Received: 25 August 2023 | Revised: 4 January 2024 | Accepted: 5 February 2024

DOI: 10.1002/ejp.2251

EJP European Journal of Pain

REVIEW ARTICLE

Systematic review and co-ordinate based meta-analysis to summarize the utilization of functional brain imaging in conjunction with human models of peripheral and central sensitization

Sophie Clarke[1] | **Richard Rogers**[1] | **Vishvarani Wanigasekera**[1] | **Francesca Fardo**[2,3] | **Hossein Pia**[2] | **Zahra Nochi**[2] | **Nicolas Macian**[4] | **Vincent Leray**[4] | **Nanna Brix Finnerup**[2] | **Gisèle Pickering**[4,5] | **André Mouraux**[6] | **Andrea Truini**[7] | **Rolf-Detlef Treede**[8] | **Luis Garcia-Larrea**[9,10] | **Irene Tracey**[1]

[1]Nuffield Department of Clinical Neurosciences, Wellcome Centre for Integrative Neuroimaging, FMRIB, University of Oxford, Oxford, UK

[2]Department of Clinical Medicine, Danish Pain Research Center, Aarhus University, Aarhus, Denmark

[3]Department of Clinical Medicine, Center of Functionally Integrative Neuroscience, Aarhus University, Aarhus, Denmark

[4]Platform of Clinical Investigation, Inserm CIC 1405, University Hospital Clermont-Ferrand, Clermont-Ferrand, France

[5]Inserm 1107, University Clermont Auvergne, Clermont-Ferrand, France

[6]Institute of Neuroscience (IoNS), Université Catholique de Louvain (UCLouvain), Ottignies-Louvain-la-Neuve, Belgium

[7]Department of Human Neuroscience, Sapienza University, Rome, Italy

[8]Department of Neurophysiology, Mannheim Center for Translational Neurosciences, Heidelberg University, Mannheim, Germany

[9]NeuroPain Lab, Lyon Centre for Neuroscience Inserm U1028 and University Claude Bernard, Lyon, France

[10]Pain Center Neurological Hospital (CETD), Hospices Civils de Lyon, Lyon, France

Correspondence
Sophie Clarke, Nuffield Department of Clinical Neurosciences, Wellcome Centre for Integrative Neuroimaging, Wolfson Building, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK.
Email: sophie.clarke@ndcn.ox.ac.uk

Funding information
Innovative Medicines Initiative 2 Joint, Grant/Award Number: 777500; European Union's Horizon 2020; EFPIA

Abstract

Background and Objective: Functional magnetic resonance imaging, in conjunction with models of peripheral and/or central sensitization, has been used to assess analgesic efficacy in healthy humans. This review aims to summarize the use of these techniques to characterize brain mechanisms of hyperalgesia/allodynia and to evaluate the efficacy of analgesics.

Databases and Data Treatment: Searches were performed (PubMed-Medline, Cochrane, Web of Science and Clinicaltrials.gov) to identify and review studies. A co-ordinate based meta-analysis (CBMA) was conducted to quantify neural activity that was reported across multiple independent studies in the hyperalgesic condition compared to control, using GingerALE software.

Results: Of 217 publications, 30 studies met the inclusion criteria. They studied nine different models of hyperalgesia/allodynia assessed in the primary (14) or secondary hyperalgesia zone (16). Twenty-three studies focused on neural correlates

of hyperalgesic conditions and showed consistent changes in the somatosensory cortex, prefrontal cortices, insular cortex, anterior cingulate cortex, thalamus and brainstem. The CBMA on 12 studies that reported activation coordinates for a contrast comparing the hyperalgesic state to control produced six activation clusters (significant at false discovery rate of 0.05) with more peaks for secondary (17.7) than primary zones (7.3). Seven studies showed modulation of brain activity by analgesics in five of the clusters but also in four additional regions.

Conclusions: This meta-analysis revealed substantial but incomplete overlap between brain areas related to neural mechanisms of hyperalgesia and those reflecting the efficacy of analgesic drugs. Studies testing in the secondary zone were more sensitive to evaluate analgesic efficacy on central sensitization at brainstem or thalamocortical levels.

Significance: Experimental pain models that provide a surrogate for features of pathological pain conditions in healthy humans and functional imaging techniques are both highly valuable research tools. This review shows that when used together, they provide a wealth of information about brain activity during pain states and analgesia. These tools are promising candidates to help bridge the gap between animal and human studies, to improve translatability and provide opportunities for identification of new targets for back-translation to animal studies.

1 | INTRODUCTION

There is an unmet need for effective treatments for chronic pain, which is due to numerous drug development challenges (Davis et al., 2020). Poor translation of promising candidate analgesics from animal studies to human studies indicates a need to improve the use of animal data for decision-making and to develop animal models that utilize more clinically relevant endpoints (Denayer et al., 2014). Also, the endpoints studied in pain clinical trials are primarily self-reported ratings, which are shown to have low sensitivity even with training (Smith et al., 2016), especially when baseline pain is variable (Farrar et al., 2014; Harris et al., 2005). Pain ratings are non-specific; while ratings can be modulated by the analgesic, they can also be modulated by other factors such as expectation, attention, anxiety and emotional responses (Berna et al., 2010; Bingel et al., 2011; Ploghaus et al., 1999, 2001; Wiech et al., 2008; Wiech & Tracey, 2009). In a clinical trial setting, it cannot be determined if a decrease in pain ratings is attributable to the drug alone. While behavioural data are highly valuable, there is a need for objective measures to assess target engagement in humans and accurately evaluating the efficacy, to help improve go/no-go decision-making in bringing new analgesics to large-scale clinical trials (Tracey et al., 2019). Brain imaging could prove to be one of the tools that enables this (Borsook et al., 2011a, 2011b).

Hyperalgesia, defined as increased pain in response to a painful stimulus, and allodynia, defined as a sensation of pain in response to a usually non-painful stimulus, arise as a result of tissue injury (IASP, 2011). These characteristics occur at the site of injury (primary hyperalgesia) primarily due to peripheral sensitization, and surrounding the injured tissue itself (secondary hyperalgesia) primarily due to central sensitization (Hardy et al., 1950; Latremoliere & Woolf, 2009; Woolf, 1983, 2011). Peripheral sensitivity at the primary injury site (primary hyperalgesia) primarily mediates an increased sensitivity to heat stimuli, whereas central sensitization in the area outside the injury site (secondary hyperalgesia) primarily results in sensitivity to mechanical stimuli only (Latremoliere & Woolf, 2009; Treede et al., 1992). Patient studies indicate an important role in central sensitization to the phenotype of chronic pain in multiple pain conditions, resulting in distressing symptoms for patients (Jensen & Finnerup, 2014). Peripheral and central sensitization symptoms of pain hypersensitivity can be temporarily induced in healthy humans using multiple experimental methodologies (Quesada et al., 2021). These techniques produce changes in the brain response, which can be studied using imaging techniques such as functional magnetic resonance imaging (fMRI). Crucially, models aimed to trigger central sensitization have also been shown to be responsive to effective analgesics

targeting central sensitization mechanisms (Quesada et al., 2021; Wanigasekera et al., 2016).

Functional neuroimaging has been instrumental in gaining an understanding of pain processing (Lee & Tracey, 2013). Across multiple imaging modalities, fMRI offers a range of temporal resolutions, good spatial resolution and the ability to evaluate whole-brain activity and cognitive processing (Morton et al., 2016). More recent co-ordinate based meta-analysis (CBMA) techniques have enabled statistical inference to identify brain regions that are consistently activated in multiple related fMRI studies, mitigating potential limitations of individual studies such as small sample sizes (Eickhoff et al., 2009; Samartsidis et al., 2017; Tanasescu et al., 2016; Wager et al., 2009). Using fMRI techniques in conjunction with experimental models of central sensitization in early-phase clinical trials could support improved translatability of new medicines, by demonstrating target engagement in humans at an early stage prior to progressing to larger clinical trials (Cho et al., 2021; Olesen et al., 2012).

To consolidate the existing literature, this review of studies conducted on healthy human participants aims to answer the following research questions:

1. How has fMRI, in conjunction with models of peripheral and/or central sensitization, been used to characterize the neural mechanisms of hyperalgesia and allodynia?
2. How has fMRI, in conjunction with models of peripheral and/or central sensitization, been applied to study the efficacy of various analgesics?

2 | METHODS

This review has been written by contributors to the IMI-PainCare BioPain RCT4 project; a study that aims to further validate fMRI as a biomarker of pharmacodynamic efficacy. The review builds on a previous review by BioPain contributors that provides an overview of research using experimental models of central sensitization in humans (Quesada et al., 2021). The review has been conducted in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 statement and guidance (Page et al., 2021). The CBMA section of this review has been conducted following guidelines specific to neuroimaging meta-analyses (Müller et al., 2018).

2.1 | Systematic search methodology

The primary search was conducted using four electronic databases: PubMed-Medline, Cochrane, Web of Science and Clinicaltrials.gov. We used the search terms "[hyperalgesia AND human AND pain AND brain AND imaging AND fMRI]". Duplicates were removed.

The results ($n = 226$) were then assessed based on set inclusion and exclusion criteria:

> *Inclusion criteria*: Studies primarily focussed on fMRI studies using a human experimental model of peripheral and/or central sensitization were included.
> *Exclusion criteria*: Studies primarily focussed on animal models, patient cohort or case studies, human studies exploring other features of pain (e.g., placebo/nocebo effects) and review articles were excluded from further analysis.

This assessment was completed by screening each article against the specified criteria, which was completed by S.C. After this primary search, a number of secondary searches were conducted using PubMed to ensure publications on certain specific topics were not missed. One secondary search focussed on spinal cord imaging, using the terms: "[hyperalgesia AND human AND pain AND model AND spinal cord AND imaging]" to identify additional spinal cord studies. This search identified one additional study that fit the inclusion criteria and was therefore included in the results for further analysis. Further individual searches for less common human experimental models were conducted, using the following format: "[*model* AND neuroimaging]" (with just the two search terms). The models run in this series of searches included menthol, mustard oil, cinnamaldehyde, nerve growth factor injection, hypertonic saline injection, and incisional models. One additional study was identified in the results from the menthol search that fit the inclusion criteria, so it was included in the results for further analysis. None of the other model-specific searches identified publications that fit the inclusion criteria. We also conducted a manual search for studies listed in literature references but not detected automatically in the electronic searches. This manual search did not identify any further studies that met the inclusion criteria. Studies identified in the searches are outlined in Figure 1.

Searches were limited to the English language only, since the database inception on 10 October 2022.

2.2 | Data extraction and analysis

For all studies included in the final list, we extracted the date of publication, sample size, experimental model used, site of model application, stimulation area relative to model application site (determining whether stimuli were applied in the primary or secondary hyperalgesia area), type of stimuli applied, whether the study

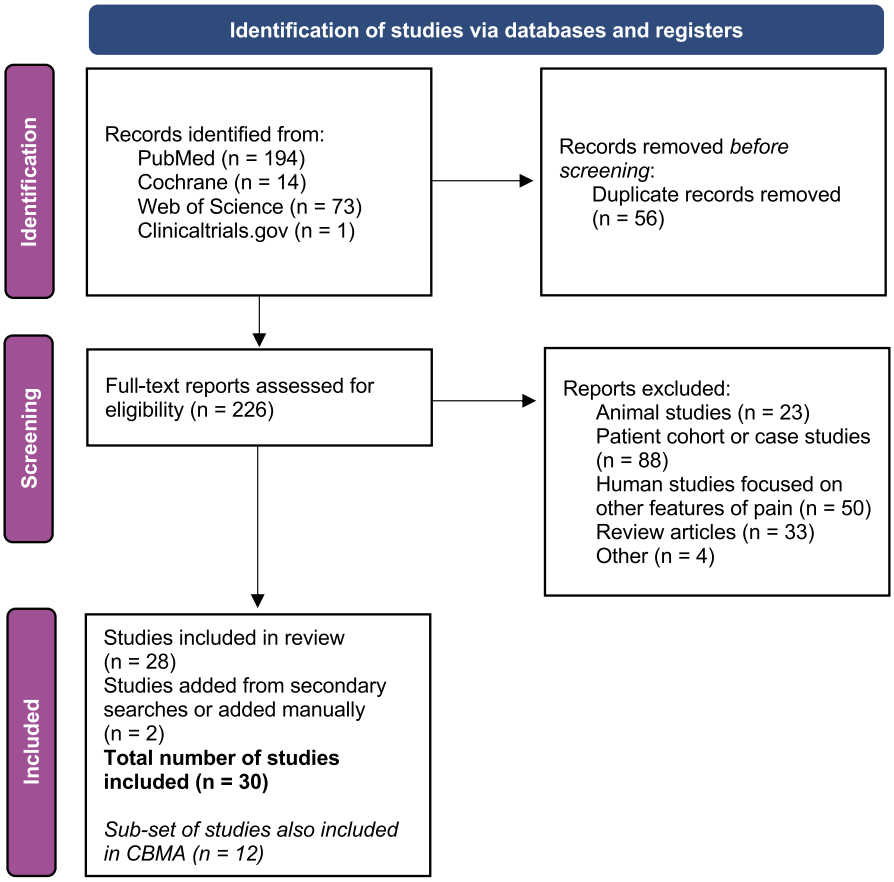

FIGURE 1 Systematic search results. The primary electronic search was conducted using PubMed-Medline, Cochrane, Web of Science and Clinicaltrials.gov databases. Duplicates were removed, and full-text reports were evaluated against pre-determined inclusion and exclusion criteria, with reports meeting the inclusion criteria ($n = 28$) taken forwards for data extraction and analysis. Further secondary searches were conducted to identify studies using spinal cord imaging and those using less common human models of central sensitization, identifying a further two studies. There were 12 studies that also met the criteria to be included in the co-ordinate based meta-analysis (CBMA), which required reporting of activation coordinates for the contrast of interest (hyperalgesia state and control or non-hyperalgesia state). Template flow diagram adapted from PRISMA 2020 statement (Page et al., 2021).

included a drug/analgesia component and whether or not the paper reports activation coordinates for the contrast of interest (hyperalgesia state and control or non-hyperalgesia state) to be included in the CBMA. This was completed by S.C.

We verified that all included studies reported behavioural data demonstrating that hyperalgesia had developed and that the time at which the imaging results were obtained occurred within a model-specific suitable time frame after the application of the model. This was to ensure that the experimental models were successfully inducing hyperalgesia and that results were obtained neither too early (before hyperalgesia has developed) or too late (once hyperalgesia has worn off).

For all identified studies, the risk of bias was assessed using the Cochrane tool for assessing the risk of bias in randomized trials (Higgins et al., 2011). Some tool domains were adapted to adequately assess the identified studies, as many were observational research studies rather than randomized trials. Risk of bias was independently assessed by two authors (S.C. and R.R.) for each study, across the five main domains of the tool. The information on the study design needed for risk of bias assessment was widely and variably distributed in manuscripts, so searches were conducted in the PDF text for keyword stems (including random-, control-, blind-, hypothe- and aim-). Any discrepancies were discussed and resolved to produce a final assessment for each study, and summarized results were reported for each domain.

