAGING (MRI)
RADIOLOGY DEPARTMENT
Hawkesbury Road, Westmead
Ph: 9845 7200 **Ext:** 57200 **Fax:** 9633 3107

Dear _____

An appointment has been made for you by Dr Aimie Peek to have an MRI examination.

Date / / Time _____

Please arrive 30 minutes before your appointment time with this form completed, to allow preparation for your examination.

Please answer all questions accurately.

Weight _____ kg Occupation _____

Do you have: *Please tick* ☑

Pacemaker or defibrillator implant ?	☐ Yes	☐ No
Neurostimulator ?	☐ Yes	☐ No
Cochlear or stapes (ear) implant ?	☐ Yes	☐ No
Cerebral aneurysm clip/s ?	☐ Yes	☐ No
Artificial heart valve or any stents ?	☐ Yes	☐ No
Have you ever had any metal fragments in your eyes ?	☐ Yes	☐ No
Are you or could you be pregnant ?	☐ Yes	☐ No
Are you breastfeeding ?	☐ Yes	☐ No

Important If you have answered YES to any of the above questions please ring as soon as possible to allow for implants to be checked prior to scanning. This is important for **SAFETY** reasons. Please call 02 9845 7200 and the staff will answer any questions.

The following items may interfere with the MRI examination. Do you have or have you:

Magnetic dentures	☐ Yes	☐ No	Shrapnel injury/foreign body	☐ Yes	☐ No
Hearing aids	☐ Yes	☐ No	Tattoos or permanent eye liner	☐ Yes	☐ No
Body piercings	☐ Yes	☐ No	Metal rods, screws, plates	☐ Yes	☐ No

Please list ALL previous surgery below:

_____

_____

_____

Form completed by: ☐ Patient ☐ Relative _____
Print name and contact number

☐ Physician or other Aimie Peek
Print name and contact extension number or page no.

Signature of person completing form _____ Date / /

PLEASE TURN OVER FOR IMPORTANT INFORMATION REGARDING YOUR SCAN

1/10

319

MRI Safety form

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MRI Safety form

What is MRI ?

Magnetic Resonance Imaging (MRI) is an advanced scanning method that uses a strong magnetic field and radiowaves to produce images of the body. **No x-rays are used**. This allows very high detailed images to be produced safely and painlessly.

Is there any preparation?

Preparing for your scan is relatively simple. You may eat normally unless we advise otherwise and take any prescribed medication. Plan to arrive 30 minutes before your appointment with this form filled out.

Occasionally some people suffer from claustrophobia which can be treated with sedation. This is not a reason to cancel your appointment. Please contact the MRI department as soon as possible so we can make alternative arrangements on another day when sedation is provided.

Metal devices cause interference with the MRI machine and their presence during the MRI procedure may cause injury to you. It is therefore important that you remove any metal objects, jewellery, hairpins, glasses, watches, credit cards, keys, wigs, makeup and dentures. Please ask the MRI staff before your scan if you have any questions.

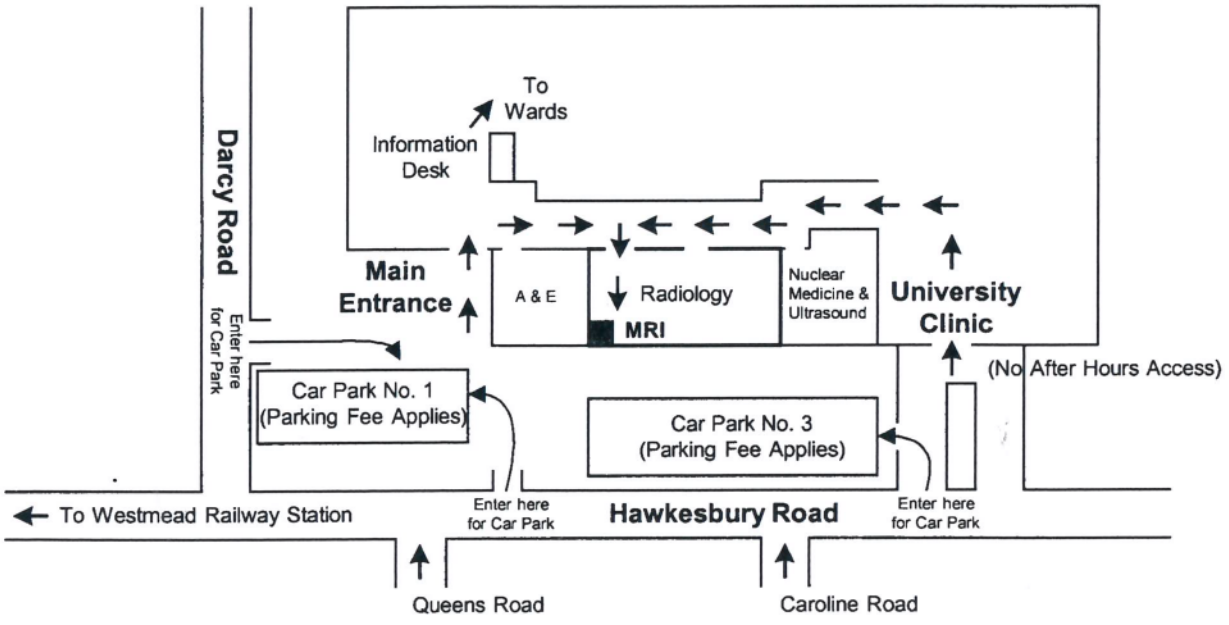
During the MRI scan

You will be asked to change into a gown and positioned on the scan table. The table will then slide into the magnet. During the scan the machine will make a lot of noise (this is the only sensation you will feel). You will be provided with earplugs, earphones and eye covers if you wish. You will also be given a contact buzzer so that you can contact us during the scan and talk to us. However, we ask that you only do this if absolutely necessary as this can extend the scan time. We will talk to you and observe you throughout the exam. The MRI scan generally takes between 20 mins to 1 hour depending on the part of the body being examined. It is extremely important that you keep still at all times during the scan. In some cases the doctor might require you to have an injection of contrast. This enhances the details of the MRI image which helps the doctor in the diagnosis of the image.

After the exam

Resume your normal daily activities. There are no after effects from this examination. The images will be reviewed by a radiologist and a report will be sent to your doctor in a few days.

