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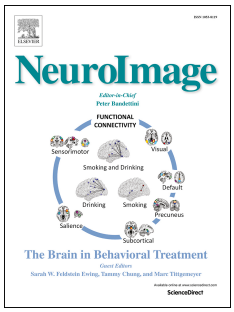

Brain regions preferentially responding to transient and iso-intense painful or tactile stimuli

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Brain regions preferentially responding to transient and iso-intense painful or tactile stimuli

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Abstract

How pain emerges from the cortical activity remains an unresolved question in pain neuroscience. A first step toward addressing this question consists in identifying brain activities that occur preferentially in response to painful stimuli in comparison to non-painful stimuli. A key confound that has affected this important comparison in many previous instances is the intensity of the stimuli generating painful and non-painful sensations. Here, we compared the brain activity during iso-intense painful and tactile sensations sampled by functional MRI in 51 healthy participants. Specifically, the perceived intensity was recorded for every stimulus and only the stimuli with rigorously matched perceived intensity were selected and compared between painful and tactile conditions. We found that all brain areas activated by painful stimuli were also activated by tactile stimuli, and vice versa. Neural responses in these areas were correlated with the perceived stimulus intensity, regardless of stimulus modality. More importantly, among these activated areas, we further identified several brain regions showing stronger responses to painful stimuli than to tactile stimuli when perceived intensity was carefully matched, including the bilateral opercular cortex, the left supplementary motor area and the right frontal middle and inferior areas. Among these areas, the right frontal middle area still responded more strongly to painful stimuli even when painful stimuli were perceived less intense than tactile stimuli, whereas other regions now showed stronger responses to tactile stimuli. In contrast, the left postcentral gyrus, the visual cortex, the right parietal inferior gyrus, the left parietal superior gyrus and the right cerebellum were found to have stronger responses to tactile stimuli than to painful stimuli when perceived intensity was carefully matched. When tactile stimuli were perceived less

intense than painful stimuli, the left postcentral gyrus and the parietal inferior gyrus still responded more strongly to tactile stimuli while other regions now showed similar responses to painful and tactile stimuli. These results suggest that different brain areas may be engaged differentially when processing painful and tactile information, although their neural activities are not exclusively dedicated to encoding information of only one modality but are also determined by perceived stimulus intensity regardless of stimulus modality.

Introduction

Transient nociceptive stimuli causing pain elicit robust responses in a set of brain regions widely distributed in the brain, mainly including the thalamus, the primary and secondary somatosensory areas, the insula, the cingulate cortex and also some areas in the frontal and parietal lobes [3; 5; 24; 30; 33; 51; 59-63]. Many of these studies explicitly suggest that pain perception is consequent to the neural activity of these brain areas [2; 6; 16; 34; 40; 53]. However, none of these brain areas is exclusively involved in nociceptive processing as they are all also activated by non-nociceptive sensory stimuli that do not cause painful percepts [41], and even in pain-free patients [56]. This evidence suggests that the function of this set of brain regions is largely unrelated to pain perception, but is instead related to the detection of sudden environmental events that require immediate attention, regardless of the sensory channel through which these events are conveyed [13-15; 27; 28; 36]. These two interpretations are not mutually exclusive. Rather, it is likely that they reflect different facets of the complex functions served by these brain regions. In fact, many studies have attempted to identify the neural correlates of pain using a variety of brain imaging techniques and suggested neural activities that might be preferentially involved in pain processing. For example, it has been claimed, on the basis of recordings using intracerebral local field potentials (LEPs), scalp electroencephalography (EEG), functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), that the secondary somatosensory cortex (S2) [50], the insula (including both posterior [32; 50] and anterior part [50]) and the anterior cingulate cortex (ACC) [38] might contain neural activities selective to pain. However, in these studies an important confound was neglected: when comparing brain responses to

painful stimuli with those to non-painful stimuli stimulus intensity was not matched. Indeed, as the amplitude of neural activity in many brain areas were found to correlate with stimulus intensity [8; 26], it remains unclear whether these previously identified brain areas truly responded to pain preferentially or simply because painful stimuli was more intense. Therefore, in the present study, we first formally tested whether the amplitude of neural activity in the brain areas responding to painful and tactile stimuli correlated with the perceived stimulus intensity. This first test proves the necessity of matching perceived intensity when comparing brain responses to painful nociceptive stimuli and brain responses to non-painful tactile stimuli. We then performed such comparison, using carefully matched painful and tactile stimuli, to identify brain regions preferentially responding to painful stimuli than to tactile stimuli. Thus we were able to rule out the possibility that differences in fMRI responses evoked by painful and tactile stimuli are due to difference in their perceived intensity. Finally, we further tested whether modality preference of the identified brain regions could still be detected when the perceived stimulus intensity of the preferred modality was lower than that of the non-preferred modality.

Materials and Methods

Participants

51 healthy young adults (mean age: 24±2.29 years; 34 females) were recruited through college and community advertisements and paid for their participation. All participants were Chinese and right-handed. Participants were carefully screened to ensure that they had no history of brain injuries, pain disorders, any psychiatric or neurological diseases, alcohol

abuse, drug abuse, or hypertension, and that they had no contraindications to MRI examination.

All participants provided written informed consent prior to the experiment, and the experimental procedures were approved by the Medical Research Ethics Committee of Tianjin Medical University.

Experimental design

While lying in the scanner, participants received stimuli of two sensory modalities (painful and tactile) and two stimulus physical intensities (low and high). Painful stimuli were delivered using laser pulses on the right foot dorsum within the sensory territory of the superficial peroneal nerve, and tactile (i.e., non-painful) stimuli were delivered using transcutaneous electrical pulses over the superficial peroneal nerve of the right foot, similar to what were used in [41]. The two levels of physical intensities (low and high) were determined for each type of stimuli (painful and tactile) for each individual participant before the scanning using the following procedure: participants were first familiarized with a few laser stimuli; then a series of laser pulses of different energies was delivered, and participants were asked to rate the perceived intensity after each stimulus using a numerical rating scale (0 indicates no sensation and 10 indicates the worst pain imaginable); the physical intensities corresponding to the perceived intensity rating of 3 and 6 were used in the subsequent experiment during the fMRI scanning as the low and high painful stimulus intensities, respectively. This procedure was repeated for electrical stimuli to determine the low and high physical intensities for tactile stimuli (0 indicates no sensation and 10 indicates

the strongest sensation as such electrical shock). The intensity of the electrical stimuli was kept below the pain threshold in all participants to ensure a non-painful, tactile sensation elicited by the electrical stimuli. Therefore, different physical intensities of both painful and non-painful stimuli were used in different participants to account for inter-subject variability in sensory thresholds.

The experiment consisted of two sessions of fMRI data acquisition, with 24 trials organized in three 'painful' blocks and three 'tactile' blocks in each session (i.e., 4 trials in each block, 2 with high physical intensity and 2 with low physical intensity, randomly ordered). 'Painful' and 'tactile' blocks were presented alternately, and their order was balanced across sessions and participants. In each trial a 10-s stimulation period was followed by a 10-s rating period. There was a 2-s interval between the onset of the trial and the onset of the stimulation period and 3-s interval between the end of the stimulation period and the beginning of the rating period. During the 10-s stimulation period, only one brief stimulus (either painful or tactile) was delivered at a random time (uniform distribution) for a jittering effect between trials. A white fixation cross was displayed at the center of the screen during the first 15-s period. During the rating period, a visual analogue scale (VAS, ranging from 0 to 10) [25; 58; 65; 66] was presented on the screen and participants were instructed to rate the perceived intensity of the stimulus delivered in the same trial using a button box.

MRI data acquisition

MRI data of the study were acquired using a MAGNETOM Prisma 3T MR scanner (Siemens, Erlangen, Germany) with a 64-channel phase-array head-neck coil. Tight but comfortable

foam padding was used to minimize head motion, and earplugs were used to reduce scanner noise. Blood-oxygenation level dependent (BOLD) signals were collected with a prototype simultaneous multi-slices gradient echo echo-planar imaging (EPI) sequence using the following parameters to achieve a good trade-off between spatial resolution and temporal resolution with a good signal-to-noise ratio at the same time: echo time (TE) = 30 ms, repetition time (TR) = 800 ms, field of view (FOV) = 222 mm × 222 mm, matrix = 74 × 74, in-plane resolution = 3 mm × 3 mm, flip angle (FA) = 54 degree, slice thickness = 3 mm, gap = 0 mm, number of slices = 48, slice orientation = transversal, bandwidth = 1690 Hz/Pixel, PAT (Parallel Acquisition Technique) mode, slice acceleration factor = 4, phase encoding acceleration factor = 2. A high-resolution 3D T1 structural image (two inversion contrast magnetization prepared rapid gradient echo sequence, MP2RAGE) was also acquired with the following parameters: TR/TE = 4000 ms/3.41 ms, inversion times (TI1/TI2)=700 ms/2110 ms, FA1/FA2 = 4 degree/5 degree, matrix = 256 × 240, FOV =256 mm× 240 mm, number of slices = 192, in-plane resolution = 1 mm × 1 mm, slice thickness = 1 mm, slice orientation = sagittal, total duration is 6 minutes 42 seconds.

