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Thermosensory perceptual learning is associated with structural brain changes in parietal-opercular (SII) cortex

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Thermosensory perceptual learning is associated with structural brain changes in parietal-opercular (SII) cortex

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Abstract

The location of a sensory cortex for temperature perception remains a topic of substantial debate. Both parietal-opercular (SII) and posterior insula have been consistently implicated in thermosensory processing, but neither region has yet been identified as the locus of fine temperature discrimination. Using a perceptual learning paradigm in male and female humans, we show improvement in discrimination accuracy for sub-degree changes in both warmth and cool detection over 5 days of repetitive training. We found that increases in discriminative accuracy were specific to the temperature (cold or warm) being trained. Using structural imaging to look for plastic changes associated with perceptual learning, we identified symmetrical increases in grey matter density in parietal-opercular (SII) cortex. Furthermore, we observed distinct, adjacent regions for cold and warm discrimination, with cold discrimination having a more anterior locus than warm. The results suggest that thermosensory discrimination is supported by functionally and anatomically distinct temperature-specific modules in parietal-opercular SII cortex.

Significance statement

We provide behavioural and neuroanatomical evidence that perceptual learning is possible within the temperature system. We show that structural plasticity localizes to SII, and not posterior insula,

providing the best evidence to date resolving a longstanding debate about the location of putative 'temperature cortex'. Furthermore, we show that cold and warm pathways are behaviourally and anatomically dissociable, suggesting that the temperature system has distinct temperature-dependent processing modules.

Introduction

Despite significant progress in our understanding of the peripheral mechanisms of temperature sensation (Caterina et al., 1997; Bautista et al., 2007; Ran et al., 2016; Pogorzala et al., 2013; Mishra et al., 2011; Vriens et al., 2014), central mechanisms remain much less clear. That humans can detect temperature changes of a fraction of a degree (Dyck et al., 1971; Kenshalo et al., 1960; Johnson et al., 1973; Chen et al., 1996), bearing in mind the relatively broad response profile of thermoceptors, strongly points to the existence of a specific 'temperature cortex', but its anatomical location remains unresolved.

One view is that parietal-opercular cortex (SII) supports temperature perception, via ventrolateral thalamic relay of thermally responsive spinal afferents (Vriens et al., 2014). This view accords with temperature as an exteroceptive sense (an inference about the outside world) similar to other somatosensory modalities such as touch and vibration. An alternative view proposes that the posterior insula incorporates temperature cortex, via medial thalamic nuclei (including VMPo), as part of a broader interoceptive cortex that also accommodates pain, itch, and pleasant touch (Craig, 2002; Hua et al., 2005). This view draws on a view as temperature perception as an inference about the physiological state of the body, along with other sensory modalities that have intrinsic motivational value through a direct link with homeostasis (e.g. behavioural thermoregulation).

Cortical stimulation of both parietal-opercular and posterior insula can induce thermal sensations, with warmth being the more common sensation (Ostrowsky et al., 2002; Mazzola et al., 2006; Isnard et al., 2004, 2011; Mazzola et al., 2012). Human posterior insula lesions have been reported as causing thermal anaesthesia and impairing thermal detection in humans (Birklein et al., 2005; Cattaneo et al., 2007; Baier et al., 2014), but in rodents SI lesions have been shown to impair cold discrimination (Milenkovic et al., 2014), and human SI disruption with tDCS impairs bilateral cold detection (Grundmann et al., 2011; Oliviero et al., 2005). Awake electrocortical responses have suggested SII better codes warmth and posterior insula pain (Frot et al., 2007), but both regions have been observed to respond to warmth in fMRI studies(Davis et al., 1998; Bornhövd et al., 2002; Moulton et al., 2012). Good fMRI evidence exists for topographic cold responses in posterior insula (Craig et al., 2000; Hua et al., 2005), and cold responses have also been localised to posterior insula in MEG data (Maihöfner et al., 2002), although recent combined EEG-MEG data have suggested a source in SII (Fardo et al., 2017).

Taken together, these studies have led to a consensus favouring posterior insula as thermosensory cortex proper (Craig, 2002, 2011). Recently, however, high density human intracortical electrophysiology suggest that posterior insula may instead support a multi-modal sensory integration zone, rather than holding modality specific representations (Liberati et al., 2016). So whereas it may have a prominent role in homeostatic functions relating to temperature, whether or not it acts as a primary locus for discriminative thermal perception is unresolved.