PLEASE BRING ALL PREVIOUS X-RAYS, MRI, ULTRASOUND & CT SCANS



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MRI Westmead Screen

Participant ID _____

Upload Westmead Scanning Checklist

What is your pain level at this moment in time

0- No Pain — 100- Worst Imaginable Pain

(Place a mark on the scale above)

When did you last experience pain over 4/10

- ◯ Within 1 hour
- ◯ Over 1 hour under 12 hours
- ◯ Over 12 hours under 3 days
- ◯ Over 3 days under 7 days
- ◯ Over 7 days under 1 month
- ◯ Over 1 month

When was your last migraine, headache or back pain episode?

_____
(Please answer in hours or days, please specify)

Where are you on your menstrual cycle? (Females only)

- ◯ Day 1-5
- ◯ Day 6-10
- ◯ Day 11- 15
- ◯ Day 16- 20
- ◯ Day 21-25
- ◯ Day 26-30
- ◯ I am post menopausal*
- ◯ My contraception stops me having periods
- ◯ Other

(Day 1 is the first day of your period)

How many years post menopausal are you
(Only if ticked post menopausal in the above question)

- ◯ 0-5 years
- ◯ 5-10 years
- ◯ 10+ years

When was the last time you had a stimulant? (eg tea, coffee, energy drink)

_____
(Please answer in terms of hours or days, please specify)

How many units of alcohol do you consume a week

_____
(A medium glass of wine is around 2 units, A bottle of beer is 1.6 units)

When was the last time you had an alcoholic drink?

_____
(Please answer in terms of days or weeks, please specify)

When was the last time you had pain relief?

_____
(Please answer in terms of hours or days, please specify)

*Post Menopausal: You have stopped menstruating

What did you take?

_____

What other medications have you taken in the last 24 hours?

_____

MEGA G- Spectroscopy Run Sheet

Participant ID: Uploaded Cloudstor ☐

Year of Birth: Saliva Sample ☐

PACS ☐Sent ☐Received

Group:

Time point in study:

Scanning Date: Time: Start Finish

Radiographer:

PCG:

Voxel Size: AP HF LR

Flip angle:

Shim: PRESS: WREF: MPRESS WREF: MPRESS:

Comments:

Thalamus:

Voxel Size: AP HF LR

Flip angle:

Shim: PRESS: WREF: MPRESS WREF: MPRESS:

Comments:

ACC:

Voxel Size: AP HF LR

Flip angle:

Shim: PRESS: WREF: MPRESS WREF: MPRESS:

Comments:

Naming of any additional Scans:

APPENDIX TWO

Ethics Approval letters

Ethical approval for Chapters 3 and 4, including local agreements, and amendations.

The study was originally designed as a multi-centre study across three centres in New South Wales and Queensland, however, for logistical reasons the decision was made to collect data only at the New South Wales site.

Research Office File No: ***(5354)***

HREC Ref:	*AU RED HREC/17/WMEAD/429*
SSA Ref:	*AU RED SSA/17/WMEAD/*

15 December 2017

Dr Trudy Rebbeck
Discipline of Physiotherapy
University of Sydney

Dear Dr Rebbeck

Project title: An investigation of brain neurochemicals in migraine, whiplash associated disorder with persistent headache and low back pain after road traffic injury

Thank you for Aimie Peek's correspondence addressing the matters raised in the HREC's letter dated 3 November 2017 following single ethical review of the above project at its meeting held on 31 October 2017.

This HREC has been accredited by the NH&MRC as a lead HREC to provide the single ethical and scientific review of proposals to conduct research within the NSW, Victoria and Queensland public health systems under the Interstate Mutual Acceptance initiative. This lead HREC is constituted and operates in accordance with the National Health and Medical Research Council's *National Statement on Ethical Conduct in Human Research* and the *CPMP/ICH Note for Guidance on Good Clinical Practice.*

This proposal meets the requirements of the National Statement and I am pleased to advise that the HREC has now granted ethical approval of this **multicentre** research project to be conducted at:

- University of Sydney – Coordinating Chief Investigator Dr Trudy Rebbeck
- Westmead Hospital – Chief Investigator Ms Sheryl Foster
- Princes Alexandra Hospital (QLD) – Chief Investigator Prof Graham Galloway
- Royal Brisbane and Women's Hospital (QLD) – Chief Investigator Prof Graham Galloway

The following documentation has been reviewed and approved by the HREC:

- NEAF submission code AU/1/42D0317
- Scientific / Study Protocol Version 1 dated 20 September 2017
- Master Participant Information and Consent Form Version 2 dated 8 November 2017
- Baseline Questionnaires, version 2, dated 7 December 2017
- Screening Questionnaires, version 2, dated 7 December 2017
- Study Poster, version 2, dated 29 November 2017
- Data recording sheet, version 1, dated 20 September 2017

Please note the following conditions of approval:

- The Coordinating Chief Investigator will immediately report anything which might warrant review of ethical approval of the project in the specified format, including unforeseen events that might affect continued ethical acceptability of the project.
- **For clinical trials of implantable medical devices only** – The Coordinating Chief Investigator will confirm to the HREC that a process has been established for tracking the participant, with consent, for the lifetime of the device and will immediately report any device incidents to the Therapeutic Goods Administration (TGA).
- The Coordinating Chief Investigator will immediately report any protocol deviation / violation, together with details of the procedure put in place to ensure the deviation / violation does not recur.
- The Coordinating Chief Investigator will provide to the HREC in the specific format, proposed amendments to the research protocol or conduct of the research which may affect the ethical acceptability of the project, must be provided to the HREC to review in the specific format. Copies of all amendments when approved by the HREC must also be provided to the Research Governance Officer.
- The Coordinating Chief Investigator must notify the HREC, giving reasons, if the project is discontinued at a site before the expected date of completion.
- The Coordinating Chief Investigator must provide an annual report to the HREC and a final report at completion of the study, in the specified format. HREC approval is valid for 12 months from the date of final approval and continuation of the HREC approval beyond the initial 12 month approval period is contingent upon submission of an annual report each year. A copy of the Annual / Final Research Report Form can be obtained electronically from the Research Office on request.
- The HREC has the discretion to adopt other appropriate mechanisms for monitoring depending on the complexity, design and risk perceived including
    1. Discussion of relevant aspects of the project with investigators, at any time,
    2. Random inspection of research sites, data or consent documentation,
    3. Interview with research participants or other forms of feedback from them, and
    4. Request and review reports from independent agencies such as a Data Safety Monitoring Board.
- If your research project is an interventional trial, please ensure it is registered on one of the clinical trial registries, eg http://www.actr.org.au.
- It should be noted that compliance with the ethical guidelines is entirely the responsibility of the Coordinating Chief Investigator.

