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Excitatory and inhibitory responses in the brain to experimental pain: A systematic review of MR spectroscopy studies

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ABSTRACT

Background: The role of the brain in processing pain has been extensively investigated using various functional imaging techniques coupled with well controlled noxious stimuli. Studies applying experimental pain have also used proton magnetic resonance spectroscopy (^{1}H-MRS). The advantage of MRS compared to other techniques is the capacity to non-invasively examine metabolites involved in neurotransmission of pain, including glutamate, γ-aminobutyric acid (GABA), glutamate + glutamine (Glx), and glutamine.
Objective: To systematically review MRS studies used in the context of studying experimental pain in healthy human participants.
Data sources: PubMed, Ovid Medline, and Embase databases were searched using pre-specified search terms.
Eligibility criteria: Studies investigating glutamate, GABA, Glx and/or glutamine in relation to experimental pain (e.g., heat) in healthy participants via MRS.
Appraisal criteria: Each study was evaluated with a modified quality criterion (used in previous imaging systematic reviews) as well as a risk of bias assessment.
Results: From 5275 studies, 14 met the selection criteria. Studies fell into two general categories, those examining changes in metabolites triggered by noxious stimulation or examining the relationship between sensitivity to pain and resting metabolite levels. In five (out of ten) studies, glutamate, Glx and/or glutamine increased significantly in response to experimental pain (compared to baseline) in three different brain areas. To date, there is no evidence to suggest Glx, glutamate or glutamine levels decrease, suggesting an overall effect in favour of increased excitation to pain. In addition to no changes, both increases and decreases were reported for levels of GABA+ (=GABA + macromolecules). A positive correlation between pain sensitivity and resting glutamate and Glx levels were reported across three studies (out of three). Further research is needed to examine the relationship of GABA+ and pain sensitivity.
Limitations: A major limitation of our review was a limited number of studies that used MRS to examine experimental pain. In light of this and major differences in study design, we did not attempt to aggregate results in a meta-analysis. As for the studies we reviewed, there was a limited number of brain areas were examined by studies included in our review. Moreover, the majority of studies included lacked an adequate control condition (i.e., non-noxious stimulation) or blinding, which represent a major source of potential bias.
Conclusion: MRS represents a promising tool to examine the brain in pain, functionally, and at rest with support for increased glutamate, glutamine and Glx levels in relation to pain.

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Implications: Resting and functional MRS should be viewed as complementary to existing neuroimaging techniques, and serve to investigate the brain in pain.
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1. Introduction

More than two decades of research has examined the brain's response to pain using task-related functional magnetic resonance imaging (fMRI) (Aharon et al., 2006; Becerra et al., 1999, 2001; Erpelding et al., 2012; Fulbright et al., 2001; Peyron and Fauchon, 2018; Teutsch et al., 2008; Wager et al., 2013; Bingel et al., 2004; Borsook et al., 2018; Brascher et al., 2016; Brooks et al., 2005; Cauda et al., 2014; DaSilva et al., 2018; Davis et al., 2002). Unprecedented insights into cortical and subcortical areas involved in the processing (e.g., anterior cingulate cortex and insula) and modulation of pain (e.g., periaqueductal grey matter) have been discovered, providing the foundation on which to explore the biology of analgesia, as well as the pathophysiology and anatomy of chronic pain (Borsook and Becerra, 2006). The knowledge generated in the course of characterizing the healthy brain in pain using fMRI has been synthesized in a number of comprehensive reviews (see (Peyron and Fauchon, 2018; Schweinhardt and Bushnell, 2010; Schweinhardt et al., 2006; Tracey, 2017; Zunhammer et al., 2018)).

In addition to fMRI, there are a variety of other neurophysiological and anatomical tools that are used to study the brain's response to pain. Among these, *in vivo* proton magnetic resonance spectroscopy (^{1}H-MRS) offers a unique opportunity to non-invasively measure levels of glutamate, γ-aminobutyric acid (GABA), glutamate + glutamine (Glx), and glutamine (i.e., the precursor of glutamate and GABA), among others (Novotny et al., 2003). As the primary excitatory and inhibitory neurotransmitters in the central nervous system (CNS), glutamate and GABA, respectively, are integrally involved in the transmission and generation of pain. This is evidenced in animal models, with experimental pain shifting the brain towards greater levels of excitation (Okuda et al., 2001; Silva et al., 2000; Sluka and Willis, 1998; Vetter et al., 2001). In humans, GABA agonists and glutamate antagonists demonstrate potent analgesic effects in response to experimental noxious stimulation, providing indirect evidence for a role in pain (Franklin et al., 2012; Kumru et al., 2013; Niesters et al., 2012; Rogers et al., 2004).

To date, the majority of studies applying MRS to measure neurotransmitter levels in the brain have done so at rest (Fayed et al., 2013; Gussew et al., 2011; Harris et al., 2009; Ito et al., 2017; Thiaucourt et al., 2017; Zunhammer et al., 2016). Such a static approach has proven sensitive to detect differences in brain function between patients with chronic pain and healthy controls (Gussew et al., 2011; Harris et al., 2009; Ito et al., 2017). Seminal observations also indicate MRS is useful as a functional tool (fMRS); suitable to track task-related fluctuations in neurotransmitter levels in response to experimental pain (Chiappelli et al., 2017; Cleve et al., 2014, 2017; Gussew et al., 2010; Gutzeit et al., 2011, 2013; Hansen et al., 2014; Kupers et al., 2009; Mullins et al., 2005). Unlike fMRI (Peyron and Fauchon, 2018; Schweinhardt and Bushnell, 2010; Schweinhardt et al., 2006; Tracey, 2017; Zunhammer et al., 2018) these findings have not been comprehensively reviewed.

This study aimed to systematically review experimental pain studies examining neurotransmitters using MRS. We specifically aimed to identify (1) changes in glutamate, GABA, Glx and glutamine triggered by experimental noxious stimulation, and (2) relationships between subjects' pain sensitivity at rest and levels of glutamate, GABA, Glx and glutamine.

2. Methods

2.1. Protocol registration

This review was conducted in adherence to the Preferred Reporting Items for Systematic Reviews and Meta-analysis statement (PRISMA) (Moher et al., 2009) and was registered on Prospero (CRD42018112917).

2.2. Electronic literature search

A systematic search of the literature was performed in the PubMed, Ovid Medline, and Embase databases. The results were formatted in accordance with the PRISMA checklist (Appendix 1). The search included all publications up until October 31st, 2019. Key terms included: magnetic resonance spectroscopy (H-MRS, functional MRS, fMRS), neurotransmitters (glutamate, GABA, Glx, glutamine), and experimental pain (pain). The full search strategy and search terms are outlined in Appendix 2. Reference lists of included studies were searched to ensure key studies had not been overlooked.

2.3. Eligibility criteria

- Population: the study included healthy human subjects (i.e., as defined by authors; no pre-existing health condition)
- Intervention: any type of noxious stimulus applied directly to the participant
- Comparison: non-noxious stimulation (when available)
- Outcome: glutamate, GABA, Glx, or glutamine levels in the brain, measured by ^{1}H-MRS at a field strength of at least 3 T (T).

Studies were screened for language (i.e., English only) and must have been published in a peer-reviewed journal, indexed in PubMed, Ovid Medline, or Embase. All study designs were considered, such as correlational (i.e., reporting on neurotransmitters in relation to pain sensitivity), cross-sectional (i.e., a type of observational study analyzing data at one specific time point), or interventional (i.e., examining changes before, during or after noxious stimulation). Reviews and case reports and other forms of spectroscopy (i.e., carbon or phosphorous MRS) were excluded. Studies using MRS at a field strength of 1.5 T were also excluded on the grounds that metabolite levels are more reliably measured at 3 T (or greater) due to reduced spectral overlap and improved signal-to-noise ratio (Wilson et al., 2019). This is especially important for neurotransmitters such as glutamate, GABA, Glx and glutamine (Wilson et al., 2019). Further, studies investigating neurotransmitters in animals, specialized clinical conditions (without a healthy control group), or *in-vitro* were excluded (Fig. 1).

