Late responses in the anterior insula reflect the cognitive component of pain: evidence of nonpain processing

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Late responses in the anterior insula reflect the cognitive component of pain: evidence of nonpain processing

Nami Taniguchi[a,b,*], Naruhito Hironaga[a], Takako Mitsudo[a], Shunsuke Tamura[c], Ken Yamaura[b], Shozo Tobimatsu[a,d]

Abstract

Introduction: Pain is a complex experience influenced by sensory and psychological factors. The insula is considered to be a core part of the pain network in the brain. Previous studies have suggested a relationship between the posterior insula (PI) and sensory processing, and between the anterior insula (AI) and cognitive–affective factors.

Objectives: Our aim was to distinguish sensory and cognitive responses in pain-related insular activities.

Methods: We recorded spatiotemporal insular activation patterns of healthy participants (n = 20) during pain or tactile processing with painful or nonpainful movie stimuli, using a magnetoencephalography. We compared the peak latency between PI and AI activities in each stimulus condition, and between pain and tactile processing in each response. The peak latency and amplitude between different movies were then examined to explore the effects of cognitive influence. A visual analogue scale was used to assess subjective perception.

Results: The results revealed one clear PI activity and 2 AI activities (early and late) in insular responses induced by pain/tactile stimulation. The early response transmitted from the PI to AI was observed during sensory-associated brain activity, whereas the late AI response was observed during cognitive-associated activity. In addition, we found that painful movie stimuli had a significant influence on both late AI activity and subjective perception, caused by nonpainful actual stimulation.

Conclusions: The current findings suggested that late AI activation reflects the processing of cognitive pain information, whereas the PI and early AI responses reflect sensory processing.

Keywords: Pain, Cognition, Anterior insula, Posterior insula, Neuroimaging

1. Introduction

Pain is a complex experience that is caused not only by sensory input but also by contextual processes that are influenced by cognition, emotion, anticipation, and memories.[44] Subjective pain perception can vary according to the situation.[52,58,61] Many studies have reported placebo (nocebo) effects induced by positive (negative) expectation[5,7,8,13] and the effectiveness of cognitive behavioral therapy for chronic pain.[8,46] However, identifying the psychological factors of pain is still difficult because they are not visualized and objective biomarkers have not yet been determined.

Pain assessment using brain imaging techniques has been expected to provide objective indicators[47,51] and biomarkers[49] of pain. Pain-related brain activity has been explored,[3,6,48,50,55] and

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[a] Department of Clinical Neurophysiology, Neurological Institute, Faculty of Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, [b] Department of Anesthesiology and Critical Care Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, [c] Department of Informatics, Graduate School of Information Science and Electrical Engineering, Kyushu University, Fukuoka, Japan, [d] Department of Orthoptics, Faculty of Medicine, Fukuoka International University of Health and Welfare, Fukuoka, Japan

**Corresponding author. Address: Department of Clinical Neurophysiology, Neurological Institute, Faculty of Medicine, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel.: +81-92-642-5543; fax: +81-92-642-5545. E-mail address: n-tani@med.kyushu-u.ac.jp (N. Taniguchi).*

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the regions have been proposed 2 aspects; a sensory-discriminative pathway in the primary and secondary somatosensory cortices and the posterior insula (PI), and the cognitive–affective dimensions in the anterior insula (AI), the anterior cingulate cortex, the prefrontal cortex, amygdala, and thalamus.[1,38] Pain perception is considered to occur as a result of complex pain processing,[6,50] and the insula is a key structure for sensory and psychological components.

The PI is thought to underlie the connection between sensory and motor tasks, whereas the AI is considered to have a stronger relationship with cognition, emotion,[39,54] and higher-order networks such as processing of salience, integration, and awareness.[16,60] In pain processing, PI activity has been reported to correlate with the objective intensity of a pain stimulus, and AI activity with subjective pain perception.[15,35] Furthermore, AI activation is thought to reflect psychological factors of pain.[2,22,23,41] Because most of these studies have used functional magnetic resonance imaging (fMRI),[35,39,60] it is currently unclear how and when pain processing occurs in the PI and AI, and the identification of sensory and psychological components of pain-related brain activity remain to be clarified.

Recently, our magnetoencephalography (MEG) study revealed that pain-related PI activity and pain perception were suppressed by tactile stimulation.[26] These results suggest that sensory input can modulate the sensory part of pain processing. We assumed that AI activity would be influenced by cognitive factors of pain. In this study, we measured participants' brain responses elicited by pain/tactile stimulation while watching different movies as cognitive stimulation because movie inputs enable us to assess multiple aspects of visual information[40] and to easily elicit sensations such as pain or touch.[48] The aim of this study was 3-fold: (1) Identification of the spatiotemporal profiles of pain processing in the PI and AI compared with a nonpainful stimulus condition. (2) Examination of whether different cognitive information modulates insular activity. (3) Exploration of the relationships between insular activity and whole-brain activity. Thus, we sought to test 3 corresponding hypotheses that: (1) activity patterns in the PI and AI would be differentiated in the pain and tactile conditions; (2) AI activity would differ between painful and nonpainful movies; and (3) there would be a relationship between the PI and sensory-associated areas and between the AI and cognitive-associated areas.

2. Methods

2.1. Participants

Twenty healthy participants (10 females; 33.4 ± 7.3 years) took part in our experiment. However, 2 participants were excluded because of no apparent somatosensory responses. All participants were right-handed and had no history of chronic pain or neurological conditions. Informed consent was obtained from each participant according to the latest version of the Declaration of Helsinki. The study was approved by the institutional ethics committee of Kyushu University and registered in a publicly accessible database in the University Hospital Medical Information Network (UMIN) in Japan (UMIN ID: UMIN000035966).

2.2. Sensory and visual stimuli

We used intraepidermal electrical stimulation and mechanical tactile stimulation devices following our previous study.[26] The details are provided in supplementary materials (available at http://links.lww.com/PR9/A146). The pain stimulus intensity was adjusted for each participant to a tolerable pinprick sensation corresponding to 30 to 40/100 on a sensory visual analogue scale (VAS) extending from 0 (no pain) to 100 (worst imaginable pain),[27] and thus, the mean stimulus intensity was 0.39 ± 0.07 mA.