You are reminded that this letter constitutes *ethical approval only*. You must not commence this research project at a site until separate authorisation from the Chief Executive or delegate of that site has been obtained. Copies of this letter, together with any approved documents as enumerated above, must be forwarded to all site investigators for submission to the relevant Research Governance Officer.

Should you have any queries about the HREC's Terms of Reference, Standard Operating Procedures or membership, please contact the Executive Officer through the Research Office on 8890 9007 or emailing WSLHD-ResearchOffice@health.nsw.gov.au. In all future correspondence concerning this study, please quote Research Office File Number ***(5354).*** The HREC wishes you every success in your research.

[redacted]

cc Research Governance Office

WSLHD Research Governance Officer
Room 2050 Research & Education Network Building
Westmead Hospital
Cnr Hawkesbury and Darcy Roads
Westmead NSW 2145
Telephone: (02) 8890 9007
Facsimile: (02) 8890 9636
Email: wslhd-rgo@health.nsw.gov.au

11 January 2018

Ms Sheryl Foster
Department of Radiology
Westmead Hospital

WSLHD Research Office number: 5354
HREC reference number: HREC/17/WMEAD/429
SSA reference number: SSA/17/WMEAD/534
Project title: An investigation of brain neurochemicals in migraine, whiplash associated disorder with persistent headache, and low back pain after road traffic injury
Protocol number: Version 1, dated 20 September 2017.

Dear Ms Foster

Thank you for submitting an application for site authorisation of this project. I am pleased to inform you that site authorisation has been granted for this study to take place at the following site:

- Westmead Hospital

The approved information and consent documents for use at this site are:

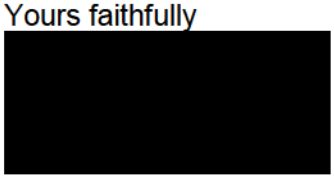
- Participant Information Sheet and Consent Form, Version 2 dated 8 November 2017.

The following conditions apply to this research project. These are additional to those conditions imposed by the Human Research Ethics Committee that granted ethical approval:

1. Proposed amendments to the research protocol or conduct of the research, which may affect the ethical acceptability of the project, and are submitted to the lead HREC for review, are copied to the research governance officer;

2. Proposed amendments to the research protocol or conduct of the research which may affect the ongoing site acceptability of the project, are to be submitted to the research governance officer.

Yours faithfully



Amelia Assareh
WSLHD Acting Research Governance Officer

Research Office File No: **(5354)**

HREC Ref: *HREC/17/WMEAD/429*
SSA Ref: *SSA/17/WMEAD/534*

10 April 2018

Dr Trudy Rebbeck
Discipline of Physiotherapy
University of Sydney

Dear Dr Rebbeck

Research title: An investigation of brain neurochemicals in migraine, whiplash associated disorder with persistent headache and low back pain after road traffic injury

We acknowledge your request for amendment dated 20 February 2018 in relation to the above study which was discussed at an out of session HREC Sub-Committee meeting.

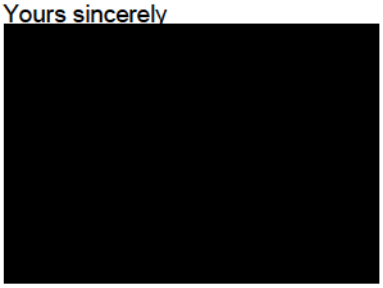
The HREC Sub Committee is constituted and operates in accordance with the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research and the CPMP/ICH Note for Guidance on Good Clinical Practice.

Your request to add Westmead Children's Hospital as a site with Dr Sushil Bandokar as an investigator to the study was reviewed and approved by Research Ethics and authorised by Research Governance at Western Sydney Local Health District

HREC approval is valid for 12 months from the date of the original approval and continuation of the HREC approval beyond the initial 12 month approval period is contingent upon submission of an annual report each year. A current version of the Annual / Final Research Report Form can be obtained electronically from the Research Office on request.

We appreciate your keeping us informed and look forward to receiving your next annual report.

Yours sincerely



Mrs Pat Fa
Secretary
WSLHD Human Research Ethics Committee

The Sydney children's Hospitals Network

care, advocacy, research, education

Site Authorisation Letter

Contact for this correspondence:
Research and Development
Name: Amelia Assareh
Phone: (02) 9845 3084
Facsimile: (02) 9845 1317
Email: Amelia.assareh@health.nsw.gov.au

Corner Hawkesbury Road
and Hainsworth Street
Locked Bag 4001
Westmead NSW 2145
Sydney Australia
DX 8213 Parramatta
Tel +61 2 9845 0000
Fax +61 2 9845 3489
http://www.schn.health.nsw.gov.au/
ABN 53 188 579 090

24 May 2018

Dr Sushil Bandokar
Department of Biochemistry
The Children's Hospital at Westmead

HREC reference number:	**HREC/17/WMEAD/429**
SSA reference number:	**SSA/18/SCHN/158**
Project title:	Brain neurochemicals in headaches and other pain conditions
Site:	**Children Hospital at Westmead**

Dear Dr Bandokar,

Thank you for submitting an application for authorisation of this project. I am pleased to inform you that authorisation has been granted for this study to take place at the above site.

The following conditions apply to this research project. These are additional to those conditions imposed by the Human Research Ethics Committee that granted ethical approval:

1. Please advise us of the date when the project starts at this site.
2. Proposed amendments to the research protocol or conduct of the research which may affect the ethical acceptability of the project, and which are submitted to the lead HREC for review, are copied to the research governance officer.
3. Proposed amendments to the research protocol or conduct of the research which may affect the ongoing site acceptability of the project are to be submitted to the research governance officer.
4. A copy of the annual report submitted to the lead HREC must be provided to this office after receipt of HREC acknowledgement.

All site post-authorisation reports and amendment applications should be sent at first instance to the SCHN governance inbox. Please visit our intranet or internet site for more information.