2.3 | Co-ordinate based meta-analysis

2.3.1 | Research question, co-ordinate extraction and pre-processing

Coordinate-based meta-analysis is a statistical method to synthesize findings from multiple neuroimaging

studies and identify consistent patterns of brain activity associated with a specific task or condition. The CBMA included in this review was conducted to answer the following research question; what areas of the brain are activated during pain perception when hyperalgesia is present, compared to control (hyperalgesia not present), in studies using experimental models of peripheral and/or central sensitization in healthy human participants?

The reports included in this systematic review were screened to identify those suitable for inclusion in the CBMA. The inclusion criteria required there to be activation co-ordinates reported for a hyperalgesic state versus a control state (without hyperalgesia present). Co-ordinates were required to be reported as 3D co-ordinates in the format *x*, *y* and *z*, within either Montreal Neurological Institute (MNI) or Talairach space.

If present, activation co-ordinates were extracted manually for the hyperalgesia state versus control/non-hyperalgesia state contrast. Differences in the standard space used (Talaraich vs. MNI) were accounted for by converting all co-ordinates to MNI space using the BrainMap icbm2tal transform (https://www.brainmap.org/icbm2tal/). For studies that reported co-ordinates, only activations were included since deactivations were rarely reported.

2.3.2 | CBMA statistical methodology

To carry out the CBMA, we used the GingerALE BrainMap application version 3.0.2 (available at: https://www.brainmap.org/ale/). Extracted co-ordinates from the different studies were inputted, following which the GingerALE algorithm applies an activation likelihood estimate (ALE) technique. The reported coordinates are represented by 3D Gaussian distributions, defined by a specified full-width half-maximum, to provide an estimate of the likelihood of activation of a particular location across all studies. It generates voxel-wise maps indicating the likelihood of activation across the brain based on the overlap of these Gaussian distributions (Eickhoff et al., 2009, 2012; Turkeltaub et al., 2012). Statistical thresholding is then applied, here; the CBMA was performed using the false discovery rate (FDR) method assuming independence or positive dependence, with an FDR of 0.05 (Laird et al., 2005). The output of this analysis will provide a map highlighting brain regions where there is a higher likelihood of consistent activation across studies related to a hyperalgesia state compared to a control state.

3 | RESULTS

The primary search conducted using electronic databases provided 282 results. These results were processed by removing duplicates ($n = 56$) and then by assessing each publication to determine whether they met the predetermined inclusion and exclusion criteria. After this assessment, there were 28 publications identified.

This list was then finalized with the addition of publications identified in secondary searches ($n = 2$, see Section 2 for detail). There were no additional publications identified during the manual review of the reference lists in the literature and other sources. The final list of studies that met the inclusion criteria ($n = 30$) was taken forward for data extraction and analysis. Search results are outlined in Figure 1.

All studies used a within-subjects design, and all had a direct statistical comparison with a control condition that aimed to address their primary research question. For all included studies, risk of bias assessment was completed independently by two authors (S.C., R.R.) using the Cochrane tool for assessing risk of bias in randomized trials (Higgins et al., 2011). Additional phrasing was added to the five domains to allow the parallel analysis of observational studies with the interventional studies. The five assessed domains were selection bias (randomization and allocation concealment, or the inclusion of a control condition in observational studies), performance bias (whether participants and researchers were blinded to the test condition, such as capsaicin vs. sham, or the intervention, such as drug vs. placebo), detection bias (whether outcome assessment was blinded), attrition bias (whether studies reported outcome data completeness), and reporting bias (whether studies reported a specific testable hypothesis or research question that the experiment was addressing, or whether the study was pre-registered). Summarized results are shown in Figure 2.

Regarding selection bias, the assessment of the presence of a control condition is likely to be an over-simplification, as control conditions must be carefully designed, and the use of an inappropriate control may introduce bias. The blinding status of the outcome assessment was often not reported; in these cases, if researchers were unblinded during data collection, it was assumed that the outcome assessment was also unblinded and, therefore, categorized as high risk. Studies that were blinded during data collection that did not mention blinding of the outcome assessment were categorized as unclear risk. Regarding attrition, many studies simply reported the total number of subjects included but did not state if this was the total number that was originally screened or included in data collection; in these cases, the risk of bias was categorized

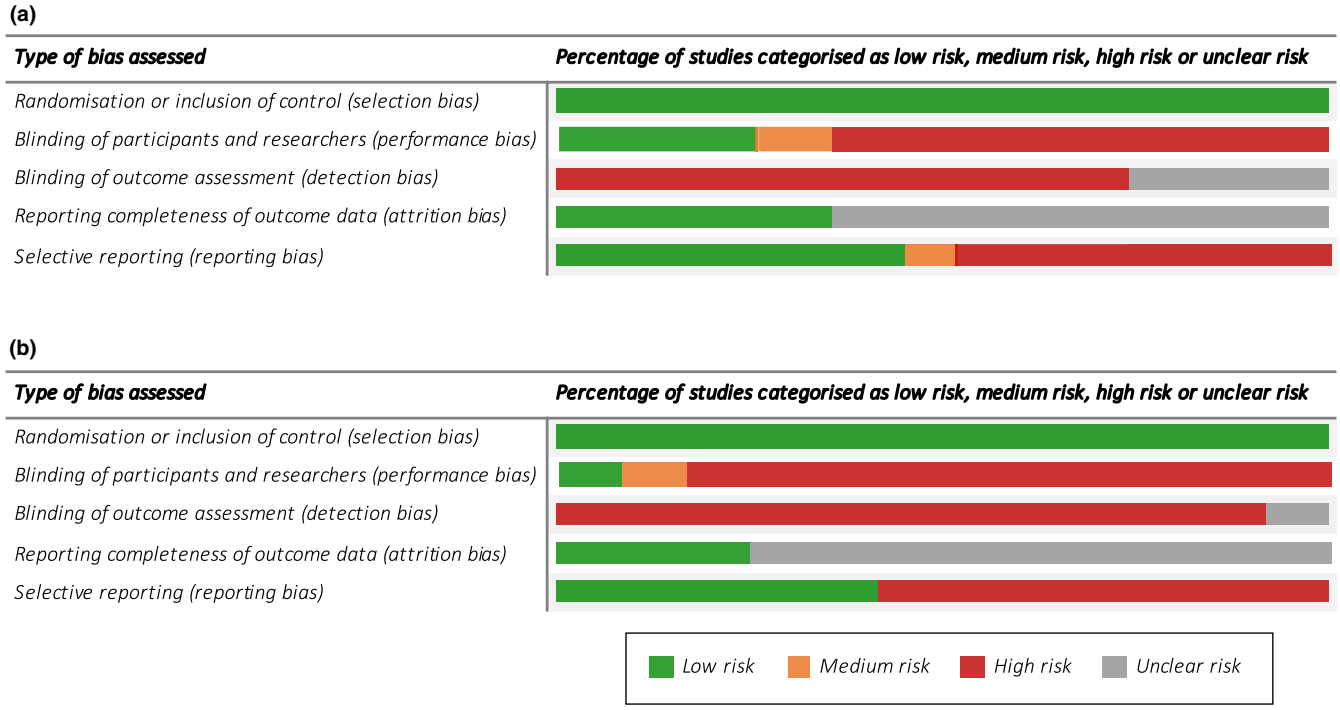

FIGURE 2 Risk of bias assessment for all included studies (a) and the subset of studies included in the CBMA (b). For each study, the risk of bias was assessed based on five standardized domains; selection bias, performance bias, detection bias, attrition bias and reporting bias. Assessment criteria for each domain were adapted for the types of study included in this review. The percentage of studies categorized as low risk (green), medium risk (orange), high risk (red) or unclear risk (grey) for each domain are shown with the coloured bars. (a) Includes all studies ($n = 30$), and (b) includes only the sub-set of studies included in the CBMA ($n = 12$). CBMA, co-ordinate-based meta-analysis.

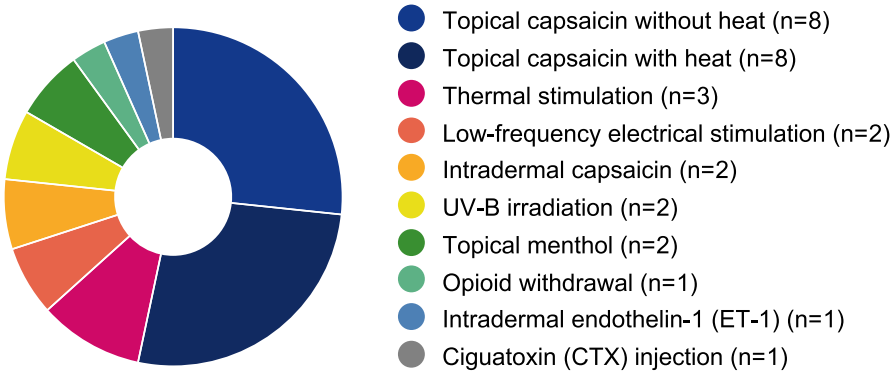

FIGURE 3 Experimental models utilized by the studies identified in this systematic literature review. The review identified 30 studies in total, across which there were nine different human experimental models used. The most common model by far was the use of topical capsaicin (with or without additional heat application with a thermode), which was used in 18 of the studies identified. All other central sensitization models identified were used in 1–2 studies each.

as unclear. This is particularly important given not all participants respond to some models of hyperalgesia; it is essential to know the proportion of non-responders, and whether these were excluded from analysis. Pre-registration has become increasingly common and is very useful in assessing reporting bias and determining if the analysis was pre-specified. In the absence of pre-registration, reporting bias was assessed to be low risk if there were defined disprovable hypotheses, as it suggests that results could be interpreted in a logical framework. Overall, the small sample sizes typically used in this type of fMRI study (due to the cost and technicality of data collection) may introduce reporting bias in the number of foci that are false positives. More lenient analysis methods can be used to increase the number of foci, or region of interest (ROI) analysis methods can be designed post-hoc

to identify significant results that were not present in the whole-brain analysis (David et al., 2013).

3.1 | Summary of experimental models of central and/or peripheral sensitization

In this review, we identified studies that utilized nine different human experimental models of central and/or peripheral sensitization in total. There were 18 studies using a capsaicin model (2 using intradermal injection and 16 using the topical application, either with or without heat application with a thermode). There were two studies that utilized a UV-B model, two studies that utilized an electrical stimulation paradigm, one study that used opioid withdrawal to induce hyperalgesia, one study that used intradermal endothelin-1 (ET-1) and 1 study that utilized thermal stimulation. There were two studies that utilized a menthol model and 1 study utilized a ciguatoxin (CTX) model, both to induce cold allodynia. The range of experimental models identified in the studies, and the frequency at which they were used, are shown in Figure 3.

The majority of studies focussed on characterizing the brain activation involved in processing primary or secondary hyperalgesia. Two studies compared stimulus responses in the primary and secondary hyperalgesia conditions, and two studies investigated whether the area of secondary hyperalgesia that develops is correlated with anatomical volumes of pain-related brain regions. These are discussed in the first part of this review.

In total, seven studies identified in this review included assessment of some form of analgesia, including pharmacological treatments (five studies), application of cold stimulus (one study), or application of anodal motor cortex (M1) transcranial direct current stimulation (tDCS) (one study). Pharmacological treatments assessed include gabapentin, ibuprofen, cyclooxygenase inhibitors (parecoxib and acetylsalicylic acid [ASA]), lidocaine and delta-9-tetrahydrocannabinol (THC). These are discussed in the second part of this section.

As per the inclusion criteria for the review, all studies included fMRI data acquisition. The strength of the MRI scanner used varied; 13 out of the 30 studies used a 1.5 tesla (1.5T) scanner, while the remaining 17 studies used a 3 tesla (3T) scanner.

All studies included in the systematic literature review analysis are summarized in Table 1, which includes the number of participants, the model of central sensitization used, the stimulus modality, the site of stimulus application relative to the model application site, and whether or not the study includes an analgesic component. Studies are grouped by the experimental model used.

3.2 | Narrative review of studies characterizing neuronal responses during hyperalgesia

First, we will discuss studies that focussed on characterizing responses during hyperalgesia. Each study is discussed in the narrative review section, and then a sub-set of studies that reported activation co-ordinates of interest (for the hyperalgesia vs. control contrast) are included in the CBMA. The CBMA approach was utilized to summarize consistently reported results for this contrast from across the multiple independent studies included.

3.2.1 | Capsaicin-induced hyperalgesia models

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is a transient receptor potential vanilloid-1 (TRPV1) agonist (Caterina et al., 1997; Nelson, 1919). It is commonly used in pain studies; it causes a burning sensation when applied topically or intradermally and produces an area of secondary mechanical hyperalgesia outside the primary application site, which is similar to the mechanical hyperalgesia often seen in patients with neuropathic pain. Three types of capsaicin models have been used in functional imaging studies identified in this review: intradermal capsaicin injection, topical capsaicin application, and topical capsaicin application with heat applied by a thermode (most commonly 45°C, either before or after capsaicin application to the skin).

The two studies using the intradermal capsaicin injection model both aimed to characterize the neural response to secondary hyperalgesia. The first compared an identical mechanical stimulus on both forearms, one without capsaicin (perceived as non-painful) and one following injection of 20 μL of 0.5% capsaicin solution to evoke secondary hyperalgesia (perceived as painful). fMRI data was acquired using a 1.5T scanner. The study identified that, during hyperalgesia, the left middle frontal gyrus and the left inferior frontal gyrus showed significantly higher activation compared to during mechanical non-painful stimulation. However, the authors discuss that the number of active voxels was highly variable between individuals, meaning subtle changes may have been missed and that changes in the brainstem and thalamus that could be involved in mechanical hyperalgesia were not addressed (Baron et al., 1999). The low sample size of nine participants and a 1.5T field strength may have also contributed to the variability in the findings and the lack of activation in other well-known pain-processing brain regions shown in later studies.

TABLE 1 Studies included in the systematic literature review analysis.