Data preprocessing

The fMRI data were firstly preprocessed using the software package Statistical Parametric Mapping (SPM8, *http://www.fil.ion.ucl.ac.uk/spm*) with the following steps: realignment (correction for head motion-induced inter-volume displacement); normalized to the Montreal Neurological Institute (MNI) space using the unified normalization-segmentation procedure via the structural images; and spatially smoothed using a Gaussian kernel of 5-mm

166 full-width at half-maximum (FWHM). The default high-pass temporal filtering (1/128 Hz
167 cut-off) in SPM8 was also applied to remove low-frequency noise and signal drifts from the
168 fMRI time course of each voxel.

169

170 **Matching perceived intensity between painful and tactile stimuli**

171 To make sure that any detected difference in brain activity between painful and tactile
172 conditions was not driven by differences in perceived stimulus intensity, a subset of painful
173 and tactile trials with matched perceived intensity was selected using the following
174 procedure: for a given laser stimulus with perceived intensity rating of *r*, all electrical stimuli
175 with perceived intensity within the range of [*r*-0.5, *r*+0.5] were identified, and the electrical
176 stimulus with the closest rating was selected to pair with that particular laser stimulus; if no
177 electrical stimulus was identified within this range, the laser stimulus was labelled as
178 unmatched. In this way, the selected pairs of the laser and electrical stimuli were matched on
179 a trial-by-trial basis in terms of their perceived intensity. The differences in brain activity
180 between the intensity-matched painful and tactile stimuli are of our interest in the present
181 study. Note that, without otherwise defined, the term “intensity-matched” or “iso-intense”
182 in the present study refers to the fact that the *perceived* intensities were matched between
183 painful and tactile stimuli.

184

185 **Analysis (1): Identification of brain areas where the neural activity correlate with perceived**
186 **stimulus intensity regardless of stimulus modality**

The rationale behind the necessity of matching perceived stimulus modality when comparing brain responses to painful and tactile stimuli is that the amplitude of these brain responses depends on the perceived intensity. To formally test this, we performed a general linear model (GLM) analysis to identify brain areas where the neural activity correlated with the perceived stimulus intensity regardless of stimulus modality. In the GLM, the occurrence of all painful and tactile stimuli was collapsed into a single regressor with parametric modulation by their perceived intensity (i.e., stimulus subjective ratings). Six head motion parameters were included as covariates in the GLM. The contrast maps corresponding to the subjective ratings of all stimuli in the first-level analysis were further entered into a second-level one-sample t test to obtain group level results. A non-parametric permutation test (n=5,000) and corrected at cluster level or voxel level based on family-wise-error (FWE) method with a whole brain mask was used to determine the statistical significance (P <0.05 corrected). This permutation and multiple correction procedure was performed using the software package SnPM13(*http://warwick.ac.uk/snpm*).

Analysis (2): General linear model of iso-intense painful vs. tactile stimuli

For each participant, first-level statistical parametric maps were obtained using a GLM with regressors modeling the stimulus occurrence of each of five event types: intensity-matched painful stimuli, intensity-matched tactile stimuli, the remaining painful stimuli, the remaining tactile stimuli and the rating period. The temporal derivatives of the five conditions and the six head motion parameters (estimated from the realignment step during fMRI data preprocessing) were also included in the GLM as additional regressors. Three contrast

analyses were performed in each participant: (1) activation by intensity-matched painful stimuli, (2) activation by intensity-matched tactile stimuli, (3) differences in activation between intensity-matched painful and tactile stimuli. These individual contrast maps were fed into second-level analyses (one-sample t-test) to generate corresponding group-level results of the three contrast analyses. The statistical significance was then determined for each of the three group-level contrast results using the following methods.

As it has recently been shown that the GLM results are heavily dependent on the methods used for determining the statistical significance [17], we reported four sets of results obtained using four different methods for correcting multiple comparisons problem. In this way we provide a systematic investigation of the GLM results and meanwhile evaluate the robustness of the results. The four sets of GLM results were obtained using statistical *P* values determined by different multiple comparisons correction methods: (1) non-parametric permutation test and corrected at voxel level using the software package SnPM13 (Results Set 1), (2) non-parametric permutation test and corrected at cluster level using SnPM13 (Results Set 2), (3) random field theory (RFT) and corrected at voxel level using the software package SPM8 (Results Set 3), (4) RFT and corrected at cluster level using SPM8 (Results Set 4). All the above correction methods were based on FWE method. For all sets of results, the statistical significance level was set to $P < 0.05$ after correction. For cluster-level corrections, the cluster-defining threshold was set to $P < 0.001$ before correction. For the non-parametric permutation test, we performed 5,000 permutations. In each of these 5,000 permutations we randomly changed the sign of the voxel value of each subject and then performing one-sample t-test. Note that, a whole-brain mask was used for obtaining the

group-level activation map by painful sensation (i.e., Contrast 1) and the group-level activation map by tactile sensation (i.e., Contrast 2). Once the group-level painful and tactile activation maps were obtained, a union mask was created by taking the union of the thresholded painful and tactile activation maps and then used as a mask for determining the corrected *P* values of each voxel of the group-level difference map (i.e., Contrast 3). In addition, we also generated a conjunction map based on the thresholded group-level painful activation map and tactile activation map by taking the overlap of the two thresholded maps.

To further visualize the differences in fMRI responses to painful and tactile stimuli in the brain areas detected by the above Contrast 3, we extracted the time courses of raw fMRI signals (after preprocessing) of each identified cluster of each trial and then averaged across trials and participants for painful and tactile conditions separately. Although the interval between the stimulus and the rating period was randomized (between 3 s and 13 s) within a trial, the fMRI signals elicited by the sensory stimuli may temporally overlap with the fMRI signals elicited by the rating process (e.g., button press to indicate the rating on the VAS) in some brain areas. To remove these overlapped responses caused by rating process from the time courses of the stimulus-elicited fMRI responses, we also calculated an average time course of fMRI signals of the rating period for each identified brain area and then removed it from the time course of the fMRI signals of each condition for the given brain area. Only intensity-matched painful and tactile stimuli were used in this time course analysis.

Analysis (3): Model-free assessment of the time courses of BOLD signals during iso-intense

painful and tactile stimulation

Although the above voxel-wise GLM analysis has better spatial resolution, it faces more severe multiple comparisons problem, and, more importantly, it depends on the assumed haemodynamic response function (HRF) which might bias the results. Therefore, a region-wise model-free analysis was also performed to compare the time courses of fMRI signals between intensity-matched painful and tactile conditions. Specifically, the whole brain was divided into brain regions using pre-defined brain atlases. The same procedure for extracting the time courses of raw fMRI signals described above was then used to obtain the time courses of raw fMRI signals of each condition and each brain region defined by an atlas. The area-under-the-curve (AUC) was calculated for the time course of fMRI signals of each condition, each brain region and each participant. The AUC, as a measure of the fMRI responses to the stimuli, were then statistically compared between painful and tactile conditions using paired t test. The statistical significance was determined using non-parametric permutation test (n=5,000) and corrected for multiple comparisons using FWE (P <0.05 corrected). Here, two brain atlases were used to define brain regions. The first atlas was the combination of the Human Brainnetome Atlas (HBA) [18] (*http://atlas.brainnetome.org*) and the AAL-cerebellum atlas (i.e., the cerebellar regions in the AAL atlas). The HBA divides the cerebrum into 246 regions but does not include the cerebellum. By combining the HBA and the AAL-cerebellum atlas, we created a whole-brain atlas (labelled as 'HBA-AAL-cerebellum Atlas'). We noticed that the clusters identified in the GLM analysis were relatively small compared to the regions defined in this atlas. Therefore, a second atlas, which divides the whole brain into 1000 regions by splitting each of the AAL atlas regions into smaller regions (labelled as 'AAL-1000 Atlas') [49] and thus has a much

275 higher spatial resolution (i.e., more and smaller regions defined in this atlas), was also used.
276 Results obtained from both atlases were reported to provide compensatory information as
277 brain areas that are much smaller than the 'HBA-AAL-cerebellum Atlas' regions could be
278 missed in the first atlas while the second atlas faces more severe multiple comparisons
279 problem. This model-free analysis does not rely on any assumption about the shape, latency and
280 duration of the HRF which has been shown to vary across different brain regions [35; 54] and
281 different types of stimuli [39; 41].
282
283 **Analysis (4): testing the effect of perceived stimulus intensity on the responses of the identified**
284 **'modality-preferential' regions**
285 The Analyses 2 and 3 were to identify brain regions showing preferential responses to a given
286 modality (either pain or tactile sensation) while the perceived stimulus intensity was
287 carefully matched between the two modalities. To further test how the perceived stimulus
288 intensity would influence the responses of these brain regions showing modality preference,
289 we performed the fourth analysis (Analysis 4) to compare the responses of these brain
290 regions when the perceived stimulus intensity of the preferred modality was lower than that
291 of the non-preferred modality. That is, for the 'pain-preferential' brain regions identified
292 from the Analyses 2 and 3, we compared their responses to 'low-perceived-intensity' painful
293 stimuli with the responses to 'high-perceived-intensity' tactile stimuli. Similarly, for the
294 'tactile-processing-preferential' brain regions, we compared their responses to
295 'low-perceived-intensity' tactile stimuli with the responses to 'high-perceived-intensity'
296 painful stimuli. The painful and tactile stimuli were labelled as 'high perceived intensity' or