[redacted]

Amelia Assareh
Research Governance Officer

Research Office File No: **(5354)**

HREC Ref: *HREC/17/WMEAD/429*
SSA Ref: *SSA/17/WMEAD/534*

10 April 2018

Ms Sheryl Foster
Radiology Department
Westmead Hospital

Dear Ms Foster

Research Title: An investigation of brain neurochemicals in migraine, whiplash associated disorder with persistent headache, and low back pain after road traffic injury

We acknowledge the email from Ms Aimie Peek received on 28 March 2018 in response to our letter dated 19 March 2018 in relation to the above study which was discussed by an out of session HREC Sub-Committee meeting.

The HREC Sub Committee is constituted and operates in accordance with the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research and the CPMP/ICH Note for Guidance on Good Clinical Practice.

I am pleased to advise that the HREC Sub Committee has granted ethical approval of the Request for Amendment / Modification for this **multicentre** research project being conducted at:

- University of Sydney – Coordinating Chief Investigator Dr Trudy Rebbeck
- Westmead Hospital – Chief Investigator Ms Sheryl Foster
- Princes Alexandra Hospital (QLD) – Chief Investigator Professor Graham Galloway
- Royal Brisbane and Women's Hospital (QLD) – Chief Investigator Professor Graham Galloway
- Westmead Children's Hospital (NSW) – Chief Investigator Dr Sushil Bandodkar

The following documentation has been reviewed and approved by the HREC Sub Committee:

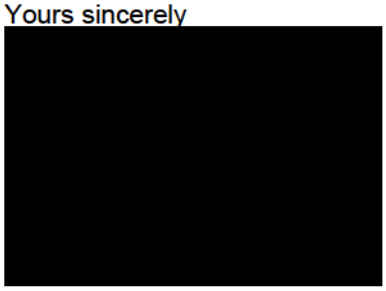
- Protocol – version 2 dated 15 February 2018
- PICF Master version 4 dated 20 March 2018
- PICF Westmead version 4 dated 20 March 2018
- PICF Master Tissue Banking version 2 dated 20 March 2018
- Patient Pain Diary- Weekly version 1 dated 1 March 2018
- Follow up Questionnaire version 2 dated 20 March 2018
- Letter from Dr Philip Vladica Acting Director Radiology Department Westmead Hospital dated 26 March 2018

This amendment has also been reviewed and authorised by the Research Governance Officer for Western Sydney Local Health District

HREC approval is granted for a period of 12 months and ongoing approval is contingent upon annual submission. Annual Reports for all studies should be submitted in November, they will be review, approved, and notified to the HREC. A copy of the Annual / Final Research Report Form can be obtained electronically from the Research Office on request.

We appreciate your keeping us informed and look forward to receiving your next annual report.

Yours sincerely



Mrs Pat Fa

WSLHD Human Research Ethics Committee

From: WSLHD-ResearchOffice
To: Aimie Laura Peek
Cc: amleaver@outlook.com; "Trudy Rebbeck"
Subject: Amendment Approved: 5354 - HREC/17/WMEAD/429 - SSA/17/WMEAD/534
Date: Thursday, 4 April 2019 9:32:52 AM
Attachments: Amendment_3_corrected.doc

Dear Dr Rebbeck,

Research Proposal: An investigation of brain neurochemicals in migraine, whiplash associated disorder with persistent headache, and low back pain after road traffic injury

I am pleased to advise approval has been granted for the Request for Amendment / Modification for this multicentre research project being conducted at:

- University of Sydney – Coordinating Chief Investigator Dr Trudy Rebbeck
- Westmead Hospital – Chief Investigator Ms Sheryl Foster
- Princes Alexandra Hospital (QLD) – Chief Investigator Professor Graham Galloway
- Royal Brisbane and Women's Hospital (QLD) – Chief Investigator Professor Graham Galloway
- Westmead Children's Hospital (NSW) – Chief Investigator Dr Sushil Bandodkar

The following documentation has been reviewed and approved.

- Patient Diary – 2019- Pain HA - version 1, dated 07 March 2019
- Patient Diary – 2019- Pain LBP - version 1, dated 07 March 2019
- Questionnaire / Survey: Day of Scanning questionnaire – version 1, dated 20 February 2019
- Recruitment Poster - version 3, dared 17 March 2019
- Recruitment Flyer_Brain neurochemicals_LBP - version 2, dated 29 March 2019
- Recruitment Flyer_Brain neurochemicals_Whiplash - version 2, dated 29 March 2019
- Recruitment Flyer_Brain neurochemicals_Migraine - version 2, dated 29 March 2019
- Recruitment Flyer_Brain neurochemicals_Control - version 2, dated 29 March 2019
- Migraine Checklist – version 2, dated 29 March 2019
- Visual Inc exc– version 1, dated 07 March 2019

This amendment has also been reviewed and authorised by the Research Governance Officer for Western Sydney Local Health District. For non-Western Sydney Local Health District sites please ensure that the Research Governance Officer for each site named on this study receives the updated approved documentation in relation to this amendment.

HREC approval is granted for a period of 12 months and ongoing approval is contingent upon annual submission. Annual Reports for all studies should be submitted in November, they will be review, approved, and notified to the HREC. A copy of the Annual / Final Research Report Form can be obtained electronically from the Research Office on request.

We appreciate your keeping us informed and look forward to receiving your next annual report.

Yours sincerely

[redacted]

Thank you.

Kind Regards,

Kavita Bhimsaria
Research Office Administrative Officer | **WSLHD Research & Education Network**
Westmead Hospital, Cnr Hawkesbury & Darcy Rds, Westmead NSW 2145
Tel 02 8890 9007 | Fax 02 9845 9636 | WSLHD-ResearchOffice@health.nsw.gov.au

NSW GOVERNMENT Health
Western Sydney
Local Health District

30 APRIL 2019

ANY STUDY STARTED IN ONLINE FORMS MUST BE FINALISED (APPROVED AND AUTHORISED)

ANY OUTSTANDING RESPONSES MUST BE FINALISED (APPROVED AND AUTHORISED) OR WITHDRAW. ANY STUDY NOT FINALISED (APPROVED AND AUTHORISED) WILL HAVE TO BE RESTARTED IN REGIS

If you have a study that is partially submitted or has outstanding responses please contact the research office as a matter of urgency to finalise or withdraw your project. Projects that have not been responded to by 30 April 2019 will be withdrawn and archived.

Amendments and any reporting should continue to be submitted via email to wslhd-researchoffice@health.nsw.gov.au until all data has been migrated to REGIS.

