Temporal modulation of brain responses during ongoing hot stimulation in burning mouth syndrome

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Major in Oral Diagnostic Sciences

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This thesis is based on the following article with additional new unpublished data on temporal changes in perceived pain intensity during ongoing hot stimulation (Fig.6).

Ryutaro Kohashi, Takahiro Shinozaki, Naohiko Sekine, Kosuke Watanabe, Daiki Takanezawa, Chisa Nishihara, Kana Ozasa, Mariko Ikeda, Noboru Noma, Akiko Okada-Ogawa and Yoshiki Imamura. Time-dependent responses in brain activity to ongoing hot stimulation in burning mouth syndrome. J Oral Sci (in press).

Abstract

Burning mouth syndrome (BMS) is classified into idiopathic orofacial pain conditions. Although central and peripheral neuropathic mechanisms are thought to be involved, the etiology is not still well known. These features include predilection for postmenopausal women, association with psychological conditions such as depression, hypochondria and cancer phobia, and taste disturbance. The participants were 15 right-handed female patients who were diagnosed as primary BMS and 15 age and gender-matched, right-handed female controls. BMS patients were diagnosed with the criteria of the Third Edition of the International Classification of Headache Disorders, beta version. Peripheral and systemic diseases that could manifest pain and burning sensation in the oral mucous membrane were ruled out accordingly. All the participants were enrolled at the Orofacial Pain Clinic in Nihon University Dental Hospital and imaging data were acquired at Nihon University Itabashi Hospital. In Study 1, temporal brain responses to the ongoing hot stimulus was studied to investigate the pain modulation system in BMS patients. The thermal stimulation sequence comprised of baseline (32°C, 40 sec) to warm (40°C, 32 sec) to baseline (32°C, 40 sec) to hot (49°C, 32 sec) was repeated four times with a Peltier thermode. These warm and hot stimuli were applied to the right palm and right lower lip in two separate sessions. Functional magnetic resonance imaging data were acquired by recording echo-planar images with a block design. Brain activity induced by

purely hot stimulation (49°C vs 40°C) applied to the palm was more pronounced than that induced by the lip stimulation, and in BMS patients as compared with controls. Comparison of brain activity in the first 16 sec and second 16 sec of the stimulus revealed pronounced time-dependent facilitation in BMS patients during lip stimulation. The findings indicate that the pain modulating system in BMS patients is dysregulated and BMS brain is highly sensitized to noxious information originating from the trigeminal system.

In Study 2, the perceived pain profile was investigated while ongoing hot stimulus was being applied to the palm and the lower lip in healthy volunteers. The results showed a significant temporal summation of perceived pain intensity during the palm stimulation, and a tendency towards temporal pain suppression during the lip stimulation. It is known that these two different nerve territories have different pain threshold and stimulus intensity that is necessary to provoke responses may be different.

These findings let to the following conclusions.

1. A significantly activated brain areas during palm stimulation in BMS patients included the secondary somatosensory cortex (S2), the dorsolateral prefrontal cortex (dlPFC), the insular cortex (IC), the visual cortex (VC), the posterior cingulate cortex (PCC), the hippocampus, the parahippocampal gyrus, and the cerebellum.

2. A significant increase of brain activity during painful hot stimulation at the lower lip in BMS patients included the premotor cortex (PMC), the orbitofrontal cortex (OFC), the medial PFC (mPFC), dlPFC, the anterior cingulate cortex (ACC), IC, VC, the caudate nucleus, and the midbrain.

3. During painful hot stimulation, the brain activation was further facilitated in the second half periods than in the first half periods both in BMS patients and in controls. This temporal facilitation of the brain activity was more apparent in BMS patients than in controls, and during lower lip stimulation than palm stimulation. BMS brain showed a time-dependent facilitation in the secondary visual cortex (V2), PMC, the thalamus, dlPFC and mPFC during lip stimulation and in the supramarginal gyrus, the pons and the cerebellum during palm stimulation.

4. Time-dependent facilitation evoked by sustained lip stimulation was more significant in BMS patients than in controls in the following brain areas: primary motor cortex (M1), PMC, IC, and PFC, as well as ACC. However, this BMS-specific time-dependent increase in brain activation was not seen during palm stimulation.