A key lacunae in the evidence to date is any neuroanatomical mapping of *fine* temperature discrimination. As the prototypical feature of cortical sensory processing, it almost certainly depends on cortical information processing across a population of thermoceptors with different tuning functions (Pogorzala et al., 2013). In a similar manner to other discriminative sensory modalities such as vision and hearing, fine discriminative processing of sensory afferent signals can be considered the primary function of a putative 'thermosensory cortex'. One method to identify a cortical locus of discrimination is to look for structural changes associated with perceptual learning (Zatorre et al., 2012). Although thermosensory perceptual learning has not been previously described, in the visual domain it has been shown that as little as 5 days of repetitive training can lead to behavioural improvements and associated grey matter increases in the corresponding cortical sensory area (Ditye et al., 2013). Following this approach, we trained subjects to discriminate very small changes in either warm or cold temperatures, and probed corresponding anatomical brain changes with structural neuroimaging.

Materials and Methods

Participants

Twenty-four healthy subjects completed the study (8 females, age: 24.5 ± 6.03). This does not include 10 subjects who started the experiment but could not complete training due to technical failure of the thermal stimulator during perceptual training (requiring a replacement stimulator to be shipped from abroad), and these subjects were therefore excluded. All subjects had normal or corrected vision and were screened for a history of psychiatric or neurological conditions. All subjects gave a written informed consent which was approved by the ethics committee of Advanced Telecommunication Research Institute International (ATR), Kyoto, Japan and National Institute of Information and Communications Technology (NICT), Tokyo, Japan.

Thermal Stimuli

We used a contact thermal stimulator (ATS PATHWAY; Medoc Ltd., Ramat Uoshay, Israel) to deliver thermal stimuli. The thermode was attached to the lateral aspect of the left or right upper calf using a Velcro strap, and the stimulation sites were marked on the first day and the same site used for all subsequent experimental sessions. Between experimental sessions, the thermode was kept at a resting temperature of 30°C, and changed to the baseline temperature (25°C or 39°C) for just before each experimental session.

Experimental procedure

Each of the 24 subjects attended the experiment on 9 separate days: pre-training MRI scanning (day 1), pre-training behavioural test session (day 2), five days of training sessions (days 3-7), post-training behavioural test session (day 8), and a post-training MRI scanning (day 9). Training and test sessions were completed within a maximum of 14 days, so as to minimise forgetting effects in perceptual learning. Some subjects performed pre/post training behavioural test and scanning on the morning and afternoon of the same day, for logistical reasons.

Thermal discrimination task We performed a one-interval thermal-pulse detection task, in which subjects were required to report the presence of a small reduction (from the 25°C cool baseline) or increase (from the 39°C warm baseline) in temperature, for cold and warm detection respectively (Figure 1). These thermal pulses occurred on 50% of trials, and across 4 different magnitudes i.e. making 4 different levels of difficulty.

At the beginning of each trial, subjects heard small tone through their headphones, accompanied by a visual message 'Press the button if you feel a pulse' displayed on a computer monitor for 500 ms. Then, the thermode either delivered the pulse stimulus, or continued at baseline. If they felt a thermal pulse, they responded by pressing a button within 3.5 sec. If they felt no pulse, then they were instructed not to press the button. There was no feedback (i.e. whether or not the detection was correct) given to the subjects. Each session had 200 trials, consisting 100 trials with thermal pulse delivery (25 for each level of difficulty) and 100 trials with no pulse. The order of pulse and no-pulse trials was pseudo-randomised. Each session took approximately 15 mins.

Calibration across subjects The 4 levels of difficulty were set individually for each subject before the experiment was performed. This is because there is significant between subject variability in discriminative performance, so we aimed to approximately equate performance across subjects. In this calibration procedure, subjects received a range of thermal pulses from 0.2°C to 1.5°C (0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5). We chose the 4 adjacent temperatures that gave an accuracy (i.e. sensitivity index d^t, see below) closest to 1.5 (typically this corresponds to roughly 75% correct, with 50% being chance). The most common set of temperatures pulses was 0.5, 0.7, 0.9, 1.1°C.

Pre-training testing After the calibration procedure, subjects then performed the pre-training behavioural testing, of both warming and cooling on both right and left leg. Specifically, they performed 2 sessions of cold testing on the left leg, 2 sessions of cold on the right leg, 2 sessions of warm training on left and 2 on the right. The order of performing each was balanced and randomised across subjects, but identical in the post-training session.