We used 3 movies to induce cognitive perception.[40,48] We played a movie of a needle penetrating a left ventral forearm as painful imaginary percept, similar to pain perception caused by intraepidermal electrical stimulation. In contrast, a movie of a cotton-swab touching the skin was used as the nonpainful imaginary percept that corresponded to the sensation induced by mechanical tactile stimulation. A video with a static hand was also used as a control trial. Before the experiment, each participant rated whether they could imagine the intended perception and emotion from the movie, using a sensory VAS and emotional VAS ("pleasantness" = −50, "fair" = 0, and "unpleasantness" = 50).

2.3. Experimental procedure

Figure 1 shows a schematic illustration of the experimental design. Hereafter, PAIN and TACTILE indicate sensory stimulus conditions, while Needle, Cotton-swab and Static indicate the movie types. Using Psychopy (ver. 1.84.2), 2 sensory stimuli and 3 types of movies were pseudorandomly presented to participants (**Fig. 1A**). The actual stimulation delivery was adjusted for the timing of the needle prick or cotton-swab touch in a movie. The experiment comprised 10 sessions, each consisting of 25 trials (5 conditions × 5 times). To avoid any attenuation of responses and habituation of the subjective pain perception, each session was separated by 2 to 3 min of rest and the same sensory stimulus was not repeated more than 4 times in a row. We collected sensory and emotional VAS scores after the experiment to avoid causing any preconceptions. We monitored participants' behavior and evoked somatosensory responses during MEG measurement.

2.4. Data acquisition

We used a 306-channel Neuromag Vectorview MEG system (Elekta, Helsinki, Finland), and anatomical images were obtained using a 3.0-T high-resolution MRI scanner (Achieve; Philips N.V. Eindhoven, the Netherlands; TE, 60 ms; TR, 100 ms; voxel size, $1.5 \times 1.5 \times 1.5\ mm^3$). A noncontact 3D camera system (VIVID 9i; Konica Minolta, Tokyo, Japan) based on laser scanning technology was used for accurate MEG-MRI co-registration.[30] A sampling rate was set to 1000 Hz with a bandpass filter (0.1–330 Hz) during online processing.

2.5. Signal processing and source reconstruction

We extracted 2 stages of processing: an analysis related to simple pain/touch response (analysis 1) and an analysis related to cognitive modulation by painful/nonpainful movie stimuli in each pain/tactile condition (analysis 2). Averaged MEG signals were obtained from 99.4 ± 1.4 (mean ± SD) responses for analysis 1 and from 49.4 ± 0.6 responses for analysis 2 (see supplementary Table 1, available at http://links.lww.com/PR9/A146). The cortical surface of each participant was reconstructed using FreeSurfer software.[17,21] A reconstructed MRI contour was co-registered with the MEG head coordinate system accurately.[30] We applied Maxfilter,[57] bandpass filter (1–58 Hz), and independent component analysis to remove human artifacts.[29] Trials were excluded during the averaging process if gradiometers >5000 fT/cm and magnetometers >8000 fT. In this study, we followed our previous minimum norm estimates-based (include dynamic statistical parametric