5. Subtraction of brain activity of the second half periods from the first half periods revealed no areas that showed significant changes.

6 Painful hot stimulation at the palm, a numerical rating score (NRS) was increased during second half period than the first half period.

Introduction

Persistent idiopathic orofacial pain often disables patients. Burning mouth syndrome (BMS) is one of the most typical idiopathic orofacial pain conditions diagnosed after exclusion of all possible conditions in which continuous pain in intraoral soft tissues manifests (1–3). Although the etiology of BMS not well known, some common characteristic features are reported. These features include predilection for postmenopausal women (4,1), association with psychological conditions such as depression, hypochondria and cancer phobia (5–7), and taste disturbance (8–10). Studies have tried to elucidate its etiology from the immune and endocrine responses (11–13), and a neuropathic changes in the peripheral and the central nervous systems (14–17).

Recent studies have suggested that there is an alteration in the pain modulation system in the BMS brain (18–22). Shinozaki et al. (22) has demonstrated that the perceived pain was more intense in patients with BMS as compared to controls while receiving repeated painful hot stimuli at the lower lip, and this increased pain perception was not observed while the hot stimulation was repeated at the palm. During this stimulation, the anterior cingulate cortex (ACC), the prefrontal cortex (PFC) and the insular cortex (IC) known as the main components of the medial pain pathway are highly activated in BMS patients. It is assumed that one of the reasons of this impairment in

the pain modulating system lay in the loss of pain habituation in C fibers that should be induced by repetition of noxious stimulation (22). This study investigated whether the same responses were provoked by the ongoing painful hot stimulation in BMS patients (Study 1). Further, we studied whether this thermal sequence model of ongoing painful hot stimulation is appropriate in observing temporal summation of the perceived pain in healthy volunteers (Study 2).

Methods

1. Study 1

1) Participants

The participants were 15 right-handed female patients (52.6 ± 6.3 y ; mean SD) who were diagnosed as primary BMS and 15 age and gender-matched, right-handed female controls (49.0 ± 8.4 y). BMS patients were diagnosed with the criteria of the Third Edition of the International Classification of Headache Disorders, beta version (23). Peripheral and systemic diseases that could manifest pain and burning sensation in the oral mucous membrane were ruled out accordingly (13).

2) Setting

All the participants were enrolled at the Orofacial Pain Clinic in Nihon University Dental Hospital and imaging data were acquired at Nihon University Itabashi Hospital. Verbal and written consent was provided by all participants. The study was conducted according to the Helsinki Declaration. This study has been reviewed and approved by the Ethical Board of Nihon University School of Dentistry (EP16D020).

3) Thermal stimulation

The thermal stimuli sequence that was used in the functional magnetic resonance imaging (fMRI) session had been preliminarily introduced to the participants and they experienced the protocol before they actually received the test stimuli for the MR data acquisition. Thermal stimulation was delivered by a thermal generator (Intercross-210, Intercross, Tokyo, Japan) with an MRI compatible Peltier thermode (10 × 10 mm). Two sites were selected for application of thermal stimulation sequence described below; first at the skin of the right palm and then at the mucosa of the right lower lip. The stimulation sequence was started with the 30°C adaptation temperature, followed by warm and hot stimulation sets. A warm stimulation set comprised of a 40 sec 32°C baseline temperature and a 32 sec 40°C warm stimulation. In a hot stimulation set, a 40 sec baseline temperature was followed by 32 sec 49°C painful hot stimulation. A pair of warm and hot stimulation sets were repeated four times in a session. The thermode was programmed to return to the adaptation temperature (40 sec 30°C) after the end of the protocol. To avoid the influence of preceding palm stimulation, a 3-minute break was scheduled before lip stimulation. After this rest, the same session protocol was repeated at the right lower lip (Fig.1).