297 'low perceived intensity' for each participant using the follow procedure: all painful and
298 tactile stimuli were first pooled together and then median split into two groups – all stimuli
299 with perceived intensity higher than the median value were labelled as 'high perceived
300 intensity' and all stimuli with perceived intensity lower than the median value were labelled
301 as 'low perceived intensity'. The number of painful stimuli and the number tactile stimuli
302 that were being compared were also equalized by removing some stimuli (near the median
303 value) from the group that had more stimuli. For each of the 'pain-preferential' brain regions,
304 the time courses of fMRI responses to 'low-perceived-intensity' painful stimuli and the time
305 courses of fMRI responses to 'high-perceived-intensity' tactile stimuli were extracted and
306 then the corresponding AUCs were calculated, respectively, for each participant. Similarly, for
307 each of the 'tactile-processing-preferential' brain regions, we also obtained the AUC of the
308 time courses of fMRI responses to 'low-perceived-intensity' tactile stimuli and the AUC of the
309 time courses of fMRI responses to 'high-perceived-intensity' painful stimuli for each
310 participant. The AUCs of the two conditions (i.e., painful and tactile) were then statistically
311 compared using paired t test. The statistical significance was determined using the same
312 permutation test (n=5,000) and corrected for multiple comparisons using FWE (P<0.05
313 corrected) as described in Analysis 3.
314
315 **Results**
316 **Behavioral Data**
317 The physical and perceived intensities for painful and tactile stimuli at two levels (low vs.
318 high physical intensity) across all participants used in the present study are summarized in

Table 1. To rigorously match the perceived intensity between painful and tactile stimuli, a subset of stimuli was selected in each participant and the number of selected stimuli across participants were summarized as a histogram in Fig. 1a. The percentage of matched stimuli for every subject was also provided in Supplemental Fig. S1. The distribution of subjective intensity ratings of all painful and tactile stimuli from all participants before and after 'intensity matching' are displayed in Figs. 1b and 1c, respectively. The histograms showed that, after the 'intensity matching' procedure, the perceived intensities were well matched between painful and tactile stimuli.

Analysis (1): brain areas where the neural activity correlate with perceived stimulus intensity

Using a voxel-wise GLM analysis modelling the perceived stimulus intensity regardless of stimulus modality, we found that a broad network of brain areas in which the amplitude of fMRI responses correlated with the perceived stimulus intensity. The results obtained using cluster-level correction method and voxel-level correction method are shown in Fig. 2a and Supplemental Fig. S2a, respectively. Within this widely distributed network, the most pronounced brain areas are the primary sensorimotor cortex, the secondary somatosensory cortex, the supplementary motor area, the ACC, the insula, the visual cortex and some cerebellar areas (Fig. 2a). Most of these areas are the core regions often found to be activated by painful stimuli and indicated in the so-called "pain matrix" [24; 27; 28; 61]. The distribution of this brain network is very similar with the activation maps by painful and tactile stimulation obtained in Analysis 2 (Fig. 3a&b; see below for detailed results of Analysis

2). The conjunction analysis between these intensity-correlated brain areas (Fig. 2a) and the common areas activated by both painful and tactile stimuli (Fig. 3c) further confirmed that the neural activity of virtually all brain areas activated by both painful and tactile stimuli also correlated with the perceived stimulus intensity, regardless of stimulus modality (Fig. 2b).

Analysis (2): brain areas commonly and differentially activated by 'intensity-matched' painful and tactile stimuli using voxel-wise GLM analysis

We performed a second GLM analysis to identify the brain areas commonly (by conjunction analysis) and differentially (by contrast analysis) activated by painful and tactile stimuli while the perceived stimulus intensities were matched. The results of the different contrast and conjunction analyses obtained using the non-parametric permutation test are shown in Fig. 3a-c (corrected at P <0.05 cluster-level) and in Fig. 4a-c (corrected at P <0.05 voxel-level): (1) the activation by intensity-matched painful stimuli (Figs. 3a&4a), (2) the activation by intensity-matched tactile stimuli (Figs. 3b&4b), (3) the conjunction of the activation by both intensity-matched painful and tactile stimuli (yellow areas in Figs. 3c&4c), and (4) the differences in activation between intensity-matched painful and tactile stimuli (red and blue areas in Figs. 3c&4c).

The activation maps by painful and tactile sensations (Figs. 3a&b, 4a&b), as well as their conjunct map (yellow areas in Figs. 3c&4c), confirmed that transient painful and tactile stimuli elicit responses in a largely similar and widely distributed network of brain areas, similar to what we reported in our previous study [41]. Importantly, differences in activation between painful and tactile stimulation were also detected in both directions (Figs. 3c&4c).

Three clusters located in the bilateral Rolandic operculum and the left supplemental motor area (SMA) showed stronger activation during painful stimulation than during tactile stimulation (red areas in Figs. 3c&4c) and one cluster located in the left postcentral gyrus showed stronger activation during tactile stimulation than during painful stimulation (blue areas in Figs. 3c&4c), both using the voxel-level and cluster-level correction methods (see also Table 2). Cluster-level correction detected five more clusters showing stronger activation during tactile stimulation than during painful stimulation and located respectively in the right calcarine cortex, the right cerebellum, the right parietal inferior gyrus, the left parietal superior gyrus and the right frontal middle gyrus which (blue areas in Fig. 3c, Table 2). Similar results were also obtained using other correction methods based on conventional RFT at cluster-level or voxel-level, and are shown in Supplemental Figs S3-S4.

We further extracted the time courses of the fMRI signals of the nine clusters (Fig. 5a-i) detected by the non-parametric permutation test combined with cluster-level correction (Fig. 3c) to examine how differently they responded to painful and tactile stimuli. The results showed that all clusters responded to both painful and tactile stimuli but the response amplitude and/or duration, were different in the two conditions. In general, the fMRI response elicited by painful stimuli had larger amplitude and lasted longer in the three clusters detected to respond more strongly to pain (Fig. 5a-c). The fMRI response elicited by tactile stimuli had larger amplitude in all clusters detected to respond more strongly to tactile stimuli (Fig. 5d-h) except the cluster in the frontal middle gyrus of which the fMRI signals elicited by painful and tactile stimuli had similar amplitude but remained at high level

for painful condition (Fig. 5i). The fMRI responses at each time point (Fig. 5) and the AUC of the time courses (Fig. 7a) were also compared between the painful and tactile conditions for each cluster using paired t test.

Furthermore, it is notable that the peak of the responses to painful stimuli occurred later than that of the responses to tactile stimuli by one or two time points in five clusters located respectively in the bilateral Rolandic Operculum, the left SMA, the left postcentral gyrus and the cerebellum (Fig. 5a-e). This difference in peak time between responses to painful and tactile stimuli is likely to be due to the difference in conduction time of peripheral nervous system between nociceptive and tactile information [31; 42; 52]. More interestingly, the peak of the responses to both painful and tactile stimuli occurred later in most of the 'tactile-processing-preferential' areas (Fig. 5f-i) than the 'pain-processing-preferential' areas (Fig. 5a-c).

Analysis (3): brain areas differentially activated by 'intensity-matched' painful and tactile stimuli using region-wise model-free analysis

All regions detected by the model-free analyses showed stronger responses (i.e., higher amplitude) to painful stimuli than to tactile stimuli (Fig. 6). Using the 'HBA-AAL-cerebellum' atlas, three regions were detected to respond more strongly to painful and tactile stimuli: the right frontal middle gyrus, the right frontal inferior orbital gyrus and the right insula (see Fig. 6a-c for their exact spatial locations). Using the 'AAL-1000' atlas, two regions were detected: the right Rolandic operculum and the right insula (see Fig. 6d-e for their exact spatial locations). The time courses of the fMRI signals of these regions were shown in Fig. 6.

Similarly to what was observed in the GLM analysis, fMRI responses to painful stimuli had larger amplitude, longer duration and peaked later than fMRI responses to tactile stimuli (Fig. 6). No regions were detected to respond more strongly to tactile stimuli than to painful stimuli.

Analysis (4): responses of 'modality-preferential' regions were affected by perceived stimulus intensity

Eight clusters in total were identified as 'pain-preferential' areas in Analyses 2 and 3. Their fMRI time courses elicited by 'low-perceived-intensity' painful stimuli and by 'high-perceived-intensity' tactile stimuli were shown in Fig. 8, and the results of statistical comparisons were shown in Fig. 10a. We observed that only one cluster in the right insula showed significant difference in the AUC between painful and tactile conditions ($p=0.024$; Fig. 10a). However, most (seven out of eight) areas showed a trend of higher responses to 'high-intensity' tactile stimuli than to 'low-intensity' painful stimuli, indicating that the responses of these areas were mainly determined by stimulus intensity and their preference to pain can only be observed when stimulus intensity were matched. The only exception is the cluster located in the frontal middle gyrus which still showed higher and longer-lasting responses to painful stimuli than to tactile stimuli even when painful stimuli were perceived less intense than tactile stimuli, although the difference in AUC did not reach the significance level.