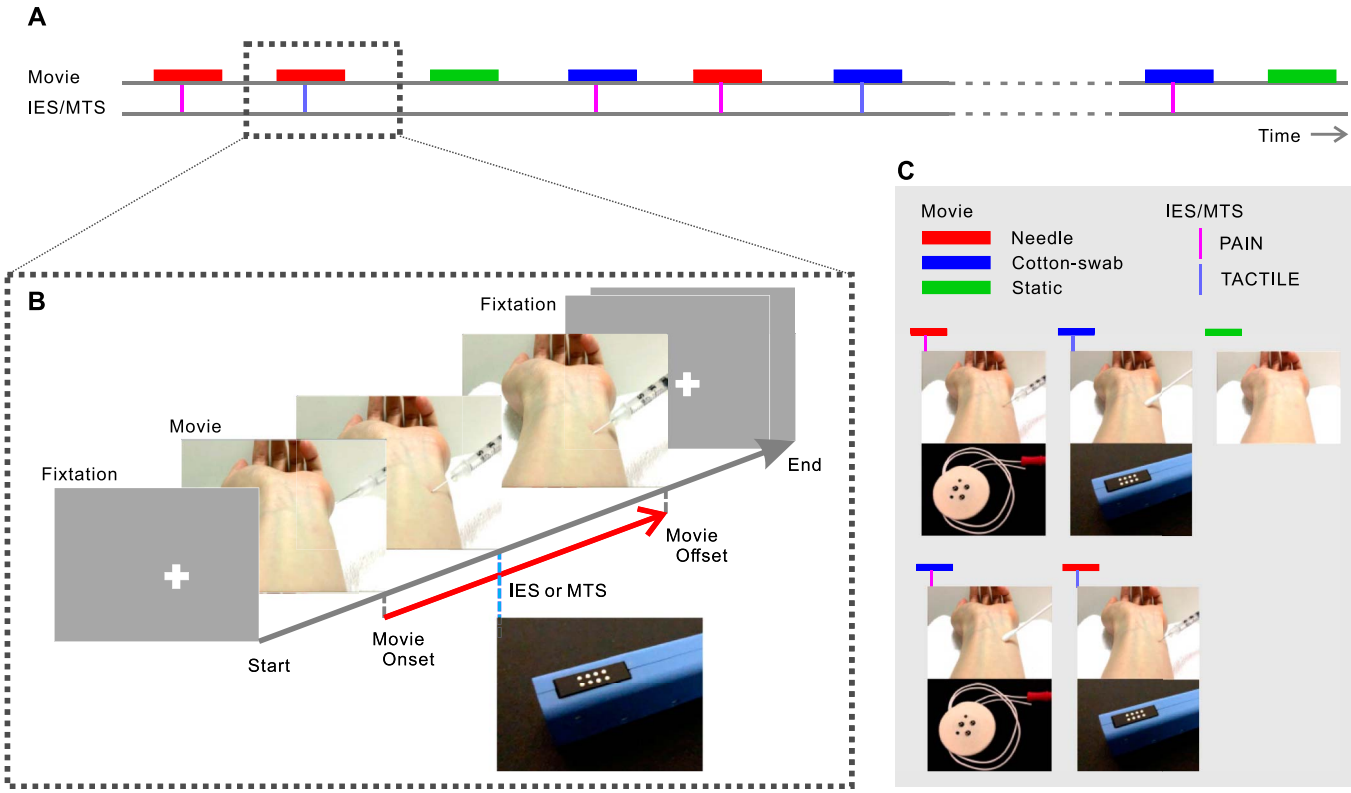

Figure 1. A schematic illustration of the stimulus patterns and movie materials. Our experiment consisted of a combination of cognitive movies and sensory inputs. Visual analogue scale assessment was performed after the experiment. (A) Three types of movies (Needle [red rectangles], Static [green], and Cotton-swab [blue]) were pseudorandomly presented on a monitor. Needle or cotton-swab stimulation was applied to the left ventral forearm. Intraepidermal electrical stimulation (IES, magenta) or mechanical tactile stimulation (MTS, cyan) was delivered to the forearms of the participants as sensory input during movie presentation. (B) The details of one trial with one movie presentation and one sensory stimulation. All movies lasted for 2.2 seconds, and sensory stimulation was delivered 0.9 seconds after the movie onset. The timing of the sensory stimulation matched with the timing of needle penetration or cotton-swab touch shown in the movie. The length of one trial was randomized and varied from 4 to 5 seconds. A fixation point (cross) was presented between the movies. (C) The contents of the movie and sensory stimulation. The 5 combinations were as follows: Needle + PAIN, Cotton-swab + PAIN, Cotton-swab + TACTILE, Needle + TACTILE, and Static.

mapping[18,24,25]) source signal analysis method.[26,31,45] A noise covariance matrix was created using entire raw data. To compensate for individual differences, we used a standardized brain (MNI-305, fsaverage; Montreal Neurological Institute[12]). We identified the insula using anatomically well-defined annotation labels provided by FreeSurfer software (l(r)h.aparc.annot based on Desikan-Killiany Atlas) to avoid double dipping.[37] Five labels (G_Ins_lg_and_S_cent_ins, G_insula_short, S_circular_insula_ant, S_circular_inf, and S_circular_insula) were imported and merged as the (whole) insula regions of interest (ROIs)[20,26] and the whole insula was divided into the PI and AI (**Fig. 2**). The center locations using MNI Talairach of MNI305 were as follows: PI = (39.20, −6.52, −0.62) and AI = (32.01, 19.97, 0.43). To the best of our knowledge, the absolute definition of the PI and AI division is still disputed, but these geometric points were in good agreement with previous reports.[19,59] The relationship between the right AI and cognitive–emotional processing has been pointed out in many studies.[14,16,33,35] To focus on identifying sensory and cognitive aspects of pain-related insular activities, we targeted right hemispheric activities in the current study.

2.6. Group analysis

We extracted the source waveforms from the ROIs by setting a baseline correction of 200 milliseconds before the movie onset. These extracted individual signals were then group-averaged with normalization.[31] Normalization was performed to the entire waveform dividing by the maximum amplitude in each stimulus condition before applying group-averaging. Maximum values were selected among the first peaks (PI/AI) in analysis 1, whereas the maximum values were selected from the 4 conditions (PI/AI × Needle/Cotton-swab) in analysis 2. In this way, all signals ranged from 0 to 1. For the peak estimation, we first identified the main peaks of the 3 responses (PI, early AI, and late AI) from the group-averaged signal. Then, we set a range of ±30 milliseconds for early peaks and ±60 milliseconds for late peaks from group-averaged signals. In principle, we selected a maximum peak from each individual waveform within these set ranges (see supplementary materials, available at http://links.lww.com/PR9/A146). However, in cases in which we could not find the peaks within these ranges, we marked the closest peak.

After selecting the peak in each ROI, we compared the peak latencies to identify latency differences among the 3 responses (PI, early AI, and late AI) and between the stimulus conditions (PAIN/TACTILE). Next, we conducted a comparison of each regional activity and latency between the movie types to explore the influence of cognitive factors (Needle/Cotton-swab). For statistical evaluation, 20-millisecond intervals around the peak times were chosen. We also performed distributed source analysis for the obtained individual peak latencies. First, we focused on how local activation patterns spread and were transmitted. Finally, we conducted comparisons between the activation in the insula and global activation. We assessed the

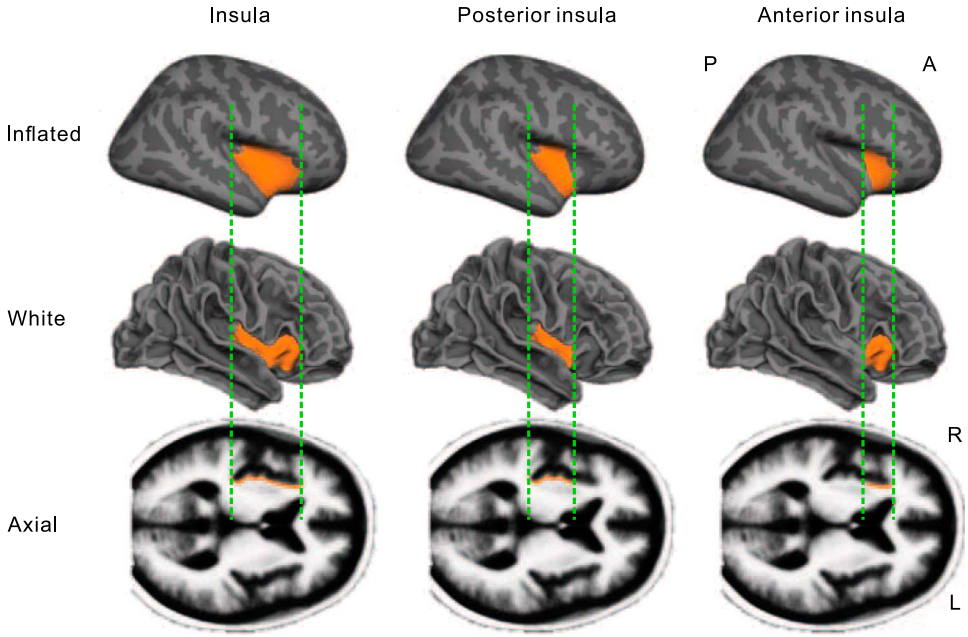

Figure 2. The regions of interest of the insula and anatomical structures in the right hemisphere. The ROI of the insula (left column) and its subdivisions of the posterior insula (middle column) and anterior insula (right column) are shown. To confirm the anatomical geometric structures, 3 views (including 2 surfaces—inflated [top row] and white [middle row]—and one anatomical view [axial view, bottom row]) are shown. Each ROI is filled with orange, and the green dotted lines indicate the boundary of the posterior–anterior insula. P, posterior; A, anterior; R, right; ROI, region of interest; L, left.