Training For the training sessions, subjects were randomly assigned to be trained on one of four task conditions. Randomization was determined before the start of the entire experiment, but blinded to experimenters until after pre-training test, to avoid bias (the pre-test discriminative accuracy of the trained temperature/laterality was not different from the non-training temperature/lateralities). On each training day, subjects performed 4 sessions of their allocated temperature/laterality over 5 days (i.e. 800 trials per day in total, lasting about 1 hour)

Post-training testing After training, the subjects performed post-training task on both temperatures and lateralities, exactly as in the pre-training test.

MRI acquisition

Structural brain images were obtained in an MRI scanner before and after the experimental task sessions. Resting-state fMRI scans were also collected, during which subjects were instructed to keep looking at a central fixation point, to keep still and stay awake. We also performed an fMRI task with small fixed pulses in warm and cool temperatures. Post-experimental analysis revealed the presence of RF noise introduced by the operation of the thermal stimulator, creating artifact that corrupted the images in a way that was correlated with the task, and so this data was discarded. We also collected diffusion-weighted images. This was intended to generate pilot data for a future study of white matter changes associated with learning.

All scanning was performed on a 3.0-T MRI Scanner (3T Magnetom Trio with TIM system; Siemens, Erlangen, Germany) equipped with echo planar imaging (EPI) capability and a standard 12-channel phased array head coil. Subjects remained supine and wore MR-compatible headphones.

A six-minute resting-state functional MRI (rsfMRI) scan consisted of 145 volumes was acquired using a single-shot EPI gradient echo T2*-weighted pulse sequence with the following parameters: TR=2,500 ms, TE=30 ms, FA=80 degrees, BW=2367 Hz, FOV=192 × 192 mm (covering the whole brain), acquisition matrix= 64 × 64, 37 to 41 axial slices with a ascending slice order of 2.5 mm slice thickness with 0.5 mm inter-slice gap. In parallel with the rsfMRI scan, cardiac pulsation and respiratory waveform were monitored with a photoplethysmography probe attached to the distal end of a finger on the left hand, and with a respiration belt strapped around the upper abdomen, and recorded with a sampling rate of 50 Hz.

A high-resolution three-dimensional volumetric acquisition of T1-weighted structural MRI scan

was collected using a MPRAGE pulse sequence: TR=1.07 ms, TE =3.06ms, time of inversion=900 ms, FA=9 degrees, BW=230 Hz, FOV=256 × 256 mm, 208 sagittal slices of 1mm slice thickness with no inter-slice gap, acquisition matrix= 256 × 256.

Data analysis

Behavioural analysis

Accuracy was measured by calculating the d^t in the standard manner: $d^t = Z(\text{hit rate}) - Z(\text{false alarm rate})$. The d^t was then used a summary statistic in ANOVA and *t*-tests as appropriate.

Voxel-based Morphometry (VBM) analysis

VBM analysis were performed with the statistical parametric mapping, SPM8 (Wellcome Trust Centre for Neuroimaging, UCL, London, UK; http://www.fil.ion.ucl.ac.uk/spm/) and its default plug-in toolbox: diffeomorphic anatomical registration using exponentiated Lie algebra (DARTEL) (Ashburner, 2007) and their extension VBM8 (Christian Gaser, Department of Psychiatry, University of Jena, Germany; ;http://dbm.neuro.uni-jena.de/vbm/) on Matlab (Mathworks, Sherborn, MA, USA). T1-weighted images were fed into this analysis pathway, and we applied a specialized framework for longitudinal analysis in VBM8 consisting of the following procedures: 1) With a view to study changes across time within the same subject, the obtained subject specific images from pre- and post-training MRI scanning were registered in the individual subject space and the mean image was generated. The original images were realigned into the mean image to avoid the occurrence of potential bias due to asymmetry in pairwise image registration. 2) A correction for intensity inhomogeneity was performed for the realigned images. 3) The derived images were segmented into grey matter (GM) and white matter (WM) based on an adaptive Maximum A Posterior (MAP). 4) The GM and WM images were spatially normalised and registered to IXI550 MNI152 space (IXI-database; http://brain-development.org/ixi-dataset/) with a manner of high-dimensional deformation. These images were smoothed with a 8 × 8 × 8 mm FWHM Gaussian kernel, and utilised for the further statistical analyses.