Six clusters in total were identified as 'tactile-processing-preferential' areas in Analyses 2 and 3. Their fMRI time courses elicited by 'low-perceived-intensity' tactile stimuli and by 'high-perceived-intensity' painful stimuli were shown in Fig 9, and the results of statistical

comparisons were shown in Fig. 10b. We observed that none of these clusters had significantly different AUC between painful and tactile conditions. Indeed, five out of six areas had similar time courses of fMRI responses to 'high-intensity' tactile stimuli and to 'low-intensity' painful stimuli, indicating that the response preference of these areas were canceled out by the difference in perceived stimulus intensity. The only exception is the cluster located in the postcentral gyrus which still showed a trend of higher responses to tactile stimuli than to painful stimuli even when tactile stimuli were perceived less intense than painful stimuli.

Discussion

The main objective of the present study was to characterize the differences in the brain responses elicited by transient painful and tactile stimuli. When comparing the responses elicited by transient and fast-rising stimuli, a major confound consists in difference in perceived stimulus intensity [8; 26]. Thus, here we ensured that perceived stimulus intensity was strictly matched between the two modalities on a trial-by-trial basis. FMRI data were explored using both voxel-wise GLM analysis and region-wise model-free analysis, and the robustness of the GLM results were also tested using different multiple comparisons correction methods. We found four main results. First, brain areas activated by transient painful stimuli were also activated by transient tactile stimuli (Figs. 3c&4c, yellow), confirming a number of previous findings [2; 41; 59; 61; 62]. Second, the amplitude of neural activity in all these activated areas correlated with the perceived stimulus intensity, highlighting the importance of matching perceived intensity when comparing brain

451 responses to painful and tactile stimuli. Third, when perceived intensity was rigorously
452 matched between painful and tactile stimuli, several brain areas were found to respond
453 differentially to painful and tactile stimuli, including areas responding more strongly to
454 painful stimuli (Figs. 3c&4c, red) and areas responding more strongly to tactile stimuli (Figs.
455 3c&4c, blue). Fourth, the responses of these identified 'modality-preferential' brain areas
456 were determined by both stimulus modality and stimulus intensity. These results indicate
457 that, although sudden painful and tactile stimuli activate the same set of brain areas and the
458 perceived stimulus intensity is an important determining factor of their neural responses,
459 different areas may have different preference in processing painful vs. tactile sensations.
460
461 **Identification of pain-preferential neural activities requires rigorous matching of stimulus**
462 **intensity**
463 To identify brain areas that respond preferentially to pain, it is mandatory to compare brain
464 responses to painful and non-painful stimuli. Here, we chose painful nociceptive stimuli and
465 non-painful tactile stimuli because both belong to somatosensory domain but only the
466 former elicits painful sensation. However, a key confound in such analyses is the perceived
467 stimulus intensity which has been often neglected in previous studies (for example,
468 comparing brain responses elicited by high-temperature painful heat with those elicited by
469 low-temperature warmth; [8; 62]). Indeed, our results showed that responses in virtually all
470 brain areas activated by painful and tactile stimuli depend on perceived stimulus intensity
471 (Figs. 3c&4c, yellow). This result highlights the necessity of matching perceived intensity
472 when performing such comparisons. Here, we aimed to match perceived stimulus intensity

473 rather than physical intensity because it has been shown that there is a large inter-subject
474 variability of sensory sensitivity [7; 44]. In other words, two stimuli of identical physical
475 intensity could be perceived very differently by different participants. Note that, the pain
476 elicited neural activity has also been related to salience processing. Although 'perceived
477 stimulus intensity' and 'stimulus salience' are two different concepts and can be perceptually
478 (and psychophysically) distinguished from each other in several contexts [26; 55], these two
479 measures are highly correlated and indistinguishable in most scenarios such as the present
480 experimental design. It should also be noted that, to ensure a rigorous match of stimulus
481 intensity between painful and tactile conditions, we had to discard some trials in each
482 participant, which resulted in unequal number of trials across participants and might have
483 affected the statistical significance of our results.
484
485 **Transient painful and tactile stimuli largely activate the same set of brain areas**
486 Our finding that the brain areas activated by painful stimuli can also be activated by tactile
487 stimuli (yellow areas in Figs. 3c&4c) confirmed our previous finding with a different dataset
488 [41]. Furthermore, all clusters identified to respond differentially to painful and tactile stimuli
489 (red and blue areas in Figs. 3c&4c) were located inside the conjunct activated areas. This
490 finding indicates that, although these brain areas were detected to respond differentially to
491 intensity-matched painful and tactile stimuli (see the results of Analysis 3), they were not
492 exclusively responding to either modality, but responding to both modalities. This suggests
493 that pain-specific information may not be encoded in any exclusively dedicated brain region.
494 However, it should be noted that only transient stimuli were tested in the present study, and

thus the results cannot be generalized to longer-lasting painful stimuli.

Certain brain areas responding more strongly to painful stimuli

Several brain areas were detected to respond differentially to painful and tactile stimuli even when perceived stimulus intensity was strictly matched between the two conditions. Among these brain areas, the bilateral parietal operculum, the left SMA, the right insula and the bilateral prefrontal areas were found to respond more strongly to painful stimuli than to tactile stimuli (Table 2, Figs. 5&6).

The involvement of the parietal operculum (largely corresponding to the secondary somatosensory area, S2) and the insula in somatosensory processing is well known and reported in a large number of studies [11; 22; 23; 29; 32; 50]. The particular involvement of the operculoinsular areas in human pain processing has been suggested in a previous study utilizing a variety of neuroimaging techniques including PET, fMRI, ERP (event-related potentials) from scalp EEG and intracerebral recordings of evoked potentials [50]. It has also been shown that electrical stimulation in the operculoinsular cortex could elicit pain sensations [1; 32; 46]. However, adequate control stimuli with matched intensity were lacking in these previous studies. Our current results obtained from intensity-matched stimuli provided more solid evidence supporting a preferential role of operculoinsular cortex in pain processing in the human brain.

The SMA contralateral to the stimulated site was also found here to respond more strongly to painful than to tactile stimuli. The SMA is traditionally associated with motor-related functions, especially more complex movements, such as movement sequence planning and

517 motor learning [43]. Stimulation of the SMA could evoke movements or even just the urge to
518 move or movement inhibition [20; 21]. Therefore, the observed stronger activation in the left
519 SMA (i.e., contralateral to the stimulated side) during painful stimulation on the right foot
520 may be related to an intrinsically closer relationship between pain and survival: although
521 there is no explicit movement directly related to painful or tactile stimuli in the present
522 experiment, painful stimuli could implicitly elicit, consciously or subconsciously, an urge for
523 an escape action to a greater extent than tactile stimuli, even though the perceived stimulus
524 intensity is strictly matched between the two conditions.
525 The model-free regional analysis further identified two lateral prefrontal areas such as the
526 frontal middle gyrus and the frontal inferior orbital gyrus to have higher and longer
527 responses to painful than to tactile stimuli. Even when painful stimuli were less intense than
528 tactile stimuli, the frontal middle gyrus still showed somewhat stronger and longer response
529 to painful than to tactile stimuli (Fig. 8c). These lateral prefrontal areas are thought to be at
530 higher hierarchical levels in cognitive functions, associated with working memory [37; 47-49],
531 episodic memory [12; 57], attention [9; 10; 19] and emotional processing [4; 45; 64]. As pain
532 is multidimensional, including not only sensory components but also affective and cognitive
533 components, the initial sensory components of pain sensations could further elicit a series of
534 higher cognitive activities which might underlies the higher and longer responses we
535 observed in these lateral prefrontal areas. However, it is also worth noting that the painful
536 sensation elicited by laser stimuli might last longer than the tactile sensation elicited by
537 electrical stimuli. However, the difference in the duration of fMRI responses was not
538 observed ubiquitously in the activated brain areas, suggesting that our results cannot be

539 explained by the difference in the duration of sensory input alone.

540 Interestingly, the cluster located in the right frontal middle gyrus was detected by the GLM
541 analysis to be a 'tactile > painful' area. However, this cluster actually had longer responses to
542 painful than to tactile stimuli (Fig. 6i). The reason that GLM detected this area to respond
543 more strongly to tactile stimuli is, at least partly, due to the fact that the GLM analysis relies
544 on the assumption of the shape of the HRF: the waveform of BOLD signals elicited by tactile
545 stimuli followed a regular increasing-decreasing changes (i.e., bell shape) and thus fit better
546 with the assumed shape of the HRF; whereas the waveform of BOLD signals elicited by
547 painful stimuli remained at high level after reaching the peak.