First author, year	*N*	Experimental model	Site of application	Stimulus modality	Stimulation area relative to model application site	Analgesic component
Maihöfner, 2004	11	Topical capsaicin + heat	Left forearm	Mechanical	Secondary	-
Maihöfner, 2005	12	Topical capsaicin	Left forearm	Thermal and mechanical	Primary and secondary	-
Zambreanu, 2005	12	Topical capsaicin + heat	Right lower leg	Mechanical	Secondary	-
Mainero, 2007	12	Topical capsaicin + heat	Right ophthalmic division (V1) of the trigeminal nerve	Mechanical	Primary and secondary	-
Moulton, 2007	12	Topical capsaicin	Left maxillary division (V2) of the trigeminal nerve	Thermal and mechanical	Primary	-
Mohr, 2008	17	Topical capsaicin	Right leg	Thermal	Primary	-
Shenoy, 2011	12	Topical capsaicin	Left forearm	Thermal	Primary	-
Liljencrantz, 2013	18	Topical capsaicin + heat	Left leg	Tactile	Secondary	-
Rempe, 2014	16	Topical capsaicin + heat	Right forearm	Mechanical	Secondary	-
Rempe, 2015	16	Topical capsaicin + heat	Right forearm	Thermal	Primary	-
Löken, 2017	19	Topical capsaicin	Forearm	Tactile	Primary	-
Asghar, 2015	40	Heat (contact thermode)	Non-dominant leg	Mechanical	Primary and secondary	-
Hansen, 2018	118	Heat (contact thermode)	Right leg	-	-	-
Hansen, 2019	115	Heat (contact thermode)	Right leg	-	-	-
Stammler, 2008	12	Electrical stimulation	Right forearm	Mechanical	Secondary	-
Baron, 1999	9	Intradermal capsaicin	Dominant forearm	Mechanical	Secondary	-
Lee, 2008	15	Intradermal capsaicin	Right leg	Mechanical	Secondary	-
Seifert, 2008	14	UV-B	Right forearm	Thermal and mechanical	Primary	-
Seifert, 2007	12	Menthol	Forearm	Thermal (cold)	Primary	-
Forstenpointner, 2019	8	Menthol or nerve block	Right forearm	Thermal (cold)	Primary	-
Wanigasekera, 2011	33	Opioid withdrawal	Right forearm	Thermal and mechanical	N/A (injury-free model)	-
Hans, 2013	9	Intradermal endothelin-1	Forearm	Mechanical	Secondary	-
Eisenblätter, 2017	12	Ciguatoxin (CTX) injection	Foot	Thermal	Primary	-
Iannetti, 2005	12	Topical capsaicin + heat	Leg	Mechanical	Secondary	Gabapentin and placebo
Mohr, 2008	15	Topical capsaicin	Right hand	Thermal	Primary	Cold stimulus
Lee, 2013	15	Topical capsaicin	Right leg	Mechanical	Secondary	Delta-9-tetrahydrocannabinol (THC) and placebo

TABLE 1 (Continued)

First author, year	*N*	Experimental model	Site of application	Stimulus modality	Stimulation area relative to model application site	Analgesic component
Wanigasekera, 2016	24	Topical capsaicin	Right leg	Tactile and mechanical	Secondary	Gabapentin, ibuprofen and placebo
Meeker, 2019	27	Topical capsaicin + heat	Left leg	Mechanical	Secondary	Anodal motor cortex (M1) transcranial direct current stimulation (tDCS)
Seifert, 2009	12	Electrical stimulation	Right foot	Mechanical	Secondary	Lidocaine and placebo
Maihöfner, 2007	14	UV-B	Left forearm	Mechanical	Primary	Parecoxib and acetylsalicylic acid (ASA) and placebo

Note: There were 30 studies identified in total from the literature searches. Data extracted from each study is shown, including the number of participants (*N*), the experimental model used, site of application, stimulus modality used, stimulation area relative to the model application site, and whether the study included the assessment of analgesics. Studies are grouped by the experimental model used, and studies involving an analgesic intervention are listed at the end.

The second study builds on this, using a crossover design to investigate the neural activation to central sensitization elicited following intradermal injection of capsaicin (50 μg of dissolved in 0.1 mL of Tween 80). fMRI data was acquired using a 3T scanner. Punctate stimulation matched for the perception of pain intensity in the hyperalgesia state, and the no hyperalgesia state revealed significantly higher activity in the brainstem (specifically the mesencephalic pontine reticular formation) and the thalamus during hyperalgesia compared to no hyperalgesia. The study primarily concludes a role for the brainstem in maintaining a centrally sensitized state, while activity in the somatosensory cortex is associated with the perception of increased pain intensity that is present during central sensitization (Lee et al., 2008). This finding is aligned with an earlier result in a topical capsaicin study described below, which also identified a role for brainstem regions—the nucleus cuneiformis (NCF) and the rostral periaqueductal grey (PAG) and superior colliculi—in facilitating central sensitization.

The topical capsaicin model, both with or without heat applied using a thermode, has been the most extensively used hyperalgesia model used in imaging studies. Studies using topical capsaicin (without heat) most commonly used 1% capsaicin (range 0.075%–2.5%), either in cream format or in 70% ethanol solution. The capsaicin was left on the skin for at least 15 min in all studies before fMRI data acquisition, with some studies removing the capsaicin prior to scanning and some leaving it on for the duration of the scan. Studies using topical capsaicin with heat primarily used a heat/capsaicin sensitization model previously described (Petersen & Rowbotham, 1999). Skin is heated to 45°C for 5 min, before capsaicin is applied topically. Capsaicin concentration ranged from 0.075% to 10% and was also either in cream format or ethanol solution.

In many of the studies using topical capsaicin (with or without heat) the primary aim of the study was to characterize different aspects of the neural signature of peripheral or central sensitization, by applying thermal or mechanical stimuli to the areas of primary or secondary hyperalgesia. Topical capsaicin models also result in ongoing unprovoked pain in addition to any pain stimuli applied.

One study looked specifically at the role of the brainstem in central sensitization, based on data from animal studies that have shown evidence for supraspinal contributions to the development and maintenance of central sensitization and secondary hyperalgesia (Zambreanu et al., 2005). fMRI data was acquired using a 3T scanner to record brain responses to punctate stimulation with capsaicin-induced secondary hyperalgesia compared to control. Two distinct activation regions showing significantly increased activity during secondary hyperalgesia

compared to control were observed in the brainstem, identified as the NCF and the rostral PAG and superior colliculi. The results support a role for these brainstem regions in facilitating central sensitization.

One study investigated whether brain activations for primary and secondary hyperalgesia may differ (Maihöfner & Handwerker, 2005). The study looked at differential coding of thermal hyperalgesia (primary hyperalgesia) and mechanical hyperalgesia (secondary hyperalgesia), using a block design consisting of randomized 21-s blocks. Stimuli consisted of mechanical stimuli (pin-prick stimuli with frequency 1 Hz) and thermal stimuli (thermode heated to 2°C below the individual's heat pain threshold). While there was no significant difference in pain intensity ratings, the study results show a significant difference in pain unpleasantness ratings between the two types of hyperalgesia. Individual differences in unpleasantness ratings were shown to correlate with individual differences in the contralateral anterior insular cortex, cingulate cortex and middle frontal cortex. Overall, the study concludes there are different brain activations produced by thermal and mechanical hyperalgesia, due to different psychophysical properties.

Three studies looked at features of allodynia specifically; the neural activation during dynamic-mechanical allodynia (DMA) (Maihöfner et al., 2004), the contribution of C-tactile afferents in dynamic-tactile allodynia (Liljencrantz et al., 2013) and the modulation of the unpleasantness of capsaicin-induced DMA by Aβ afferent firing rate (Löken et al., 2017). Stimuli to test allodynia were applied by stroking a soft brush or cotton wool swab on the skin; in the first two studies, the stimuli were applied to the secondary area (i.e., not the same site as capsaicin was applied), whereas in the third study, the stimuli were applied to the primary area of capsaicin application. The first study demonstrated significant increases in the BOLD response in the contralateral primary somatosensory cortex, parietal association cortex, inferior frontal cortex and bilateral secondary somatosensory cortices (S2) and insular cortex during painful brush stroking in allodynia compared to non-painful brush stroking (Maihöfner et al., 2004). Allodynia is due to central sensitization enabling low threshold mechanoreceptors (LTMs) to signal to sensitized nociceptive neurons in the spinal dorsal horn, and the LTMs responsible for allodynia are considered to be Aβ afferents. However, the second study indicates an additional role for C-tactile afferents. C-tactile afferents signal the pleasantness of gentle stroking in normal conditions. Imaging results showed multivoxel pattern differences in the dorsal posterior insular cortex—the primary receiving area for small fibre signalling (Craig, 2011; Dum et al., 2009)—to stroking during allodynia compared to control. There was also a decrease in activation of the medial prefrontal cortices, which are involved in processing pleasure from C-tactile fibre input. Overall, this suggests there is a decrease in C-tactile afferent processing during DMA. However, the study also included two patients lacking Aβ afferents who did not develop allodynia, indicating the development of allodynia is reliant on Aβ signalling (Liljencrantz et al., 2013). The final allodynia study demonstrates an unexpected relationship during DMA between the frequency of Aβ firing rates and the unpleasantness of the sensation elicited, with low-frequency firing (low brush stroke velocity) associated with higher unpleasantness compared to high-frequency firing. Since the size of the secondary area outside the area of application varies among participants, the authors describe that to engage sufficient spatial summation in the neurons of interest, and to reliably achieve a clear brush-evoked allodynia along with robust brain activity, brush strokes were applied to the primary area but engaged the secondary effects expressed on sensitized skin with the soft brush stroke. Imaging data showed increased cortical activity in the somatosensory cortex and insular cortex in response to low Aβ firing rates. These regions were previously associated with pain processing during brushing of sensitized skin but not normal skin. Overall, the study concludes a dual role for Aβ afferents during the injured state, with both pleasant and unpleasant sensations evoked depending on stroking stimulus frequency. There could be a confounding effect from the number of brush traversals in each block, as the study design applied brush stroking velocities of 0.3, 1, 3, 10, or 30 cm/s during blocks of 20 s, therefore there would be a different total number of strokes at each velocity. The ongoing burning sensation elicited by capsaicin treatment was rated throughout the scan session, and pain ratings were a significant covariate of activity in a large number of brain areas during brushing on sensitized skin but not on normal skin (Löken et al., 2017).

Two studies applied capsaicin to areas of the face to investigate sensitization in the trigeminal nociceptive pathway, with one investigating thermal hyperalgesia (using two painful heat stimuli of different intensities) and allodynia (using brush stimuli) (Moulton et al., 2007). All stimuli (brush and thermal) were applied to the site of capsaicin application (primary hyperalgesia). The three types of stimuli were applied in three fMRI sessions, with data collected on a 3T scanner. During each session, the stimuli were alternated between capsaicin and control sites, with the intensity of the thermal stimuli adjusted to be perception-matched at each site. The study identified differences between capsaicin and control sites across the trigeminal nociceptive

pathway in all stimulus conditions, with higher activation in the trigeminal ganglion and nucleus, thalamus and somatosensory cortex for capsaicin compared to control, as well as significant changes in the bilateral dorsolateral prefrontal cortex and amygdala. The other study focussed on DMA, aiming to compare primary versus secondary DMA using soft brush to apply stimuli to either normal skin or the primary and secondary areas of DMA (Mainero et al., 2007). Brush stimuli were applied in four 30-s blocks, each followed by 30 s rest, during two fMRI sessions (capsaicin session and control session) in which data were acquired using a 3T scanner. The results showed significantly increased activity in the ipsilateral spinal trigeminal nucleus in primary and secondary DMA compared to control. Some activity in supraspinal areas was shown to be increased in primary DMA compared to secondary DMA, including in the rostroventromedial medulla, pons reticular formation and dorsolateral PAG, whereas the medial reticular formation of the caudal medulla was more active during secondary DMA compared to primary DMA. There was a significant negative correlation between the mean signal change in the ventrolateral PAG and pain ratings during mechanical stimulation of the primary area, with more deactivation in the ventrolateral PAG associated with higher pain intensity ratings.

Two studies looked specifically at spinal cord activations in response to mechanical hyperalgesia (Rempe et al., 2014) and to thermal hyperalgesia (Rempe et al., 2015). The studies employed similar experimental protocols involving fMRI data acquisition using a 3T scanner in two sessions, with heat or mechanical stimuli applied before sensitization and then applied after sensitization with capsaicin. For mechanical hyperalgesia, stimuli were applied to an area outside the area of capsaicin application (secondary hyperalgesia), whereas for thermal hyperalgesia, stimuli were applied to the primary area of capsaicin application (primary hyperalgesia). Both studies showed changes in the spinal and supraspinal activity in the sensitized condition compared to the control. There was increased activity in the ipsilateral dorsal grey matter of the spinal cord during secondary mechanical hyperalgesia compared to mechanical stimulation before sensitization, which was shown to be correlated with a decrease in activity in some supraspinal centres including the dorsolateral pontine tegmentum (DLPT), rostral ventromedial medulla (RVM) and subnucleus reticularis dorsalis (SRD). The study suggests that during hyperalgesia, nociception is facilitated by decreased descending endogenous inhibition (Rempe et al., 2014). In contrast, during thermal hyperalgesia, there was increased activation in similar areas (including bilateral rubber nuclei, contralateral DLPT, RVM and SRD), that were now positively correlated with activations in the ipsilateral dorsal horn of the spinal cord. No correlations were observed between rated pain intensity and activations in the spinal cord or brainstem (Rempe et al., 2015).

One study focussed on how cognitive factors may result in differences in the neural response to self- versus externally administered thermal hyperalgesia (Mohr, Leyendecker, & Helmchen, 2008). During data acquisition with a 1.5T scanner, thermal stimuli of four different intensities (30, 37, 40, 43°C) were applied to capsaicin-treated skin on the participant's leg using a rope and lever system, with one rope controlled by the investigator and the other rope controlled by the participant. The 30°C stimulus served as a high-level baseline for each condition, used to eliminate components such as the motor response to move the rope. fMRI data showed graded activity in the anterior insular and anterior cingulate cortex (ACC) during thermal hyperalgesia depending on stimulus intensity (once the high-level baseline was subtracted), in addition to stronger activity in the posterior insular during self-administration and stronger activity in the prefrontal cortex during investigator-administration.

One study used fMRI to characterize the response to the Contact Heat Evoked Potential Stimulator (CHEPS) applied to an area of capsaicin-induced hyperalgesia, with the aim to develop topical capsaicin in conjunction with CHEPS and fMRI as a useful model of neuropathic pain (Shenoy et al., 2011). The study design consisted of two scanning sessions, one baseline (pre-capsaicin) and one following the application of capsaicin. During each session, a 51°C CHEPS stimuli for a duration of 800 ms was applied 32 times, during data acquisition using a 1.5T scanner. Participant's mean pain ratings were significantly increased in the post-capsaicin session compared to the pre-capsaicin session, and this was accompanied by increased BOLD response in the contralateral posterior cingulate gyrus, precentral and postcentral gyrus, the superior frontal gyrus, left anterior cingulate gyrus, middle frontal gyrus, and cuneus with bilateral activation in the caudate nucleus. ROI analysis showed a significant increase in BOLD signal in the contralateral insular during the post-capsaicin session compared to pre-capsaicin.

3.2.2 | UV-B models

One study investigated brain activations during UVB-induced thermal hyperalgesia and mechanical hyperalgesia (Seifert et al., 2008). The minimal erythema dose (MED) for UV-B irradiation was established in advance. An area of skin was then irradiated with UV-B at a dosage of three times the MED 24 h prior to the experimental

session itself. UV-B models are not associated with ongoing unprovoked pain. Stimuli were applied to the site of irradiation. UV-B models do not induce secondary hyperalgesia, so this was not investigated. The study design was a 2×2 factorial block design, in which the factors were sensitization (hyperalgesia or no hyperalgesia) and stimulus modality (heat pain or mechanical pain). Thermal stimuli were applied with a thermode, and mechanical stimuli were applied using a mechanical impact stimulator and all stimulations for fMRI measurements were perception matched to a numeric rating scale rating for pain intensity of 40. fMRI data was acquired using a 1.5T scanner. fMRI data demonstrated that there are differences in the neural activity associated with mechanical hyperalgesia versus heat hyperalgesia in response to stimuli of perception-matched intensities, suggesting different pathways may be involved in the different types of hyperalgesia. For heat hyperalgesia, there was increased activation in the prefrontal cortex and parietal association (PA), whereas for mechanical hyperalgesia, there was increased activation in the S2 and posterior insular cortex.