Interaction between the differences in trained task condition (cool and warm pulse detection) and training effect (pre- and post-training) were tested for statistical significance in a flexible factorial ANOVA with a threshold at uncorrected $p < 0.001$ after application of a small volume correction encompassing bilateral SII (OP1, OP2, OP3, and OP4) and posterior insula (Ig1, Ig2 and Id1) regions as defined in the SPM Anatomy toolbox (Eickhoff et al., 2005).

We also did a post-hoc analysis of the effect of laterality, by using cold and warm masks (at $p<0.005$ uncorrected) to directly contrast contralateral minus ipsilateral effect sizes. This allowed us to group the effects of left and right trained subjects for each temperature. Finally, we also

considered whether there might be warm or cold specific responses in SI cortex, so we performed a supplementary analysis using a mask from the probabalisitc atlas from (Geyer et al., 1999).

RS-fMRI seed-based correlation analysis

Resting-state fMRI data were analysed with SPM8 and the FMRIB Software Library (FSL; http://fsl.fmrib.ox.ac.uk/fsl/). The first five images were discarded to allow for T1 equilibration and the remaining images were corrected for physiological noise, cardiac and respiratory artifacts, by applying RETROICOR method (Glover et al., 2000). Slice timing was adjusted to the intermediate slice and all the images were realigned to the first image of each scan with the estimated 6 rigid-body head motion parameters with SPM8. Additionally, a wavelet-based de-spiking method (Patel et al., 2014) was applied to all the realigned images to attenuate a range of spurious variance related to abrupt head motions. Non-brain structures such as skull and scalp surfaces were removed (Smith, 2002; Jenkinson et al., 2002) prior to the performance of Boundary-Based Registration (Greve and Fischl, 2009) between the first image of the functional images and the corresponding T1 weighted structural image, followed by spatial normalisation to Linear ICBM Average Brain (ICBM152) Stereotaxic Registration Model (Mazziotta et al., 1995, 2001b,a) with 12 degrees-of-freedom linear affine transformation. Smoothing was applied with a 8 × 8 × 8 mm FWHM Gaussian kernel, and a temporal band-pass filter ranging from 0.01 to 0.08 Hz was applied.

Next, seed-based correlation analysis was applied. The seed ROIs for cold and warm condition were defined by the VBM results on T1 weighted images (see VBM result section). Based on the average time-course within each of the ROIs, connectivity was calculated as Pearson's correlation coefficient for all other voxels in the brain, and then Fisher's *Z*-transformation was applied. Statistical analysis was performed to compare pre- and post-training effect for the cold seed ROI in the cold-trained subjects together with that for the warm seed ROI in the warm-trained subjects.

Results

Behavioural results

Twenty-four subjects performed a thermosensory perceptual learning experiment to identify improvements in accuracy in fine temperature discrimination in a one-interval detection task (without feedback) within warm (from 39°C) and cold (from 25°C) temperature domains. Thermal stimuli were delivered by a contact peltier thermode applied to the left or right leg, and subjects were required to identify the presence of a transient change in baseline temperature (cooling in the cold domain, and warming in the warm domain) that occurred with 50% probability, across 4 levels

of difficulty determined by the magnitude of the phasic temperature change. At the start of the experiment, subjects were tested on discriminative accuracy for both warm and cold conditions, on both right and left legs. Then, subjects were randomised into two groups: 12 subjects were trained to discriminate brief increases from a warm baseline temperature (39°C), and 12 subjects were training with to detect transient decreases from a cool baseline (25°C) (Figure 1). Within these groups, subjects were randomised to be trained on either the left or right leg. Subjects performed the task for about an hour on 5 days (over the course of about a week) on their respective temperature and laterality. After training, they were re-tested on both temperatures and lateralities, so we could identify improvements in discriminative accuracy (d^t) as a specific function of training. MRI scanning was done before and after the experiment to look for evidence of neural plasticity (see below).

Accuracy was improved as a function of training, with a significant increase in the d^t (Δd^t) of 0.44 across all subjects when comparing pre- and post-training performance on the temperature and laterality on which they were trained (one-sample *t*-test, $n = 24$, $p = 0.0005$) (Figure 2). The effect was more clear in the cold training group ($n = 12$, $\Delta d^t = 0.49$, $p = 0.005$) than warm subjects ($n = 12$, $\Delta d^t = 0.40$, $p = 0.042$).

