Supplementary Material

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at 10.1016/j.jid.2019.03.1153.

TO THE EDITOR

Perceptually, itch is clearly discernible from pain, yet both sensations exhibit a substantial anatomical overlap with common peripheral transmission and recruited brain regions. For example, recent functional magnetic resonance imaging (fMRI) studies have observed activations in the pain-processing network during cowhage- or histamine-induced itch in the thalamus (Leknes et al., 2007; Mochizuki et al., 2009), insular cortex (Herde et al., 2007; Leknes et al., 2007), cingulate cortex (Mochizuki et al., 2007), prefrontal cortex (Mochizuki et al., 2009), postcentral gyrus (Herde et al., 2007; Ishiui et al., 2009; Pappou et al., 2012), parietal operculum (Mochizuki et al., 2009; Pappou et al., 2012), parahippocampal gyrus (Pappou et al., 2012), and basal ganglia (Mochizuki et al., 2007). However, the differences in brain processing of these two types of sensation have yet to be satisfactorily determined.

Abbreviations: ALE, activation likelihood estimation; fMRI, functional magnetic resonance imaging

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CA Roberts et al.

Meta-Analysis of Experimentally-Induced Itch fMRI Studies

Where Is Itch Represented in the Brain, and How Does it Differ from Pain? An Activation Likelihood Estimation Meta-Analysis of Experimentally-Induced Itch

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TO THE EDITOR

Perceptually, itch is clearly discernible from pain, yet both sensations exhibit a substantial anatomical overlap with common peripheral transmission and recruited brain regions. For example, recent functional magnetic resonance imaging (fMRI) studies have observed activations in the pain-processing network during cowhage- or histamine-induced itch in the thalamus (Leknes et al., 2007; Mochizuki et al., 2009), insular cortex (Herde et al., 2007; Leknes et al., 2007), cingulate cortex (Mochizuki et al., 2007), prefrontal cortex (Mochizuki et al., 2009), postcentral gyrus (Herde et al., 2007; Ishiui et al., 2009; Pappou et al., 2012), parietal operculum (Mochizuki et al., 2009; Pappou et al., 2012), parahippocampal gyrus (Pappou et al., 2012), and basal ganglia (Mochizuki et al., 2007). However, the differences in brain processing of these two types of sensation have yet to be satisfactorily determined.
limited and, as noted above, confined to a small number of imaging studies which show somatosensory, limbic, and motor-related activity similar to that evoked by noxious stimuli (for a detailed overview, see Lee et al., 2016).

To contribute to our understanding of brain processing of itch compared with pain, we conducted activation likelihood estimation (ALE) meta-analysis of experimentally induced itch from the published fMRI literature and generated a comparison ALE map of experimental pain (using the previously reported coordinates of Tanasescu et al., 2016), to conduct meta-analytic conjunction/contrast analyses between the two sensations.

Analyses were performed using Brainmap GingerALE, version 2.3.6 (Research Imaging Institute, San Antonio, TX). We adhered to the ALE method devised by Eickhoff et al. (2009, 2012), with the correction devised by Turkeltaub et al. (2012). The $P$-values in our analyses were generated by 10,000 permutations. We used a cluster-level family-wise error correction at $P < 0.05$ to correct for multiple comparisons, following an initial cluster forming threshold of uncorrected $P < 0.001$ (see Supplementary Materials and Methods online for more information).

### Experimental itch
ALE meta-analysis included all studies reporting whole brain fMRI analysis of experimentally induced itch (histamine, cowhage, or electrical stimulation). Data were pooled from a total of 11 experiments (from 10 papers, with a total of 117