3.2.3 | Electrical stimulation models

One study utilized electrical stimulation models to induce secondary hyperalgesia. It applied two different electrical stimulation paradigms to the forearm, each over 35 min; the first paradigm consisted of continuous noxious low-frequency (0.5 Hz) stimulation to induce hyperalgesia, and the second paradigm consisted of continuous noxious high-frequency (20 Hz) stimulation to induce hypoalgesia/hypoesthesia (Stammler et al., 2008). fMRI data was acquired on a 1.5T scanner. The scan paradigm used a block design in which pin-prick (both sessions) and von Frey (hypoesthesia session only) mechanical stimuli were applied at a frequency of 1 Hz for 21 s per block. In the pin-prick hyperalgesia session (which is most relevant for this review), activated brain regions included the somatosensory cortices, parietal cortices, insular, dorsolateral and ventrolateral prefrontal cortices, cuneus, anterior and posterior parts of the ACC, basal ganglia and the thalamus. These activations were greater in the hyperalgesic state compared to baseline (pre-stimulation). A weighted generalized linear model with the individual perceptual changes included as co-regressors showed that for pin-prick hyperalgesia, cerebral activations in many of these brain regions were positively correlated with the extent of the individually induced sensory gain (i.e. decrease of mechanical pain threshold and extent of hyperalgesic area).

3.2.4 | Opioid withdrawal-induced hyperalgesia

One study used an injury-free model of central sensitization by inducing hyperalgesia with opioid withdrawal (Wanigasekera et al., 2011). The opioid used was remifentanil. Out of 23 participants included in the study, 12 participants demonstrated hyperalgesia to thermal stimuli ('responders') and only 3 participants developed hyperalgesia to punctate stimuli. Due to this small number, punctate data was not further analysed. Participants who developed hyperalgesia were identified using within-subject data. A significant increase in intensity scores in at least one of the post-infusion stimulation blocks of the opioid visit when compared with the corresponding stimulation blocks in the saline visit ($p < 0.05$; two-tailed paired t-test) was assumed to be evidence of opioid withdrawal-induced hyperalgesia (OIH) to thermal or punctate stimuli. fMRI data was collected with a 3T scanner.

The fMRI data from responders showed a significant increase in neuronal response (BOLD activity) in a cluster of voxels in the mesencephalic-pontine reticular formation (MPRF) during the opioid withdrawal period compared to baseline. This area had been demonstrated in a previous fMRI study in humans to be involved in maintaining capsaicin-induced central sensitization (Lee et al., 2008). A correlation analysis demonstrated a significant negative correlation between ($r = 0.61$, $p = 0.03$) between the opioid withdrawal-induced increase in pain perception and the increase in BOLD activity in the MPRF region. The authors conclude that this relationship indicates a net descending inhibitory response of the MPRF during OIH.

3.2.5 | Intradermal ET-1 model

One study used an intradermal ET-1 sensitization model to induce secondary mechanical hyperalgesia (Hans et al., 2013). ET-1 is a potent vasoconstrictor that has been shown to induce pain in humans. Prior to scanning, 40 µL of a 10^{-6} M ET-1 solution was intradermally injected into the volar surface of the forearm. To assess the development of mechanical hyperalgesia, punctate stimuli were applied using a von Frey monofilament at 10 and 30 min post-injection, on both the injected and non-injected arms, and the hyperalgesic area was defined as a region in which participants reported a distinct change in the quality of the sensation. All participants reported a hyperalgesia state at both 10 and 30 min timepoints, with a significant ($p < 0.05$) increase in responsiveness in the injected arm compared to the non-injected arm. There was also a low intensity of ongoing (spontaneous) pain.

After the 30 min timepoint, fMRI data was acquired using a 1.5T scanner in a block design, with two conditions (punctate stimulus and baseline), and stimulus blocks alternating between the injected and non-injected arms. The authors report that the fMRI results show that, during hyperalgesic stimulation, the most active cortical regions include the primary and secondary somatosensory cortices, the insular, the inferior parietal lobe, the superior and inferior frontal cortices and the ACC. They report an increase in fMRI signal in activated regions, and an increase in the number of regions activated, during the hyperalgesic condition (injected arm) compared to non-painful tactile stimulation (non-injected arm).

3.2.6 | Thermal stimulation models

In three studies, a brief thermal sensitization (BTS) model was used to induce first-degree burn injury (Asghar et al., 2015; Hansen et al., 2018, 2019).

The first study aimed to assess whether there are differences in the neuronal activation in participants who were categorized based on the size of the area of secondary hyperalgesia they develop (as either high- or low-sensitization responders) (Asghar et al., 2015). The size of the area is based on their phenotypic expression of secondary hyperalgesia. Following the screening, at which secondary hyperalgesia was induced and participants categorized based on the size of the hyperalgesia area, an MRI-compatible contact thermode (47°C, 7 min, 9 cm^2) was used to induce a first-degree burn injury on the lower leg. During the scans, BOLD responses were measured for pre-burn injury painful pin-prick stimulation, post-burn injury in the secondary hyperalgesia area and post-burn injury in the primary hyperalgesia area, with a 3T scanner. In total, 40 participants were included (20 in each group). There were no statistical differences between the groups with regard to age, sex, height or weight ($p > 0.05$). The pin-prick stimulus applied was matched to the participants' mechanical pain threshold, and there were no statistical differences in the mechanical pain thresholds between the two groups. There was also no difference in the pain scores between groups in any experimental condition (pre-burn injury, post-burn secondary hyperalgesia or post-burn primary hyperalgesia). BOLD data did show significantly higher activation of the right post-central gyrus, left precuneus, left posterior cingulate cortex, right parahippocampal gyrus, right caudate nucleus and lower activation of the right precuneus in high-sensitization responders compared to low-sensitization responders, in response to pin-prick stimulation of the secondary hyperalgesia area, indicating the two groups are processing pain differently.

The subsequent studies built on this one, aiming to explore whether there is an association between the area of secondary hyperalgesia developed and the anatomical volumes of pain-relevant brain regions, such as the caudate nuclei (Hansen et al., 2018), and explore whether there is an association between the area of secondary hyperalgesia and brain connectivity in known resting-state networks (Hansen et al., 2019). A similar BTS model was used (contact thermode applied to the leg, area 2.5 × 5 cm heated to 45°C for 3–5 min) to induce secondary hyperalgesia, and the area of hyperalgesia was measured using a von Frey filament.

In the first publication, 3T anatomical MRI data was used to quantify the volume of pain-relevant brain structures. There were no significant associations between the area of secondary hyperalgesia and the volume of the right and left caudate nucleus (primary analysis) or the volume of other cortical and sub-cortical structures (secondary analysis), including primary somatosensory cortex, ACC and mid cingulate cortex, putamen, nucleus accumbens, globus pallidus, insular or the cerebellum's white matter and cortex (Hansen et al., 2018).

In the second, 3T resting state data identified that area of secondary hyperalgesia is associated with increasing and decreasing connectivity in multiple networks (sensorimotor network, fronto-parietal network, and default mode network), suggesting that differences in the phenotypic secondary hyperalgesia areas may be expressed as differences in the resting-state central neuronal activity (Hansen et al., 2019).

3.2.7 | Models to induce cold allodynia

There were three studies that used models to induce cold allodynia. In all three studies, stimuli were delivered to the primary site at which the experimental model was applied (primary hyperalgesia). The first study used 200 μL of 40% menthol solution applied to the forearm to induce cold allodynia (Seifert & Maihofher, 2007). The study design consisted of a block design fMRI paradigm. Prior to fMRI data acquisition, the cold pain threshold was identified for each participant, then the experiment included three blocks; innocuous cold (5°C above cold pain threshold—not painful), noxious cold (5°C below cold pain threshold—painful), and then, following the application of menthol for 15 min, cold allodynia (5°C above cold pain threshold—now painful due to menthol-treatment).

Functional magnetic resonance imaging data was acquired using a 1.5T scanner. The results of contrast analyses comparing cerebral activations in each of the three conditions showed stronger activation in the anterior

insular, bilateral dorsolateral prefrontal cortices, the ACC and bilateral inferior frontal cortex in the cold allodynia condition compared to perception-matched noxious cold.

The second study, aiming to extend the previous study, induced reversible cold allodynia using shallow intracutaneous injection of 1.5 nM CTX into the dorsal surface of the right foot (Eisenblätter et al., 2017). fMRI data was acquired on a 3T scanner. The experimental paradigm consisted of a block design with four sections; section 1 was warm stimulation (42–44°C) on the CTX site, section 2 was cold stimulation (18°C) on the CTX site, section 3 was warm stimulation (42–44°C) on the control site, and section 4 was cold stimulation (18°C) on the control site. For each stimulation, there was a 4 s dynamic period, when increasing or decreasing to the target temperature, followed by a 15 s constant temperature period. There was a 20 s rest between each stimulation.

Functional magnetic resonance imaging data showed bilateral brain responses in the medial insular, medial cingulate cortex, secondary somatosensory cortex, frontal areas, and cerebellum during cold allodynia. When compared to the innocuous cold, the cold allodynia predominantly activated the mid-anterior parts of the insular cortex. There were more robust changes in the BOLD response, and more brain areas activated, during the dynamic period when the cold stimulus was applied to the CTX site compared to the constant temperature period. This was not seen in the control site data.

The final study explored two different experimental models to induce cold allodynia (topical menthol application and mechanical block of peripheral A-fibre input; Wahren et al., 1989), each repeated in every participant over two sessions. In one session, menthol was topically applied to the skin on the back of the right hand (causing peripheral nociceptor sensitization, resulting in increased C fibre input), whereas in the other session, the superficial radial nerve at the right forearm was mechanically blocked (causing inhibition of A-fibre input, reducing inhibition of central pain processing) (Forstenpointner et al., 2019). fMRI data was acquired using a 1.5T scanner. A block design was used, with alternating blocks of neutral thermal stimulation (32°C) or cold thermal stimulation (adjusted to the participants cold pain threshold with four temperatures; +6, +3, −3 and −6°C above or below threshold). Spontaneous pain was not reported during the fMRI sessions by any subjects.

Functional magnetic resonance imaging data demonstrated that the two different types of cold allodynia represented with the two experimental models induce activity in different brain areas representative of the different underlying mechanism of each type. Contrast analysis showed that menthol produced significantly stronger activation of the contralateral lateral thalamus and primary and secondary somatosensory cortices, whereas nerve block produced significant BOLD signal increases in the contralateral medial thalamus, ACC and bilateral medial and superior frontal cortex.

3.2.8 | Comparison of pain ratings and fMRI activity

Out of the total number of publications included in this review, there were 10 studies that included some form of assessment of pain ratings co-varying with imaging data. In general, this either consisted of modelling pain ratings as regressors in the first-level imaging analysis or a correlation analysis with signal change in a specific ROI. Overall, eight of the studies that included this analysis reported that pain ratings (in the form of pain intensity, unpleasantness, or pleasantness for cold analgesia) did correlate with the fMRI measure explored (Lee et al., 2008, 2013; Löken et al., 2017; Mainero et al., 2007; Mohr, Leyendecker, Mangels, et al., 2008; Seifert et al., 2009; Stammler et al., 2008; Wanigasekera et al., 2011). Brain regions in which fMRI activity was shown to correlate with pain ratings included the PAG, MPRF and, prefrontal cortex and the right amygdala during cannabinoid-induced analgesia. The remaining two studies did not show statistically significant correlations with pain ratings; the first which looked at correlations between pain ratings and activations in the brainstem and spinal cord during thermal stimulations (Rempe et al., 2015), and the second of which looked at the correlation between gabapentin-induced suppression of hyperalgesia evoked BOLD responses and pain reports (Wanigasekera et al., 2016). In summary, the majority of studies that completed this analysis showed that there was some form of relationship between pain ratings and neural activity. Statistical analysis showing that specific fMRI activity is correlated with participant pain ratings may enable greater interpretability of the fMRI results.

3.3 | CBMA results: Summarizing the consistently observable neural response to stimulation in the hyperalgesic state compared to control across multiple studies

During further screening of the reports for eligibility to be included in the CBMA, we identified 12 studies that met these inclusion criteria and reported activation coordinates of interest, with a total of 14 contrasts for the hyperalgesia state versus control/non-hyperalgesia state. Co-ordinates for each contrast were manually extracted. Differences in the standard space used (Talairach vs. MNI) were accounted for by converting Talairach coordinates (reported

by Eisenblätter et al., 2017; Maihöfner et al., 2004; Maihöfner & Handwerker, 2005; Mainero et al., 2007; Seifert et al., 2008, 2009; Seifert & Maihofher, 2007) to MNI space. The peripheral and/or central sensitization models used in the eligible studies covered a range of the total spectrum of models included in this review; seven studies used the topical capsaicin model, one study used the intradermal capsaicin model, one used menthol, one used UV-B, one used electrical stimulation and one used CTX injection. The site and laterality of stimulation varied across studies; eight studies stimulated on the right and four stimulated on the left, and the most common site of stimulation was the volar forearm (five studies). Studies included in the CBMA are shown below in Table 2.

From the 12 studies included in the CBMA, a total of 168 activation foci were reported. Across studies, the foci commonly appeared in the key regions associated with hyperalgesia that are discussed above, including the primary and secondary somatosensory cortices, insular cortex, cingulate cortex, parietal cortex, thalamus, NCF and PAG. All reported co-ordinates for peak activations were transformed to a 2.5 mm spherical mask and plotted onto the MNI512 1 mm standard brain image, shown in Figure 4.

Co-ordinate based meta-analysis performed on the co-ordinates extracted for the contrast hyperalgesia state versus control/no hyperalgesia state ($n = 14$) produced six clusters that were statistically significant at the FDR of 0.05. The cluster locations, in order of size from largest to smallest, were the right anterior insular cortex, left anterior insular cortex, left cingulate gyrus, right thalamus, right mid-insular cortex and right inferior parietal lobe. These regions summarize the most commonly reported regions showing increased pain response to secondary hyperalgesia across the 12 independent studies included. The activation clusters are shown in Figure 5.

There were seven contrasts where stimulation had been applied to the primary hyperalgesia zone and seven where stimulation had been applied to the secondary hyperalgesia zone. Although not enough contrasts for a CBMA analysis to compare these conditions, a simple comparison showed that there was no significant difference in the number peak co-ordinates reported for contrasts in the secondary zone compared to contrasts in the primary zone (13.6 ± 5.4 and 10.4 ± 6.4 peaks respectively, $p = 0.3385$, unpaired t-test).

3.4 | Narrative review of studies characterizing neuronal responses during analgesic interventions

There were eight studies included in this review that focussed on characterizing neural responses to various analgesic interventions during hyperalgesia. Five of these studies used the topical capsaicin model, two studies used an electrical stimulation model and one study used the UV-B model. Studies investigating the effects of an analgesic intervention are summarized below and in Table 3.