4) Imaging acquisition

A 1.5-T MRI scanner (Ingenia, Phillips, Amsterdam, Netherlands) with a conventional

bird-cage head coil was used in collecting anatomical and functional images. The following setting was applied to a T2-weighted gradient-echo planar imaging (EPI) sequence (TR: 4000 msec, TE: 50 msec, flip angle: 90°, Matrix = 256 × 256, FOV = 256 mm). Data acquisition was started at the fifth scan because the magnetization required time to become steady. T1-weighted images were acquired for an anatomical reference in localizing the functional MR images with the following settings (TR = 2000 msec, TE = 3.2 msec, flip angle = 15°, Matrix = 256 × 256, FOV = 256 mm).

5) Statistical analysis

The acquired functional MRI data were analyzed with a statistical image analyzer software (SPM 12, The Wellcome Department of Cognitive Neurology, London, UK) with MATLAB 6.5.1 (The Mathworks, Natick, MA, USA). The EPI images were generated by processing the participant's functional images through reorientation, realignment, co-registration, and normalization. Consequently, the obtained images were smoothed with a Gaussian kernel (22). Statistical analysis was performed using the general linear model on an individual basis. Low-frequency noise was took out by high-pass–filtering (set to 256 sec) and the obtained data were temporally smoothed. A statistical parametric map was generated by the voxel-by-voxel comparison using the t-statistic, and then a group analysis was conducted on these individual data using the random-effects model. Each 32-sec period

of stimulation was divided into a 16-sec first half period and a 16-sec second half period. The following issues were compared by computing blood oxygenation level–dependent (BOLD) signals.

i. Pathognomonic brain activation and suppression in BMS patients were calculated by subtracting the brain activity between two groups (Fig. 2, ΔP: BMS patients-controls, and vice versa).

ii. Time-dependent facilitation and suppression in brain activity were calculated by subtracting the brain activity between two groups (Fig. 2, ΔB & ΔC: second half periods-the first half periods, and vice versa).

iii. BMS-specific time dependent facilitation and suppression were further calculated (Fig. 2, ΔG: differences of the brain activity between two groups, ΔB – ΔC and vice versa).

In the statistical analyses, the analysis of variance followed by a post-hoc Bonferroni's test was employed for the multiple comparisons between groups. The comparison of data between two groups was analyzed with the *t*-test. The threshold for statistical significance was initially set at $P < 0.01$ (uncorrected) for voxel-level analysis and $P < 0.05$ (family-wise error correction) was set for cluster-level analysis.

2. Study 2

A psychophysical test was conducted to evaluate the perceived pain intensity in 13 female healthy volunteers (43.1 ± 10.7 y) during the test session that was employed in the study 1. Participants received thermal stimulation sequence using the same as thermode and in a totally same as protocol to study 1. During this protocol, participants were requested to show their perceived pain intensity using NRS (0: pain free, 5: the most painful as imaginable) by indicating the number of their left fingers. Data were collected at every time point of 13 sec from the start of the first or second half period during warm and hot stimulation (Fig.1). Because verbal communication was difficult due to the operating noise of the MR machine, a cue was indicated on the monitor in front of the participant to tell the correct evaluating time point. A mean NRS score was calculated for every first and second half period of warm and hot stimulation, and the data were statistically compared between the first and second half periods for the warm and hot stimulation, respectively. A paired *t*-test was employed for the statistical analysis in comparison of mean values between two groups. The threshold for statistical significance was set at $p < 0.05$.

Results

1. Study 1

1) Pathognomonic brain activation in BMS patients

Subtraction of brain activity evoked by painful hot stimulation at the palm of the control group from that of the BMS group revealed a significantly activated brain areas during palm stimulation in BMS patients as compared to controls. These areas included the secondary somatosensory cortex (S2), the dorsolateral prefrontal cortex (dlPFC), IC, the visual cortex (VC), the posterior cingulate cortex (PCC), the hippocampus, the parahippocampal gyrus, and the cerebellum. Further, the statistical analysis of another data set of brain activity during lower lip stimulation in both groups showed a significant increase of brain activity during painful hot stimulation at the lower lip in BMS patients as compared to controls. These brain areas included the premotor cortex (PMC), the orbitofrontal cortex (OFC), the medial PFC (mPFC), dlPFC, ACC, IC, VC, the caudate nucleus, and the midbrain (Fig. 3).