548

549 **Brain areas responding more strongly to tactile stimuli**

550 We found five clusters located in the left postcentral gyrus, the calcarine cortex, the right
551 cerebellum, the right parietal inferior gyrus and the left parietal superior gyrus, respectively,
552 and to respond more strongly to tactile stimuli than to painful stimuli using voxel-wise GLM
553 analysis, which was further confirmed by their waveforms of the BOLD signals (Fig. 5d-h).
554 Furthermore, these areas did not show stronger responses to painful stimuli even when
555 painful stimuli were perceived more intense than tactile stimuli (Fig. 9), especially for the left
556 postcentral gyrus which still showed a trend of stronger responses to tactile stimuli with low
557 perceived intensity (Fig. 9a). Another interesting finding is that the peak of the responses to
558 both painful and tactile stimuli occurred later by in the 'tactile-processing-preferential' areas
559 (i.e., at the 7^{th} and 6^{th} TR after stimulus onset for painful and tactile stimulation, respectively;
560 Fig. 5d-h) than the 'pain-processing-preferential' areas (i.e., at the 8^{th} and 7^{th} TR after

561 stimulus onset for painful and tactile stimulation, respectively; Fig. 5a-c). This peak time
562 difference observed between the two different groups of brain areas also suggests that these
563 brain areas serve different functions in processing painful and tactile information. These
564 observations are somewhat unexpected and requires further investigation.
565 Note that 'tactile-processing-preferential' areas were only detected using voxel-wise GLM
566 analysis but not using other analysis approaches. Many factors can contribute to this
567 discrepancy. For example, different approaches may be sensitive in detecting different types
568 of information: GLM approach does not require *a priori* definition of brain regions and thus
569 can detect clusters of any shape in the brain, but this approach is based on the assumption
570 of haemodynamic response function (HRF) and thus is only sensitive in detecting brain areas
571 where the temporal dynamics of the fMRI responses is well fitted with the presumed HRF.
572 On the contrary, the model-free analysis does not require any assumption of the HRF and
573 thus is more sensitive in detecting brain areas with arbitrary temporal dynamics of fMRI
574 responses, but it is less powerful in detecting clusters with arbitrary spatial shapes that do
575 not fit the brain parcellation predefined by an atlas.
576
577 **Conclusion**
578 By rigorously matching the perceived stimulus intensity and comparing the brain responses
579 to painful and tactile stimuli, we confirmed that iso-intense painful and tactile stimulation
580 activate the same set of brain areas, indicating that brain regions exclusively dedicated to
581 encoding pain-specific information is unlikely to exist. Furthermore, although activated by
582 both types of stimuli, when perceived intensity is rigorously matched some of these areas

583 respond more strongly to painful sensation and others respond more strongly to tactile
584 sensation, suggesting that different brain areas may preferentially process painful or tactile
585 information. It should be noted that our current findings were derived from very artificial
586 experimental pain and thus may be limited in clinical translations. Further investigations are
587 needed to understand how clinical acute, subacute and chronic pain are specifically
588 represented in the human brain.

589

590

591 **Conflict of interest**

592 The authors declare no competing financial interests.

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602

603 **Data and code availability statement**

604 The code supporting the findings of this study are available from the corresponding author

upon request.

Ethics statement

All participants provided written informed consent prior to the experiment, and the experimental procedures were approved by the Medical Research Ethics Committee of Tianjin Medical University.

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787

788 # Table

789

790 **Table 1.** The physical and perceived intensities of all painful and tactile stimuli at two levels

791 across participants.

	Painful stimuli		Tactile stimuli	
	Low physical level (Mean±SD; Range)	High physical level (Mean±SD; Range)	Low physical level (Mean±SD; Range)	High physical level (Mean±SD; Range)
Physical intensity	3.87±0.93J; 1.75 – 5.75J	4.57±0.93J; 2.25 – 6.25J	6.27±4.40mA; 1.00 –20.00mA	13.07±8.11mA; 2.80 – 33.00mA
Perceived intensity	2.76±1.54; 0.00 – 7.93	5.59±1.51; 2.00 – 9.90	3.06±1.14; 0.38 – 7.05	5.65±1.26 1.65 – 10.00

792

793

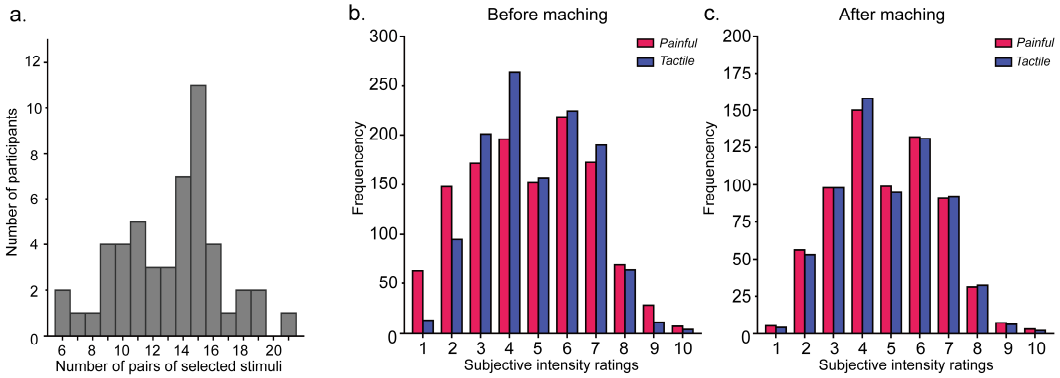
794 **Table 2.** Clusters showing significantly different responses to intensity-matched painful and
795 tactile stimuli identified by non-parametric permutation test and corrected at cluster level. L:
796 left; R: right.

Regions	Cluster size (voxels)	Peak intensity (T / *P* value)	Coordinates (x, y ,z)
Painful > Tactile			
Rolandic Operculum (R)	84	6.838/ 5.379E-9	60, 6, 9
Rolandic Operculum (L)	70	7.389/ 7.401E-10	-57, 3, 9
Supplemental Motor Area (L)	100	5.403/ 9.116E-7	-9, -9, 69
Painful < Tactile			
Postcentral Gyrus (L)	320	-5.544/ 5.544E-7	-54, -27, 54
Calcarine (L, R)	495	-4.769/ 8.210E-6	24, -51, -15
Cerebellum (R)	53	-5.499/ 6.499E-7	21, -51, -18
Parietal Inferior Gyrus (R)	72	-4.267/ 4.405E-5	30, -48, 39
Parietal Superior Gyrus (L)	100	-4.470/ 2.251E-6	-27, -57, 54
Frontal Middle Gyrus (R)	55	-4.820/ 6.899E-6	30, 54, 0

797

798 **Figure**

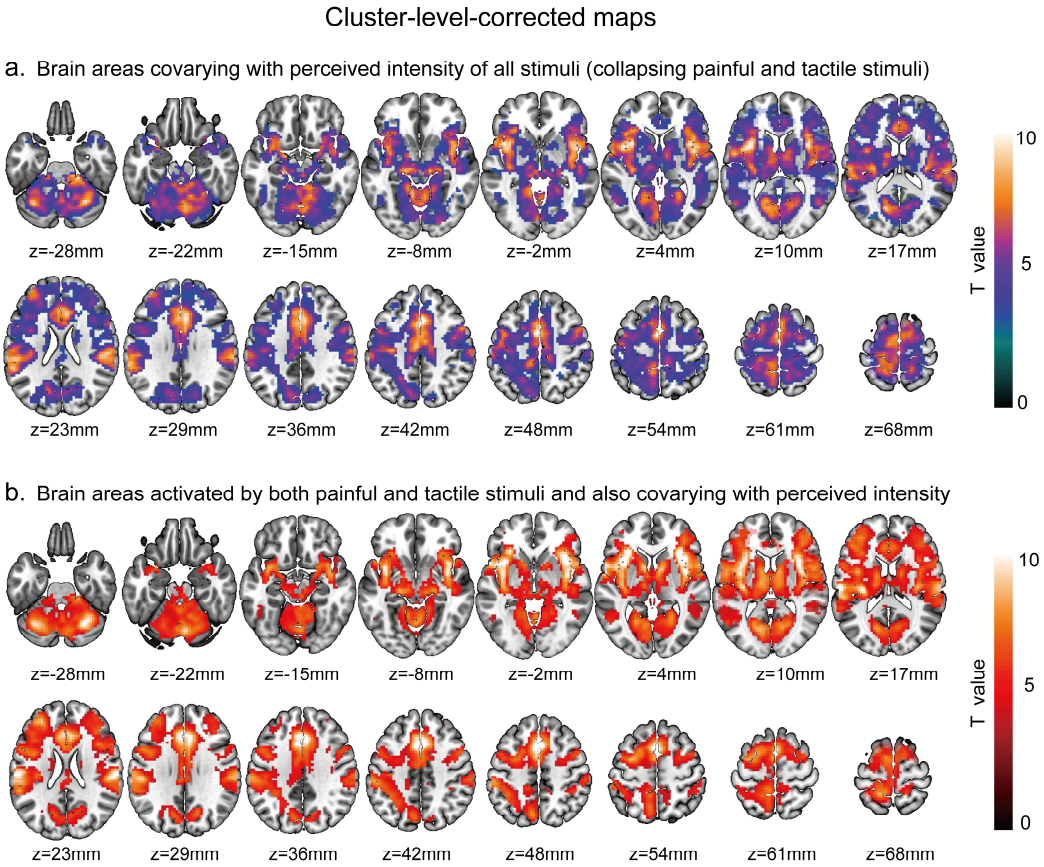
799



800

801 **Figure 1.** The histogram of the number of selected stimuli with matched perceived intensity
802 (a), the histograms of subjective intensity ratings of *all* painful and tactile stimuli (b) and the
803 histograms of the subjective intensity ratings of the *selected* painful and tactile stimuli with
804 matched perceived intensity (c). In b and c, the histograms for painful stimuli are shown in
805 red and the histograms for tactile stimuli are shown in blue.