3.4.1 | Capsaicin models

In five capsaicin studies, the primary aim was to characterize the neural response to various analgesics. The first explored pharmacological modulation by *gabapentin* during a normal state and a centrally sensitized state (Iannetti et al., 2005). The study design consisted of a four-way crossover design, with four sessions consisting of gabapentin (180 mg) or placebo and centrally-sensitized state or normal state, with the order of the drug or placebo conditions randomized. In each session involving central sensitization, thermal stimulation and capsaicin were applied to induce central sensitization 2 h after gabapentin/placebo were administered. Three hours after drug/placebo administration, during fMRI data acquisition using a 3T scanner, 20 mechanical stimuli were applied to each leg using a 60 g von-Frey hair. The three main findings were that gabapentin reduced activations in the bilateral operculo-insular cortex independent of whether central sensitization was present, that gabapentin reduced activation in the brainstem during central sensitization only and that gabapentin reduced stimulus-induced deactivations during central sensitization only, an effect that was more robust than the effect on brain activation. The study concludes that gabapentin has a major effect during the centrally-sensitized state and a reduced but measurable effect during the normal state.

One study investigated the use of *cold stimuli* to relieve capsaicin-induced pain (Mohr, Leyendecker, Mangels, et al., 2008). Four thermal stimuli (0, 20, 30 and 43°C) were applied to the skin 20 times each in a random order, using a contact thermode applied for 4 s. This was repeated in two fMRI sessions, one pre-capsaicin and one post-capsaicin, with data acquired using a 1.5T scanner. Visual analogue scale (VAS) ratings showed that participants perceived the 0°C stimuli as painful in the pre-capsaicin session, but as pleasant in the post-capsaicin session. BOLD responses in the pre-frontal cortex and PAG were increased in the post-capsaicin session compared to the pre-capsaicin session, and were positively correlated with VAS ratings for perceived pleasantness in a simple regression analysis. Connectivity analysis also showed contributions from the prefrontal cortex activity and PAG that were cold-dependent during the post-capsaicin session, leading the

TABLE 2 Studies included in the co-ordinate based meta-analysis (CBMA).

First author, year	*N*	Model of central sensitization	Stimulus modality	Stimulation zone	Stimulation site	Contrast included	Number of peak co-ordinates
Maihöfner, 2004	11	Topical capsaicin + heat	Mechanical	Secondary	Left forearm	Allodynia minus brush-related activations	7
Maihöfner, 2005	12	Topical capsaicin	Thermal + mechanical	Primary + secondary	Left forearm	[A] Pin-prick hyperalgesia minus pin-prick stimulation	7
						[B] Thermal hyperalgesia minus thermal stimulation	15
Zambreanu, 2005	12	Topical capsaicin + heat	Mechanical	Secondary	Right lower leg	Secondary hyperalgesia compared with control	15
Mainero, 2007	12	Topical capsaicin + heat	Mechanical	Primary + secondary	Right ophthalmic division (V1) of trigeminal nerve	Brush to the secondary area vs. brush to the untreated skin	11
Mohr, 2008	15	Topical capsaicin	Thermal	Primary	Right hand (dorsum)	Thermal stimulation on capsaicin-treated skin vs. untreated skin	11
Liljencrantz, 2013	18	Topical capsaicin + heat	Tactile	Secondary	Left thigh (ventral)	Allodynia > control	17
Löken, 2017	19	Topical capsaicin	Tactile	Primary	Left forearm	Sensitized > normal skin	4
Lee, 2008	15	Intradermal capsaicin	Mechanical	Secondary	Right lower leg	Punctate stimulation hyperalgesic state vs. normal state	17
Seifert, 2007	12	Menthol	Thermal (cold)	Primary	Right forearm	Cold allodynia vs. noxious cold	3
Seifert, 2008	14	UV-B	Thermal + mechanical	Primary	Right forearm	[A] Thermal hyperalgesia vs. normal thermal pain	12
						[B] Mechanical hyperalgesia vs. normal mechanical pain	21
Seifert, 2009	12	Electrical stimulation	Mechanical	Secondary	Right foot (dorsum)	Areas more activated during hyperalgesia	21
Eisenblätter, 2017	12	Ciguatoxin (CTX) injection	Thermal	Primary	Right foot (dorsum)	Cold allodynia vs. innocuous cold	7

Note: There were 12 studies identified that included activation co-ordinates meeting the inclusion criteria. Data extracted from each study is shown, including the number of participants (*N*), model of central sensitization used, stimulus modality used, contrast for which activation co-ordinates are reported, and the number of peak co-ordinates reported. Studies are grouped by the model used.

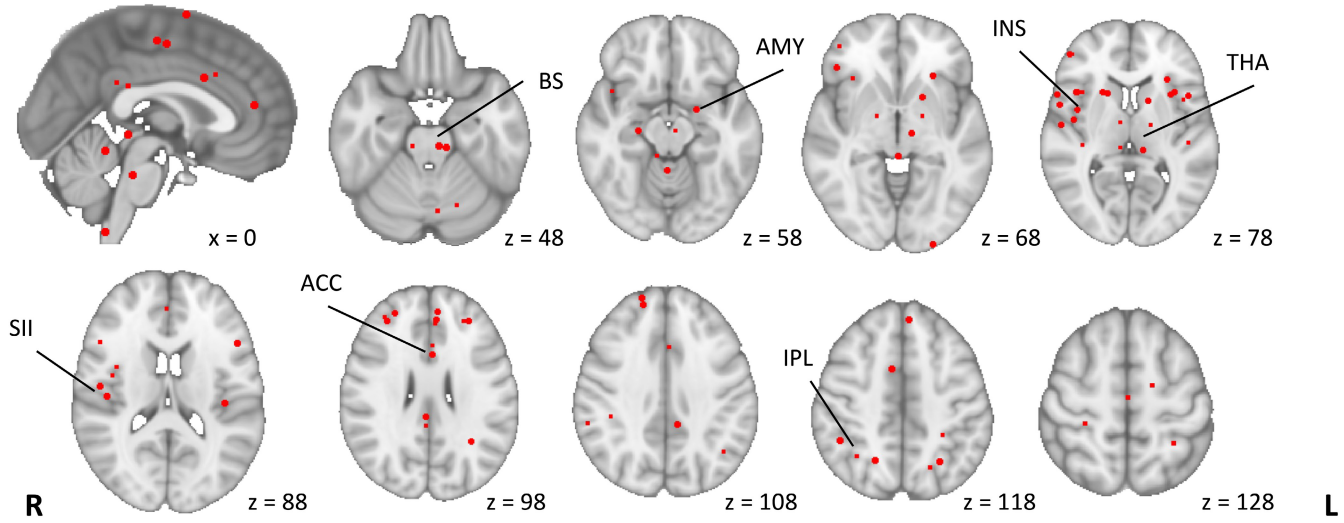

FIGURE 4 Extracted peak co-ordinates included in the co-ordinate based meta-analysis. Co-ordinates for peak activation voxels were manually extracted from each study. Each was made into a peak voxel mask then transformed into a 2.5 mm spherical mask. The spherical masks for all peaks are shown here on the MNI512 1 mm brain. Slices shown are (L–R, top row first): sagittal section, axial sections taken at $z = 48, 58, 68, 78, 88, 98, 108, 118, 128$. ACC, anterior cingulate cortex; AMY, amygdala; BS, brainstem; INS, insular cortex; IPL, inferior parietal cortex; SII, secondary somatosensory cortex; THA, thalamus.

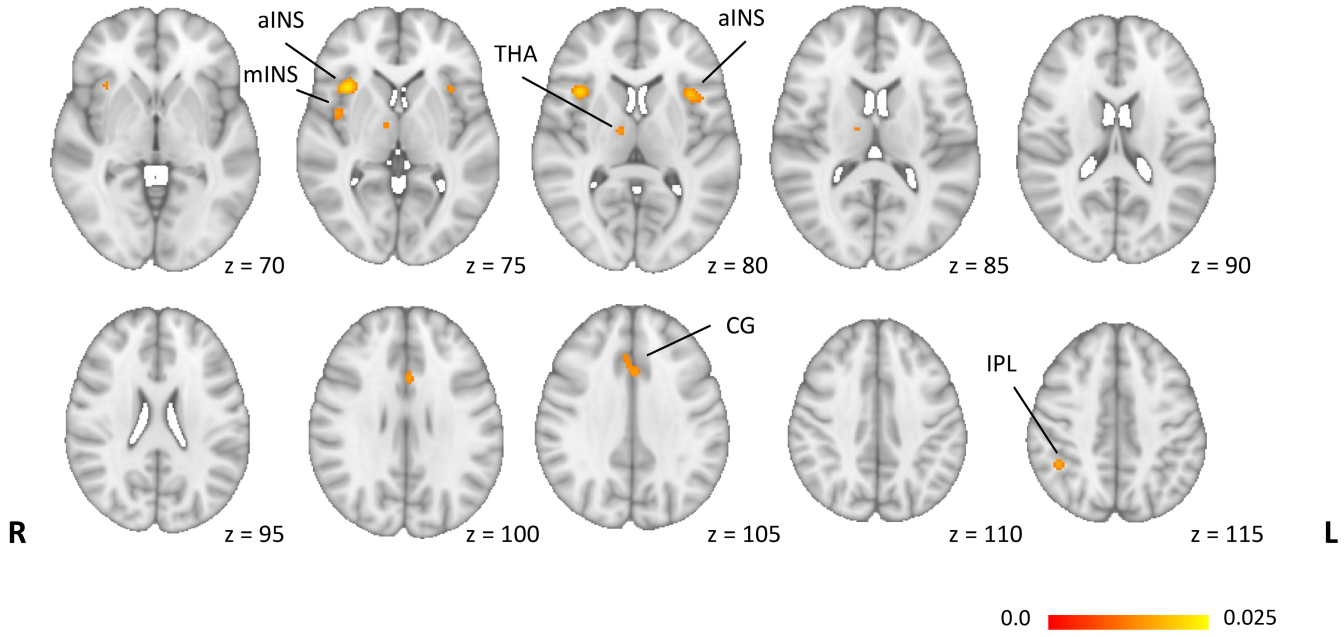

FIGURE 5 Activation clusters for CBMA of hyperalgesia state versus control/no hyperalgesia state. ALE cluster map showing the six clusters identified in the CBMA. Cluster locations, in descending size order, are right anterior insular cortex (aINS), left anterior insular cortex, left cingulate gyrus (CG), right thalamus (THA), right mid-insular cortex (mINS) and right inferior parietal lobe (IPL). ALE clusters are shown here on the MNI512 1 mm brain. Slices shown are (L–R, top row first): axial sections taken at $z = 70, 75, 80, 85, 90, 95, 100, 105, 110, 115$. ALE, activation likelihood estimate; CBMA, co-ordinate based meta-analysis.

authors to propose that the pain relief perceived during the cold stimuli results in part from activation of endogenous descending inhibition.

One study investigated the pharmacological effects of a *cannabinoid*—delta-9-THC—on the pain response in hyperalgesia (Lee et al., 2013). Participants completed four study sessions under different conditions; participants were administered either 15 mg THC or placebo (orally), repeated in either a normal state or capsaicin-induced hyperalgesia. Capsaicin or placebo cream was applied to the skin 2 h post-dose, and fMRI was then performed 3 h post-dose. During each session, fMRI data was acquired

TABLE 3 Summary table outlining regions in which activity was modulated by each analgesic intervention.

Analgesic intervention	Brain regions in which modulation of activity (vs. placebo) was reported	Reference
Gabapentin	Contralateral insula, SII, ipsilateral NCF/ brainstem	Iannetti et al. (2005), Wanigasekera et al. (2016)
Delta-9-tetrahydrocannabinol (THC)	ACC, right amygdala	Lee et al. (2013)
Ibuprofen	-	Wanigasekera et al. (2016)
Lidocaine	Contralateral primary motor cortex, and thalamus, bilateral SII, insula, ACC, mPFC, dlPFC, PA and midbrain	Seifert et al. (2009)
Cyclooxygenase inhibitors	Contralateral SI, PA, insula, middle and inferior frontal cortices, bilateral SII and ACC	Maihofner et al. (2007)
Cold stimulus	Prefrontal cortex and PAG[a]	Mohr, Leyendecker, and Helmchen (2008)
Transcranial direct current stimulation (tDCS)	Medial prefrontal cortex, pregenual ACC, and PAG	Meeker et al. (2019)

Note: There were seven studies included in this review that included assessment of an analgesic intervention, covering five pharmacological interventions (gabapentin, delta-9-THC, ibuprofen, lidocaine and cyclooxygenase inhibitors) and two non-pharmacological interventions (cold stimulus and anodal motor cortex [M1] tDCS). This table summarizes the brain regions in which activity was modulated by each analgesic. Only one analgesic (gabapentin) was included in two studies.

Abbreviations: ACC, anterior cingulate cortex; AG, periaqueductal grey; dlPFC, dorsolateral prefrontal cortex; mPFC, medial prefrontal cortex; NCF, nucleus cuneiformis; PA, parietal association; PAG, periaqueductal grey; SI, primary somatosensory cortex; SII, secondary somatosensory cortex.

[a]For the cold stimulus study this was a comparison between response to cold in capsaicin treated versus untreated skin (not to a placebo or sham like the other studies).

using a 3T scanner. Scans consisted of 5 min of resting state (no task), 15 min punctate mechanical stimuli (21 stimuli applied to the area of secondary hyperalgesia with a handheld probe) and 5 min of a visual control task (black and white checkerboard). Intensity and unpleasantness of ongoing and provoked pain was rated using a VAS. Behavioural data showed that during secondary hyperalgesia, THC significantly reduced the perceived unpleasantness of ongoing and provoked pain compared to placebo, but had no effect on the perceived intensity of ongoing or provoked pain. BOLD data showed that there was increased activity in the ACC and thalamus during capsaicin-induced hyperalgesia, and that there was a significant reduction in this ACC activity during the THC session compared to placebo, but no significant change in the thalamus activity. ROI analysis also showed that THC significantly increased BOLD response in the right amygdala compared to placebo, and that the effect of THC on reducing pain unpleasantness was positively correlated with this effect of THC on the capsaicin-induced activity in the right amygdala compared to placebo. THC also significantly decreased the functional connectivity between the right amygdala and primary sensorimotor region during capsaicin-induced ongoing pain, and this reduction was positively correlated with the difference in drug effects on unpleasantness and intensity of the ongoing pain.