2.4. Study selection

Abstracts identified in the initial search were imported into EPPI-Reviewer 4 software. Duplicates were removed, and eligibility criteria were applied based on titles and abstracts in order to determine relevant manuscripts for full-text review. Two authors (JA and AE) independently screened the studies. The full text of each article was then analyzed by two authors (JA and AE) to determine suitability for final inclusion, with discrepancies resolved by discussion with a third reviewer (JLK).

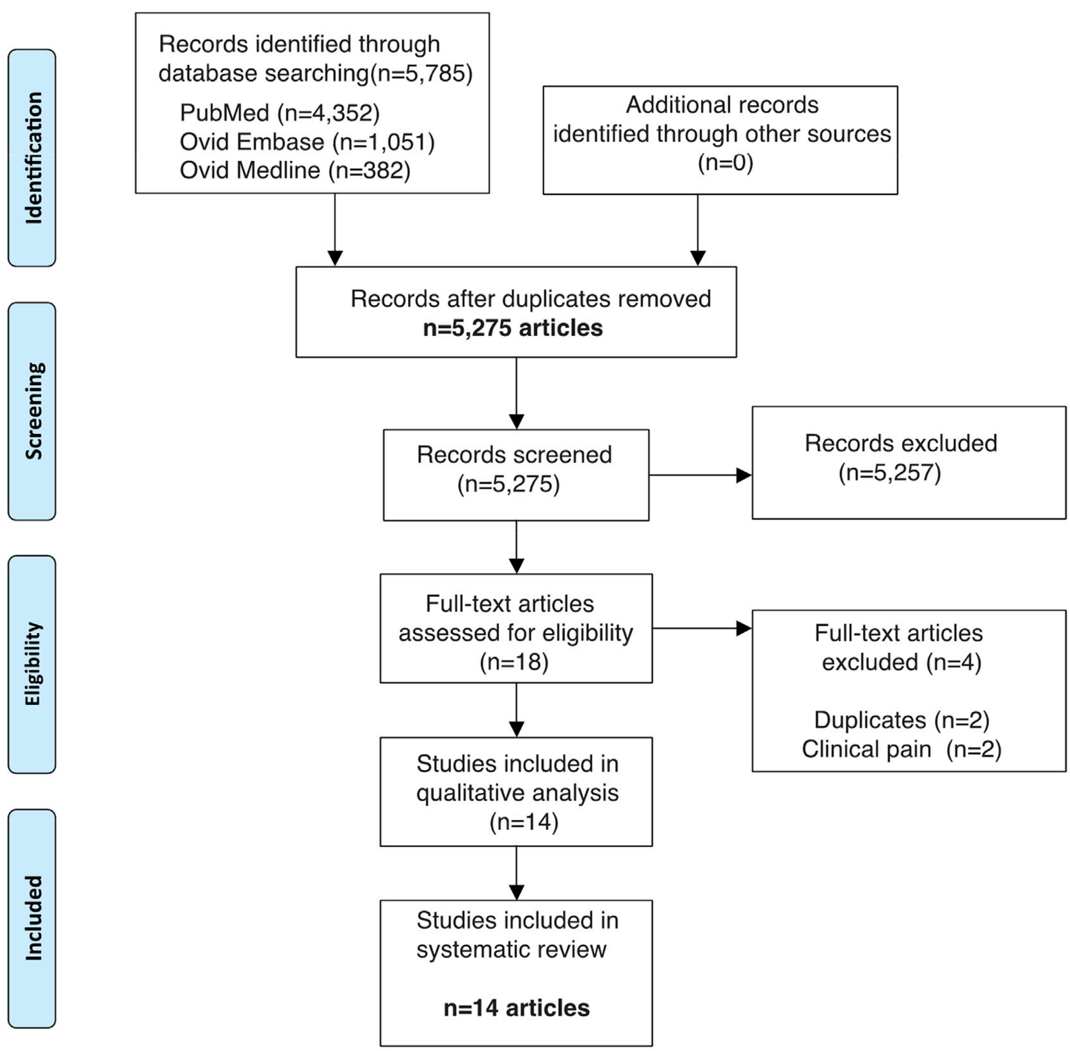
2.5. Study appraisal and risk of bias assessment

All included studies were subject to quality and risk of bias assessments. Critical appraisal criteria were adapted from a previous study (Campbell et al., 2013), which has been applied in neuroimaging systematic reviews (Jutzeler et al., 2015; Seixas et al., 2014). Two questions relevant to MRS were added (i.e., question seven and eleven). Further, question five (“Does the study have a proper control condition?“) refers to a non-noxious control condition, either of another sensory modality or no stimulation (Table 1). The risk of bias assessment focused on reporting and detection bias. Reporting bias refers to the selective reporting of some outcomes but not others, depending on the nature and direction of the results (Boutron et al., Altman). Detection bias refers to systematic differences between conditions in how outcomes are determined. Blinding of outcome assessors may reduce the risk of biasing the outcome measurement (Boutron et al., Altman) (Table 2). These criteria were assessed by two authors independently (JA and CRJ), and disagreements were resolved at a consensus meeting with a third reviewer (JLK) (Tables 1 and 2).

3. Quality assessment criteria questions

1) Does the study have a clear defined research objective?
2) Does the study adequately describe the inclusion/exclusion criteria?
3) Does the study report on the population parameters and demographics?
4) Does the study report details on noxious stimulation paradigms?
5) Does the study have a proper control condition?
6) Does the study provide details of imaging protocol?
7) If quantifying GABA, was an edited sequence applied?
8) Are subjects selected to participate in the study likely to be representative of the target population?
9) Is it unlikely that subjects received an unintended intervention (contamination or co-intervention) that may influence the results?
10) Does the study adequately report on the strength of effect (i.e., ways of calculating effect size, reporting of confidence intervals)?
11) Do the authors report on quality criteria metrics for MRS results (i.e., SNR, LW, CRLB)?
12) Do the authors report on the limitations of the study?

Signal to-noise-ratio (SNR); Line width (LW); Cramer-Rao minimum lower bounds (CRLB). See Box 1 for definitions (Kreis, 2004).



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *Meta-A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Fig. 1. PRISMA flowchart illustrating study inclusion.

Table 1
Quality assessment of studies.

	1	2	3	4	5	6	7	8	9	10	11	12
Mullins et al., 2005r	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	Y	Y
Kupers et al., 2009r	Y	Y	Y	Y	N	Y	N	Y	Y	Y	Y	N
Gussew et al., 2010r	Y	Y	Y	Y	Y	Y	NA	N	Y	Y	Y	Y
Gutzeit et al., 2011r	Y	Y	Y	Y	Y	Y	NA	N	Y	Y	N	Y
Gutzeit et al., 2013r	Y	Y	Y	Y	N	Y	NA	N	Y	Y	N	Y
Cleve et al., 2014r	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y
Hansen et al., 2014r	Y	Y	Y	Y	N	Y	NA	Y	Y	Y	Y	N
Cleve et al., 2017r	Y	Y	Y	Y	N	Y	Y	N	Y	Y	Y	Y
de Matos et al., 2017	Y	Y	Y	Y	N	Y	Y	N	Y	Y	Y	Y
Chiappelli et al., 2017r	Y	Y	Y	Y	N	Y	NA	Y	Y	N	Y	Y
Harris et al., 2009r	Y	Y	Y	Y	N	Y	NA	N	N	Y	N	Y
Zunhammer et al., 2016r	Y	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y
Thiaucourt et al., 2017r	Y	Y	Y	Y	N	Y	Y	N	N	Y	Y	Y
Gradinger et al., 2019r	Y	Y	Y	Y	N	Y	Y	N	N	Y	N	N

Y = yes, N = no, NA=Not applicable.