To probe the specificity of this effect, we compared the improvement in accuracy for the temperature/laterality on which they were trained, with those on which they were not. Across all subjects, a two-way ANOVA (based on using the post-training minus pre-training contrast as the summary statistic) revealed a main effect of temperature ($F = 5.66$, $p = 0.019$), but no significant main effect of laterality ($F = 1.77$, $p = 0.1863$) and a non-significant interaction ($F = 3.26$, $p = 0.074$) (Figure 3). That is, the improvement in discriminative accuracy was restricted to the temperature - cold or warm - being trained.

To study this effect in more detail, we then looked separately at the cold and warm trained subjects. Cold subjects showed a main effect of temperature ($F = 5.71$, $p = 0.021$), no effect of laterality ($F = 0.36$, $p = 0.549$), and a marginally significant temperature × laterality interaction ($F = 4.08$, $p = 0.0494$) (Figure 3 (right panel)). Warm subjects showed no main effect of temperature ($F = 0.21$, $p = 0.375$), no effect of laterality ($F = 0.478$, $p = 0.187$), and no significant temperature × laterality interaction ($F = 0.052$, $p = 0.661$) (Figure 3, right panel). This suggests that the training effect is more robust for cold than warm temperatures.

Response times were significantly faster for cold detection (mean = 1,459ms) than warm detection (mean = 2,026ms)(*t*-test, $p < 1e-14$), which is consistent with the notion that cold detection relies on myelinated A-delta fibers, whereas warm detection relies on unmyelinated C fibers. Figure 4A shows the response times as a function of difficulty, illustrating that only cold detection shows longer response times for correctly identifying the smaller, more difficult stimuli than the easier, larger stimuli. With respect to training, there was no difference in overall response times between

the pre-training and post-training tests (ΔRT), when looking at all subjects and conditions (ΔRT = 8.1ms, p = 0.8668), or in just warm trained subjects (ΔRT = ˇ21.96ms, p = 0.6820) or cold trained subjects (ΔRT = 38.15ms, p = 0.4752). Figure 4B looks specifically at response times as a function of training, and although the overall pattern suggests a reduction in RT mirroring improvements in accuracy, these effects don't reach significance (see figure legend for stats).

In conclusion, there was evidence for perceptual learning across both warm and cold trained subjects. Overall this was specific to the temperature being trained, and this effect was primarily driven by more robust learning in the cold trained subjects, with learning present but less robust in the warm trained subjects.

Neuroimaging results

We next sought to identify brain regions associated with perceptual learning by comparing grey matter density from structural T1 MRI scans before and after training, using voxel-based morphometry (VBM) (Ashburner and Friston, 2000). An initial contrast of post-training minus pre-training scans across all subjects did not identify any differences within an atlas-based mask that comprised bilateral parietal opercular (SII) and posterior insula (PI) cortex as our regions of interest (see methods), or at whole brain level (with appropriate corrections for multiple comparisons). Based on the behavioural observation that learning was temperature specific, we therefore directly contrasted post- minus pre-training VBM maps between cold-trained and warm-trained subjects (i.e. to identify an interaction between the effect of training and temperature), regardless of laterality.

In the cold-trained subjects, we observed symmetrical increases in VBM grey matter signal in parietal opercular cortex (SII), illustrated in Figure 5 at an uncorrected threshold of $p < 0.005$. This survived correction for multiple comparisons using the bilateral parietal opercular (SII) and posterior insula (PI) cortex ROI mask. Based on the anatomical atlas, this increase in grey matter density fell primarily with areas OP4 and OP3 (see figure legend for details).

In the warm-trained subjects, we identified symmetrical increases in grey matter density in more posterior region of parietal opercular cortex at an uncorrected threshold of $p < 0.005$ (Figure 6). Probabilistic anatomical localisation isolated these areas as primarily within area OP1 (see figure legend). Some caution should be noted, however, as this result did not quite reach significance when corrected for multiple comparisons across bilateral parietal opercular (SII) and PI cortices.