2) Pathognomonic brain suppression in BMS patients

Subtraction of brain activity in BMS patients from that in controls represents less activated areas in BMS patients than in controls. This statistical analysis revealed that there were few areas showing significant decrease of brain activation during either palm or lip stimulation in BMS patients as compared to controls (data not shown).

3) Time-dependent facilitation in brain activity

During painful hot stimulation, the brain activation was further facilitated in the second half periods than in the first half periods both in BMS patients and in controls. This temporal facilitation of the brain activity was more apparent in BMS patients than in controls, and during lower lip stimulation than palm stimulation. BMS brain showed a time-dependent facilitation in the secondary visual cortex (V2), PMC, the thalamus, dlPFC and mPFC during lip stimulation and in the supramarginal gyrus, the pons and the cerebellum during palm stimulation (Fig. 4, Table 1).

4) BMS-specific time-dependent brain activation

Subtraction of brain activity in the first half periods from that in the second half periods revealed brain areas that showed a time-dependent facilitation. This time-dependent facilitation evoked by sustained lip stimulation was more significant in BMS patients than in controls in the following brain areas: primary motor cortex (M1), PMC, IC, and PFC, as well as the ACC. However, this BMS-specific time-dependent increase in brain activation was not seen during palm stimulation (Fig. 5).

5) Time-dependent suppression in brain activity

Subtraction of brain activity of the second half periods from the first half periods revealed no areas that showed significant changes.

2. Study 2

Painful hot stimulation at the palm generated pain as expressed with NRS 2.1 ± 1.6 during the first half period and 2.6 ± 2.1 during the second half period. There was a significant increase in the perceived pain intensity according to ongoing painful hot stimulation (p = 0.03). Contrarily, NRS during the lower lip stimulation showed no significant change in pain intensity from 2.7 ± 2.8 in the first half to 2.2 ± 2.5 in the second half period (p = 0.07, Fig. 6).

Discussion

The hot pain thresholds in BMS patients are reported higher in the lip than in the hand (24). In this study, a fixed–temperature (49°C) stimulus was applied to both palm and lower lip, thus it is supposed that the perceived stimulus was stronger when it was applied to the palm than at the lower lip. Therefore, due to the site specific difference in pain threshold, the magnitude of the brain response was greater while the stimulation was applied to the palm than at the lower lip. Contrarily, to my knowledge, there are no previous studies on quantitative sensory tests (QST) that reported significant differences in pain thresholds between BMS patients and controls. That means the difference in magnitude of brain activity between BMS patients and controls does not depend on the difference in pain thresholds. Grushka et al. (25) and Ito et al. (26) reported that there was a decreased pain tolerance in BMS patients as compared to controls. These findings suggest that the difference in brain activity between BMS patients and controls seems to reflect the difference in responses of the central nervous system of both groups, which is the main target of this study.

Subtraction of the magnitude of the brain activity (BMS patients – controls, and vice versa) revealed brain areas of facilitation and suppression in BMS patients in comparison with controls, respectively. The brain areas that showed further activation in

BMS patients than in controls during palm stimulation included the somatosensory areas (S2 cortex and supramarginal gyrus), the VC, the cerebral limbic system (hippocampus and parahippocampal gyrus), and the cerebellum, which are mainly associated with pain perception. Contrarily, the further activated areas in the BMS brain during lip stimulation included the motor-related areas (M1 and PMC), the cognito-affective areas (ACC, IC, mPFC, dlPFC, and OFC), the VC, the caudate nucleus, and the midbrain (Fig. 3), which are deeply involved in pain modulation (27–29) and emotions (27,30–32). These results are consistent with previous reports (33,22). Changes in grey matter volume or concentration in these areas associated with pain modulation and emotions were reported in persons with BMS patients (34,35) as well as other chronic pain conditions (30,36), and these changes probably reflect the relationship between BMS and the psychological distress induced by chronic pain. Recently, using resting-state fMRI, it is reported that connectivity between these areas is more intense in the BMS group than in the control group and was related to depression severity (35,37). These findings suggest that BMS pathogenesis is closely related to depression and anxiety, and my data support this hypothesis.