806



807

808 **Figure 2.** The brain areas in which the neural activity correlated with perceived stimulus

809 intensity regardless of stimulus modality (a) and the conjunct areas activated by both painful

810 and tactile stimuli and at the same time correlated with the perceived stimulus intensity (b).

811 These results were corrected at cluster level.

812

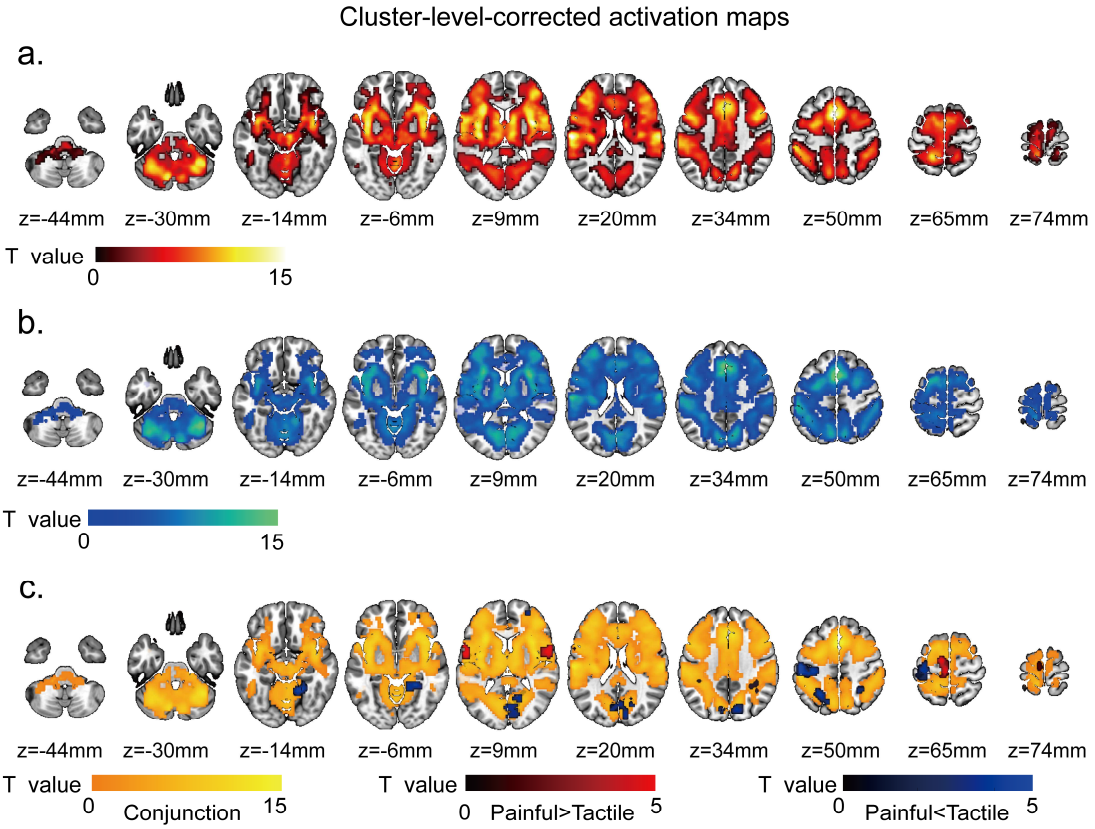
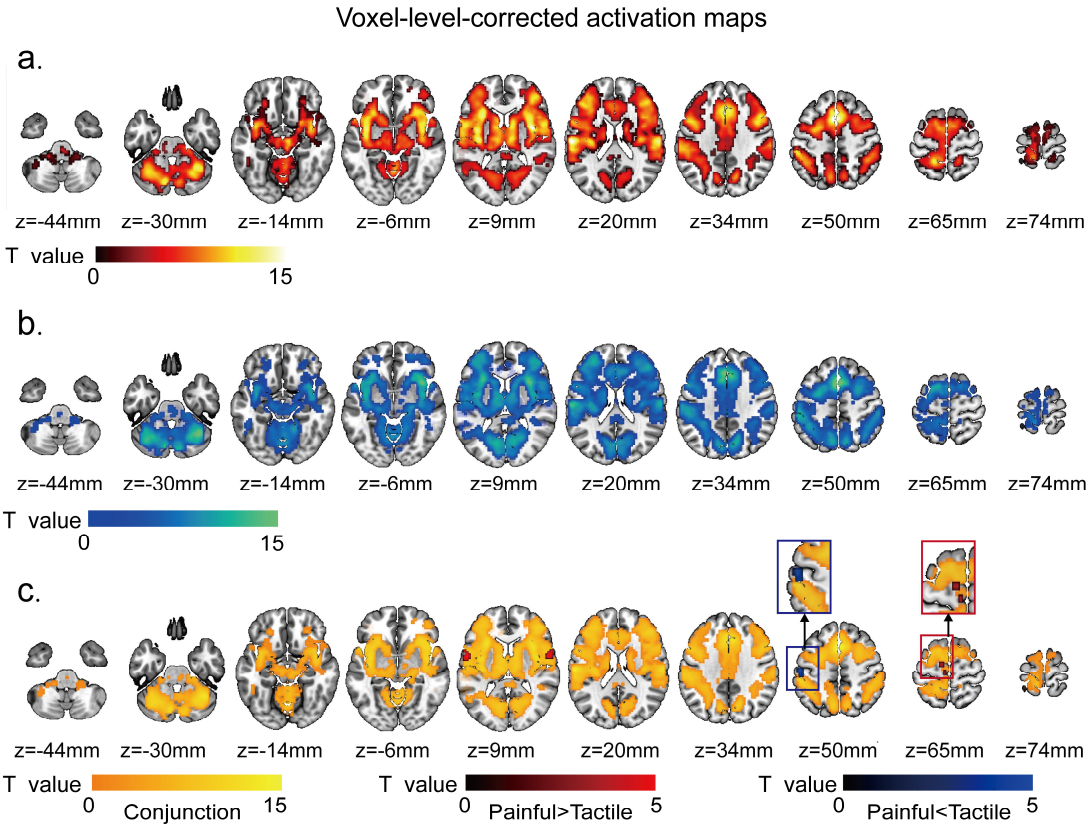


Figure 3. Results of GLM analyses obtained using non-parametric permutation test and corrected using FWE at cluster level (P <0.05 corrected; cluster defining threshold P <0.001): (a) activation map by 'intensity-matched' painful sensation, (b) activation map by 'intensity-matched' tactile sensation, (c) conjunct activation map (yellow areas) and the areas activated more strongly by painful stimuli than by tactile stimuli (red areas) and the areas activated more strongly by tactile stimuli than by painful stimuli (blue areas).



821

822 **Figure 4**. Results of GLM analyses obtained using non-parametric permutation test and

823 corrected using FWE at voxel level (*P* <0.05 corrected): (a) activation map by

824 'intensity-matched' painful sensation, (b) activation map by 'intensity-matched' tactile

825 sensation, (c) conjunct activation map (yellow areas) and the areas activated more strongly

826 by painful than by tactile stimuli (red areas) and the areas activated more strongly by tactile

827 than by painful stimuli (blue areas).