It could be argued that SI might also be expected to show temperature specific responses, so in a supplementary analysis we applied a SI mask (Geyer et al., 1999) and repeated the analysis above. In a post-training minus pre-training contrast across all subjects, a just-significant peak was identified in right SI cortex (1 voxel at coordinates: 44,-34,45), but we found no significant differences in the temperature-specific contrasts. In addition, we considered whether there might be laterality

differences in the VBM data in SII. The study is under-powered to look directly at anatomical effects of laterality within the trained temperatures, primarily because of the asymmetry of the brain in this region. However, we did perform an ROI analysis of the effect sizes of contralateral versus ipsilateral within masks defined by the cold and warm regions presented above. However, this did not identify significant differences: cold proportional increase contralateral = 0.00863 and ipsilateral = 0.00583 (p = 0.55); warm proportional increase contralateral = 0.00250 and ipsilateral = 0.00110 (p = 0.66).

We also acquired resting state fMRI data before and after training, to identify whether a broader network of regions might be involved in perceptual learning. This analysis is more exploratory, since there are few prior studies on which to inform which brain regions might be involved in up-stream/down-stream aspects of fine temperature discrimination. With this in mind, we looked across all subjects used a seed defined by the VBM results (the anterior bilateral SII region for cold-trained subjects, and the posterior SII region for the warm-trained subjects). Specifically, we looked across all subjects to identify increases in connectivity in post- compared to pre-training scans, and used a whole-brain FWE correction. This analysis identified increased connectivity in post-central gryus, medial prefrontal cortex, and a region of visual cortex (looking purely at warm or cold trained groups alone did not identify brain regions surviving whole-brain FWE correction)(Figure 7).

Discussion

The data provide three new findings about human discriminative thermosensation. First, we show that fine, sub-degree discrimination of temperature can be enhanced through perceptual learning with repetitive training over a period of days. Second, we show that this improvement in performance is temperature specific (i.e. cool versus warmth), indicating a functional dissociation within thermosensation. Finally, we show that perceptual learning correlates with putatively anatomically distinct temperature specific modules in parietal-opercular (SII) cortex.

The debate about thermosensory cortical localisation has tended to focus on data of neural responding to coarse-grained thermal stimuli, at the cost of clearly defining the information processing function of cortical regions. Discrimination is the prototypical function of primary sensory cortex across modalities. In vision, for instance, perceptual learning for orientation has been shown to involve primary visual cortex (Shibata et al., 2011). In thermosensation, although relatively computationally undemanding compared to other modalities, acuities of 0.3°C or less must almost certainly require both heterogeneity in the thermal response profiles of peripheral thermoceptors, and inference over a broad population of such thermoceptors in the cortex.

The finding of dissociable modules for warm and cold discrimination in SII suggests that these

pathways remain at least partially distinct not only in peripheral nerve, spinal projection and thalamus (Lenz and Dougherty, 1998; Bushnell et al., 1993; Chen et al., 1996; Yarmolinsky et al., 2016; Burton et al., 1979), but also include the cortex. Compatible with this functional dissociation, it has also been observed that putatively enhancing cold responses into the warm domain using menthol doesn't improve discrimination, suggesting that people cannot spontaneously integrate warm and cold afferents to improve discrimination (Barber et al., 2017). However, although warm and cold responses can be dissociated, this does not necessarily mean they are independent, and it remains entirely possible that warm-responsive afferents can contribute to cold discrimination and vice-versa (Pogorzala et al., 2013). In particular, we did not include a test condition in which warm baseline temperatures were reduced, or cold temperatures were increased (primarily because of the prohibitive duration of the test sessions). Therefore, we do not know, for instance, if training on temperature reductions from a cool baseline would generalise to increases from a cool baseline, or decreases from a warm baseline.

Across both behavioural and imaging results, perceptual learning for cold temperatures appeared more robust. This may be unsurprising, since the presumed dependence of warm discrimination primarily on unmyelinated C-fiber afferents, compared to myelinated A-delta afferents for cold discrimination on, would suggest lesser fidelity of afferent information transmission (Ran et al., 2016; Bautista et al., 2007; Craig et al., 2000). There are other functional differences in these pathways: cold-responsive spinal cord neurons, which receive input from TRPM8-expressing dorsal-root ganglion (DRG) neurons, tend to show more adaptation to baseline temperature which may allow them to more sensitively respond to small temperature changes in contrast to warm sensitive spinal neurons, which receive input from TRPV1-expressing DRG neurons. Thermosensing TRPM8 receptors may contribute to this adaptivity by showing baseline adaption response properties (Fujita et al., 2013). Peripheral pathways are also complicated by the fact that some afferents respond to both warming and cooling (Ran et al., 2016), and their contribution to discrimination is unclear.