To study the details of this difference in brain responses, temporal changes in brain activity during sustained painful hot stimulation in both groups were investigated. The results revealed a significant time-dependent facilitation (Fig. 4) with little inhibition

in both groups. Previous studies have reported that ongoing painful hot stimulation induces temporal summation of pain intensity (38). This time-dependent facilitation was more apparent in BMS patients than in controls and during the lower lip stimulation than the palm stimulation, and the brain areas that showed time-dependent facilitation were those involved in pain modulation (Fig. 5B). Findings in association with the temporal summation may represent pathognomonic features of BMS pathophysiology. First, time-dependent facilitation may reflect the brain activity to modulate the temporal summation evoked by sustained painful stimulation (39). The brain of BMS patients has behaved to exert the pain modulating function more significantly than that of controls, and the BMS brain kept facilitating the function without waning for 32 sec. Secondly, time-dependent facilitation was observed more significantly when the stimulation was applied to the lower lip. This finding suggests that there is a site-specific, peripheral mechanism. As shown in Fig. 3, the magnitude of brain response to the fixed temperature was stronger during palm stimulation than lip stimulation. Thus, the time-dependent facilitation of pain intensity did not occur with brain-response intensity dependently. This finding suggests that BMS brain is highly sensitized to pain signals originating from the trigeminal system. It is known that in BMS patients, small nerve fiber atrophy is observed in the oral mucous epithelium (20,40), and such a peripheral pathology may be involved in the sensitization of the BMS brain.

In study 2, healthy volunteers showed inconsistent results in temporal changes of perceived pain intensity between the two stimulation sites. Palm stimulation with an ongoing hot stimulus revealed a significant time-dependent increase in pain intensity (P = 0.03), namely temporal summation of hot pain. In contrast to this result, lower lip stimulation showed no significant changes but a tendency toward a pain suppression (P = 0.07). Shinozaki et al. reported a temporal suppression of hot pain induced by the repeated hot stimuli with a fixed temperature at the lower lip (22). Although this repeated stimulation did not show any significant changes when applied to the palm, there was a tendency towards an increase that did not reach the significance level (22). Thus, these two different types of hot stimuli (ongoing & repetitive) induced increase and decrease of pain sensation when they were applied to the palm and the lower lip, respectively. These results suggest that the trigeminal system may behave differently from the spinal system to the same thermal stimulus. Although it is not easy to fully explain this difference, appropriate size of Peltier thermode may be different. It is reported that the pain threshold is lower in the palm than in the lip (24), which suggests that the received energy as pain information during fixed temperature stimuli may be greater in the palm stimulation. This issue should be investigated in the future study.

Conclusion

An fMRI study of BMS patients and healthy controls revealed pathophysiological changes in the brain of BMS patients. Specific brain responses to changes in stimulus magnitude (innocuous vs noxious) and duration (early vs. late stimulation) probably reflect BMS pathophysiology. Stimulus-site–specific and time-accumulative changes revealed evidence of extreme responses in somatosensory areas during hand pain and the absence of such responses during perioral pain. The brain areas associated with motor and cognito-affective functions (M1, PMC, PFC, and ACC) appeared to have a pivotal role in pain processing/modulation in BMS.