3.1. Data extraction process

From each study, we extracted (i) the method applied to quantify pain sensitivity (e.g., thresholds), (ii) the direction of the relationship between pain sensitivity and glutamate, GABA, Glx, and/or glutamine, and (iii) an estimate of the strength of the relationship (as reported by the authors of the original study). Functional MRS studies were further characterized as block or event-related. Block designs comprised studies that incorporated a single, long-duration noxious stimulus, which was then compared to rest. Event-related designs incorporated repeated presentation of short-duration stimuli, which was then averaged to determine changes from rest (i.e., periods of time with no noxious stimulation). For both functional designs, the type of noxious stimulation, direction, and significance of change were extracted for all neurotransmitters (glutamate, GABA, Glx, and glutamine), and then recorded. GABA is referred to as GABA + to recognize the contamination of macromolecules (Near et al., 2011). Additional study details, including the number of subjects, and sex of subjects, were also extracted (Table 3). The extraction of technical specifications related to data acquisition procedures focused on MR manufacturer and field strength, MRS acquisition parameters [repetition time (TR), echo time (TE), number of averages, region of interest (ROI)], analysis methods (software, units reported), and spectral quality metrics (SNR, LW, CRLB) were also extracted (Table 4). Data extraction was performed by two reviewers (JA and AE) to minimize bias. WebPlotDigitizer was used to evaluate data presented in figures.

4. Results

4.1. Included/excluded studies

The literature search yielded a total of 5275 candidate publications (Fig. 1). Following the review of titles and abstracts, eighteen studies were retained for full-text review. The full-text review led to the exclusion of four studies. Of the remaining fourteen studies, a total of 250 subjects (~46% male) were examined. Using the quality assessment criteria listed in Table 1, the most common quality concern among included studies was the lack of a control group (Chiappelli et al., 2017; Cleve et al., 2017; Gutzeit et al., 2013; Hansen et al., 2014; Kumru et al., 2013; Kupers et al., 2009; de Matos et al., 2017a; Thiaucourt et al., 2017; Zunhammer et al., 2016). Based on communication with the authors, one study (Thiaucourt et al., 2017) was excluded because of overlap in subjects who were included in the target analysis in a larger, subsequent study (Gradinger et al., 2019). Therefore we included thirteen studies in this review. When assessing the risk of bias (Table 2), only one study was found to have neither reporting or detection bias. Detailed information on the included studies is provided in Tables 3–5.

4.2. MRS methods and spectral quality results

All of the reviewed studies applied single voxel MRS. Detailed information, including scanner information, localization sequences, TE, TR, ROI, and voxel size are presented in Table 4. LCModel fitting was most frequently utilized for analysis (Chiappelli et al., 2017; Cleve et al., 2014; Gradinger et al., 2019; Gutzeit et al., 2011, 2013; Hansen et al., 2014; Harris et al., 2009; Kupers et al., 2009; de Matos et al., 2017a; Zunhammer et al., 2016), and creatine ratios were the most common method of quantification (Cleve et al., 2014, 2017; Gutzeit et al., 2011; Hansen et al., 2014; Zunhammer et al., 2016) (Table 4). The majority of reviewed studies were focused on the cingulate and/or insular cortices. Voxel size variation ranged from 2.5 to 27 mL in the brain. Only two other brain areas were investigated - the occipital cortex, and the brainstem nuclear complex (Cleve et al., 2014; de Matos et al., 2017a). Five (out of thirteen) studies did not report any spectral quality metrics (Cleve et al., 2014; Gradinger et al., 2019; Gutzeit et al., 2011, 2013; Harris et al., 2009). However, one study set *a priori* spectral quality criteria: full width at half maximum (i.e., FWHM< 0.07 ppm; SNR_{NAA} >20 (Cleve et al., 2014). One study reported no significant change in FWHM and SNR during rest and stimulation but did not provide exact values (Kupers et al., 2009).

When quantifying the neurotransmitters in the region of interest, only five studies (out of thirteen) corrected for the individual estimated brain tissue fraction (i.e. grey matter, white matter and cerebral spinal fluid) for each subject (Chiappelli et al., 2017; Cleve et al., 2014, 2017; Gussew et al., 2010; Harris et al., 2009). Of the five studies reporting GABA+, none adopted macromolecular suppression (Cleve et al., 2014, 2017; Gradinger et al., 2019; Kupers et al., 2009; Zunhammer et al., 2016) (see: *Detection of GABA+: Spectral editing techniques)*.

4.3. Neurochemical changes in response to experimental pain

Ten of thirteen studies applied noxious stimulation during the MRS scan and assessed changes during stimulation relative to baseline/rest. The types of painful stimuli included heat (Chiappelli et al., 2017; Cleve et al., 2014, 2017; Gussew et al., 2010; Hansen et al., 2014; Kupers et al., 2009; Zunhammer et al., 2016) and cold (Mullins et al., 2005) delivered at noxious intensities for varying lengths of time (Mullins et al., 2005; Zunhammer et al., 2016), as well as electric (Gutzeit et al., 2011, 2013; de Matos et al., 2017a), pressure (Harris et al., 2009), and mechanical stimulation (Gradinger et al., 2019; Zunhammer et al., 2016). Additional details of the noxious interventions are shown in Table 3. Changes in glutamate and Glx both ranged from 0 (Chiappelli et al., 2017; Cleve et al., 2017; Hansen et al., 2014; Kupers et al., 2009) to approximately +20% relative to baseline (Cleve et al., 2014; Gussew et al., 2010; Gutzeit et al., 2011, 2013;

Table 2
Risk of bias assessment.

Study	Reporting Bias	Detection Bias		Notes
	Results presented in accordance with pre-specified plan	Blinding	Randomization	
Mullins et al., 2005	Yes	• Yes	Yes	–
Kupers et al., 2009	Probably no	• No	• No	Unclear pre-specified analysis plan (e.g., unknown justification for presenting ratios between stimulation and rest).
Gussew et al., 2010	Yes	• No	No	–
Gutzeit et al., 2011	Yes	• No	No	–
Gutzeit et al., 2013r	Yes	• No	• No	–
Cleve et al., 2014	Probably no	• No	• No	Missing results (i.e., pain ratings not reported).
Hansen et al., 2014	Yes	• No*	• No*	*No control condition for pain (for the objective of this randomized, double-blind cross-over study there is likely no detection bias).
Cleve et al., 2017	Yes	• No	• No	–
de Matos et al., 2017	Yes	• No	• No	–
Chiappelli et al., 2017	Yes	• No	No	*No control condition for pain (the objective of this study was to assess differences in patients and controls).
Harris et al., 2009	Yes	• No	No	–
Zunhammer et al., 2016	• Yes	• No	No	–
Thiaucourt et al., 2017r	Yes	• No	No	–
Gradinger et al.,. 2019	Probably no	• No	No	Creatine ratios are the most commonly reported outcome measure. This study found no significant results using this metric, but found significance when reporting glutamate/GABA + ratio (not predetermined). Out of 5 pinprick levels tested only the significant results were reported.

GABA: ϒ-aminobutyric acid; BOLD: blood oxygen level dependent; 1H-MR: proton magnetic resonance; NMR: nuclear magnetic resonance.

Mullins et al., 2005). Significant changes are shown in Table 5. The change in GABA + ranged from −10.88% (Cleve et al., 2014, 2017; de Matos et al., 2017a) to +15% (Kupers et al., 2009). Where reported, changes in glutamine ranged from 0 (Mullins et al., 2005) to +55% (Gutzeit et al., 2011, 2013). The percent change for each study is shown in Table 5 and directions of change are summarized in Fig. 2.