simultaneous brain activations across the whole cortex to determine the relationship between the insula and co-activated areas, predominantly in the somatosensory area and frontal cortex, at the millisecond level. Because this final step accompanies our new insight and can potentially only be achieved using MEG with high temporal and spatial resolution, we used a simple method and compared the activation map in both the insula and the global cortex at the same latencies that were marked in the insula responses, as described above.

2.7. Statistical analyses

All statistical analyses were performed using SPSS Statistics v21 (IBM Inc., Armonk, NY). We conducted Wilcoxon signed rank tests to compare the VAS scores (**Tables 1 and 2**). In analysis 1, a 1-way analysis of variance (ANOVA) and post hoc Bonferroni corrections for multiple comparisons were performed for detecting differences in 3 responses (PI, early AI, and late AI) in each stimulus condition. A paired *t* test was used for analyzing the modality differences (PAIN/TACTILE) at each peak. In analysis 2, paired *t*-tests were also applied for the comparison of the signal strength and latency of each peak activity between movie types (Needle/Cotton-swab). For ANOVA, the partial eta-squares (η_p^2) were calculated to quantitatively compare effect sizes. In multiple comparisons and paired *t*-tests, *r* was provided for the effect sizes.

3. Results

3.1. Behavioral results

Table 1 summarizes the behavioral results of the VAS scores with Wilcoxon signed rank tests while watching movie stimuli without delivering actual sensory stimulation. A Wilcoxon signed rank test revealed a significant difference in mean VAS scores between the Needle and Cotton-swab movies (sensory score: $P < 0.01$, emotional score: $P < 0.01$): the mean VAS scores for the Needle movie were significantly higher than those for the Cotton-swab movie. This result suggests that the Needle movie caused participants to imagine a pain-like sensation. In **Table 2**, both the sensory and the emotional mean VAS scores of the tactile stimulation while watching the painful movie (Needle + TACTILE condition) were significantly higher than those of the Cotton-swab + TACTILE condition (sensory score: $P = 0.02$; emotional score: $P < 0.01$). No significant differences were found between the 2 movie types in the PAIN condition. These results indicate that the painful movie stimuli significantly influenced sensory and emotional scores in the TACTILE condition but not in the PAIN condition.

3.2. Neuromagnetic brain activity

3.2.1. Analysis 1: Spatiotemporal profile of insular activity during pain and tactile processing

We extracted the source waveforms for pain and tactile responses from target insula ROIs. In this analysis, the type of movie was not taken into account, so that each source waveform was created with the data irrespective of the movie type. **Figure 3** shows the time course of each ROI in the PAIN and TACTILE

Table 1

Visual analogue scale scores of sensory and emotional ratings from movie imagination, and *P* values obtained by Wilcoxon signed rank test to compare between movie types (Needle and Cotton-swab).

	Sensory rating	Emotional rating
Needle	52.8 ± 22.8	28.56 ± 12.0
Cotton-swab	6.06 ± 9.6	−9.7 ± 14.9
P	$2.9e^{-4}$*	$2.9e^{-4}$*

Values are expressed as mean ± SD in this and subsequent tables.
* $P < 0.01$.

Table 2

Visual analogue scale scores of sensory and emotional ratings in all conditions, and *P* values obtained by Wilcoxon signed rank test to compare between movie types (Needle and Cotton-swab) in each stimulus condition.

	Sensory rating		Emotional rating	
	PAIN	TACTILE	PAIN	TACTILE
Needle	41.1 ± 8.3	25.3 ± 15.5	26.1 ± 9.6	13.3 ± 19.7
Cotton-swab	40.3 ± 16.5	19.6 ± 10.9	18.4 ± 23.4	−5.8 ± 12.9
P	0.96	$2.0e^{-2}$*	0.57	$3.8e^{-3}$†

* $P < 0.05$.
† $P < 0.01$

conditions (**Fig. 3A and B**) and corresponding source activation maps (**Fig. 3C**). Group-averaged responses revealed one early peak in the PI (see **Fig. 3A** left and **Table 3**), whereas an early peak and a late peak were observed in the AI in both stimulus conditions (**Fig. 3A** middle and **Table 3**). This trend was also confirmed at the individual level (Supplementary Fig. 1, available at http://links.lww.com/PR9/A146). In the control condition (STATIC), the waveforms were almost flat compared with the other 2 conditions and removed from further analysis.

We first compared the latency between PI and AI activities in each stimulus condition. The peaks of PI responses slightly but clearly preceded those of the early AI responses in both stimulus conditions (**Fig. 3B**). The mean time lags between the PI and early AI peaks were 11.7 ± 6.7 milliseconds (mean ± SD) and 19.7 ± 5.9 milliseconds in the PAIN and TACTILE conditions, respectively. A 1-way ANOVA showed a significant main effect of the peak latencies in each stimulus condition ($F = 57.98$, $P < 0.01$,