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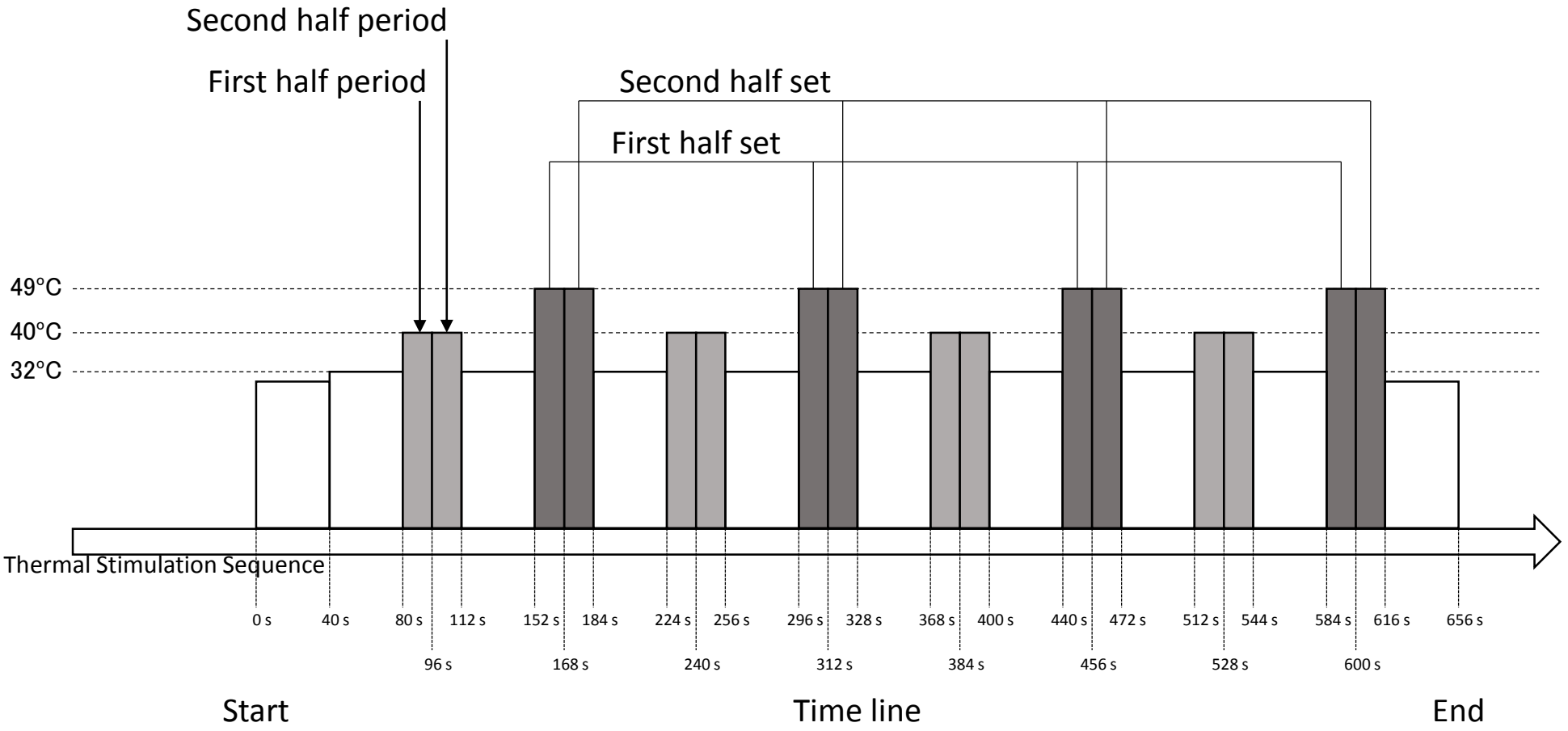

Fig. 1 Schematic diagram of the experimental protocol

Baseline Warm Hot

The protocol repeated 4 cycles of the thermal stimulation sequence comprised of three different temperatures, baseline (32ºC) – warm (40ºC) – baseline – hot (49ºC). Each warm and hot stimulation period was divided into first half (16 sec) and second half (16 sec) periods, respectively. Brain activity was calculated for whole warm or hot stimulation periods, and then for the first half or the second half periods, respectively. Brain activity during the second half periods (second half set) was subtracted from that during the first half periods (first half set) and vice versa. Subtraction of the second half set from the first half set revealed brain areas that showed time-dependent facilitation, and reversed subtraction revealed time dependent inhibition.