828

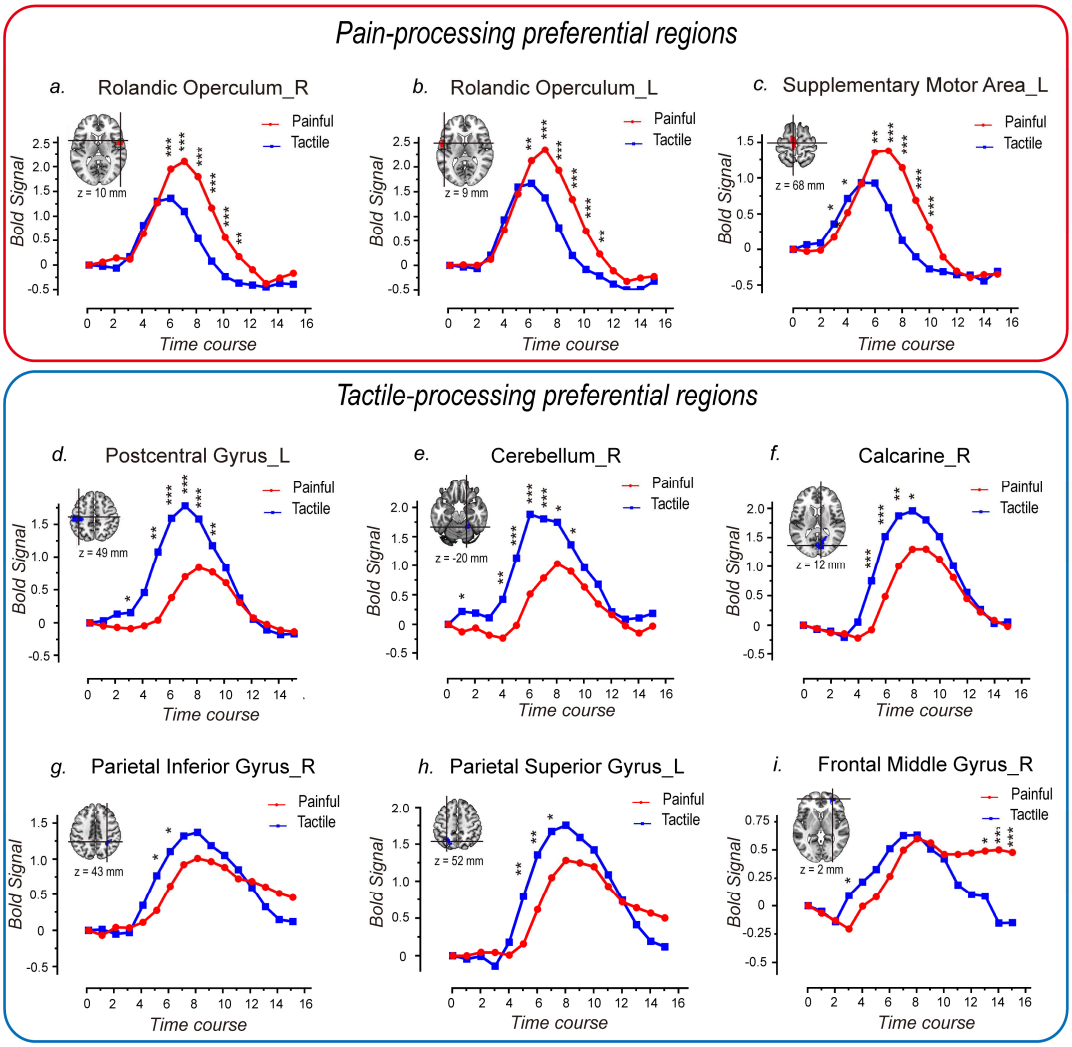


Figure 5. The time courses of the fMRI signals extracted from the nine clusters activated differently by painful and tactile stimuli detected using voxel-wise GLM analysis (red: painful; blue: tactile). Three clusters were identified as 'painful>tactile' (a-c): they were located in the right Rolandic operculum (a), the left Rolandic operculum (b), and the left supplemental motor area (c) and showed greater signal amplitude and longer duration for painful sensation than for tactile sensation. Six clusters were identified as 'tactile>painful' and located in the left postcentral gyrus (d), the right cerebellum (e), the right calcarine (f), the right parietal inferior gyrus (g), the left parietal superior gyrus (h) and the right frontal middle gyrus (i), respectively. The first five clusters showed greater signal amplitude for tactile than

for painful sensation (d-h). For the sixth cluster located in the right frontal middle gyrus (i), although detected as 'tactile>painful' by GLM, the fMRI signals increased to a similar amplitude after both painful and tactile stimuli but did not return to baseline for painful stimulation. Paired t test was also performed to compare the signal amplitude between painful and tactile conditions for each time point, and the time points at which the fMRI signal amplitudes were significantly different are indicated by asterisks. *, P <0.05; **, P <0.01; ***, P <0.001.

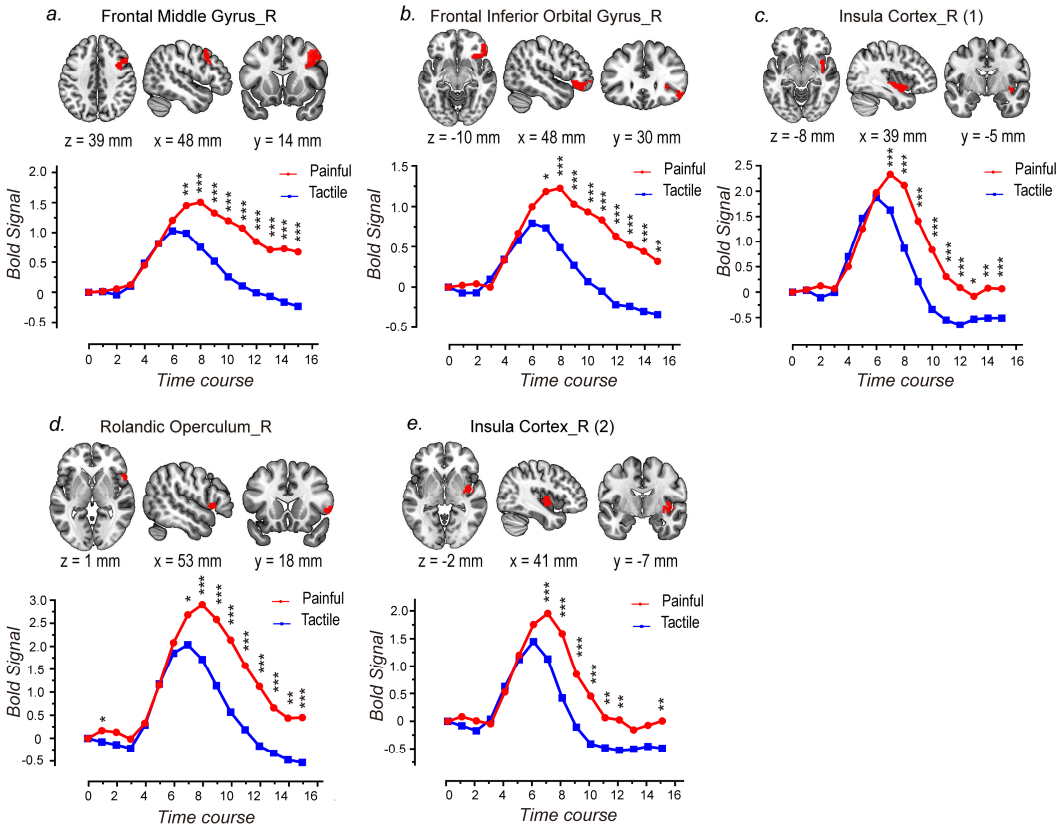


Figure 6. The locations of the brain regions responding more strongly to painful than to tactile stimuli, along with their time courses of the fMRI responses under the two conditions, identified by the region-wise model-free analysis using the 'HBA-AAL-cerebellum' atlas (a-c) and using the 'AAL-1000' atlas (d-e). Paired t test was also performed to compare the signal amplitude

852 between painful and tactile conditions for each time point, and the time points at which the
853 fMRI signal amplitudes were significantly different are indicated by asterisks. *, P <0.05; **, P
854 <0.01, ***, P <0.001.

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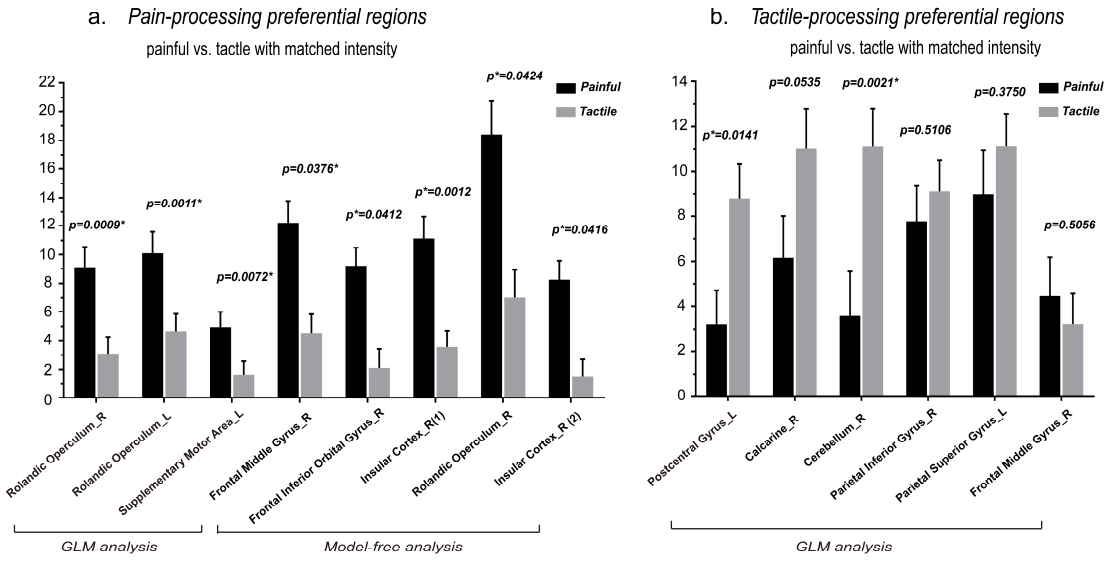


856

857

858 **Figure 7.** Comparisons of the area under curve (AUC) of the time courses of the fMRI signals
859 between intensity-matched 'painful' condition and 'tactile' condition for the eight clusters
860 that were identified as 'painful > tactile' (a) and for the six clusters that were identified as
861 'tactile > painful' (b). The AUCs were compared between painful and tactile conditions using
862 paired t test. The error bars indicate the standard error of mean.