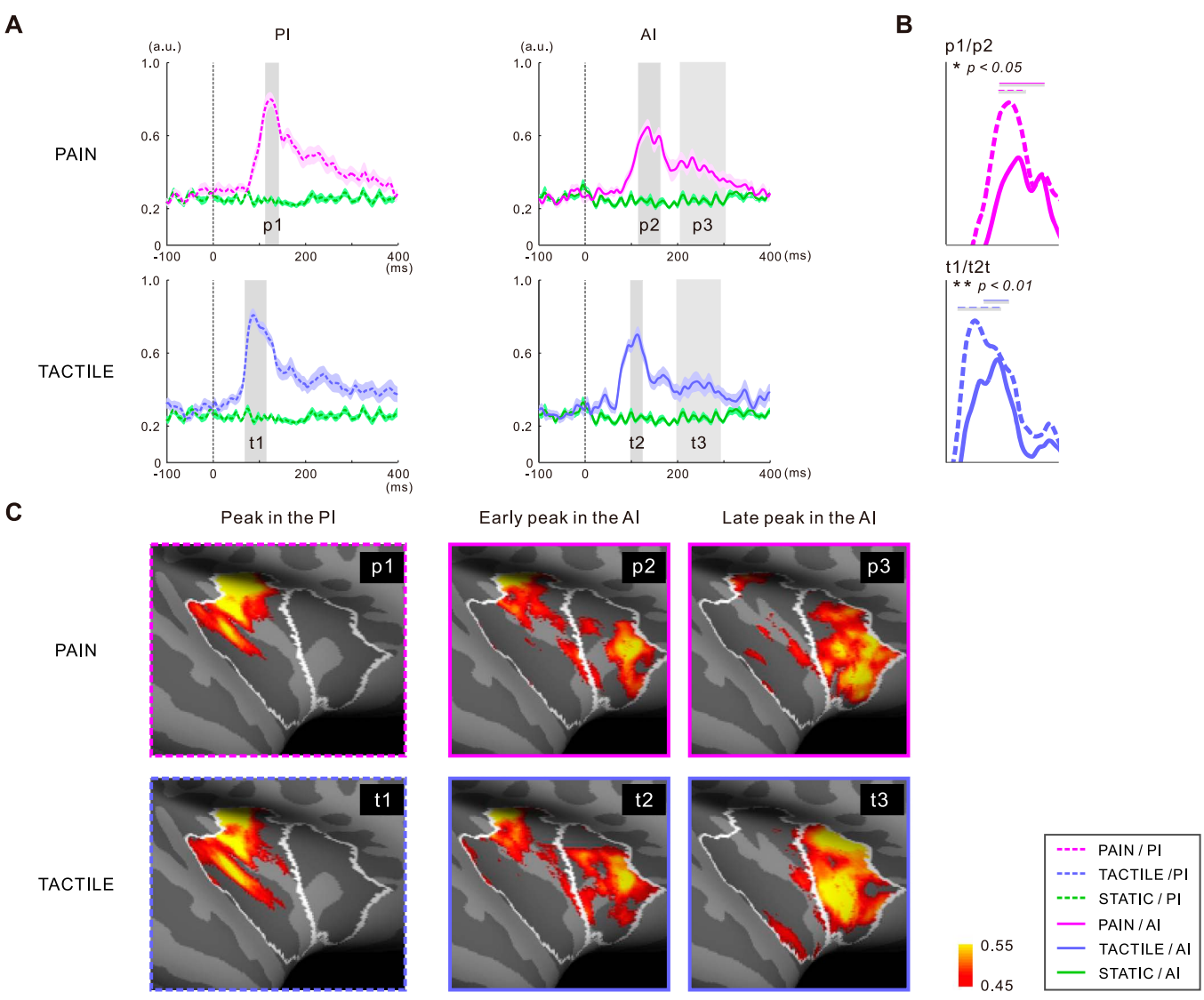

Figure 3. Spatiotemporal profiles of activation patterns induced by pain stimulation (PAIN) and tactile stimulation (TACTILE) in the target ROIs of the AI and PI. (A) Grand averaged source waveforms of PAIN (magenta), TACTILE (cyan), and STATIC (green) conditions in the PI (dotted lines) and AI (solid lines). 0 on the *x*-axis is the sensory stimulus onset. The center lines of waveforms represent the mean activations across each individual while color-matched transparent areas represent the standard errors of the mean (SEM). Each gray shaded area indicates the time range of mean latency and SEM at each peak. p1/p2/p3 represent the pain-related peak in the PI and the early and late peaks in the AI, respectively. Similarly, t1/t2/t3 represent 3 tactile-related peaks. (B) An enlarged graph showing a clear difference in the early peak latencies between the PI and AI in each stimulus condition: p1 and p2 (upper) and t1 and t2 (lower). Horizontal colored bars indicate the significant peak time ranges corresponded to the gray shaded area of (A), and matched color of PAIN (magenta) and TACTILE (cyan) and line type for PI (dotted line) and AI (solid line). (C) Activation pattern maps of the PI (left) and AI (middle: early; right: late) for the PAIN and TACTILE. Each map corresponds to the group-averaged activity on the insula at the individual peaks of PI and AI. The group-averaged brain activation images were projected onto a standard brain. The PI and AI are outlined by white lines. **$P < 0.01$; *$P < 0.05$ (for latency analysis, see Table 3 for the details). AI, anterior insula; a.u., arbitrary unit; PI, posterior insula.

Table 3

Latency values of 3 peak responses (PI, early AI, and late AI) in the 2 stimulus conditions (PAIN and TACTILE).

	PI	Early AI	Late AI
PAIN	127.3 ± 23.6	139.1 ± 24.5	254.3 ± 48.1
TACTILE	91.6 ± 14.9	111.3 ± 13.7	245.2 ± 46.6
P	2.1e^{-8}†	6.6e^{-5}†	0.54
r	0.92	0.79	0.15

	PAIN			TACTILE		
	PI − e AI	PI − l AI	e AI − l AI	PI − e AI	PI − l AI	e AI − l AI
P	3.1e^{-2}*	1.0e^{-8}†	2.1e^{-8}†	2.7e^{-4}†	1.7e^{-10}†	7.8e^{-10}†
r	0.51	0.91	0.92	0.66	0.94	0.95

For the modality difference at each peak response, *P* values were obtained by a paired *t* test. *P* values obtained by post hoc tests of a 1-way ANOVA for the difference of the peak responses in each stimulus condition. In both cases, *r* was provided for the effect sizes.

* $P < 0.05$.

† $P < 0.01$.

AI, anterior insula; ANOVA, analysis of variance; e AI, early AI; l AI, late AI; PI, posterior insula.

$\eta_p^2 = 0.87$ for PAIN; $F = 98.78$, $P < 0.01$, $\eta_p^2 = 0.91$ for TACTILE). Then, post hoc tests revealed that the peak PI activities were significantly earlier than the peak AI early responses in the PAIN ($P = 0.03$) and TACTILE ($P < 0.01$) conditions. The latency of the late AI peaks was much longer compared with those of the 2 early responses (PAIN, $P < 0.01$; TACTILE, $P < 0.01$).