One study focussed on the ability of fMRI in conjunction with central sensitization induced by capsaicin to differentiate between effective analgesia for neuropathic pain (*gabapentin*) and ineffective analgesia for neuropathic pain (*ibuprofen*), both compared to placebo, with a view to validate the use of fMRI to guide decision making in drug development (Wanigasekera et al., 2016). The study design comprised of three study visits at which participants received either gabapentin (1200 mg), ibuprofen (600 mg) or placebo orally, followed by application of capsaicin 90 min after dosing, and fMRI data acquisition using a 3T scanner 150 min after dosing. During each scan, 15 stimuli were applied using a soft brush (for allodynia) and 18 stimuli were applied using a punctate probe (for mechanical hyperalgesia). Stimuli were applied in the secondary hyperalgesia area adjacent to the site of capsaicin application. Participants were asked to rate pain intensity and unpleasantness using a VAS. Gabapentin, but not ibuprofen, significantly reduced pain intensity and unpleasantness ratings compared to placebo. Gabapentin, but not ibuprofen, also significantly reduced the BOLD response associated with hyperalgesia in the right NCF, left insular and left secondary somatosensory cortex (SII) compared to placebo. Resting-state functional connectivity between the left thalamus and left SII was also reduced by gabapentin compared to placebo, but not by ibuprofen. This study demonstrates the ability of resting-state and task MRI techniques to differentiate between effective and ineffective analgesics. Analysis was also conducted to explore whether there was a correlation between

gabapentin-induced behavioural reports and the neural activity, which showed that there were no statistically significant relationships between gabapentin-induced suppression of hyperalgesia evoked BOLD response from the right NCF, left anterior and posterior insula and left SII and pain reports, or between left thalamus-SII connectivity and ongoing pain.

The final study aimed to elucidate the neural mechanism underlying the analgesic effects of motor cortex stimulation using *tDCS* (Meeker et al., 2019). Following the determination of pain thresholds at a screening visit, participants attended three sessions, which included anodal, cathodal and sham tDCS in a randomized cross-over design. Following capsaicin application, anodal tDCS was shown to moderately reduce secondary hyperalgesia (measured using pain intensity in response to pin-prick mechanical stimuli) compared to cathodal or sham tDCS. fMRI data acquired using a 3T scanner showed that the BOLD response following anodal tDCS was normalized to the baseline level in the medial prefrontal cortex (mPFC), ACC and PAG; key areas in the descending pain modulatory network.

3.4.2 | Electrical stimulation models

One study investigated the effects of pharmacological modulation by *lidocaine*, a sodium channel blocker, compared to placebo, using pin-prick stimuli applied during fMRI acquisition with a 1.5T scanner (Seifert et al., 2009). A transcutaneous low-frequency electrical stimulation model was used to induce secondary mechanical hyperalgesia. The model consisted of two electrodes mounted on the skin to deliver electrical pulses of 0.5 ms duration at a frequency of 1 Hz, with the current initially adjusted to a pre-defined pain rating target and then kept constant for the rest of the session. This model applies a repeated painful stimulus for the duration of the session, therefore, ongoing acute pain is present in addition to the secondary hyperalgesia (Koppert et al., 2001).

To investigate pharmacological modulation by lidocaine, a 2×2 factorial analysis—in which factor 1 was pain sensitization (pin-prick hyperalgesia vs. normal pin-prick) and factor 2 was pharmacological modulation (lidocaine vs. placebo) was performed. The lidocaine (or placebo) infusion was started 20 min after the start of the electrical stimulation. Initially, 12 mg/kg was infused intravenously for 10 min, followed by 2 mg/kg/h for 15 min, and the fMRI data was then collected 15 min after the start of the infusion. Psychophysics results indicated that lidocaine was anti-hyperalgesic, with significantly reduced pain intensity ratings to the electrical stimulation and to the pin-prick stimuli applied in the hyperalgesia area, as well as a significantly smaller area of hyperalgesia, with lidocaine compared to placebo. The main fMRI finding of the study was that activity in the mPFC was reduced by lidocaine. The mPFC activity during hyperalgesia was shown to inversely correlate with the extent of hyperalgesia in each individual, and to be predictive for the responsiveness to lidocaine analgesia in each individual (Seifert et al., 2009).

3.4.3 | UV-B models

One study investigated UV-B-induced mechanical hyperalgesia at three separate study visits, at which subjects received 5-min intravenous infusions of *cyclooxygenase inhibitors* (either 40 mg *parecoxib* or 1000 mg *ASA*) or placebo (0.9% saline) (Maihofner et al., 2007). The UV-B was applied on the forearm to induce primary hyperalgesia. This study established the MED for UV-B irradiation in advance, it irradiated an area of skin with UV-B at a dosage of three times the MED 24 h prior to the experimental session itself. fMRI data was acquired during each visit using a 1.5T scanner. The experiment consisted of a block design with three conditions; mechanical impact hyperalgesia (stimuli applied to UV-B site), acute mechanical pain and tactile stimuli (both applied approximately 5 cm away from the UV-B site). Testing was conducted before and 30 min after drug/placebo infusion. fMRI data showed that in the mechanical impact hyperalgesia and acute mechanical pain conditions there were activations of many brain regions involved in pain perception, including the primary (S1) and secondary (S2) somatosensory cortices, PA cortex, inferior parietal lobule, orbitofrontal cortex, superior, middle and inferior frontal cortices, ACC, insular and supplementary motor cortex. With both parecoxib and ASA, there were smaller activations in certain regions, notably in S1, PA, S2, insular, ACC and prefrontal cortices. When comparing the hyperalgesic condition to the acute pain, there was more drug-induced modulation of the PA and inferior frontal cortex during mechanical hyperalgesia, and more drug-induced modulation of the bilateral S2 during acute mechanical pain.

4 | DISCUSSION

This review outlines the use of fMRI in conjunction with models of peripheral and/or central sensitization to investigate two important aspects of pain research in healthy human participants; firstly, the neural signature associated with primary and/or secondary hyperalgesia, and secondly, its modulation by various analgesics.

4.1 | Comparison of different peripheral and/or central sensitization models

We identified functional imaging studies using nine different experimental models in humans. This represents a subset of experimental models; in a recent review, there were more than 15 different models described (Quesada et al., 2021). The nine models include the most commonly used models (capsaicin, UV-B and thermal stimulation), with one notable exception being the use of cutaneous high-frequency electrical stimulation. This typically involves stimulation via a small circular array of surface electrodes delivering high-frequency (100 Hz) 1 s duration trains repeated five times at 10 s interval. Although there is limited data on use of this model with pharmacological modulation, it does have desirable features for imaging studies, including fast and straightforward induction of secondary hyperalgesia and maintenance for several hours (Klein et al., 2004; Pfau et al., 2011; Quesada et al., 2021). These features make it a promising model to be utilized in future fMRI studies.

The most common model used in the studies identified by this review was topical capsaicin, which was used in 16 studies. Half used topical capsaicin with application of heat using a thermode, whereas the other half used topical capsaicin alone. Both methodologies work well, with the major advantage of the heat and capsaicin model being a longer duration of sensitivity, with stable areas of secondary hyperalgesia lasting up to 4 h with heat rekindling (Petersen & Rowbotham, 1999). This is advantageous in fMRI studies as it is important that the hyperalgesia induced by the model remains stable for the duration of the data acquisition, which can be 1–2 h depending on the study design. One challenge with using capsaicin models is that the response to capsaicin can be variable, with some developing very low hyperalgesia or allodynia response (Liu et al., 1998). It would be important to account for this in the experiment design to either conduct pre-screening to exclude non-responders or ensure a large enough sample size to achieve adequate power with the non-responders included in analysis, which is challenging as MRI data acquisition is expensive and time-consuming.

The other experimental models were used in only 1–3 studies per model. It is therefore challenging to make conclusions about the advantages and disadvantages of their use in imaging experiments. They have been shown to be feasible for use in imaging studies with spatial and temporal properties conducive to allow fMRI data acquisition. Similar to capsaicin as discussed above, the use of opioid withdrawal was shown to induce hyperalgesia in only a sub-set of participants, which is consistent with previous evidence (Jensen et al., 2009; Wanigasekera et al., 2011), and would need to be accounted for in the experiment design. One consideration for comparison across studies using different models is the scale of the hyperalgesia response, as some of these models trigger widespread hyperalgesia (such as the OIH and endotoxin injection), while others create very localized symptoms. This variation is likely to produce different responses and should be considered to ensure an optimum model is used in each study design depending on the research question being addressed.

Across all 30 studies, 14 included testing in the primary hyperalgesia zone (reflecting both peripheral and central sensitization) and 16 included testing in the secondary hyperalgesia zone (reflecting central sensitization). This includes three studies that tested in both zones. There were three studies that didn't test in either zone (one as the model is injury-free so doesn't have specific zones, and the others focussed on quantifying the size of the hyperalgesia zone itself). In studies included in the CBMA there was no significant difference in the number of peak co-ordinates reported for primary or secondary zones.

4.2 | Functional imaging as an objective measure of pain perception and analgesic efficacy

Despite variation in the sensitization models used, experiment designs, stimulus modalities and stimulus application sites, the fMRI results across studies describe consistent patterns of neural activation in the sensitized state. This is validated by the CBMA conducted in this review (see Figures 3 and 4), demonstrating that across multiple experimental conditions, fMRI has provided consistent information about underlying neural activations in the hyperalgesia state with six activation clusters identified. Although these regions are consistently activated in response to experimental pain models there remains uncertainty about whether it can be considered a neural 'signature' for enhanced pain perception, with a need for experiment control arms to be carefully designed to match unpleasantness, salience and relevance of the pain stimuli (Mouraux & Iannetti, 2018). However, this does not reduce the potential utility of fMRI for evaluation of pain perception in experimental settings. It provides a wealth of information about the whole-brain response to pain stimuli and enables objective measurement of neural target engagement for analgesics.

The experimental models in conjunction with fMRI are useful for assessing analgesic efficacy in placebo-controlled studies, as demonstrated by the studies that include assessment of gabapentin, parecoxib and ASA,

lidocaine, delta-9-THC, and ibuprofen. These studies showed modulation of brain activity by the analgesic interventions in the insular cortex, prefrontal cortex, ACC, thalamus, secondary somatosensory cortex, PAG and NCF/brainstem. The cannabinoid (THC) also modulated the activity in the amygdala, which was not seen with the other analgesics included and may reflect the drug mechanism for that analgesic (Lee et al., 2013). Five out of the six activation clusters associated with hyperalgesia that were identified in the CBMA were modulated by analgesics, only the inferior parietal lobe was not. Additional modulations occurred in the prefrontal and somatosensory cortices, amygdala and brainstem, and by comparing activations due to hyperalgesia and modulation by analgesics, it is observed that there is substantial but incomplete overlap. There are limitations relating to the comparability of these studies with clinical applications; for example, all studies provide only a single dose of analgesic. In addition, experimental models are not fully representative of the ongoing pain characteristic of chronic pain conditions, although they do provide a useful surrogate (Quesada et al., 2021).

Although fMRI can provide information about analgesic efficacy, this alone does not necessarily meet the need to improve the translation of novel therapeutics from animal models to clinical trials. A challenge remains that due to differences in experiment designs and data analysis techniques used, it is not easy to compare across studies and identify consistent brain activity that indicates analgesic efficacy. More recently, multivariate pattern analysis techniques have enabled progress in developing pain signatures using neuroimaging data from multiple studies (Wager et al., 2013), and analgesic signatures characterizing drug effect on pain perception, either following sensitization with experimental models or in patients (Duff et al., 2015). The latter has the potential to detect pharmacodynamic effects of novel analgesics if fMRI data with the new analgesic is demonstrated to match the analgesia signature (Tracey et al., 2019).

4.3 | Strengths and limitations of this review and future directions

One potential limitation with this systematic literature review is the diversity of methods used in the studies included, which makes it challenging to draw comparisons. The studies focus on different specific research questions and therefore use different experimental designs, with a large range of stimuli applied (including mechanical, tactile and thermal modalities). However, these designs may be sorted according to testing in the treated zone (primary hyperalgesia invoking both peripheral and central sensitization) or in the surrounding untreated zone (secondary hyperalgesia, invoking central sensitization in the spinal cord and potentially more rostral central nervous system sites). Further, the imaging protocols are not well aligned, with differences in hardware and sequences used, and also will have developed significantly over the 20-year period we have included studies from 1999 to 2019 (Bandettini, 2012), so this does impact the comparability of the studies and consistency of the results. Finally, the results are also dependent on the analysis techniques applied to the data, which can lead to variability (Carp, 2012). However, these factors could be considered a strength, as the fact that activation is reported in a largely consistent set of brain regions despite this diversity of experimental designs, protocols and analysis techniques is reassuring, since it is important that findings are generalizable across different stimuli and experimental methods (Nichols et al., 2017).

The limitations described above emphasize the need for more standardization of experiment designs, imaging protocols and analysis pipelines to produce consistent results in the future. To make progress in the assessment of novel analgesics, there is also a need to validate protocols using a wider range of drugs with different mechanisms of action. This further validation will aid the continued development of robust biomarkers for clinical efficacy, against which novel analgesics can be assessed.

Meta-analysis techniques provide an opportunity to evaluate the consistency of results across a group of studies and, therefore can address the challenges outlined above (Wager et al., 2009). However, there were also limitations associated with the CBMA section of this review. While one of the key advantages of the CBMA approach is that the analysis only requires *x*, *y* and *z* co-ordinates, in this particular sample of 30 neuroimaging studies identified in the literature review, only 12 studies reported activation co-ordinates that met the criteria for this CBMA. Some further studies did report *x*, *y* and *z* co-ordinates for other contrasts (such as reporting co-ordinates for the control condition and hyperalgesic condition separately). In future, reporting of *x*, *y* and *z* co-ordinates for all contrasts of interest should be a standard requirement for neuroimaging studies of hyperalgesia or drug actions. With regard to the CBMA itself, the laterality of stimulation was not accounted for as this was not a focus of the current study, but this may have shown further information allowing comparison between contralateral and ipsilateral responses.

The scope of this review included only fMRI studies to specifically understand the response to hyperalgesia seen in fMRI data, as this work aimed to inform the IMI-PainCare BioPain RCT4 study, which included primarily fMRI endpoints. However, research into the neural response to hyperalgesia has also been conducted using

other functional imaging techniques such as positron emission tomography (PET) and arterial spin labelling (ASL). For example, PET studies using capsaicin to induce heat and mechanical allodynia have shown changes in activity in brain regions consistent with those shown in the fMRI studies reviewed, such as the thalamus, insula, ACC, and PAG (Iadarola et al., 1998; Lorenz et al., 2003). Further, one of these studies showed a positive correlation between imaging data activity and pain intensity and unpleasantness ratings, which is again aligned tporesults seen in the fMRI papers reviewed (Lorenz et al., 2003). ASL has also been applied to elucidating the neural basis for hyperalgesia, particularly alongside experimental models for tonic, ongoing pain (Loggia et al., 2019). One such study showed that pain ratings for continuously varying capsaicin-induced heat pain were strongly correlated to cerebral blood flow in the dorsolateral insular (Segerdahl et al., 2015), which again is aligned to the fMRI studies reviewed which reported changes in the insular cortex during hyperalgesia. In addition, similar research has also been conducted in patient populations. Since experimental models of hyperalgesia are meant to mimic pathological conditions, it would be valuable in future work to explore studies on neuropathic hyperalgesia in patient populations. These studies might give further insight into the validity of experimental models relative to the pathological states they are meant to reproduce.