4.4. Baseline neurotransmitters and reported pain sensitivity

Three studies assessed the relationship between baseline neurotransmitters and pain sensitivity (Gradinger et al., 2019; Harris et al., 2009; Zunhammer et al., 2016). Pain sensitivity was characterized based on three different approaches. One applied pressure pain thresholds via a computerized device – whereby higher values indicate less sensitivity to pain (Harris et al., 2009). Zunhammer et al., 2016 adopted a conventional quantitative sensory testing (QST) protocol (Rolke et al., 2006), including hot, cold and mechanical pain thresholds. Results of QST were z-transformed, from which an aggregate measure was calculated. According to this measure, higher scores reflected greater sensitivity to pain (e.g., lower heat pain thresholds) (Zunhammer et al., 2016). The remaining study used mechanical thresholds (pinprick stimuli), with lower thresholds indicating greater sensitivity.

All three studies provided evidence of a positive correlation between baseline Glx (Harris et al., 2009; Zunhammer et al., 2016) or glutamate (Gradinger et al., 2019) and pain sensitivity, with r-values ranging from 0.26 to 0.52 (Table 6). A significant relationship was consistently reported in one study across multiple brain areas (individual brain areas shown in Table 6) with partial r-values ranging from 0.38 to 0.50 (Zunhammer et al., 2016). In the same study, positive but non-significant correlations were also reported in the anterior cingulate cortex (ACC), mid-cingulate cortex (MCC), and insula for pain sensitivity and GABA+ (partial r-values ranging from 0.14 to 0.27; all nonsignificant) (Zunhammer et al., 2016). This contrasts a negative relationship reported elsewhere (r-values range −0.24 to −0.27; all nonsignificant) (Gradinger et al., 2019). One study also reported a significant positive correlation between glutamate/GABA + ratio and pain sensitivity, with r-values ranging from 0.43 to 0.45 (512 and 256 nM, respectively) (Gradinger et al., 2019).

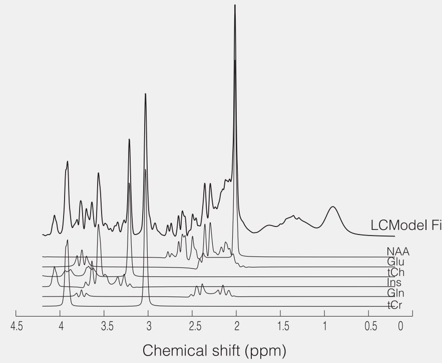
5. Discussion

A systematic review was performed of studies that used MRS and examined responses to experimental pain in healthy subjects. The first use of fMRS in the context of experimental pain was published in 2005 (Mullins et al., 2005), with similar studies emerging over the next twelve years (Chiappelli et al., 2017; Cleve et al., 2014, 2017; Thiaucourt et al., 2017; Zunhammer et al., 2016; Gussew et al., 2010; Gutzeit et al., 2011, 2013; Hansen et al., 2014; Harris et al., 2009; Kupers et al., 2009; de Matos et al., 2017a). In fMRS studies, glutamate, Glx and/or glutamine either significantly increased (Cleve et al., 2014; Gussew et al., 2010; Gutzeit et al., 2011, 2013; Mullins et al., 2005) or did not change in response to experimental pain (Chiappelli et al., 2017; Cleve et al., 2017; Hansen et al., 2014; Kupers et al., 2009; Mullins et al., 2005). To date, there is no evidence to suggest that glutamate, Glx or glutamine levels significantly decrease in the brain (Chiappelli et al., 2017; Cleve et al., 2014, 2017; Gussew et al., 2010; Gutzeit et al., 2011, 2013; Hansen et al., 2014; Kupers et al., 2009; Mullins et al., 2005) or brainstem (de Matos et al., 2017a). Consistent with the notion of increased excitatory activity, two of three studies (applying spectral editing to evaluate GABA+) reported decreased levels of inhibitory neurotransmission (Cleve et al., 2014; de Matos et al., 2017a). At rest, higher levels of Glx were consistently associated with greater sensitivity to painful stimuli (Gradinger et al., 2019; Zunhammer et al., 2016). Collectively, these observations demonstrate the feasibility of resting and fMRS as experimental pain research tools.

Box 1 | MRS Data Quality Metrics

SNR: is calculated by taking the height of the largest peak and dividing it by the the root mean squared amplitude of the noise in a signal (and artifact) free part of the spectrum (Kreis, 2004).

LW: is defined as the full width at half maximum peak height (FMHM) in the frequency domain (Kreis, 2004), usually calculatedfor N-Acetyl aspartic acid (NAA).



CRLB: reflects the uncertanty of fitting each metabolite which is helpful to determine the ability to distinguish each metabolite in the spectrum (Kreis, 2004).This metric reflects both the SNR and the LW.

5.1. Functional MRS: effect of noxious stimulation in healthy subjects

Glutamate is the primary excitatory neurotransmitter in the CNS and areas of the brain responsive to pain express a variety of glutamate receptors, including ionotropic (e.g., AMPA, kainate, NMDA) and metabotropic receptors (Zhuo, 2006). While our review of generally supports that MRS captures an increase in glutamate and Glx, there is a notable degree of heterogeneity across studies. For example, in the ACC, there are two studies reporting an increase (Cleve et al., 2014; Mullins et al., 2005) and three reporting null effects (Chiappelli et al., 2017; Hansen et al., 2014; Kupers et al., 2009). Independent of the brain region, when significant increases were reported, the effect sizes are large, approximately three to ten-fold greater than in other task-related MRS studies (e.g., applying visual stimulation) (Mullins, 2018; Stanley and Raz, 2018). Further supporting a tendency for increased excitation in response to pain, no study, in any brain area, reported significant reductions in glutamate, Glx or glutamine levels.

Several methodological factors may contribute to a high degree of heterogeneity. Based on our review, an obvious challenge was that numerous analytical methods are applied to quantify neurotransmitters with MRS. In studies using ratios, the assumption is that the denominator, often creatine, is stable over time (Li et al., 2003). The extent to which this assumption is valid, particularly in the case of task-related MRS is, at this point, unclear. Interestingly, both studies applying absolute quantification (in the brain) reported an increase in glutamate and Glx levels (Gussew et al., 2010; Gutzeit et al., 2013). In principle, this could reflect increased sensitivity to track task-related effects of pain compared to other methods of quantification (e.g., ratios). Differences in stimuation parameters is another likely source of heterogeneity. The largest increases (measured as percent change) in glutamate were reported in studies applying very brief noxious phasic stimulation (~1 s in duration) (Cleve et al., 2014; Gussew et al., 2010). Short duration phasic stimuli may be advantageous in terms of maintaining attention and be accompanied by less habituation, leading to larger effects than tonic stimuli. Beyond these potential sources, differences in the scanner manufacturer and preprocessing analysis steps could also lead to variations in reported percent changes.

5.2. Detection of GABA+: spectral editing techniques

Only 4 studies reported GABA + levels during task-related MRS in response to noxious stimulation (Cleve et al., 2014, 2017; Kupers et al., 2009; de Matos et al., 2017a). This is likely attributed to difficulties in acquiring and quantifying GABA in cortical areas, where levels are generally low (typically 0.8–2 mM) (de Graaf, 2019). Moreover, accurate detection and quantification of GABA by conventional MRS is difficult because of the overlap with large peaks that originate from other metabolite resonances, which are present in much greater concentrations (e.g. phosphocreatine, glutamate, glutamine, and macromolecules) (de Graaf, 2019; Mullins et al., 2014; Near et al., 2011). For optimal detection, specialized editing is required (Harris et al., 2017). Spectral editing exploits the known scalar coupling between protons in the GABA molecule by selectively refocusing the signal from one proton group and then observing the effect on a coupled proton group (de Graaf, 2019; Gülin, 2016; Mullins et al., 2014). The most widely applied spectral editing technique available is MEGA-PRESS (for details on technique see (Mescher et al., 1998)). A well-known drawback of measuring GABA with spectral editing is macromolecular contamination, which has been estimated to contribute up to 50% of the acquired signal (Near et al., 2011). The resonance frequency and scalar coupling properties of some macromolecules are similar to those of GABA, thus the resulting GABA signal from a conventional spectral editing measurement is referred to as GABA+ (=GABA + macromolecules). A limitation of the CRLB in the case of a GABA + edited measurement is that they do not reflect any of the macromolecular contributions which have been attributable to GABA. Spectral editing acquisitions also suffer from subtraction errors and result in increased scanning time. Although MEGA-PRESS is the most commonly applied editing technique, current implementation methods are diverse across vendors (i.e., different shape and timing of radio frequency pulses are used for localization and editing). This too can lead to differences in the intensity of the detected GABA + signal (Mullins et al., 2014; Saleh et al., 2019).