Compared with each peak latency between PAIN and TACTILE, peaks of the 2 early responses in the PAIN appeared with an apparent delay compared with those in the TACTILE condition. Paired *t*-tests revealed a significant modality difference between latencies of the early response in the PI ($P < 0.01$) and AI ($P < 0.01$). In contrast to the early activation, the peak latencies of the late activity in the AI were not significantly different between the PAIN and TACTILE ($P = 0.54$).

Figure 3C shows the spatial activation patterns in the PAIN and TACTILE conditions corresponding to the marked latency in **Figure 3A**. Each image corresponds to the group-averaged map of individual peaks for PI, early AI, and late AI activation (see supplementary Fig. 2 for individual maps, available at http://links.lww.com/PR9/A146). The activations show very identical patterns between the 2 stimulus conditions. The PI peak responses in both the PAIN and the TACTILE conditions were concentrated in the dorsal part of the PI (**Fig. 3C**, left). Brain activity corresponding to the early AI peak was seen in the anterior AI region with dorsal activation in PI (**Fig. 3C**, middle). The late AI peak activities were mainly concentrated in the AI (**Fig. 3C**, right). In conclusion, the spatiotemporal features of the insular sources demonstrated that pain and tactile processing shared core activation patterns but exhibited different activation times.

3.2.2. Analysis 2: Cognitive influence on sensory-induced insular activity

We next compared the sensory-induced insular activity between the different movie types to explore the influence of cognitive factors. **Table 4** summarizes the peak values and the statistical results for the different movie conditions. The latency of the PI peak evoked by tactile stimulation while watching the Needle movie stimulus was slightly shorter than that for the Cotton-swab movie stimulus (paired *t* test, $P = 0.045$). There were no significant differences between movie types in the other conditions. In contrast, the signal strength of late AI activity while watching the Needle movie stimulus was higher than that while watching the Cotton-swab movie stimulus in the TACTILE condition ($P = 0.015$). No significant differences were found between the movie types on the other peaks, in both the PAIN and the TACTILE conditions (**Table 4**). **Figure 4** shows late AI activity, comparing cognitive effects between the different movie types in AI in the PAIN and TACTILE conditions. As observed in the mean source waveforms of the AI and bar graphs created from the mean amplitudes of late AI peaks, the late AI response did not exhibit a movie-related difference in the PAIN condition ($P = 0.89$). However, late AI activity in the TACTILE condition clearly differed between the movie types ($P = 0.015$) (**Figs. 4A and B**). From a spatial perspective, the significant difference between the movie types in activation maps for the late AI activations was less clear in the PAIN condition. However, in the TACTILE condition, the AI showed greater activation while watching the Needle movie than the Cotton-swab movie, in accord with alteration of the source waveform (**Fig. 4C**).

3.3. From sensory inputs to the cognitive processing of pain

The co-activated brain areas in relation to pain-related insular activation were further determined. **Figure 5** shows the simultaneously co-activated brain areas during pain processing that correspond to the insular responses. Using the temporal profiles, we identified 3 stages of brain activation. A representative example of the activation pattern from the Needle + PAIN condition showed that the peak of PI activity was observed in

Table 4

Values of latency and signal strength of 2 movie types (Needle and Cotton-swab) at 3 peak responses (PI, early AI, and late AI) in the 2 stimulus conditions (PAIN and TACTILE).

	Latency			Signal intensity		
	PI	Early AI	Late AI	PI	Early AI	Late AI
PAIN						
Needle	124.7 ± 21.5	137.9 ± 21.6	256.2 ± 39.8	29.3 ± 5.4	25.8 ± 5.5	20.0 ± 7.3
Cotton-swab	126.2 ± 19.9	138.7 ± 22.2	255.2 ± 42.1	30.0 ± 4.3	24.6 ± 4.6	20.2 ± 6.1
P	0.34	0.77	0.83	0.65	0.51	0.89
r	0.08	0.07	0.05	0.11	0.16	0.03
TACTILE						
Needle	87.6 ± 13.2	112.7 ± 21.4	246.2 ± 46.6	29.9 ± 4.5	25.8 ± 4.0	23.2 ± 9.6
Cotton-swab	93.3 ± 15.3	115.4 ± 18.9	245.1 ± 50.3	27.4 ± 4.8	24.9 ± 6.6	18.5 ± 5.6
P	0.045*	0.53	0.77	0.054	0.48	0.015*
r	0.47	0.15	0.07	0.45	0.17	0.55

To analyze differences between movie types at each peak response in each stimulus condition, *P* values were obtained by paired *t* tests and *r* was provided for the effect sizes.

* $P < 0.05$.

AI, anterior insula; PI, posterior insula.