| Cluster | Brain Region | Peak Voxel Coordinates | Cluster Size (mm$^3$) | ALE Value ($\times 10^{-2}$) | No. of Contributing Experiments |
|---------|--------------|------------------------|----------------------|-------------------------------|---------------------------------|
| 1 Thalamus L | -8 -16 10 | 3,936 | 1.68 | 7 63.64 |
| 0 -18 4 | 1.60 |
| -6 -4 6 | 1.57 |
| 10 -4 2 | 1.36 |
| -8 -14 -2 | 1.31 |
| -10 -18 8 | 1.20 |
| 2 Frontal operculum / anterior insula / central operculum L | -46 12 2 | 2,016 | 1.58 | 5 45.45 |
| -36 14 -2 | 1.31 |
| -46 2 8 | 1.25 |
| -54 2 2 | 1.16 |
| 3 Frontal operculum / anterior insula R | 40 16 4 | 1,424 | 1.72 | 4 36.36 |
| 38 10 -2 | 1.33 |

**Abbreviations:** ALE, activation likelihood estimation; L, left; MNI, Montreal Neurological Institute; R, right

Coordinates are reported in MNI space. Analysis used whole brain data from Herde et al. (2007); Ishiiji et al. (2009); Kleyn et al. (2012); Leknes et al. (2007); Mochizuki et al. (2007); Mochizuki et al. (2009); Mochizuki et al. (2014); Papoiu et al. (2012); Valet et al. (2008), and Walter et al. (2005). Studies that reported coordinates in the Talairach space were converted into MNI coordinates using GingerALE before analysis.

![Figure 1](image_url)

**Figure 1.** Localization of significant ALE clusters from the experimentally-induced itch ALE meta-analysis. GingerALE output overlaid onto a standard template (Colin27_T1_seg_MNI.nii) in Montreal Neurological Institute (MNI) space.

Thalamus: $x = -8, y = -16, z = 10$

Left frontal operculum / Anterior insular:
$x = -46, y = 12, z = 2$

Right frontal operculum / Anterior insular:
$x = 40, y = 16, z = 4$
participants and 313 reported foci (Supplementary Figure S1). Significant clusters were observed in the thalamus, left frontal operculum cortex/insular cortex, and right frontal operculum cortex/insular cortex (Table 1, Figure 1).

Experimental pain
Significant clusters resulting from experimental pain meta-analysis are fully described by Tanasescu et al. (2016); therefore, our ALE map of experimental pain was used only in the conjunction and contrast analysis described next.

Conjunction and contrast analyses
The conjunction analysis between itch and pain showed three significant clusters, located in the left thalamus and the left and right frontal operculum/insula.

We chose a minimum cluster size of 500 mm$^3$ for contrast analyses. For itch – pain, we identified five clusters: left and right thalamus, left anterior insula/frontal operculum, right central operculum, and right supramarginal gyrus. The reverse pain – itch contrast revealed four clusters: right parietal operculum/postcentral gyrus, right frontal pole (anterior frontal gyrus), left frontal pole (middle frontal gyrus), and right supramarginal gyrus.

Experimental itch was associated with activations in the thalamus and anterior parts of the left and right insula/frontal operculum. There was a high degree of overlap in brain activation for itch and pain in the conjunction analysis. However, areas of the thalamus, anterior insula/frontal operculum, central operculum, and supramarginal gyrus showed significant differences in activation convergence in an itch – pain contrast. This finding suggests that somatosensory processing that is specific to itch resides in these areas, but further work is necessary to resolve this possibility with precision.

Our meta-analysis confirms that the thalamus is the most consistently activated brain region in fMRI studies of experimental itch (Herde et al., 2007; Leknes et al., 2007; Mochizuki et al., 2009; Mochizuki et al., 2014; Papoiu et al., 2012; Valet et al., 2008), and corroborates positron-emission tomography imaging studies that highlight the thalamus as important in the subjective appraisal of itch (Mochizuki et al., 2003). As with itch, the thalamus is acknowledged as a critical area involved in the perception of pain, being consistently reported in meta-analyses of fMRI studies on experimental pain (Jensen et al., 2016; Tanasescu et al., 2016). Our conjunction analysis showed substantial overlap between itch and pain in the thalamus, with every itch brain region overlapping with pain regions. However, areas of the left and right thalamus showed significant differences in convergence between itch and pain, supporting the proposals that variation in itch and pain perception can be found in subregions of the thalamus (Drzezga et al., 2001; Mochizuki et al., 2003), and that differences in thalamus sensitivity underlie the difference between the sensations (Mochizuki et al., 2007). Clearly, convergence and divergence of thalamic activation in itch and pain are complex and require further investigation.