Royal Brisbane and Women's Hospital
Metro North Hospital and Health Service

Queensland Government

Enquiries to: Dr Merrilyn Banks, Executive Director of Research
Research Services, MNHHS-RBWH
Phone: 07 3646 8579

A/Prof Graham Galloway
Translational Research Institute
37 Kent Road
Woollongabba, QLD 4102

Dear Prof Galloway,

HREC reference no: HREC/17/WMEAD/429
Project reference no: 37871
Project title: An investigation of brain neurochemicals in migraine, whiplash associated disorder with persistent headache, and low back pain after road traffic injury

Thank you for submitting your research protocol approved by the NSW Health Western Sydney Local Health District HREC on 15 December 2017 with further amendment approved on 10 April 2018. I am pleased to inform you that authorisation has been granted for this study to be conducted at the MNHHS-RBWH. Your trial meets the principles and practices set out in the Australian Code for the Responsible Conduct of Research (2007 Universities Australia, updated 2014) and the ICH Harmonised Tripartite Good Clinical Practice (GCP) Guidelines.

The following documents approved by above mentioned HREC are specifically accepted for the MNHHS-RBWH site:

Document	Version	Date
Covering letter to RBWH RGO		27 June 2018
Human Research Ethics Application (Submission Code: AU/1/42D0317)		
Application: SSA (Submission Code: AU/3/76A7318)	3.1 (2010)	4 July 2018
Study Protocol	2.0	15 February 2018
RBWH Participant Information Sheet and Consent Form (Based on Master Participant Information Sheet and Consent Form V4.0 dated 20 March 2018)	4.0	27 June 2018
Patient Pain Diary – Weekly	1.0	1 March 2018
Follow up Questionnaire	2.0	20 March 2018
Baseline Questionnaire	2.0	7 December 2017
Screening Questionnaire	2.0	7 December 2017
Study Poster	2.0	29 November 2017
Data recording sheet	1.0	20 September 2017

Office
Royal Brisbane and Women's Hospital
Butterfield Street
Herston

Postal
Post Office
Herston 4029

Phone
(07) 3646 8111

Fax
(07) 3646 4240

Please email HREC approved amendments to RBWH-RGO@health.qld.gov.au and provide the description and the rationale. This will assist in the governance review to see if any further documentation is required for our MNHHS-RBWH site.

When the study commences please complete the Commencement form and send it to the HREC office with a copy to the Research Governance office. Forms can be found at the MNHHS Research Web site: https://www.health.qld.gov.au/metronorth/research/ethics-governance/post-approval-reporting/default.asp .

If you have any questions relating to this authorisation please contact the Research Governance Office on 3646 8579.

I wish you continued success with your research.

Yours sincerely

[redacted]

Dr Merrilyn Banks
Executive Director of Research
Research Services, MNHHS-RBWH
9/11/18

cc: A/Prof Katie McMahon, Herston Imaging Research Facility

Office
Royal Brisbane and Women's Hospital
Butterfield Street
Herston

Postal
Post Office
Herston 4029

Phone
(07) 3646 8111

Fax
(07) 3646 4240

Enquiries to: Metro South Research Governance
Phone: (07) 3443 8050
Email: MSH-RGO@health.qld.gov.au

Professor Graham Galloway
Translational Research Institute
37 Kent Street
WOOLLOONGABBA QLD 4102

SSA AUTHORISATION: METRO SOUTH HOSPITAL AND HEALTH SERVICE

HREC Reference number: HREC/17/WMEAD/429
SSA reference number: SSA/18/QPAH/491
Project Title: An investigation of brain neurochemicals in migraine, whiplash associated disorder with persistent headache, and low back pain after road traffic injury

Dear Professor Graham Galloway,

Thank you for submitting your application for authorisation of this project. On the recommendation of Metro South Research Governance Office, I am pleased to inform you that authorisation is granted for your research project to proceed at Princess Alexandra Hospital.

This approval is subject to researcher(s) compliance throughout the duration of the research with requirements as outlined in the National Statement on Ethical Conduct in Human Research 2007, Australian Code for the Responsible Conduct of Research and the Metro South Research Management Policy and Procedures. **The duration of this study approval is up until expiration of the reviewing HREC's approval.**

Site Specific Document/s Authorised	Version	Date
BDHP Agreement University of Qld		

The following conditions apply to this research proposal. These are additional to those conditions imposed by the approving HREC.

1. **PowerTrials*: Please review the Research Management – PowerTrials 2017-18 procedure to confirm if build is required.*** If you require support please contact MSH-Powertrials@health.qld.gov.au.
2. **Lapsed Approval:** If the study has not commenced within twelve months of approval, resubmission of the study to the approving HREC and RGO is necessary.
3. **Proposed amendments:** Amendments that may have a bearing on site specific documentation, financial arrangements or have legal implications (e.g. amendments to contracts) must be submitted to the Governance Office along with a copy of the HREC approval letter.
4. **Safety Monitoring:** All safety reporting should follow the requirements as set out in the NHMRC Safety Monitoring and Reporting in Clinical Trials involving Therapeutic Goods.
5. **Annual Reporting:** A copy of the annual report (due on the anniversary of HREC approval) and final report must be supplied to the governance office along with a copy of the HREC acknowledgement

We wish you every success in undertaking this research.

[redacted]

Queensland Government **Research Contracts Approval and Study Execution Form (Metro South Application Submission & Amendment Form)**

PART C - FOR COMPLETION BY METRO SOUTH RESEARCH GOVERNANCE OFFICE

Name of Contracted Party: The University of Qld **ABN:** 63942912684

Type of contract: BDHP Agreement **Contract Total Value:** $ 0

HREC/SSA Ref : HREC/18/Wmead/429 - SSA/18/QPAH/491 (Graham Galloway PAH)

Contract Start Date: Upon final signature Extension Options ☑ Yes ☐ No

Contract End Date: 1/1/2100

Does this contract indicate Metro South is providing a service (Sell) or is another institution providing Metro South with a service (Buy)? ☑ Revenue (sell) ☐ Expense (buy)

Compliance with MSH Financial Delegations Framework (Contract) – see section B of General Contracts Form

(Under the SBFA Act, a guarantee or indemnity can only be granted in limited circumstances)

Does MSH provide indemnity, guarantee in any form to the contracting party under the contract? ☐ Yes ☑ No

If yes, complete the Indemnity Register Entry Form. Must be approved by the relevant Financial Delegate (refer to the Financial Delegations Framework, Indemnity Procedure and Indemnity Guidance Note)

Legal Advice Sought

Internal/External legal advice received and contract terms/conditions accepted? ☐ Yes ☐ No ☑ Not Applicable

Based on Contract Management Framework thresholds

Comments:

Indemnity Delegate Signature (if applicable)

Please refer to Financial Delegations Manual for confirmation of appropriate delegate.