Kupers et al. (2009) did not apply spectral editing or any other approach to distinguish the signal from GABA from other molecules. This calls into question the reported increase in GABA + levels in the anterior cingulate cortex (Kupers et al., 2009). In studies applying spectral editing, GABA + levels were decreased (Cleve et al., 2014; de Matos et al., 2017a) or unchanged in response to noxious stimulation (Cleve et al., 2017). Reductions in the anterior cingulate cortex are consistent with the notion that increased excitation can be achieved by way of decreased GABAergic inhibition. However, the notion that decreases in GABA must accompany increased glutamate is likely an oversimplification of a more complex response, conceivably involving fluctuations in both excitatory and inhibitory neurotransmitters occurring on different time-scales. Simultaneously acquiring glutamate and GABA levels with optimized MRS sequences is necessary to fully elucidate the relationship between excitation and inhibition in response to pain.

Table 3
Studies included for systematic review.

Study	Design	Sample Size	Sex	Noxious Stimulation	Control Condition
Mullins et al., 2005	Block	8	m/f	Cold pressor pain. A frozen compress (0–4 °C).MRS during four 10-min conditions: rest, cold pressor or sham pain and two rest periods. [Average NRS 4.1 ± 1.5]. Location: plantar surface of the left foot.	Sham pain. Cool compress (°C not indicated). Two separate scanning sessions in a single-blind, randomized fashion.
Kupers et al., 2009	Block	13	m/f	Tonic heat pain. MR during 4-min baseline (35 °C) and 4-min tonic heat stimulation (mean 46.7 ± 0.2 °C) [Average NRS 7.3 ± 0.3]. Location: right upper leg.	no
Gussew et al., 2010	Event	6	m	Phasic heat pain. From 35 °C to individual painful stimulus, held for 1s; range = 45–49 °C lasting 9-min. Each stimulation cycle was repeated 16 times. In each repetition 2 single spectra were acquired (one during pain and one in rest- 2s after pain stimulation) [Average NRS at start = 6–7±NA at end = 2–4±NA]. Location: left forearm.	Following the pain and rest conditions, a dummy scan was performed without stimulation (same day).
Gutzeit et al., 2011	Block	20	m	Electric dental tonic pain. MRS during 3 conditions: 3-min pre-pain (1 spectrum acquired), 9-min pain (electrical dental pain; 3 spectra), 9-min post-pain (3 spectra) [Average NRS 6.8 ± 2.4]. Location: right maxillary canine tooth.	10 subjects (age and sex-matched) exposed to identical procedures minus painful stimulation. To exclude the influences of dental splint.
Gutzeit et al., 2013	Block	16	m	Electric dental tonic pain. MRS during 3 conditions lasting 3.48min. Baseline, dental pain, post-stimulation. Each condition was repeated 4 times. [Average NRS run1 = 6.8 ± 1.5 run2 = 7.1 ± 1.5]. Location: right maxillary canine tooth.	no
Cleve et al., 2014	Event	13	f	Phasic heat pain. Stimulation (46.0 °C ± 1.0 °C) held for 1s (with 5–8 s intervals). Overall 44 stimuli repeated 6 times. MRS during pain stimulation and rest (3s after pain stimuli). [Average NRS NA].Location: left forearm.	Following the pain and rest conditions, a dummy scan was performed without stimulation (same day).
Hansen et al., 2014	Block	12	m/f	Tonic heat pain. MRS at rest (5min) and during painful stimulation Stimulation (44.9 ± 0.7 °C) applied for 5-min. [Average NRS 6.8 ± 1.0].Location: 16 cm proximal to the right knee.	no
Cleve et al., 2017	Event	25	m	Phasic heat pain MRS during 12-min rest, and stimulation. 16 s period of stimulation (x22) delivered at varying intensities and duration. Mean temperature (50 ± 0.9 °C) [Average NRS start = 4.6 ± 0.6 end = 5.0 ± 1.1]. Location: right dorsal part of the hand.	no
de Matos et al., 2017	Block	13	m	Electrical dental pain. MRS at baseline and during painful stimulation.128 (1 ms) electrical pulses ISI = 10–12.5 s. Painful stimulation for 21-min. [Average NRS 4–5±NA]. Location: right upper canine.	no
Chiappelli et al., 2017	Block	21	m/f	Phasic heat pain. MRS at rest, during, and after the application of heat.10 trials (+0.5 °C pain thresholds), stimulation duration = 3–10s, ramp = 0.7–5.25 °C/s. ISI = 5–24s. Total = 5-min. [Average NRS 5.45 ± 1.7]. Location: forearm.	no
Harris et al., 2009	Correlational	14	f	Pressure pain. MRS assessed before pain. Pressure pain was matched to subjects' perception [Average NRS NA] Location: thumbnail.	no
Zunhammer et al., 2016	Correlational	39	m/f	Experimental heat, cold, mechanical pain summarized into a measure of pain, prior to MRS measures. [Average NRS NA]. Location: palmar left lower arm.	no
Gradinger et al.,. 2019	Correlational	31	f	Mechanical pain (pinprick). MRS was assessed prior to pain. Five mechanical stimuli intensities (32, 64, 128, 256, and 512 mN) in pseudorandomized order. [Average NRS for 256 mN = 4.1 ± 4.4; 512 mN = 9.3 ± 3.0]. NRS for other intensities NA. Location: right forearm.	no

m: males; f: females; ISI: Inter-stimulus interval NRS: Numeric rating scale; NA: Not available.

5.3. Interpretation of neurochemical changes in the brain based on task-related MRS

A major problem facing the widespread adoption of fMRS is a lack of knowledge regarding the physiological interpretation of the measured changes in neurotransmitter levels *in vivo*. In chronic pain patients, changes in glutamate at rest could reflect pathology that develops over time, potentially from long-term homeostatic dysregulation in the brain. In a healthy CNS, transiently increased glutamate levels in response to noxious stimulation must reflect a distinct process, bound by normal homeostatic functions (Mangia et al., 2011). In all likelihood, fMRS measures the net effect of multiple simultaneous processes, including a change in visible glutamate arising from neurotransmission (e.g., movement of vesicles to the pre-synaptic terminal and exocytosis into the synaptic cleft) (Mullins, 2018), the availability of glutamate as a precursor for the tricarboxylic acid cycle, as well as glutamate redistribution occurring in glial cells (i.e., glutamate conversion into glutamine-the primary precursor of glutamate and GABA, affecting Glx values) (Mullins, 2018; O'Gorman Tuura et al., 2019; Ramadan et al., 2013; Schousboe et al., 1997).

A strategy towards divulging more specific information on neurotransmission may be to consider effects of stimulation on glutamine. Glutamate is stored as glutamine in glial cells, which have a vital role in preserving low extracellular levels of glutamate to prevent excitotoxicity (Ramadan et al., 2013). In theory, glutamine levels represent a reasonable estimate for the rate of glial uptake of glutamate (Kanamori et al.,

Table 4
Summary of MRS acquisition, quantification, and spectral quality results.