The remaining two significant clusters from the itch ALE comprised bilateral activity in anterior parts of the insula and the frontal operculum area of the insula, confirming that these areas are consistently activated across studies of experimental itch (Herde et al., 2007; Leknes et al., 2007; Mochizuki et al., 2009; Mochizuki et al., 2014; Papoiu et al., 2012). These areas are also commonly activated by pain (Yosipovitch and Mochizuki, 2015; Jensen et al., 2016; Tanasescu et al., 2016), and this was confirmed by our conjunction analysis.

The insula is understood to process stimulus intensity in both pain and itch. For example, Papoiu et al. (2012) reported correlations between itch intensity and insula activity. Additionally, the anterior insula is associated with affective responses to stimuli, which may relate to the high rates of depression in atopic dermatitis (Gupta and Gupta, 1998). It is the affective component of itch which has led to the suggestion that psychological interventions, such as mindfulness or cognitive behavioral therapy, could prove successful in chronic itch treatment (Schut et al., 2014). Psychological interventions that reduce stress may produce positive effects on itch, as well as altering itch-associated brain activation, such as connectivity between the insular cortex and the anterior cingulate cortex (Mochizuki et al., 2017).

The pain – itch subtraction showed residual activation in the parietal operculum and postcentral gyrus that seems to be specific to pain. Possibly, pain activates somatosensory cortices more reliably than itch, as is indicated by molecular imaging studies (Mochizuki et al., 2003; Darsow et al., 2000; Drzezga et al., 2001).

The main limitation of the present analyses is our focus on experimentally induced itch in healthy individuals. However, the strength of this approach is that confounding factors such as comorbidity are carefully controlled. It is probable that chronic and acute itch differ in central nervous system activity, and our findings therefore require validation with data from clinical populations. In addition, our analysis combined data from several different experimental itch methodologies. It may be that there are different types of itch, each with a distinct neural signature. The fundamental studies to explore this possibility have yet to be conducted, and the quantity of data on neural correlates of itch is currently insufficient to independently investigate specific types of itch with meta-analysis.

We report that the thalamus and the affective areas of the anterior insula/frontal operculum are consistently activated across fMRI studies that induce itch experimentally. We propose these brain regions as targets for future exploratory neurofeedback experimentation.

Data availability statement
Data used in main analysis and supplementary analysis have been submitted with this manuscript as Supplementary Data online. These are available for replication, and building upon the current analysis.

CONFLICT OF INTEREST
CAR, AS, NF, and TCK report grants from Unilever during the conduct of this study. TG and AT are employees of Unilever.

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AUTHOR CONTRIBUTIONS
Conceptualization: CAR, TG; Formal Analysis: CAR; Funding Acquisition: TCK; Investigation:
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SUPPLEMENTARY MATERIALS AND METHODS

Data search and extraction

**Information sources and search strategy.** Systematic searches using three databases (MEDLINE, Scopus, and PsycINFO) were conducted using the following MeSH search terms: (fMRI AND itch OR pruritus) and (fMRI AND [afferent touch OR pleasant touch]). Searches were restricted to terms found in the title or abstract of the articles. No date limit was set for the searches. We adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analysis method (Supplementary Table S2; http://www.prisma-statement.org).

**Eligibility criteria.** Generic inclusion criteria were the following: (i) human functional magnetic resonance imaging (fMRI) studies published up until February 2018; (ii) original articles reported in English; (iii) published in peer-reviewed journals; (iv) fMRI coordinates reported in the paper or supplementary material in either Montreal Neurological Institute (Evans et al., 1993) or Talairach space (Talairach and Tournoux, 1988); (v) data were obtained from a healthy population (systemic disease-free); and (vi) analyses were conducted on whole brain fMRI data (region of interest analyses were excluded as such data lead to bias in activation likelihood estimation [ALE] interpretation [Eickhoff et al., 2009; Turkeltaub et al., 2012]).