Name: Position:

Signature: ..... Date:

Metro South Research Governance Office – Recommendation for Authorisation

Name: Sonia Hancock **Position:** Compliance Manager

This proposal satisfies Metro South Health Research Governance requirements and is endorsed for consideration and final authorisation. Select checkbox applicable:

☐ *No contract required or* ☑ *Research contract is required.*

☐ *This study requires the granting of an indemnity and the Indemnity Register Entry form has been completed:*

Signature : [redacted] Date: 23/10/18

Metro South Health CE/ Delegate

Name: Prof Tim Geraghty **Position:** A/Chair, CHR

My signature indicates that I authorise this research study to commence at the nominated Metro South site/s on the condition the study will be conducted as per Metro South Research Management Policy PL2017-55.

Signed [redacted] Date: 26/10/18

Comments:
New study

Documents For CE/ Delegate Signature (as tagged)

Metro South Research Governance Authorisation Letter x1 ☐

Research Agreement ☐

Medicine Australia Standard Indemnity ☐

Research Agreement Variation ☑

Confidentiality Agreement ☐

Other:

Aimie Laura Peek

From: WSLHD-ResearchOffice <WSLHD-ResearchOffice@health.nsw.gov.au>
Sent: Thursday, 18 October 2018 3:40 PM
To: Aimie Laura Peek
Cc: 'Trudy Rebbeck'; amleaver@outlook.com
Subject: 5354 Amendment Approved

Dear Aimie,

Research Proposal: An investigation of brain neurochemicals in migraine, whiplash associated disorder with persistent headache, and low back pain after road traffic injury

I am pleased to advise approval has been granted for the Request for Amendment / Modification for this multicentre research project being conducted at:

- University of Sydney – Coordinating Chief Investigator Dr Trudy Rebbeck
- Westmead Hospital – Chief Investigator Ms Sheryl Foster
- Princes Alexandra Hospital (QLD) – Chief Investigator Professor Graham Galloway
- Royal Brisbane and Women's Hospital (QLD) – Chief Investigator Professor Graham Galloway
- Westmead Children's Hospital (NSW) – Chief Investigator Dr Sushil Bandodkar

The following documentation has been reviewed and approved.

- Study Protocol version 3, dated 24 September 2018
- Participant Information Sheet/Consent Form Master– version 5, dated 14 September 2018
- Participant Information Sheet/Consent Form Westmead– version 5, dated 14 September 2018
- Recruitment Poster version 2, dated 14 September 2018
- Other- Letter from head of Physiotherapy department dated 14 September 2018

Your request to add **Alexis Curtis, Mi Hoang Amanda Dinh**, as an investigator to the study was reviewed and approved by **Research Ethics** and authorised by **Research Governance.**

Any electrical equipment to come in contact with participants (for example ECG machine) that is provided for use in the study will need to be checked and documented by WS Biomedical prior to use at Westmead Hospital. Please contact James Wong, Director WS Biomedical WSLHD Phone: 8890 7731 Email: JamesDavid.Wong@ health.nsw.gov.au.

This amendment has also been reviewed and authorised by the Research Governance Officer for Western Sydney Local Health District. For non-Western Sydney Local Health District sites please ensure that the Research Governance Officer for each site named on this study receives the updated approved documentation in relation to this amendment.

HREC approval is granted for a period of 12 months and ongoing approval is contingent upon annual submission. Annual Reports for all studies should be submitted in November, they will be review, approved, and notified to the HREC. A copy of the Annual / Final Research Report Form can be obtained electronically from the Research Office on request.

We appreciate your keeping us informed and look forward to receiving your next annual report.

Yours sincerely

[redacted]
Mrs Pat Fa
Secretary

Thank you.

Kind Regards,

Kavita Bhimaria

Project Administrator | **WSLHD Research & Education Network**
Westmead Hospital, Cnr Hawkesbury & Darcy Rds, Westmead NSW 2145
Tel 02 8890 3643 | Fax 02 9845 9636 | WSLHD-ResearchOffice@health.nsw.gov.au

APPENDIX THREE

Media Engagement

Media engagement includes a feature in the University of Sydney, Faculty of Health Science research brochure 2017. In addition interviews by Migraine Australia in 2020 and 2021.

Trudy Rebbeck and Aimie Peek featured in the Faculty of Health Sciences Research Brochure 2017

Aimie Peek
PhD candidate

In the 12 years I have worked as a physiotherapist, I have been intrigued as to why some patients recover quickly while others are left with persistent symptoms. I realised there was a need to look deeper into the neurophysiology of persistent pain conditions, specifically headache and migraine.

If we can understand why certain patients are not responding to traditional treatment approaches at a cellular level, we can better tailor treatments to their individual needs. My current research uses non-invasive brain imaging to validate a biomarker for migraine, which will hopefully improve our understanding of the condition and spur the development of new treatments.

As a physiotherapist, it is very important to produce clinically meaningful research; a vision I share with my supervisor Dr Trudy Rebbeck. I also want to expand my research to explore other pain conditions such as neck and low back pain, one of the leading causes of disability worldwide.