Study	*Field Strength/ Manufacturer*	MRS localization sequence + metabolite of interest	*TR ms*	*TE ms*	Number of averages	ROI	Fitting software	Units	*Mean SNR*	*Mean LW (Hz)*	*CRLB (%)*
Mullins et al., 2005	4T Varian	STEAM GLU & GLN	2000	20	256	Bilateral ACC 20x20x20 = 8 mL	In house software	IU	–	<12	–
Kupers et al., 2009	3T Siemens	STEAM GLX & GABA	3000	20	80	Rostral ACC 22x22x20 = 9.68 mL	LCModel	NA	–	–	Rest GABA = 22.4 ± 1.0 Pain GABA = 20.5 ± 1.1
Gussew et al., 2010	3T Siemens	PRESS GLU	5000	30	32	Anterior left insula 25x10x10 = 2.5 mL	LCModel	AbsQ	SNR_{NAA} = 12.2 ± 2.4	$FWHM_{NAA}$ = 4.6 ± 1.0	Glu = 10.1 ± 1.5
Gutzeit et al., 2011	3T Philips	PRESS ALL	2000	30	80	Left insula 20x20x37.6 = 15 mL	LCModel	/Cr	–	–	–
Gutzeit et al., 2013	3T Philips	PRESS ALL	2000	30	96	Right/left insula 20x20x24 = 9.6 mL	LCModel	AbsQ	–	–	–
Cleve et al., 2014	3T Siemens	MEGA PRESS GLX & GABA	3000	68	128	ACC 36x20x12 = 8.64 mL Occipital cortex 35x20x15 = 10.5 mL	Amares	/Cr	–	–	–
Hansen et al., 2014	3T GE	PRESS ALL	2000	30	128	ACC 20x20x20 = 8.0 mL	LCModel	/Cr	Bsl 14.8 ± 5.5 Pain 14.6 ± 3.0	Bsl FWHM = 0.042 ± 0.011 Pain FWHM = 0.046 ± 0.01	Rest Glu = 9.3 ± 2.4 Pain Glu = 8.0 ± 1.3
Cleve et al., 2017	3T Siemens	MEGA PRESS GLX & GABA	1800	68	384	Left insula 28x40x14 = 15.8 mL	Amares	/Cr	SNR_{NAA} = 104.1 ± 11.0	$FWHM_{NAA}$ = 5.8 ± 0.6	–
de Matos et al., 2017	3T Philips	PRESS MC-PRESS ALL	2500	32	512	Brainstem nuclear complex 0.8 × 1 × 1.3 = 0.001 mL	LCModel	AbsQ	Bsl_{NAA} 19.1 ± 2.7 $Pain_{NAA}$ 19.1 ± 2.3	Bsl $FWHM_{NAA}$ = 6.5 ± 1.6 Pain $FWHM_{NAA}$ = 6.3 ± 1.4	BSL GABA = 23.4 ± 6.3 tNAA = 2.8 ± 0.4 Pain GABA = 26.5 ± 8.4 tNAA = 3.2 ± 0.8
Chiappelli et al., 2017	3T Siemens	PR-STEAM GLU & GLX	2000	6.5	128	ACC 4.0x 2.0x1.5 = 7.5 mL	LCModel	IU	30.9 ± 6.5	0.033 ± 0.007	GLU = 4.1 ± 0.74
Harris et al., 2009	3T GE	PRESS GLU & GLX	3000	30	NA	Right anterior/posterior insula 20x20x30 = 12 mL	LCModel	I.U	–	–	–
Zunhammer et al., 2016	3T Philips	PRESS MEGA PRESS GLX & GABA	2000 2000	30 68	32	ACC 5x30x20 = 21.6 mL; Insula 25x45x20 = 22.5 mL; DLPFC 30x30x30 = 27 mL; MCC 35x20x20 = 21 mL; Thalamus 30x30x25 = 22.5 mL	LCModel GANNET	/Cr in z-scores	–	–	Glx = 8
Gradinger et al., 2019	3T Siemens	MEGA PRESS GABA & GLU	3000	68	192	Right posterior insula 30x40x20 = 24 mL	LCModel	/Cr and GLU/ GABA ratio in z scores	–	–	–

Region of interest (ROI); Repetition time (TR); Echo time (TE); Signal to-noise-ratio (SNR); Line width (LW); Cramer-Rao minimum lower bounds (CRLB); FHWM: Full width at half maximum; NAA: N-Acetyl aspartic acid; GLU: glutamate; GLX: glutamate + glutamine; GABA; γ-aminobutyric acid; GLN: Glutamine; tNAA: NAA + NAAG (N-Acetyl-aspartyl-glutamate).Point-resolved spectroscopy (PRESS); Stimulated echo acquisition mode (STEAM); MEshcher-GArwood Point RESolved Spectroscopy (MEGA PRESS); Phase Rotation stimulated echo acquisition mode (PR-STEAM); Metabolite Cycling Point-resolved spectroscopy (MC-PRESS). Creatine ratios (/Cr); Institutional Units (IU); Absolute quantification (AbsQ); Anterior cingulate cortex (ACC); Mid-Cingulate Cortex (MCC); Dorsolateral prefrontal cortex (DLPFC).

Table 5
Summary of reported results in relation to experimental noxious stimulation.

Study	Changes due to experimental pain			
	glutamate	GABA+	Glx	glutamine
Mullins et al., 2005	↑ = 9.3% in Bilateral ACC **(p = 0.006).**	–	↑ = 11.4% **(p = 0.04).**	↑ = 16.3% (p = 0.2).
Kupers et al., 2009	↓ = −0.2% in Rostral ACC (p = NS*).	↑ = 15% ACC **(p<0.02).**	↑ = 0.1% (p = NS*).	–
Gussew et al., 2010	↑ = 18.1% in anterior insular cortex **(p = 0.003).**	–	–	–
Gutzeit et al., 2011	↑ = 3.3% in left insula (p = 0.35).	–	↑ = 16.4% **(p = 0.03).**	↑ = 55.1% **(p = 0.01).**
Gutzeit et al., 2013	↑ = 9.4% (p = 0.05) in left anterior insula ↑ = 8.5% **(p = 0.003)** in right anterior insula ↑ = 3.1% (p = 0.5) in left posterior insula ↑ = 7.4% (p = 0.09) in right posterior insula.	–	↑ = 16.0% **(p = 0.01)** in left anterior insula ↑ = 15.2% (p = 0.05) in right anterior insula ↑ = 11.1% (p = 0.16) in left posterior insula ↑ = 13.2% **(p = 0.004)** in right posterior insula.	↑ = 27.7% (p = 0.08) in left anterior insula ↑ = 26.9% (p = 0.06) in right anterior insula ↑ = 26.0% (p = 0.13) left posterior insula ↑ = 22.4% **(p = 0.04)** in right posterior insula.
Cleve et al., 2014	–	↓ = 15.1% (p = 0.11) in ACC. ↓ = 12.7% **(p = 0.001)** in OC.	↑ = 21.5% **(p<0.001)** in ACC. ↑ = 15.7% **(p = 0.001)** in OC.	–
Hansen et al., 2014	= no significant change (p = 0.6).	–	–	–
Cleve et al., 2017	–	↑ = 0.4% (p = NS*).	≠ 0.1% (p = NS*).	–
de Matos et al., 2017	–	↓ = -10.8% **(p = 0.04).**	↓ = −3.6% (p = 0.3).	–
Chiappelli et al., 2017	≠ −1.7% (p = NS*).	–	–	–

Glx: glutamate + glutamine; GABA+: y-aminobutyric acid + macromolecules; Cr: creatine; Anterior cingulate cortex (ACC); NS*: not significant (exact value not reported).