Our study was not sufficiently powered to study the functional anatomy of the lateralisation of thermosensory learning. Behaviourally, there was some suggestion, primarily in the cold domain, that learning was lateralised i.e. we did find a temperature × laterality interaction in the improvement of accuracy (d^t). However we were not able to demonstrate this with an ROI approach to the imaging data. It remains a reasonable prediction that laterality specific changes might be found in a larger sample size, although it should be noted that there is evidence that thermal responses may involve bilateral representations to a certain extent (Robinson and Burton, 1980)

Our results require rationalisation with the clear evidence of graded thermal responses previously observed in insula cortex. One possibility is that insula acts in a behaviourally sensitive manner, and reflects the homeostatic value of thermal input. That is, that insula integrates motivationally important information with sensory information to generate motivational values that can be used

464 to guide behaviour, such as approach and avoidance. This would predict, for example, that insula
465 representations of thermal stimuli would be dependent on current homeostatic state, and that for
466 example a cooling stimulus would have a different representation depending on whether an individual
467 was hot (when it is rewarding) than cold (when it is aversive)(Hendersen and Graham, 1979). If
468 confirmed, this would imply a functional dissociation between discriminative and homeostatic
469 cortical loci in SII and insula, respectively.

470 The use of voxel-based morphometry (VBM) allows a relatively unambiguous method to localise
471 function, under the assumption that evidence of modality specific behavioural plasticity would be
472 predicted to have a corresponding change in grey matter plasticity. Experience-dependent grey
473 matter changes are unlikely to reflect fundamental changes in neuronal populations, but rather
474 subtle changes in neuronal morphology, glial cell structure, vascularization and signalling pathways
475 (Zatorre et al., 2012). In the context of perceptual learning, it has several advantages over other
476 neuroimaging methods and so provides a valuable complement to existing results. For example,
477 BOLD fMRI responses can be confounded by large vessels and changes in the haemodynamic
478 response function. Furthermore, simply observing BOLD responses opens awkward possible
479 confounds, in particular interference from the explicit memory and hence attention arising from
480 recall of training. In contrast, VBM effectively integrates over the history of perceptual learning in
481 the absence of requirement to perform a task during evaluation of the brain. Furthermore, the use of
482 an unreinforced paradigm (no feedback is given to the subjects about their performance) removes
483 other confounds such as reward conditioning.

484 The resting state network analysis identifies regions that might have a functional role in supporting
485 perceptual learning. Although the nature of that function is speculative, two regions are noteworthy.
486 First, post-central gyrus activity might suggest connectivity with thermal representations in SI,
487 although the region is not clearly within the usual topographic region of the leg. Hence the question
488 of whether the thermosensitive input to SII comes directly from thalamus or indirectly from SI
489 (both pathways exist anatomically), cannot be answered with in the current study. The activity in
490 medial PFC has been implicated in metacognitive evaluation of perceptual discrimination, and
491 might support a similar role here. Interestingly, metacognitive judgments can be dissociated from
492 discriminative performance in thermal discrimination by application of menthol (which reduces
493 accuracy but increases confidence in intermediate temperatures (Barber et al., 2017)), so this
494 hypothesis may be testable in the future.

495 Finally, our findings inform a parallel debate about the localisation of nociceptive cortex,
496 with a similar and lognstanding discussion about the relative importance of somatosensory and
497 insula cortices. There is sufficient evidence that nociceptive sensation involves fine-discriminative
498 processing to imply cortical processing (Mancini et al., 2012), and perceptual learning has recently
499 been observed for nociceptive stimuli (Mancini et al.). It is even possible that there might be

different loci for different submodalities of pain (heat, cold, mechanical, inflammatory pain and so on). However, the importance of non-painful temperature processing is illustrated in the multiple interactions between pain and temperature, not least in chronic pain conditions such as post-stroke pain, thought to arise through imbalance between different spinothalamic pathways (Craig, 2003).