863

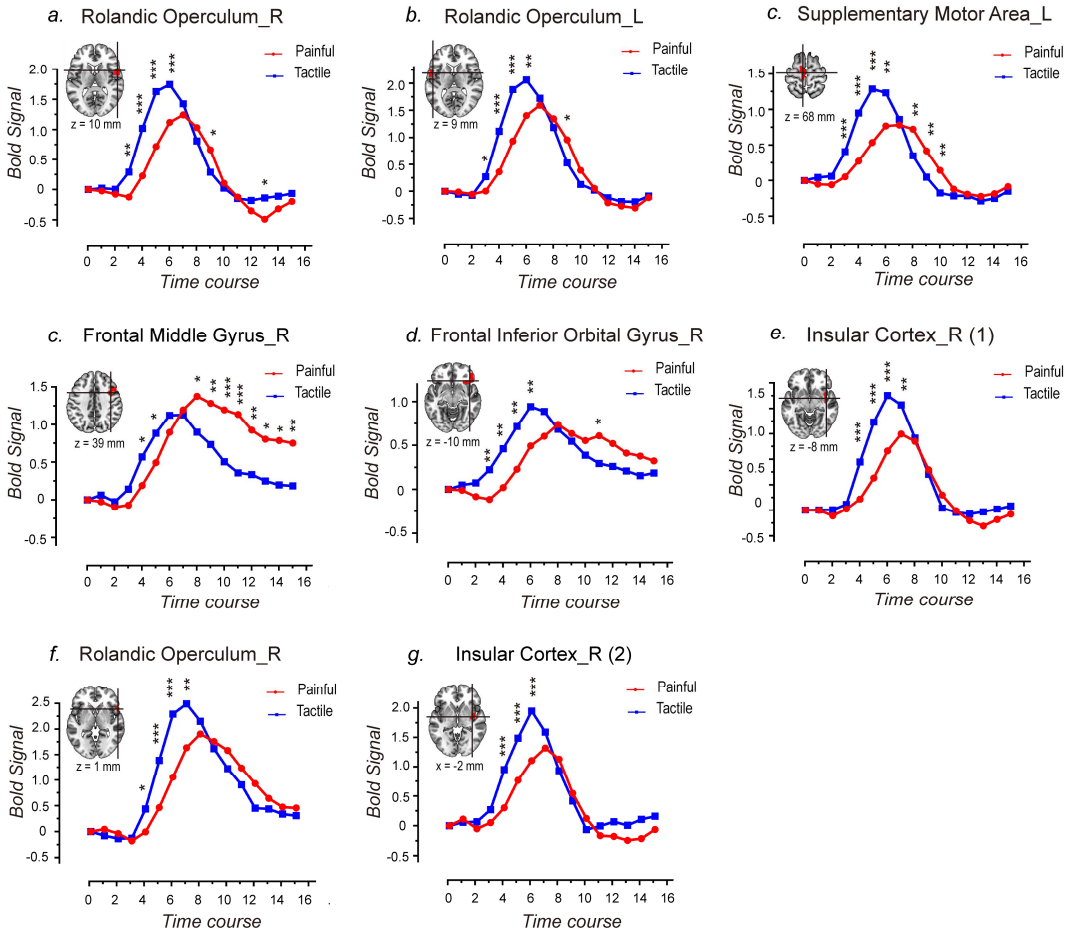
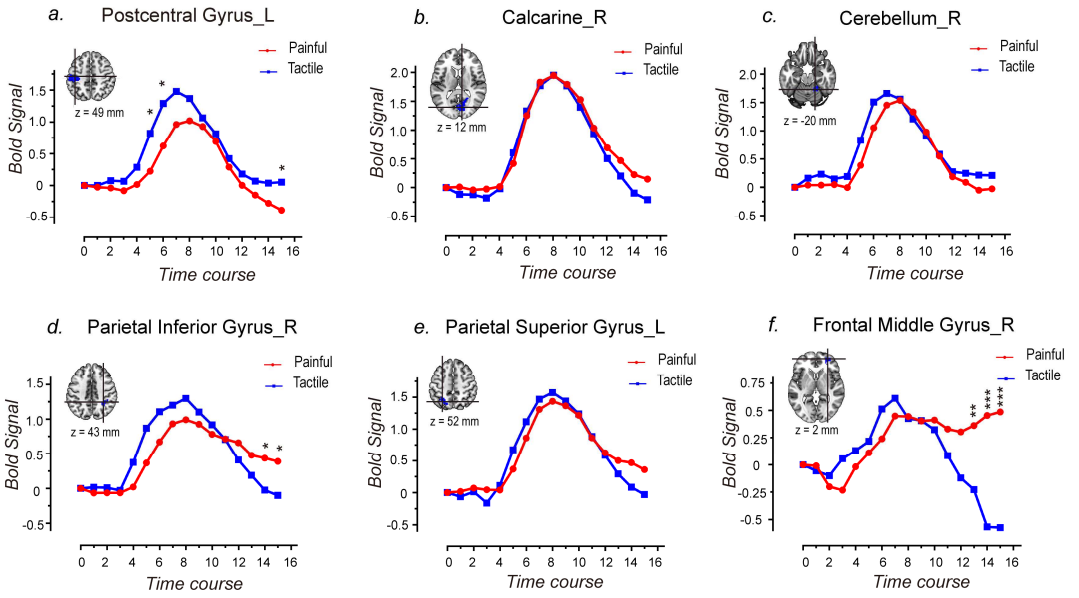


Figure 8. Comparisons of the fMRI time courses between 'low-perceived-intensity' painful stimuli (in red) and 'high-perceived-intensity' tactile stimuli (in blue) for the eight 'pain-preferential' clusters identified in Analyses 2 and 3. Paired t test was performed to compare the signal amplitude between painful and tactile conditions for each time point, and the significance are indicated by asterisks (*, P <0.05; **, P <0.01; ***, P <0.001).



871

872 **Figure 9.** Comparisons of the fMRI time courses between 'low-perceived-intensity' tactile stimuli

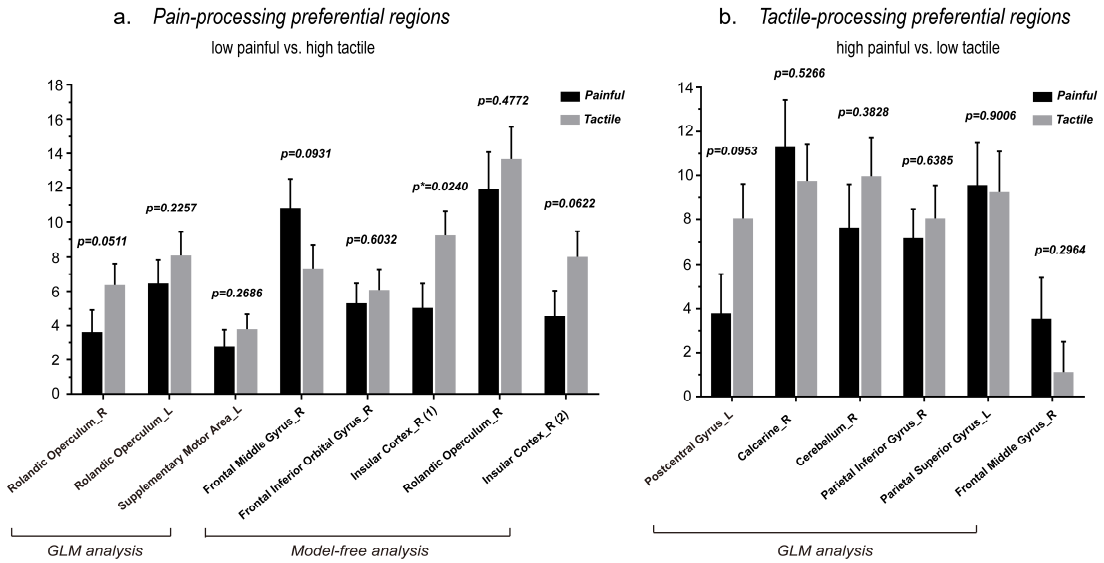
873 (in blue) and 'high-perceived-intensity' painful stimuli (in red) for the six

874 'tactile-processing-preferential' clusters identified in Analyses 2 and 3. Paired t test was

875 performed to compare the signal amplitude between painful and tactile conditions for each time

876 point, and the significance are indicated by asterisks (*, P <0.05; **, P <0.01; ***, P <0.001).

877



878

879 **Figure 10.** Comparisons of the AUC of the time courses of the fMRI signals between

880 'low-perceived-intensity painful' condition and 'high-perceived-intensity tactile' condition for the
881 eight clusters that were identified as 'painful > tactile' (a) and comparisons of the AUC of the time
882 courses of the fMRI signals between 'low-perceived-intensity tactile' condition and
883 'high-perceived-intensity painful' condition for the six clusters that were identified as 'tactile >
884 painful' (b). The AUCs were compared between painful and tactile conditions using paired t test.
885 The error bars indicate the standard error of mean.