2002), presumably as a result of increased neurotransmission (O'Gorman Tuura et al., 2019). Three studies have reported on the effect of noxious stimulation on glutamine (Gutzeit et al., 2011, 2013; Mullins et al., 2005), two of which observed a significant increase (Gutzeit et al., 2011, 2013). The challenge with detecting glutamine is that its structure overlaps with glutamate, which makes accurate quantification difficult (Ramadan et al., 2013).

5.4. MRS and the brain at rest: relationship with inter-individual variability in experimental pain sensitivity

Across three studies, three different measures were used to assess pain sensitivity and correlations with resting metabolite level. This included pressure (Harris et al., 2009), mechanical (pinprick) (Gradinger et al., 2019), and an aggregate outcome of heat, cold, and mechanical (pinprick) thresholds (Zunhammer et al., 2016). All three studies of this nature, applying different threshold measurements, support that a subject's pain sensitivity increases with higher levels of Glx (Gradinger et al., 2019; Harris et al., 2009; Zunhammer et al., 2016) and glutamate (Gradinger et al., 2019). Importantly, no study reported an opposing relationship (i.e., less excitation associated with high sensitivity to pain). Speaking further to the strength of the relationship, r-values were significant in 2 of 3 studies (Harris et al., 2009; Zunhammer et al., 2016) and always exceeded 0.3 (Gradinger et al., 2019; Harris et al., 2009; Zunhammer et al., 2016). The nature of this relationship, prevailing across modalities, argues against a specific peripheral mechanism (e.g., recruitment of A-delta versus c-fibers), potentially reflecting a supraspinal state predisposing sensitivity to afferent stimuli.

The observation that the pain sensitivity is positively correlated with excitatory levels is further supported by observations in special populations remarkably insensitive to pain (e.g., Zen meditators), whose glutamate levels in the brain are lower than average (Fayed et al., 2013; Grant et al., 2010; Grant and Rainville, 2009). On the opposing end of the spectrum, glutamate and Glx levels are reportedly increased in individuals with fibromyalgia (Fayed et al., 2012, 2014; Feraco et al., 2011; Harris, 2010; Harris et al., 2008, 2013) – a condition characterized by heightened sensitivity to noxious and non-noxious stimuli (Sluka and Clauw, 2016). Additionally, interventions known to reduce the severity of chronic pain symptoms (e.g., pregabalin and neuromodulation) reduce Glx levels in the brain (Foerster et al., 2015; Harris et al., 2013), indicating that effective management can restore "aberrant brain chemistry" by decreasing glutamate levels (Harris et al., 2013). Interestingly, a previous study reported that more sensitive individuals tended to be associated with higher concentrations of grey matter (Erpelding et al., 2012). In principle, this fits with MRS observations, as there are higher levels of neurotransmitter in grey matter, compared to white matter (Novotny et al., 2003).

Compared to resting glutamate, the relationship between GABA and pain sensitivity is much less clear. While not significant, results from one study indicated a trend for a negative relationship between GABA+ and sensitivity to pain (Gradinger et al., 2019). In an earlier study from the same laboratory, this relationship was significant in a smaller sample (excluded from our review because of overlapping study samples [personal communication]), (Thiaucourt et al., 2017). The fact that adding subjects resulted in an nonsignificant relationship would suggest the initial study was underpowered and fell consequence to type 1 error. A study better powered to detect a correlation reported small and opposing effects (i.e., a positive relationship between GABA and pain sensitivity). Keeping in mind the limitations of quantifying GABA (see above), the available evidence does not support a relationship between GABA and pain sensitivity in healthy subjects at this time.

5.5. Overall quality of studies using MRS to examine experimental pain

From a design perspective, the biggest problem we encountered with fMRS studies was the lack of a control condition. There are two obvious conditions to consider in future studies. The first is "no stimulation", whereby subjects undergo the same MRS acquisition but in the absence of noxious stimulation. This can account for a tendency of glutamate, Glx, GABA, and glutamine to spontaneously change, unrelated to pain, over time. The second and arguably more important control would be a non-noxious stimulus applied in a similar context as the painful stimulus. This approach has been adopted for other techniques (e.g., fMRI, EEG, MEG) (Brascher et al., 2016; Fardo et al., 2017; Wager et al., 2013), to establish the degree observable brain responses are specific to pain. The obvious concern is that changes in neurotransmitters may reflect generalizable response features associated with processing afferent stimuli, including a variety of cognitive functions (Mouraux and Iannetti, 2018). From our review, only three studies have considered a "no stimulation" condition (Cleve et al., 2014; Gussew et al., 2010; Gutzeit et al., 2011) and just one other has incorporated a non-noxious stimulation (Mullins et al., 2005). Importantly, all four studies were able to demonstrate a significant change relative to their respective control condition (Cleve et al., 2014;

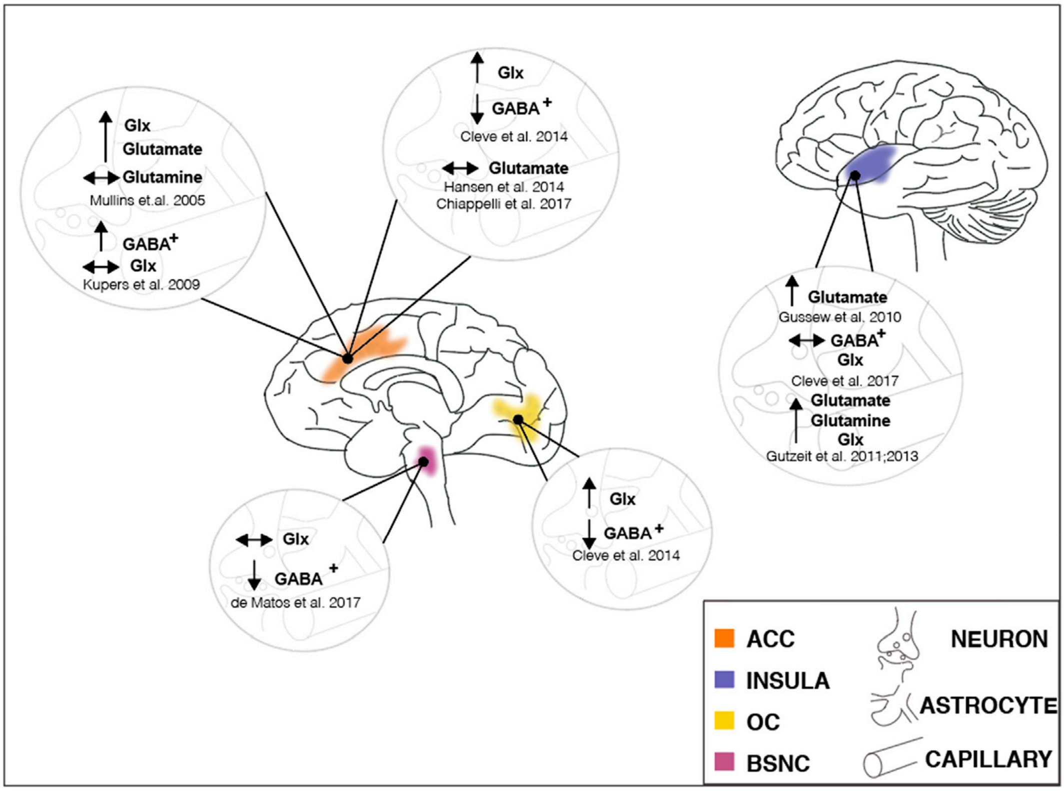


Fig. 2. Illustration of glutamate, GABA+, Glx, and glutamine alterations during experimental pain reported in the reviewed studies in different brain areas. Changes are described relative to a baseline scan (no stimulation). The prevailing trend emphasizes an increase in glutamate as well as the combination of glutamate and glutamine (Glx)) independent of quantification methods and noxious interventions (ACC: anterior cingulate cortex; OC: occipital cortex; BSNC: brainstem nuclear complex).