**Experimental itch.** Inclusion of studies of itch was restricted to those that conducted whole brain fMRI testing on acute itch induced by histamine, cowhage, allergens, or electrical stimulation of the skin, in participants who were free from pre-existing skin conditions. We included studies which contrasted itch with baseline, rest, saline, or placebo. Studies using itch ratings as regressors to predict itch-activated brain regions (e.g., Herde et al., 2007; Mochizuki et al., 2014; Walter et al., 2005) were also included. We excluded papers that observed scratching behavior if the basic contrast itch-baseline was not reported in the paper.

In addition to the formal search strategies, supplemental manual searches of the reference sections of identified papers were conducted. Additionally, a review paper on itch and fMRI studies in dermatology (Mueller et al., 2017) was screened for articles not previously identified by our formal search. These supplementary searches led to the inclusion of one additional paper for the itch meta-analyses.

**Pain.** Coordinates for activation in healthy controls subjected to acute experimental pain stimuli (electrical, thermal, and mechanical) were presented in the supplementary material of Tanasescu et al. (2016). We produced an ALE map in GingerALE, using the same thresholding preferences as those used in the itch and touch meta-analyses, for use in a contrast/comparison analysis.

**Article selection and extraction of data.** Formal database searches and supplementary searches were conducted by two authors independently (CAR and NF). CAR screened articles at the title/abstract level, and then at the full text level to determine relevance for inclusion. This process was repeated independently by NF. Final decisions over article inclusion were determined by discussion. CAR extracted the relevant data from the papers, which was cross-checked by NF.

We extracted the following information from each paper included in the analysis: author names, year of publication, contrast used, sample size, age of sample, coordinates of activation, whether coordinates were reported in Montreal Neurological Institute or Talairach space, and the statistical correction applied.

**Additional handling of data.** Studies that reported coordinates in the Talairach space were converted into Montreal Neurological Institute coordinates using GingerALE.

**ALE meta-analysis**

ALE meta-analyses were conducted to generate separate maps of itch and acute cutaneous pain. In each case, a single dataset analysis was conducted to obtain independent maps showing consistency of activated regions in response to different types of somatic sensation. Analyses were performed in Brainmap GingerALE, version 2.3.6 (Research Imaging Institute). We adhered to the ALE method (http://www.brainmap.org/ale/) devised by Eickhoff et al. (2009, 2012) with the correction devised by Turkeltaub et al. (2012), which uses a random effects model and minimizes within-experiment effects and within group effects.

 Reported ALE coordinates show the degree of convergence across studies. Each voxel is assigned an ALE value, which increases with the number of studies reporting activated peaks at (or nearby) that voxel. This process allows for assessment of consistency in voxel activation across studies. The standardized procedures for performing ALE in GingerALE are reported in the GingerALE user manual (Research Imaging Institute, 2013).

A P-value was calculated for each voxel based on the probabilities of attaining an ALE value that differed from that of the corresponding voxel on a null-distribution map via random permutation. The P-values in our analyses were generated by 10,000 permutations. We adhered to the most recent thresholding recommendations from Eickhoff et al. (2016) and used a cluster-level family-wise error correction at $P < 0.05$ to correct for multiple comparisons, following an initial cluster forming threshold of uncorrected $P < 0.001$. Cluster-level family-wise error is suggested to be more sensitive than voxel-level family-wise error because of its superior power to voxel inference (Eickhoff et al., 2016; Friston et al., 1996) while still controlling for incidental convergence. Cluster-level family-wise error thresholding provides an appropriate compromise between sensitivity and specificity. Multi-image Analysis Graphical User Interface (http://ric.uthscsa.edu/mango) was used to overlay ALE maps onto an anatomical image using Montreal Neurological Institute coordinates.