Finally, it should be noted that fMRI cannot replace patient-reported outcomes, because pain, by definition is subjective (Davis et al., 2017; Raja et al., 2020). Further, with any imaging experiment assessing the effects of an analgesic intervention, it can be difficult to determine whether changes in brain activity 'explain' the pain relief reported, or whether the changes are the consequence of such relief. However, without objective measures of brain activity, it is not possible to trace the nociceptive signal processing involved in generating the first-person percept of pain.

5 | CONCLUSION

In summary, the use of human models of peripheral and/or central sensitization in conjunction with functional neuroimaging have provided valuable insight into changes in brain activity during hyperalgesia, which is a common symptom for chronic pain patients. The techniques have also been instrumental in expanding our understanding of the neural changes associated with analgesics during hyperalgesic states. Experimental evidence amassed over the past 20 years of neuroimaging research and the ongoing development of new and improved imaging techniques positions functional imaging as a viable option to meet the need for objective biomarkers of analgesic efficacy in humans. As with any experimental model, the hyperalgesia models are not able to fully replicate the clinical features of chronic pain conditions, but they do provide a useful surrogate for key symptoms that have been shown to be modulated by drugs that have a known action in relevant pathways.

AUTHOR CONTRIBUTIONS

Database searches were conducted by SC. Articles were assessed against inclusion/exclusion criteria and risk of bias criteria by SC and RR. All authors discussed the results and commented on the manuscript.

FUNDING INFORMATION

This project has received funding from the Innovative Medicines Initiative 2 Joint undertaking under grant agreement No 777500. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program and EFPIA (www.imi.europa.eu; www.imi-paincare.eu). The statements and opinions presented here reflect the author's view and neither IMI nor the European Union, EFPIA, or any Associated Partners are responsible for any use that may be made of the information contained therein. This research was also partly supported by the NIHR Oxford Health Biomedical Research Centre (NIHR203316) and by the Wellcome Trust (203139/Z/16/Z and 203139/A/16/Z). The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest regarding this work.

REGISTRATION

This review article was not registered. The review was planned as part of the IMI-PainCare Consortium dissemination plan, and the protocol was prepared in advance of the work.

ORCID

Sophie Clarke https://orcid.org/0000-0001-6464-5578

REFERENCES

Asghar, M. S., Pereira, M. P., Werner, M. U., Mårtensson, J., Larsson, H. B., & Dahl, J. B. (2015). Secondary hyperalgesia phenotypes exhibit differences in brain activation during noxious stimulation. *PLoS One*, *10*, e0114840. https://doi.org/10.1371/journal.pone.0114840

Bandettini, P. A. (2012). Twenty years of functional MRI: The science and the stories. *NeuroImage*, *62*, 575–588. https://doi.org/10.1016/j.neuroimage.2012.04.026

Baron, R., Baron, Y., Disbrow, E., & Roberts, T. P. L. (1999). Brain processing of capsaicin-induced secondary hyperalgesia – A functional MRI study. *Neurology*, *53*, 548–557. https://doi.org/10.1212/wnl.53.3.548

Berna, C., Leknes, S., Holmes, E. A., Edwards, R. R., Goodwin, G. M., & Tracey, I. (2010). Induction of depressed mood disrupts emotion regulation neurocircuitry and enhances pain unpleasantness. *Biological Psychiatry*, *67*, 1083–1090. https://doi.org/10.1016/j.biopsych.2010.01.014

Bingel, U., Wanigasekera, V., Wiech, K., Ni Mhuircheartaigh, R., Lee, M. C., Ploner, M., & Tracey, I. (2011). The effect of treatment expectation on drug efficacy: Imaging the analgesic benefit of the opioid remifentanil. *Science Translational Medicine*, *3*, 70ra14. https://doi.org/10.1126/scitranslmed.3001244

Borsook, D., Becerra, L., & Hargreaves, R. (2011a). Biomarkers for chronic pain and analgesia. Part 1: The need, reality, challenges, and solutions. *Discovery Medicine*, *11*, 197–207.

Borsook, D., Becerra, L., & Hargreaves, R. (2011b). Biomarkers for chronic pain and analgesia. Part 2: How, where, and what to look for using functional imaging. *Discovery Medicine*, *11*, 209–219.

Carp, J. (2012). On the plurality of (methodological) worlds: Estimating the analytic flexibility of fMRI experiments. *Frontiers in Neuroscience*, *6*, 149. https://doi.org/10.3389/fnins.2012.00149

Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D., & Julius, D. (1997). The capsaicin receptor: A heat-activated ion channel in the pain pathway. *Nature*, *389*, 816–824. https://doi.org/10.1038/39807

Cho, C., Deol, H. K., & Martin, L. J. (2021). Bridging the translational divide in pain research: Biological, psychological and social considerations. *Frontiers in Pharmacology*, *12*, 603186. https://doi.org/10.3389/fphar.2021.603186

Craig, A. D. (2011). Significance of the insula for the evolution of human awareness of feelings from the body. *Annals of the New York Academy of Sciences*, *1225*, 72–82. https://doi.org/10.1111/j.1749-6632.2011.05990.x

David, S. P., Ware, J. J., Chu, I. M., Loftus, P. D., Fusar-Poli, P., Radua, J., Munafò, M. R., & Ioannidis, J. P. A. (2013). Potential reporting bias in fMRI studies of the brain. *PLoS One*, *8*, e70104. https://doi.org/10.1371/journal.pone.0070104

Davis, K. D., Aghaeepour, N., Ahn, A. H., Angst, M. S., Borsook, D., Brenton, A., Burczynski, M. E., Crean, C., Edwards, R., Gaudilliere, B., Hergenroeder, G. W., Iadarola, M. J., Iyengar, S., Jiang, Y., Kong, J.-T., Mackey, S., Saab, C. Y., Sang, C. N., Scholz, J., … Pelleymounter, M. A. (2020). Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: Challenges and opportunities. *Nature Reviews Neurology*, *16*, 381–400. https://doi.org/10.1038/s41582-020-0362-2

Davis, K. D., Flor, H., Greely, H. T., Iannetti, G. D., Mackey, S., Ploner, M., Pustilnik, A., Tracey, I., Treede, R.-D., & Wager, T. D. (2017). Brain imaging tests for chronic pain: Medical, legal and ethical issues and recommendations. *Nature Reviews Neurology*, *13*, 624–638. https://doi.org/10.1038/nrneurol.2017.122

Denayer, T., Stöhr, T., & Van Roy, M. (2014). Animal models in translational medicine: Validation and prediction. *New Horizons in Translational Medicine*, *2*, 5–11. https://doi.org/10.1016/j.nhtm.2014.08.001

Duff, E. P., Vennart, W., Wise, R. G., Howard, M. A., Harris, R. E., Lee, M., Wartolowska, K., Wanigasekera, V., Wilson, F. J., Whitlock, M., Tracey, I., Woolrich, M. W., & Smith, S. M. (2015). Learning to identify CNS drug action and efficacy using multistudy fMRI data. *Science Translational Medicine*, *7*, 274ra216. https://doi.org/10.1126/scitranslmed.3008438

Dum, R. P., Levinthal, D. J., & Strick, P. L. (2009). The spinothalamic system targets motor and sensory areas in the cerebral cortex of monkeys. *The Journal of Neuroscience*, *29*, 14223–14235. https://doi.org/10.1523/jneurosci.3398-09.2009

Eickhoff, S. B., Bzdok, D., Laird, A. R., Kurth, F., & Fox, P. T. (2012). Activation likelihood estimation meta-analysis revisited. *NeuroImage*, *59*, 2349–2361. https://doi.org/10.1016/j.neuroimage.2011.09.017

Eickhoff, S. B., Laird, A. R., Grefkes, C., Wang, L. E., Zilles, K., & Fox, P. T. (2009). Coordinate-based activation likelihood estimation meta-analysis of neuroimaging data: A random-effects approach based on empirical estimates of spatial uncertainty. *Human Brain Mapping*, *30*, 2907–2926. https://doi.org/10.1002/hbm.20718

Eisenblätter, A., Lewis, R., Dörfler, A., Forster, C., & Zimmermann, K. (2017). Brain mechanisms of abnormal temperature perception in cold allodynia induced by ciguatoxin. *Annals of Neurology*, *81*, 104–116. https://doi.org/10.1002/ana.24841

Farrar, J. T., Troxel, A. B., Haynes, K., Gilron, I., Kerns, R. D., Katz, N. P., Rappaport, B. A., Rowbotham, M. C., Tierney, A. M., Turk, D. C., & Dworkin, R. H. (2014). Effect of variability in the 7-day baseline pain diary on the assay sensitivity of neuropathic pain randomized clinical trials: An ACTTION study. *Pain*, *155*, 1622–1631. https://doi.org/10.1016/j.pain.2014.05.009

Forstenpointner, J., Binder, A., Maag, R., Granert, O., Hüllemann, P., Peller, M., Wasner, G., Wolff, S., Jansen, O., Siebner, H. R., & Baron, R. (2019). Neuroimaging of cold allodynia reveals a central disinhibition mechanism of pain. *Journal of Pain Research*, *12*, 3055–3066. https://doi.org/10.2147/jpr.S216508

Hans, G. H., Vandervliet, E., Deseure, K., & Parizel, P. M. (2013). Cerebral activation during von Frey filament stimulation in subjects with endothelin-1-induced mechanical hyperalgesia: A functional MRI study. *BioMed Research International*, *2013*, 610727. https://doi.org/10.1155/2013/610727

Hansen, M. S., Asghar, M. S., Wetterslev, J., Pipper, C. B., Mårtensson, J., Becerra, L., Christensen, A., Nybing, J. D., Havsteen, I., Boesen, M., & Dahl, J. B. (2018). The association between areas of secondary hyperalgesia and volumes of the caudate nuclei and other pain relevant brain structures-a 3-tesla MRI study of healthy men. *PLoS One*, *13*, e0201642. https://doi.org/10.1371/journal.pone.0201642

Hansen, M. S., Becerra, L., Dahl, J. B., Borsook, D., Mårtensson, J., Christensen, A., Nybing, J. D., Havsteen, I., Boesen, M., & Asghar, M. S. (2019). Brain resting-state connectivity in the development of secondary hyperalgesia in healthy men. *Brain Structure & Function*, *224*, 1119–1139. https://doi.org/10.1007/s00429-018-01819-z

Hardy, J. D., Wolff, H. G., & Goodell, H. (1950). Experimental evidence on the nature of cutaneous hyperalgesia. *The Journal of Clinical Investigation*, *29*, 115–140. https://doi.org/10.1172/jci102227

Harris, R. E., Williams, D. A., McLean, S. A., Sen, A., Hufford, M., Gendreau, R. M., Gracely, R. H., & Clauw, D. J. (2005). Characterization and consequences of pain variability in individuals with fibromyalgia. *Arthritis and Rheumatism*, *52*, 3670–3674. https://doi.org/10.1002/art.21407

Higgins, J. P., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., Savovic, J., Schulz, K. F., Weeks, L., & Sterne, J. A. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ*, *343*, d5928. https://doi.org/10.1136/bmj.d5928

Iadarola, M. J., Berman, K. F., Zeffiro, T. A., Byas-Smith, M. G., Gracely, R. H., Max, M. B., & Bennett, G. J. (1998). Neural activation during acute capsaicin-evoked pain and allodynia assessed with PET. *Brain*, *121*, 931–947. https://doi.org/10.1093/brain/121.5.931

Iannetti, G. D., Zambreanu, L., Wise, R. G., Buchanan, T. J., Huggins, J. P., Smart, T. S., Vennart, W., & Tracey, I. (2005). Pharmacological modulation of pain-related brain activity during normal and central sensitization states in humans. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 18195–18200. https://doi.org/10.1073/pnas.0506624102

IASP. (2011). *IASP terminology*. https://www.iasp-pain.org/resources/terminology/

Jensen, K. B., Lonsdorf, T. B., Schalling, M., Kosek, E., & Ingvar, M. (2009). Increased sensitivity to thermal pain following a single opiate dose is influenced by the COMT val(158)met polymorphism. *PLoS One*, *4*, e6016. https://doi.org/10.1371/journal.pone.0006016

Jensen, T. S., & Finnerup, N. B. (2014). Allodynia and hyperalgesia in neuropathic pain: Clinical manifestations and mechanisms. *Lancet Neurology*, *13*, 924–935. https://doi.org/10.1016/s1474-4422(14)70102-4

Klein, T., Magerl, W., Hopf, H. C., Sandkühler, J., & Treede, R. D. (2004). Perceptual correlates of nociceptive long-term potentiation and long-term depression in humans. *The Journal of Neuroscience*, *24*, 964–971. https://doi.org/10.1523/jneurosci.1222-03.2004

Koppert, W., Dern, S. K., Sittl, R., Albrecht, S., Schüttler, J., & Schmelz, M. (2001). A new model of electrically evoked pain and hyperalgesia in human skin: The effects of intravenous alfentanil, S(+)-ketamine, and lidocaine. *Anesthesiology*, *95*, 395–402. https://doi.org/10.1097/00000542-200108000-00022

Laird, A. R., Fox, P. M., Price, C. J., Glahn, D. C., Uecker, A. M., Lancaster, J. L., Turkeltaub, P. E., Kochunov, P., & Fox, P. T. (2005). ALE meta-analysis: Controlling the false discovery rate and performing statistical contrasts. *Human Brain Mapping*, *25*, 155–164. https://doi.org/10.1002/hbm.20136

Latremoliere, A., & Woolf, C. J. (2009). Central sensitization: A generator of pain hypersensitivity by central neural plasticity. *The Journal of Pain*, *10*, 895–926. https://doi.org/10.1016/j.jpain.2009.06.012

Lee, M. C., Ploner, M., Wiech, K., Bingel, U., Wanigasekera, V., Brooks, J., Menon, D. K., & Tracey, I. (2013). Amygdala activity contributes to the dissociative effect of cannabis on pain perception. *Pain*, *154*, 124–134. https://doi.org/10.1016/j.pain.2012.09.017

Lee, M. C., & Tracey, I. (2013). Imaging pain: A potent means for investigating pain mechanisms in patients. *British Journal of Anaesthesia*, *111*, 64–72. https://doi.org/10.1093/bja/aet174

Lee, M. C., Zambreanu, L., Menon, D. K., & Tracey, I. (2008). Identifying brain activity specifically related to the maintenance and perceptual consequence of central sensitization in humans. *Journal of Neuroscience*, *28*, 11642–11649. https://doi.org/10.1523/jneurosci.2638-08.2008

Liljencrantz, J., Björnsdotter, M., Morrison, I., Bergstrand, S., Ceko, M., Seminowicz, D. A., Cole, J., Bushnell, C. M., & Olausson, H. (2013). Altered C-tactile processing in human dynamic tactile allodynia. *Pain*, *154*, 227–234. https://doi.org/10.1016/j.pain.2012.10.024

Liu, M., Max, M. B., Robinovitz, E., Gracely, R. H., & Bennett, G. J. (1998). The human capsaicin model of allodynia and hyperalgesia: Sources of variability and methods for reduction. *Journal of Pain and Symptom Management*, *16*, 10–20. https://doi.org/10.1016/s0885-3924(98)00026-8