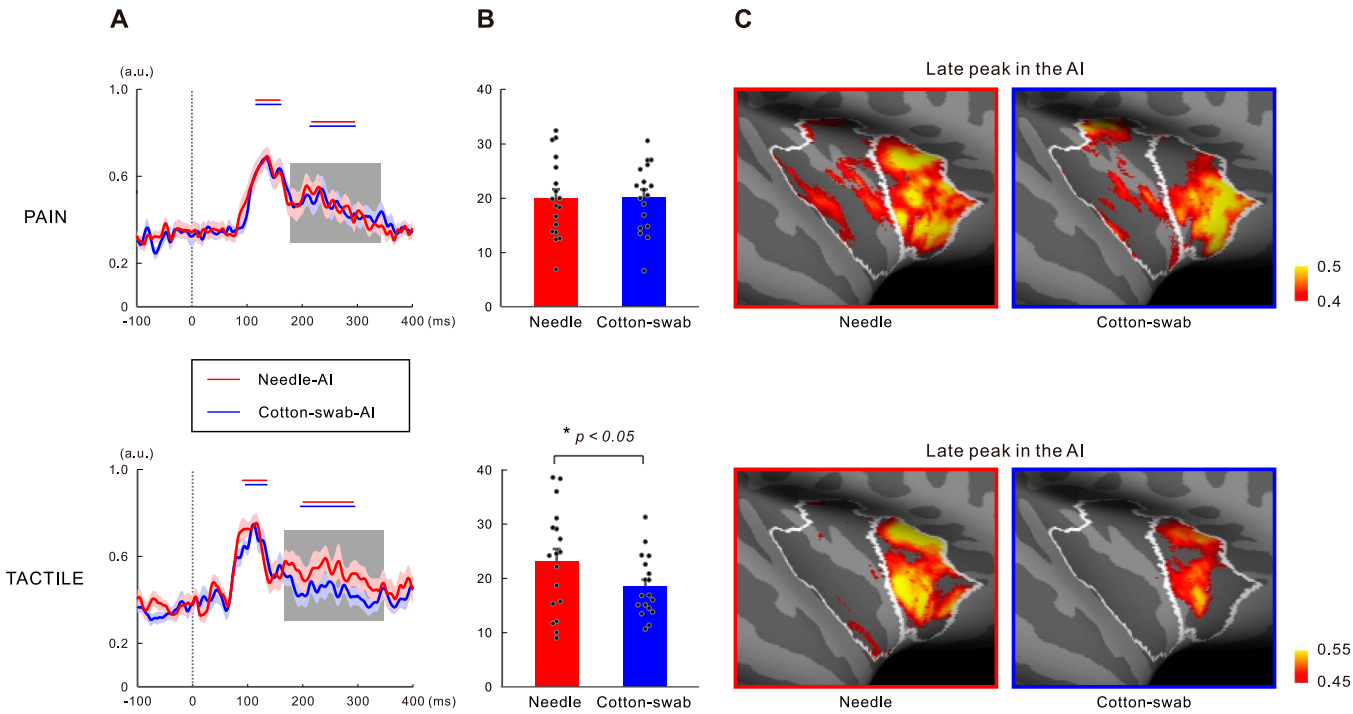

Figure 4. Spatiotemporal profiles of the magnitude of the peak responses derived from painful (Needle)/nonpainful (Cotton-swab) movies in the AI for the PAIN and TACTILE conditions. (A) Comparison of mean source waveforms between the Needle (red) and Cotton-swab (blue) movies within the AI in the PAIN and TACTILE conditions. Colored bold lines represent mean activations while color-matched transparent areas indicate the SEMs. (B) Bar graphs indicate the mean magnitude of the regional activity calculated by the individual late AI peak responses. The center line in each graph indicates the SEM and dots represent average single-participant magnitudes. (C) Spatial maps show the group-averaged insular activations created from individual maps of late AI peaks (gray rectangles of A). *$P <$ 0.05 (for amplitude analysis, see Table 4 for the details). AI, anterior insula.

association with the sensory network (I). The early response of the AI was involved in the second stage, which reflected the propagation of the sensory activity with a shift towards the anterior side (II). The late activity of the AI was connected to the third stage, which showed co-activation with frontal regions in association with the cognitive network (III) (see also supplementary Fig. 3, available at http://links.lww.com/PR9/A146).

4. Discussion

We sought to clarify the spatiotemporal profiles of pain-related insular activity and to explore the effect of cognitive modulation. We found one PI peak and 2 AI peaks as sensory-induced insular activations, and cognitive information significantly influenced the late AI activation induced by nonpainful tactile stimulation. Furthermore, the results showed whole brain responses that co-activated with each stage of insular activity (PI, early AI, and late AI) during pain and tactile processing.

Posterior insula activation preceded early AI activation in both pain and tactile conditions (**Fig. 3**). The time lag between PI and AI activity by sensory stimulus was only a few tens of milliseconds, and these temporal activation changes have not been well investigated using fMRI.[9,39,60] Bastuji et al.[3,4] reported the transition of pain signals from the PI to AI using stereotactic electroencephalography (SEEG) and the delay of 16.3 ms[4] is consistent with the current finding of a 12- to 20-millisecond difference. In addition, we identified co-activated brain areas during PI and AI activation using MEG enabling global insights into cortical activity across the whole brain with high temporal resolution. As a result, we found that the early responses in the PI and AI were clearly delineated in conjunction with somatosensory activity, and only the peak response in the insula shifted from PI to AI (**Fig. 5**). From a spatial perspective, the PI peak-related co-activated areas (**Fig. 5I**) fit closely with the fMRI-based model of "PI and sensorimotor processing areas" constructed by co-activation analyses in previous meta-analysis studies.[59,60] In brief, early insular activity reflects the sensory inputs, and the activation shift from the PI to the AI appeared to indicate the signal flow of sensory processing.

The latency difference in early activations between pain and tactile stimulation (**Fig. 3A**) is considered to reflect the difference in conduction velocity between Aδ and Aβ fibers.[26,32,34] The absence of a significant difference in the late AI response (**Fig. 3A**) indicates that late activity is largely unaffected by nerve conduction velocities, suggesting that early and late activations have different origins.

Interestingly, the late AI activity in the tactile condition was influenced by cognitive information, whereby the late AI activation was stronger during the painful movie than during the nonpainful movie in the tactile condition (**Fig. 4**). In the VAS results, subjective touch perception and emotional ratings during the painful movie were significantly higher than those of the nonpainful condition (**Table 2**). This suggests that late AI activity and subjective perception are modulated by cognitive information, at least in tactile processing. Many studies have reported that the AI exhibits a correlation with cognitive and emotional aspects of pain processing.[2,16,22,23,41,52,54] However, the timing of processing is less well understood. A laser-evoked potentials study has reported that late activity (the P300) in the frontal area was associated with the cognition of pain processing.[43] We speculate that the frontal P300 includes insular activity, given that the latency was similar to our findings of late AI activation, which occurred approximately 250 milliseconds after stimulus onset (**Tables 3 and 4**). Although it is not directly comparable, a face recognition study using MEG showed early