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Figure Legends:

Figure 1: Thermal detection task.
(A) Subjects performed a simple detection task, in which they had to press a button if they felt a small decrease (from a 25C baseline in the cold condition) or increase (from a 39C baseline in the warm condition), occurring with 50% probability. The phasic temperature changes were of 4 different magnitudes to create a range of difficulties, and was calibrated to each subject beforehand (see methods). The start of each trial was signaled by a message on the computer monitor, and the timing of the possible temperature change cued by an auditory tone 1.5 secs in advance.
(B) Experimental schedule: subjects underwent 5 days of training with a specific temperature and laterality. Before and after training, they performed behavioural testing on all temperatures/lateralities, and underwent structural and functional imaging.

Figure 2: Performance over test and training sessions.
Accuracy improved with training over time, when evaluated across all subjects (left panel), or restricted to within the cold-trained and warm-trained groups (right panel). All error bars are SEM.

Figure 3: Perceptual detection accuracy.
The left panel shows the change in accuracy (d') at the post-training test session compared to pre-training, across all subjects (n=24). The x axis refers to the temperature and laterality being tested, with 'same, ipsilateral' referring to the *trained* temperature and laterality. The right panel is the same analysis, but split into the cold-trained (n=12) and warm-trained (n=12) subjects.

Figure 4. Response times.
(A) As a function of the difficulty of successfully detected stimuli across warm and cold trials.
(B) In cold trained subjects (left panel) the mean improvement from pre- to post-training (ΔRT) was 131.7 ($p=0.1619$). Between condition ANOVA identified a non-significant main effect of temperature ($p=0.158$) and no temperature x laterality interaction ($p=0.223$). In warm trained subjects (right panel), there were no observable changes in response times ($\Delta RT = -28.5$ms, $p=0.760$, and no main effects or interactions)

Figure 5: VBM changes in cold versus warm-trained subjects.
(A). Coronal and axial sections at an uncorrected threshold of $p<0.005$. For the left cluster, peak MNI coordinate, t-statistics and p-value, and spatial extent were [–56, –6, 13], t=5.19, p=0.00002, and 161 voxels, and family-wise error (FWE) correction within SII and PI was significant at p=0.032 (extent 7 voxels). For the right cluster, corresponding statistics were [45, –13, 18], t=6.43, p=0.000001, 68 voxels, with FWE correction p=0.0031 (extent 15 voxels).
(B). The Maximum Probability Map (MPM)(Collins et al, 1994) at the same threshold as (a), illustrating bilateral SII within anatomically-defined masks of the two ROIs: SII (Eickhoff et al, 2006) and posterior insula Kurth et al, 2010. Localization probability (Eickhoff et al, 2005) as follows: left cluster, 52.8% in area OP4, 14.1% in area TE 1.2, 8.4% in area TE 3; right cluster, 42.7% in area OP3 and 10.9% in area OP4.

Figure 6: VBM changes in warm versus cold-trained subjects.
(A). Coronal and axial sections at an uncorrected threshold of $p<0.005$. In the left cluster, peak MNI coordinate, t-statistics and p-value, and extent were [–57, –27, 15], t=3.39, p=0.00035, and 157 voxels, with non-significant FWE correction of p=0.273. In the right cluster there were two peaks, with corresponding stats: [48, –30, 17], t=4.46, p=0.0001, and 40 voxels; and at [38, –7, 12], t=3.13, p=0.003, and 16 voxels. FWE corrections yielded p=0.12 and, p=0.756 respectively.
(B). The MPM shown at the same threshold as (A). On the left, probabilistic localisation was 96.5% in left area OP1 (SII) and 0.7% in left Area PFcm (IPL). On the right, the caudal and rostral clusters had corresponding localisation probabilities of 97.6% in area OP1 (SII) and 1.6% in area PFcm (IPL) (caudal right); and 97.8% in right area OP3 [VS] respectively.

Figure 7: Seed-based correlation analysis of the resting-state fMRI.
(A). Sagittal section at x=–12 and axial section at z=15 at a whole-brain FWE-corrected threshold of $p<0.05$. The peak coordinate, its t-statistics and p-value, and the extent of the cluster in the rostral medial prefrontal cortex were [–12, 57, 15], t=7.96, p=0.003, and 9 voxels.
(B). Sagittal section at x=–24 and axial section at z=77 at a FWE-corrected threshold of $p<0.05$. The peak coordinate, its t-statistics and p-value, and the extent of the cluster in the post central gyrus (primary sensory cortex) were [–24, –27, 78], t=9.57, p=0.0001, and 28 voxels. We also noted responses in occipital lobe: [–27, –93, –3], t=8.03, p=0.003, and 13 voxels.