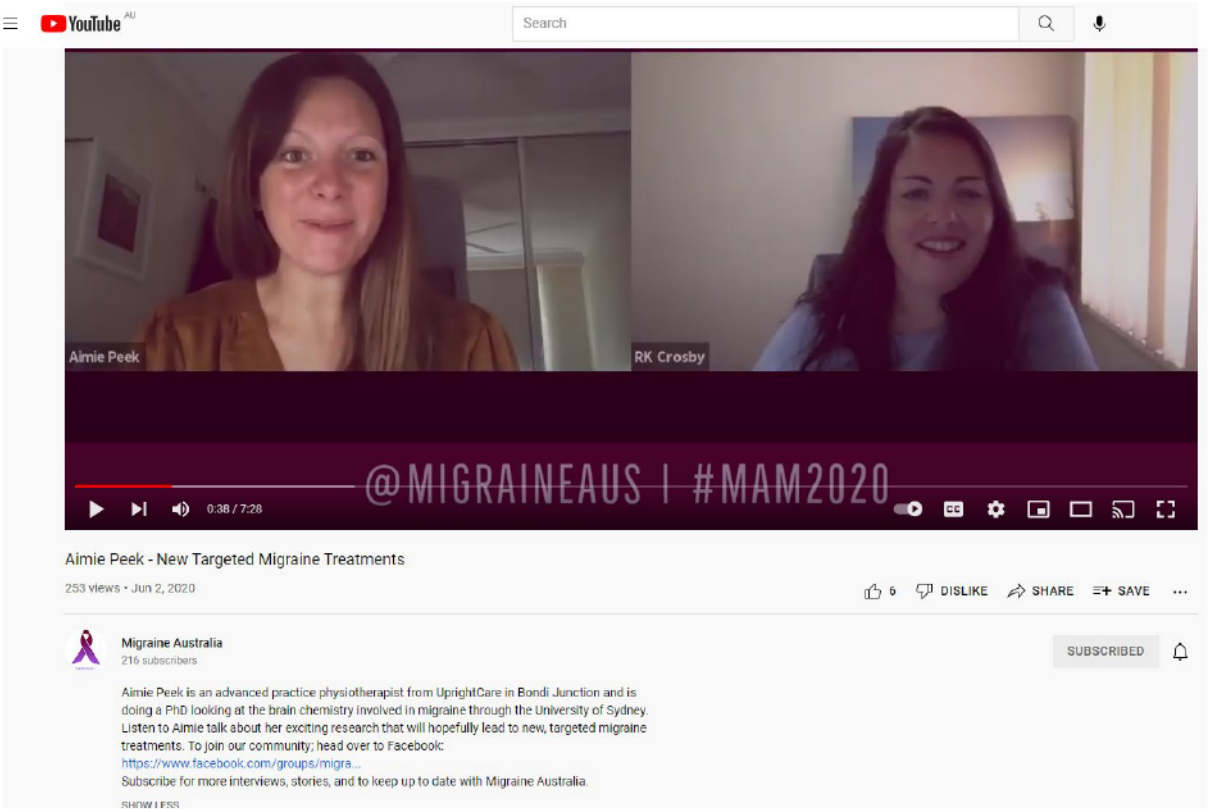
"Early in my physiotherapy career, it frustrated me that we often had no answers for distressed patients who presented with unimaginable pain."

Dr Trudy Rebbeck

Early in my physiotherapy career, it frustrated me that we often had no answers for distressed patients who presented with unimaginable pain. I was most concerned by the variety of failed treatments and different and confusing messages they received. Spurred on by this, I became a specialist musculoskeletal physiotherapist, a role in which I was able to provide comprehensive and targeted solutions and turn my patients' lives around.

I am driven to translate research into practice and teaching. In a recent breakthrough, we discovered different concentrations of brain neurochemicals (GABA) in people with migraine.

I have been privileged to work with industry throughout my career, such as partnering with the State Insurance Regulatory Authority since 2000. We published the first guidelines on how whiplash is managed in affected individuals. Since then, I have worked with multiple disciplines and institutions in educating clinicians, general practitioners, physiotherapists and medical specialists.



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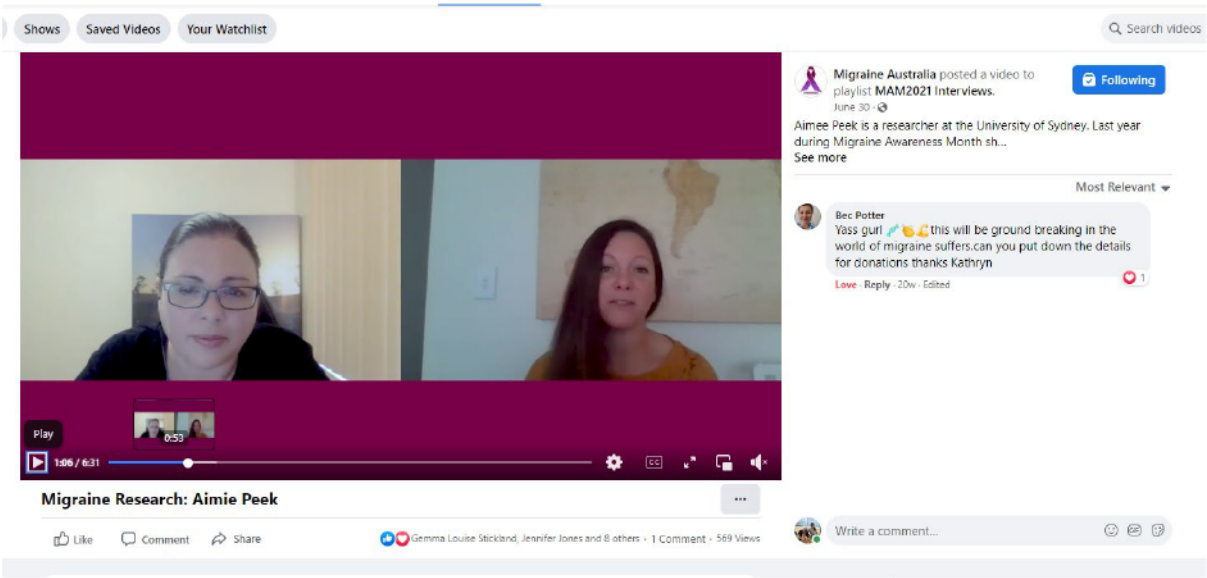

Available from https://www.facebook.com/watch/?v=1146026805804118 or https://www.youtube.com/watch?v=UNHwfGJ_JTQ

Research panel: the future for migraine research

Available from https://www.youtube.com/watch?v=f-cb5zmB-og

APPENDIX FOUR

Related project not forming part of the thesis

The systematic review (Chapter 2) lead to the invitation for collaboration in a systematic review of brain neurochemistry in alcohol use disorder using the MRS-Q. Attached in this appendix is the registered protocol of the project.

Citation

Marilena DeMayo, Kirsten Morley, Warren Logge, Glenn Hunt, Aimie Peek. Glutamate alterations in Alcohol Use Disorder: A systematic review and meta-analysis of magnetic resonance spectroscopy studies. PROSPERO 2020 CRD42020144746 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020144746

Review question

What is the nature of glutamatergic alterations in individuals with Alcohol Use Disorder?