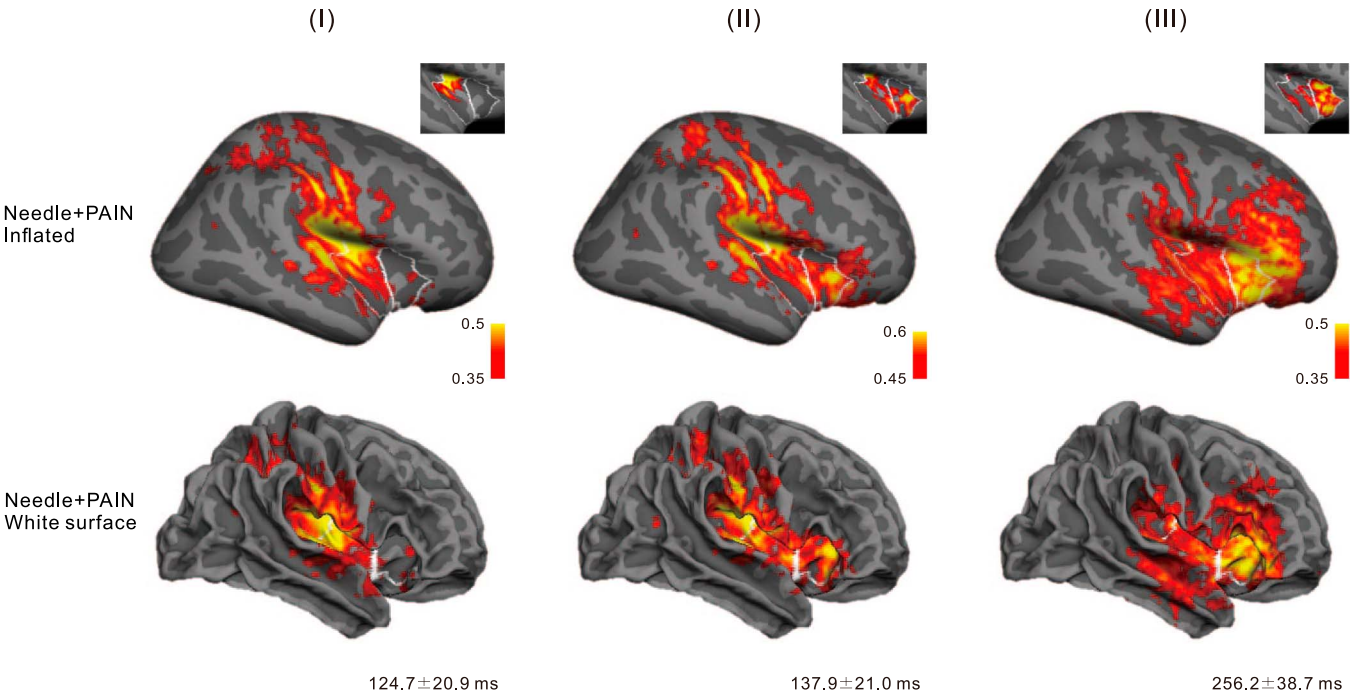

Figure 5. Co-activations of the pain-related brain areas corresponding to the insular activations. The PI peak-related co-activated areas (I), early AI peak-related co-activated areas (II), and late AI peak-related co-activated areas (III) are shown. The co-activated areas are depicted by 2 different brain surface structures—an inflated surface (upper row) and white surface (lower row), as shown in a representative example from the Needle + PAIN condition. Each stage shown in the main panel corresponds to the inset core activations within the insula (right top) and the time ranges (bottom). AI, anterior insula; PI, posterior insula.

and late activities in the insula.[11] This study suggested that the late insular activities were involved in the discrimination of emotional differences and occurred approximately 150 milliseconds after the early insular response. In our study, the time difference between early and late AI activations was approximately 130 to 150 milliseconds. These 2 previous reports support the current results and the notion that late AI activity may be associated with cognitive processing. Additionally, we found that late AI activity was co-activated with the frontal cortex (**Fig. 5** III), as reported in connection to cognitive processing revealed by fMRI.[59,60] Thus, late AI activation was suggested to reflect cognitive processing under the emergence of perception, both temporally and spatially.

Contrary to our expectations, the cognitive information had no influence on late AI activity and perception by pain stimulation (**Fig. 4**). The VAS assessments showed similar results (**Table 2**). This could be because the pain itself contained internal cognitive and emotional information,[16] and this effect was greater than that of external inputs in this study. Previous studies have reported that VAS scores vary in the balance between predicted and actual stimuli,[28,36] and our cognitive information may have been relatively weak for a pain stimulus or the intensity of the pain stimulation may have been insufficient. Although the reason for the lack of a detectable cognitive influence in the pain condition remains unclear, late AI activation was clearly observed in all conditions involving actual pain stimulation and its latencies were similar to those of the cognitive response reported in past studies.[11,43] Thus, the results also suggested that late AI activity is related to cognitive processing of pain perception.

Tactile stimulation has been reported to induce similar brain responses to those evoked by pain, and also generates insular activity.[49,56] We found that painful movie information led to enhancement of late AI activity of tactile stimulation, similar to pain, and increased the strength of touch perception. A heat allodynia study reported that some patients could feel pain sensations with even a soft touch.[42] This suggests a close interaction between pain and touch perception. Considering that the development of chronic pain has been discussed from 2 perspectives, the relationship with cognitive and affective factors[5,8,46] and the relationship with brain activation in the AI and prefrontal cortex,[10,53] the late response of AI activity may also be associated with chronic pain.

It should be noted that the sample size for this study may not have provided sufficient statistical power. The inclusion of adequate sample sizes is crucial in the field of neuroscience because of the challenges regarding replication. Future studies involving larger sample sizes may reveal a correlation between VAS scores and MEG signals. Integration with pain-related brain activity and VAS scores may be helpful for objective assessment of complex pain and selection of effective treatments such as cognitive behavioral therapy, particularly for chronic pain. In addition, further investigations of the causal relationships among brain activities should be conducted. Objective systematic evaluation of peak detection may provide robust findings than traditional visual inspection methods.

To our knowledge, this is the first study to identify sensory and cognitive aspects of pain processing in the insula with temporal changes, and indicating a connection between cognitive factors and late AI responses. Although the approach described here requires further refinement for assessment and treatment of pain in clinical settings, we are confident that our method will provide new insight in this area. Regarding prospective pain studies, future studies of late AI responses should be conducted to detect the cognitive influence in pain stimulus conditions and examine emotion and higher-order functions, such as the processing of salience, integration, and awareness.

Disclosures

The authors have no conflicts of interest to declare.

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Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PR9/A146.

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