Loggia, M. L., Segerdahl, A. R., Howard, M. A., & Tracey, I. (2019). Imaging clinically relevant pain states using arterial spin labeling. *Pain Reports*, *4*, 4. https://doi.org/10.1097/pr9.0000000000000750

Löken, L. S., Duff, E. P., & Tracey, I. (2017). Low-threshold mechanoreceptors play a frequency-dependent dual role in subjective ratings of mechanical allodynia. *Journal of Neurophysiology*, *118*, 3360–3369. https://doi.org/10.1152/jn.00977.2016

Lorenz, J., Minoshima, S., & Casey, K. L. (2003). Keeping pain out of mind: The role of the dorsolateral prefrontal cortex in pain modulation. *Brain*, *126*, 1079–1091. https://doi.org/10.1093/brain/awg102

Maihöfner, C., & Handwerker, H. O. (2005). Differential coding of hyperalgesia in the human brain: A functional MRI study. *NeuroImage*, *28*, 996–1006. https://doi.org/10.1016/j.neuroimage.2005.06.049

Maihofner, C., Ringler, R., Hermdobler, F., & Koppert, W. (2007). Brain imaging of analgesic and antihyperalgesic effects of cyclooxygenase inhibition in an experimental human pain model: A functional MRI study. *European Journal of Neuroscience*, *26*, 1344–1356. https://doi.org/10.1111/j.1460-9568.2007.05733.x

Maihöfner, C., Schmelz, M., Forster, C., Neundörfer, B., & Handwerker, H. O. (2004). Neural activation during experimental allodynia: A functional magnetic resonance imaging study. *The European Journal of Neuroscience*, *19*, 3211–3218. https://doi.org/10.1111/j.1460-9568.2004.03437.x

Mainero, C., Zhang, W. T., Kumar, A., Rosen, B. R., & Sorensen, A. G. (2007). Mapping the spinal and supraspinal pathways of dynamic mechanical allodynia in the human trigeminal system using cardiac-gated fMRI. *NeuroImage*, *35*, 1201–1210. https://doi.org/10.1016/j.neuroimage.2007.01.024

Meeker, T. J., Keaser, M. L., Khan, S. A., Gullapalli, R. P., Seminowicz, D. A., & Greenspan, J. D. (2019). Non-invasive motor cortex neuromodulation reduces secondary hyperalgesia and enhances activation of the descending pain modulatory network. *Frontiers in Neuroscience*, *13*, 467. https://doi.org/10.3389/fnins.2019.00467

Mohr, C., Leyendecker, S., & Helmchen, C. (2008). Dissociable neural activity to self- vs. externally administered thermal hyperalgesia: A parametric fMRI study. *The European Journal of Neuroscience*, *27*, 739–749. https://doi.org/10.1111/j.1460-9568.2008.06036.x

Mohr, C., Leyendecker, S., Mangels, I., Machner, B., Sander, T., & Helmchen, C. (2008). Central representation of cold-evoked pain relief in capsaicin induced pain: An event-related fMRI study. *Pain*, *139*, 416–430. https://doi.org/10.1016/j.pain.2008.05.020

Morton, D. L., Sandhu, J. S., & Jones, A. K. (2016). Brain imaging of pain: State of the art. *Journal of Pain Research*, *9*, 613–624. https://doi.org/10.2147/jpr.S60433

Moulton, E. A., Pendse, G., Morris, S., Strassman, A., Aiello-Lammens, M., Becerra, L., & Borsook, D. (2007). Capsaicin-induced

thermal hyperalgesia and sensitization in the human trigeminal nociceptive pathway: An fMRI study. *NeuroImage*, *35*, 1586–1600. https://doi.org/10.1016/j.neuroimage.2007.02.001

Mouraux, A., & Iannetti, G. D. (2018). The search for pain biomarkers in the human brain. *Brain*, *141*, 3290–3307. https://doi.org/10.1093/brain/awy281

Müller, V. I., Cieslik, E. C., Laird, A. R., Fox, P. T., Radua, J., Mataix-Cols, D., Tench, C. R., Yarkoni, T., Nichols, T. E., Turkeltaub, P. E., Wager, T. D., & Eickhoff, S. B. (2018). Ten simple rules for neuroimaging meta-analysis. *Neuroscience and Biobehavioral Reviews*, *84*, 151–161. https://doi.org/10.1016/j.neubiorev.2017.11.012

Nelson, E. K. (1919). The constitution of capsaicin, the pungent principle of *Capsicum*. *Journal of the American Chemical Society*, *41*, 1115–1121. https://doi.org/10.1021/ja02228a011

Nichols, T. E., Das, S., Eickhoff, S. B., Evans, A. C., Glatard, T., Hanke, M., Kriegeskorte, N., Milham, M. P., Poldrack, R. A., Poline, J.-B., Proal, E., Thirion, B., Van Essen, D. C., White, T., & Yeo, B. T. T. (2017). Best practices in data analysis and sharing in neuroimaging using MRI. *Nature Neuroscience*, *20*, 299–303. https://doi.org/10.1038/nn.4500

Olesen, A. E., Andresen, T., Staahl, C., & Drewes, A. M. (2012). Human experimental pain models for assessing the therapeutic efficacy of analgesic drugs. *Pharmacological Reviews*, *64*, 722–779. https://doi.org/10.1124/pr.111.005447

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., … Moher, D. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ*, *372*, n71. https://doi.org/10.1136/bmj.n71

Petersen, K. L., & Rowbotham, M. C. (1999). A new human experimental pain model: The heat/capsaicin sensitization model. *Neuroreport*, *10*, 1511–1516. https://doi.org/10.1097/00001756-199905140-00022

Pfau, D. B., Klein, T., Putzer, D., Pogatzki-Zahn, E. M., Treede, R.-D., & Magerl, W. (2011). Analysis of hyperalgesia time courses in humans after painful electrical high-frequency stimulation identifies a possible transition from early to late LTP-like pain plasticity. *Pain*, *152*, 1532–1539. https://doi.org/10.1016/j.pain.2011.02.037

Ploghaus, A., Narain, C., Beckmann, C. F., Clare, S., Bantick, S., Wise, R., Matthews, P. M., Rawlins, J. N., & Tracey, I. (2001). Exacerbation of pain by anxiety is associated with activity in a hippocampal network. *The Journal of Neuroscience*, *21*, 9896–9903. https://doi.org/10.1523/jneurosci.21-24-09896.2001

Ploghaus, A., Tracey, I., Gati, J. S., Clare, S., Menon, R. S., Matthews, P. M., & Rawlins, J. N. (1999). Dissociating pain from its anticipation in the human brain. *Science*, *284*, 1979–1981. https://doi.org/10.1126/science.284.5422.1979

Quesada, C., Kostenko, A., Ho, I., Leone, C., Nochi, Z., Stouffs, A., Wittayer, M., Caspani, O., Brix Finnerup, N., Mouraux, A., Pickering, G., Tracey, I., Truini, A., Treede, R. D., & Garcia-Larrea, L. (2021). Human surrogate models of central sensitization: A critical review and practical guide. *European Journal of Pain*, *25*, 1389–1428. https://doi.org/10.1002/ejp.1768

Raja, S. N., Carr, D. B., Cohen, M., Finnerup, N. B., Flor, H., Gibson, S., Keefe, F. J., Mogil, J. S., Ringkamp, M., Sluka, K. A., Song, X. J., Stevens, B., Sullivan, M. D., Tutelman, P. R., Ushida, T., & Vader, K. (2020). The revised International Association for the Study of Pain definition of pain: Concepts, challenges, and compromises. *Pain*, *161*, 1976–1982. https://doi.org/10.1097/j.pain.0000000000001939

Rempe, T., Wolff, S., Riedel, C., Baron, R., Stroman, P. W., Jansen, O., & Gierthmühlen, J. (2014). Spinal fMRI reveals decreased descending inhibition during secondary mechanical hyperalgesia. *PLoS One*, *9*, e112325. https://doi.org/10.1371/journal.pone.0112325

Rempe, T., Wolff, S., Riedel, C., Baron, R., Stroman, P. W., Jansen, O., & Gierthmühlen, J. (2015). Spinal and supraspinal processing of thermal stimuli: An fMRI study. *Journal of Magnetic Resonance Imaging*, *41*, 1046–1055. https://doi.org/10.1002/jmri.24627

Samartsidis, P., Montagna, S., Johnson, T. D., & Nichols, T. E. (2017). The coordinate-based meta-analysis of neuroimaging data. *Statistical Science*, *32*(580–599), 520.

Segerdahl, A. R., Mezue, M., Okell, T. W., Farrar, J. T., & Tracey, I. (2015). The dorsal posterior insula subserves a fundamental role in human pain. *Nature Neuroscience*, *18*, 499–500. https://doi.org/10.1038/nn.3969

Seifert, F., Bschorer, K., De Col, R., Filitz, J., Peltz, E., Koppert, W., & Maihöfner, C. (2009). Medial prefrontal cortex activity is predictive for hyperalgesia and pharmacological antihyperalgesia. *The Journal of Neuroscience*, *29*, 6167–6175. https://doi.org/10.1523/jneurosci.4654-08.2009

Seifert, F., Jungfer, I., Schmelz, M., & Maihöfner, C. (2008). Representation of UV-B-induced thermal and mechanical hyperalgesia in the human brain: A functional MRI study. *Human Brain Mapping*, *29*, 1327–1342. https://doi.org/10.1002/hbm.20470

Seifert, F., & Maihofher, C. (2007). Representation of cold allodynia in the human brain – A functional MRI study. *NeuroImage*, *35*, 1168–1180. https://doi.org/10.1016/j.neuroimage.2007.01.021

Shenoy, R., Roberts, K., Papadaki, A., McRobbie, D., Timmers, M., Meert, T., & Anand, P. (2011). Functional MRI brain imaging studies using the contact heat evoked potential stimulator (CHEPS) in a human volunteer topical capsaicin pain model. *Journal of Pain Research*, *4*, 365–371. https://doi.org/10.2147/jpr.S24810

Smith, S. M., Amtmann, D., Askew, R. L., Gewandter, J. S., Hunsinger, M., Jensen, M. P., McDermott, M. P., Patel, K. V., Williams, M., Bacci, E. D., Burke, L. B., Chambers, C. T., Cooper, S. A., Cowan, P., Desjardins, P., Etropolski, M., Farrar, J. T., Gilron, I., Huang, I. Z., … Dworkin, R. H. (2016). Pain intensity rating training: Results from an exploratory study of the ACTTION PROTECCT system. *Pain*, *157*, 1056–1064. https://doi.org/10.1097/j.pain.0000000000000502

Stammler, T., De Col, R., Seifert, F., & Maihöfner, C. (2008). Functional imaging of sensory decline and gain induced by differential noxious stimulation. *NeuroImage*, *42*, 1151–1163. https://doi.org/10.1016/j.neuroimage.2008.05.048

Tanasescu, R., Cottam, W. J., Condon, L., Tench, C. R., & Auer, D. P. (2016). Functional reorganisation in chronic pain and neural correlates of pain sensitisation: A coordinate based meta-analysis of 266 cutaneous pain fMRI studies. *Neuroscience & Biobehavioral Reviews*, *68*, 120–133. https://doi.org/10.1016/j.neubiorev.2016.04.001

Tracey, I., Woolf, C. J., & Andrews, N. A. (2019). Composite pain biomarker signatures for objective assessment and effective treatment. *Neuron*, *101*, 783–800. https://doi.org/10.1016/j.neuron.2019.02.019

Treede, R.-D., Meyer, R. A., Raja, S. N., & Campbell, J. N. (1992). Peripheral and central mechanisms of cutaneous hyperalgesia.

Progress in Neurobiology, *38*, 397–421. https://doi.org/10.1016/0301-0082(92)90027-C

Turkeltaub, P. E., Eickhoff, S. B., Laird, A. R., Fox, M., Wiener, M., & Fox, P. (2012). Minimizing within-experiment and within-group effects in activation likelihood estimation meta-analyses. *Human Brain Mapping*, *33*, 1–13. https://doi.org/10.1002/hbm.21186

Wager, T. D., Atlas, L. Y., Lindquist, M. A., Roy, M., Woo, C.-W., & Kross, E. (2013). An fMRI-based neurologic signature of physical pain. *New England Journal of Medicine*, *368*, 1388–1397. https://doi.org/10.1056/NEJMoa1204471

Wager, T. D., Lindquist, M. A., Nichols, T. E., Kober, H., & Van Snellenberg, J. X. (2009). Evaluating the consistency and specificity of neuroimaging data using meta-analysis. *NeuroImage*, *45*, S210–S221. https://doi.org/10.1016/j.neuroimage.2008.10.061

Wahren, L. K., Torebjörk, E., & Jörum, E. (1989). Central suppression of cold-induced C fibre pain by myelinated fibre input. *Pain*, *38*, 313–319. https://doi.org/10.1016/0304-3959(89)90218-2

Wanigasekera, V., Lee, M. C. H., Rogers, R., Hu, P., & Tracey, I. (2011). Neural correlates of an injury-free model of central sensitization induced by opioid withdrawal in humans. *Journal of Neuroscience*, *31*, 2835–2842. https://doi.org/10.1523/jneurosci.5412-10.2011

Wanigasekera, V., Mezue, M., Andersson, J., Kong, Y., & Tracey, I. (2016). Disambiguating pharmacodynamic efficacy from behavior with neuroimaging: Implications for analgesic drug development. *Anesthesiology*, *124*, 159–168. https://doi.org/10.1097/aln.0000000000000924

Wiech, K., Ploner, M., & Tracey, I. (2008). Neurocognitive aspects of pain perception. *Trends in Cognitive Sciences*, *12*, 306–313. https://doi.org/10.1016/j.tics.2008.05.005

Wiech, K., & Tracey, I. (2009). The influence of negative emotions on pain: Behavioral effects and neural mechanisms. *NeuroImage*, *47*, 987–994. https://doi.org/10.1016/j.neuroimage.2009.05.059

Woolf, C. J. (1983). Evidence for a central component of post-injury pain hypersensitivity. *Nature*, *306*, 686–688. https://doi.org/10.1038/306686a0

Woolf, C. J. (2011). Central sensitization: Implications for the diagnosis and treatment of pain. *Pain*, *152*, S2–s15. https://doi.org/10.1016/j.pain.2010.09.030

Zambreanu, L., Wise, R. G., Brooks, J. C. W., Iannetti, G. D., & Tracey, I. (2005). A role for the brainstem in central sensitisation in humans. Evidence from functional magnetic resonance imaging. *Pain*, *114*, 397–407. https://doi.org/10.1016/j.pain.2005.01.005

SUPPORTING INFORMATION

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

How to cite this article: Clarke, S., Rogers, R., Wanigasekera, V., Fardo, F., Pia, H., Nochi, Z., Macian, N., Leray, V., Finnerup, N. B., Pickering, G., Mouraux, A., Truini, A., Treede, R.-D., Garcia-Larrea, L., & Tracey, I. (2024). Systematic review and co-ordinate based meta-analysis to summarize the utilization of functional brain imaging in conjunction with human models of peripheral and central sensitization. *European Journal of Pain*, *00*, 1–26. https://doi.org/10.1002/ejp.2251