What are the effects of different stages of abstinence on glutamate concentration in Alcohol Use Disorder?

Searches

We will search PubMed, EMBASE, Ovid MEDLINE, Web of Science, Google Scholar, and PsycINFO.

The search strategy will include terms relating to alcohol use disorder and magnetic resonance spectroscopy.

Only studies published in English will be included. Studies up until the date the searches are run will be included. The searches will be re-run just before the final analyses and further studies retrieved for inclusion.

We will also examine reference lists of eligible studies and review articles.

Types of study to be included

Cross-sectional studies examining levels of glutamate in an alcohol use disorder group and a control group. Longitudinal studies that report on comparisons between alcohol use disorder and light/non-drinking controls. Randomised controlled trials that report on baseline differences between alcohol use disorder and light/non-drinking contols.

Condition or domain being studied

Alcohol Use Disorder

Participants/population

Inclusion criteria: studies that compare individuals with Alcohol Use Disorder as defined by Diagnostic and Statistical Manual criteria or similarly structured method to a light drinking or non-drinking control group on a measure of glutamate, glutamine or Glx.

Exclusion criteria: post-mortem and animal investigations. Review articles and those without a light or non-drinking comparison group will be excluded

Intervention(s), exposure(s)

Glutamate, glutamine or a combination of the two (glx) as measured by proton magnetic resonance spectroscopy (1H-MRS) within the brain will be the focus of this review.

Phosphorous MRS and indirect measures such as those taken from saliva or plasma will be excluded, as will studies that used spectroscopy on other forms of tissue such as the spinal cord. 1H-MRS studies of the brain solely investigating other metabolites will also be excluded.

Comparator(s)/control

Control group in study (light drinkers or non drinkers)

Main outcome(s)

Primary outcome is difference in glutamate, glutamine or glx level between individuals with Alcohol Use Disorder and control group.

Measures of effect

Abstinence from alcohol (time from last drink)

It is anticipated that the studies included in the the review are likely to be cross sectional, however, if intervention studies are retrieved then the baseline measures will be used.

Additional outcome(s)

None

Measures of effect

Not applicable

Data extraction (selection and coding)

Titles and/or abstracts of studies retrieved using the search strategy and those from additional sources will be screened independently by two review authors (MMD and ALP) to identify studies that potentially meet the inclusion criteria outlined above. The full text of these potentially eligible studies will be retrieved and independently assessed for eligibility by two review team members. Any disagreement between them over the eligibility of particular studies will be resolved through discussion with a third reviewer.

Data to be extracted by two reviewers independently. Data extracted will include patient demographics (including age, sex), confounders (including medication use, smoking status, time since last drink). Glutamate, Glutamine or Glx level, region of interest and voxel size.

Where data is missing the authors will be contacted twice via provided email.

Risk of bias (quality) assessment

Two quality assessments will be conducted.

The Appraisal Tool for Cross Sectional Studies (AXIS; Downes, 2016) will be used to assess the quality of the cross-sectional design and likelihood of bias in the data collection. MMD and ALP will conduct this independently and any inconsistencies will be resolved by WL.

The MRS-Q (Peek 2020) will be used to assess the quality of the glutamate spectroscopy. MMD and ALP will conduct this independently and any inconsistencies will be resolved by WL.

Strategy for data synthesis

In order to compare across different measures (mmol, IU, ratios) the standardized mean differences (with 95% confidence interval) will be calculated to allow comparison between studies. Regions will be grouped as anatomically appropriate, with sufficiently homogeneous labels collapsed (for instance, within the prefrontal cortex)

Where sufficient homogeneity exists, as calculated by the I^2 test, results will be pooled for meta-analysis using a fixed effects model. If significant heterogeneity is identified then a random effects model will be applied.

Where meta-analysis is not possible a narrative synthesis will be used, following the guidelines stipulated by the PRISMA-P tool (Rogers et al. 2009). All statistical analysis will be performed using the computer software Comprehensive Meta-analysis.

Smoking levels and medication amounts will be noted and included in the analysis as modifiers to see if they

explain variance or have a relationship/association using meta-regression

Analysis of subgroups or subsets
Data will also be collapsed across three abstinence groups:

- Acute abstinence (<24 hours)

- Recent abstinence (24+ hours to 3 weeks)

- Prolonged abstinence (3< weeks)

Smoking levels and medication amounts will be noted and included in the analysis where possible.

In a secondary analysis high quality datasets as per MRS-Q will be analysed to assess the effects of study quality.

Contact details for further information
Marilena DeMayo
marilena.demayo@sydney.edu.au

Organisational affiliation of the review
University of Sydney

Review team members and their organisational affiliations
Ms Marilena DeMayo. University of Sydney
Assistant/Associate Professor Kirsten Morley. University of Sydney, Faculty of Medicine and Health, Central Clinical School, NHMRC Centre for Research Excellence in Mental Health and Substance Use, NSW, Australia.
Dr Warren Logge. University of Sydney, Faculty of Medicine and Health, Central Clinical School, NHMRC Centre for Research Excellence in Mental Health and Substance Use, NSW, Australia.
Assistant/Associate Professor Glenn Hunt. Discipline of Psychiatry and Addiction Medicine, Concord Clinical School, University of Sydney, Hospital Rd, Concord, NSW, 2139, Australia
Aimie Peek. University of Sydney, Faculty of Medicine and Health, University of Sydney, East Street, Lidcombe, NSW, 2042, Australia

Type and method of review
Meta-analysis, Narrative synthesis, Systematic review

Anticipated or actual start date
01 March 2019

Anticipated completion date
01 July 2020

Funding sources/sponsors
None

Conflicts of interest

Language
English

Country
Australia

Stage of review
Review Ongoing

Subject index terms status
Subject indexing assigned by CRD

Subject index terms

Alcoholism; Glutamic Acid; Humans; Magnetic Resonance Imaging; Magnetic Resonance Spectroscopy

Date of registration in PROSPERO
28 April 2020

Date of first submission
04 August 2019

Details of any existing review of the same topic by the same authors
NA

Stage of review at time of this submission

Stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

The record owner confirms that the information they have supplied for this submission is accurate and complete and they understand that deliberate provision of inaccurate information or omission of data may be construed as scientific misconduct.
The record owner confirms that they will update the status of the review when it is completed and will add publication details in due course.

Versions
28 April 2020