Table 6
Summary of reported results relationship between pain sensitivity.

Study	Correlation with pain sensitivity metric and resting levels				
	glutamate,	GABA,	Glx	glutamine	other
Harris et al., 2009	–	–	Positive correlation between pressure thresholds in the posterior insula (r = 0.52, p = 0.05).	–	–
Zunhammer et al., 2016	–	Positive but no significant correlation with GABA+/Cr levels Insula: r = 0.14, p = 0.46. ACC: r = 0.25, p = 0.11. MCC: r = 0.25, p = 0.14. *No GABA measurements were obtained for other ROI due to time constraints.	Positive correlation between summary of heat, cold and mechanical thresholds Insula: r = 0.01, p = 0.95 ACC: r = 0.38, **p = 0.02.** MCC: r = 0.40,**p = 0.01.** DLPFC: r = 0.50, **p = 0.002.** Thalamus: r = 0.46, **p = 0.008.**	–	–
Gradingeret al., 2019	Positive but NS correlation 256 mN: r = 0.30, p = NS*. 512 mN: r = 0.26, p = NS*	Negative but NS correlation 256 mN: r = −0.27, p = NS*. 512 mN: r = −0.24, p = NS*.	–	–	Positive correlation between mechanical thresholds and glutamate/GABA + ratio in the right posterior insula 256 mN: r = 0.45, **p = 0.01.** 512 mN: r = 0.43, **p = 0.01.**

Glx: glutamate + glutamine; GABA+: y-aminobutyric acid + macromolecules; Cr: creatine; Anterior cingulate cortex (ACC); Mid-Cingulate Cortex (MCC); Dorsolateral prefrontal cortex (DLPFC); NS*: non-significant (exact value not reported.

Gussew et al., 2010; Gutzeit et al., 2011; Mullins et al., 2005). While further investigation is required, this may indicate a specific effect of pain.

Our systematic review focused on two forms of bias: detection and reporting. To evaluate detection bias, we evaluated if the studies incorporated a measure of blinding - namely if subjects and examiner were blinded to the experimental condition. In theory, blinding is important to account for potential confounds, including physiological responses arising from increased arousal and anxiety that come with a participant anticipating pain (Ploghaus et al., 1999, 2003). In light of the fact that the vast majority of studies did not include any control condition, it is not surprising that the lack of blinding represents a major potential for bias. However, one needs to consider that MRS, particularly fMRS applications, are in their infancy in pain research, and a number of studies were aimed at addressing feasibility (de Matos et al., 2017b). Other studies were not explicitly designed to examine the effects of pain on glutamate/GABA in healthy subjects (Chiappelli et al., 2017; Gradinger et al., 2019; Hansen et al., 2014) or were correlational in nature (Gradinger et al., 2019; Harris et al., 2009; Zunhammer et al., 2016), and thus cannot be expected to have the requisite control conditions and blinding. Compared to detection bias, reporting bias appears to be less of a concern, as the majority of studies reported results consistent with their stated aims. Nevertheless, other types of reporting bias may be pervasive such as the potential for publication bias.

5.6. Limitations and technical implications

The primary limitation of our review is the lack of studies using MRS to examine experimental pain. This, combined with a degree of variability between studies, limits the opportunity for a meta-analysis to meaningfully estimate effect sizes. From a practical standpoint, our review highlights the need to standardize the acquisition and reporting of MRS outcomes. Notably missing from many of the reviewed studies were measurements of MRS quality, such as SNR, LW, and CRLB from the neurotransmitters of interest (i.e., glutamate, Glx) (Cleve et al., 2014; Gradinger et al., 2019; Gutzeit et al., 2011, 2013; Hansen et al., 2014; Kupers et al., 2009). Indeed, only eight out of fourteen studies reported key quality metrics. Moreover, five studies (out of thirteen) corrected for fractional brain tissue volumes (Chiappelli et al., 2017; Cleve et al., 2014, 2017; Gussew et al., 2010; Harris et al., 2009). This is a major problem because MRS neurotransmitter levels can be underestimated and misinterpreted if grey and white matter tissue fractions are not considered (Gasparovic et al., 2018; Novotny et al., 2003; Wilson et al., 2019). A recent consensus statement emphasizes acquisition, pre-processing, and analysis to optimize the measurement of all metabolites for wider adoption of MRS (Wilson et al., 2019). Future studies may improve the quantification of glutamate, glutamine, and GABA + by incorporating measurements of the macromolecular baseline or employing two-dimensional spectroscopy techniques (Cudalbu et al., 2012; de Graaf, 2019), thereby providing further insights into the neurotransmitter response to noxious stimulation and its relationship to pain perception.

6. Conclusion

In summary, MRS represents a feasible experimental tool to examine the neurobiology of pain in healthy subjects. This has been demonstrated functionally, as a method to track changes related to noxious input, and at rest by examining the relationship between neurotransmitters and pain sensitivity. The lack of control conditions (i.e., non-noxious stimulation or no stimulation), as well as blinding of participant and examiner represents major sources of bias and should be addressed in future studies. Overall, MRS is well-positioned to offer new insights into mechanisms of normal and abnormal sensitivity to pain.

Author contribution statement

Jessica Archibald: Conceptualization, Methodology, Software, Validation and Writing-Original Draft. Erin L. MacMillan: Conceptualization, Writing- Review and Editing and Visualization. Alinda Enzler: Methodology, Software, Validation, Writing- Review and Editing and Visualization. Catherine R. Jutzeler: Methodology, Validation, Writing-Review and Editing and Visualization. Petra Schweinhardt: Conceptualization, Writing-Review and Editing and Visualization. John L.K. Kramer: Conceptualization, Writing-Review and Editing Visualization and Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroimage.2020.116794.

Appendix 2. Search strategy for each database

MEDLINE

MeSH	Key terms
Magnetic Resonance Spectroscopy	MRS
Proton magnetic resonance spectroscopy	H-MRS
	Functional magnetic resonance spectroscopy fMRS.mp
Glutamic Acid	Glutamate
Gamma-Aminobutyric Acid	GABA
Glutamine	Glx
	Glutamine
	Neurotranmsmitters.mp
Brain	Brain.mp
Pain	Experimental pain.mp
Experimental Pain	

1 to 4 = 9 papers.
1 and 4 = 132 papers.
2 to 4 = 241 papers.
TOTAL = 382.

EMBASE

MeSH	Key terms
Magnetic Resonance Spectroscopy	MRS
Proton magnetic resonance spectroscopy	H-MRS
	Functional magnetic resonance spectroscopy fMRS.mp
Glutamic Acid	Glutamate
Gamma-Aminobutyric Acid	GABA
Glutamine	Glx
	Glutamine
	Neurotranmsmitters.mp
Brain	Brain.mp
Pain	Experimental pain.mp
Experimental Pain	

1 to 4 = 39 papers.
1 and 4 = 338 papers.
2 to 4 = 674 papers.
TOTAL = 1051.

PUBMED

((((1. Magnetic Resonance Spectroscopy/or Proton Magnetic Resonance Spectroscopy/or 1h-MRS/or MRS/or functional Magnetic resonance spectroscopy/or fMRS)) AND (2. Glutamic Acid/or gamma-Aminobutyric Acid/or Glutamine/or glutamate/or GABA/or Glx/or Glutamine/or neuro-transmitters.)) AND (3. brain.mp. or Brain/)) AND (4. Pain/or Pain Perception/or Experimental pain).

MeSH Terms: physiology; glutamine; gamma-aminobutyric acid; brain; glutamic acid; pain perception; pain; neurotransmitter agents; proton magnetic resonance spectroscopy; magnetic resonance spectroscopy; glutamate neurotransmitter agents.

1 to 4 = 42 papers.
1 and 4 = 1057 papers.
2 to 4 = 3253 papers.
TOTAL = 4352.

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