**Conjunction and contrast analysis**

Conjunction and contrast analyses for itch and pain were conducted to assess areas consistently activated across both maps and specify activations unique to each map. Conjunction analyses were conducted according to Eickhoff et al. (2009), whereby the voxel minimum value for each individual MA map was used to produce a conjunction image for itch + pain. Simultaneously, contrast images were produced by subtracting each ALE map from one another. Contrast analyses were conducted for...
itch — pain and pain — itch. Contrast analysis clusters were thresholded at uncorrected $P < 0.05$, with 5,000 permutations and a minimum cluster size of 500 mm$^3$ in all analyses.

**Sensitivity analysis**

Because Papoiu et al. (2012) contributed the most foci to the reported clusters, for sensitivity we have also run the analysis with the exclusion of this study. This analysis showed significant activity in the same three regions which are reported in the final analysis (see Supplementary Table S1).

**SUPPLEMENTARY REFERENCES**

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### Supplementary Table S1. MNI Locations of Significant Clusters from the Sensitivity Analysis Itch ALE Map

| Cluster | Brain Region | Peak Voxel Coordinates | Cluster Size (mm³) | ALE Value (×10⁻²) | No. of Contributing Experiments |
|---------|--------------|------------------------|-------------------|-------------------|-------------------------------|
| 1       | Thalamus L   | -2 -18 2               | 1512              | 1.29              | 5                             |
|         |              | -10 -18 12             |                   | 1.13              |                               |
|         |              | -6 -14 0               |                   | 0.96              |                               |
|         |              | -6 -4 6                |                   | 0.92              |                               |
|         |              | 2 -20 10               |                   | 0.86              |                               |
| 2       | Frontal operculum / anterior insula R | 38 10 -4 | 792 | 1.28 | 3 | 30 |
|         |              | 38 16 2                |                   | 1.23              |                               |
| 3       | Frontal operculum / anterior insula / central operculum L | -46 12 2 | 752 | 1.21 | 3 | 30 |
|         |              | -44 2 6                |                   | 1.07              |                               |

Abbreviations: ALE, activation likelihood estimation; L, left; MNI, Montreal Neurological Institute; R, right.

Coordinates are reported in MNI space. Analysis used whole brain data from: Herde et al. (2007); Ishiuji et al. (2009); Kleyn et al. (2012); Leknes et al. (2007); Mochizuki et al. (2007); Mochizuki et al. (2009); Mochizuki et al. (2014); Valet et al. (2008), and Walter et al. (2005). Studies that reported coordinates in the Talairach space were converted into MNI coordinates using GingerALE before analysis.

### Supplementary Table S2. Preferred Reporting Items for Systematic Reviews and Meta-Analysis Checklist

| Section/topic                       | # | Checklist item                                                                 | Reported on page # |
|-------------------------------------|---|-------------------------------------------------------------------------------|--------------------|
| TITeL                               |   | Identify the report as a systematic review, meta-analysis, or both.            | 1                  |
| ABSTRACT                            |   | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | N/A                |
| INTRODUCTION                        |   | Describe the rationale for the review in the context of what is already known. | 2                  |
| Rationale                           | 3 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | 1                  |
| Objectives                          | 4 |                                                                               |                    |
| METHODS                             |   | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | NA                 |
| Protocol and registration           | 5 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | Supplementary materials |
| Eligibility criteria                | 6 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | Supplementary materials |
| Information sources                 | 7 |                                                                               |                    |
| Search                              | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | Supplementary materials |
| Study selection                     | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | Supplementary materials |
| Data collection process             | 10| Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | Supplementary materials |
| Data items                          | 11| List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | Supplementary materials |
| Risk of bias in individual studies  | 12| Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | Supplementary materials |
| Summary measures                    | 13| State the principal summary measures (e.g., risk ratio, difference in means).    | Table 1            |
| Synthesis of results                | 14| Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I²) for each meta-analysis. | Supplementary materials |