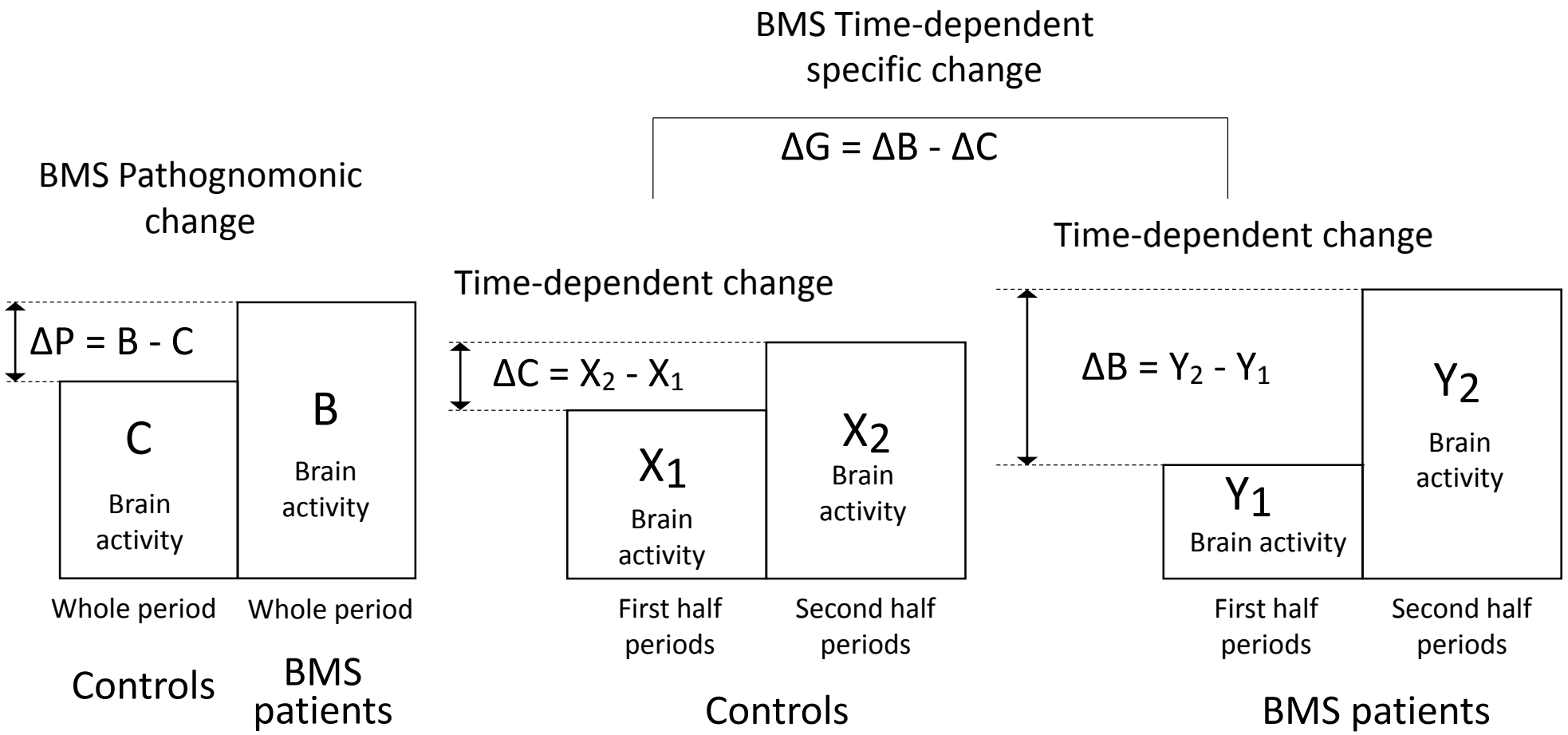

Fig. 2 Conceptual diagram of the statistics

ΔP : Pathognomonic activation (or suppression) of brain activity in BMS patients
ΔC : Time dependent facilitation (or suppression) of brain activity in controls
ΔB : Time dependent facilitation (or suppression) of brain activity in BMS patients
ΔG : BMS time-dependent specific facilitation (or suppression) of brain activity

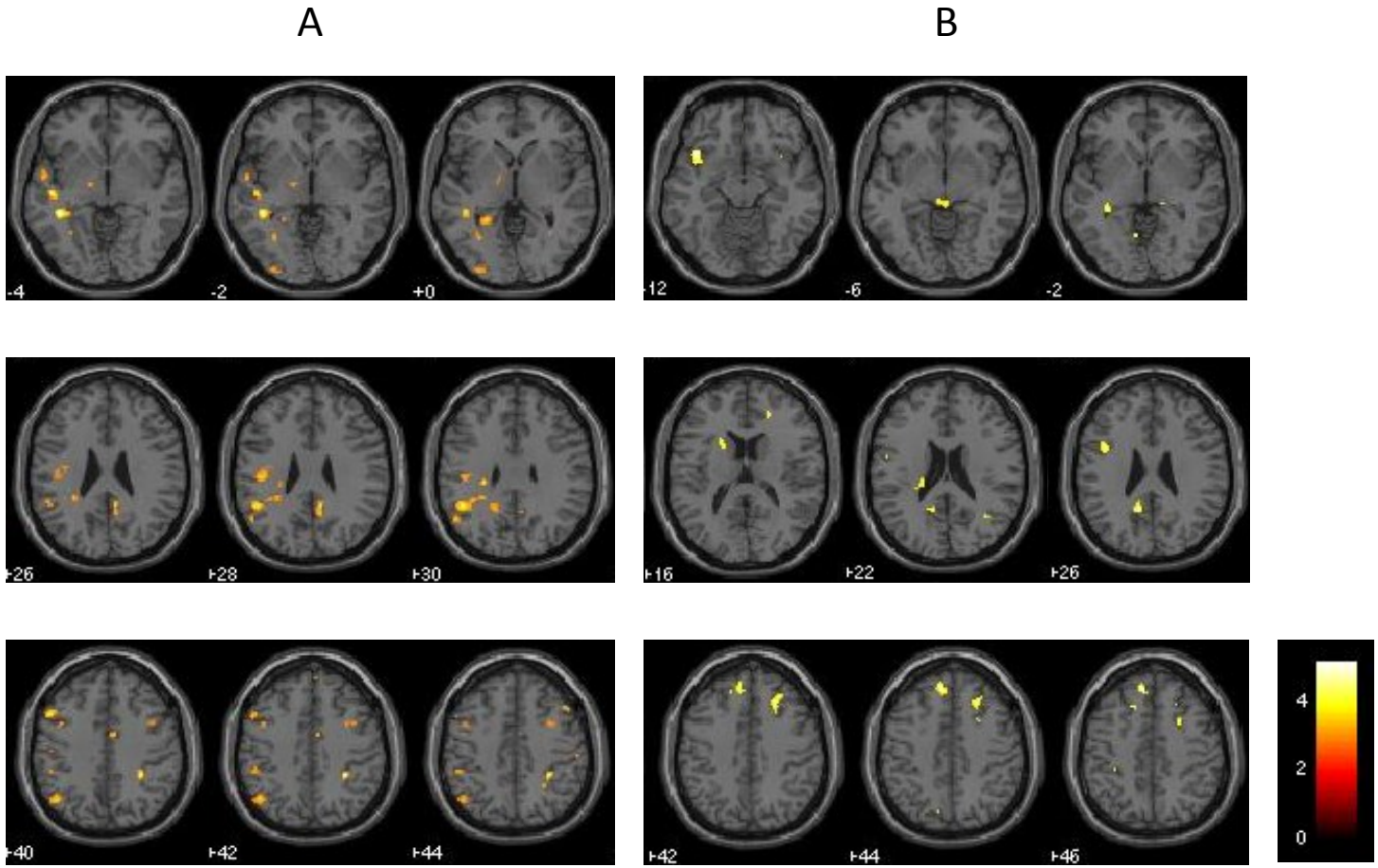

Fig. 3 Pathognomonic brain activation during painful hot stimulation

Increased activation in BMS patients as compared to controls was observed in;
A (Palm stimulation): S2 cortex, dlPFC, IC, VC, PCC, hippocampus, parahippocampal gyrus and cerebellum.
B (Lip stimulation): PMC, OFC, mPFC, dlPFC, ACC, IC, VC, caudate nucleus and midbrain.
Color bar indicates T value.

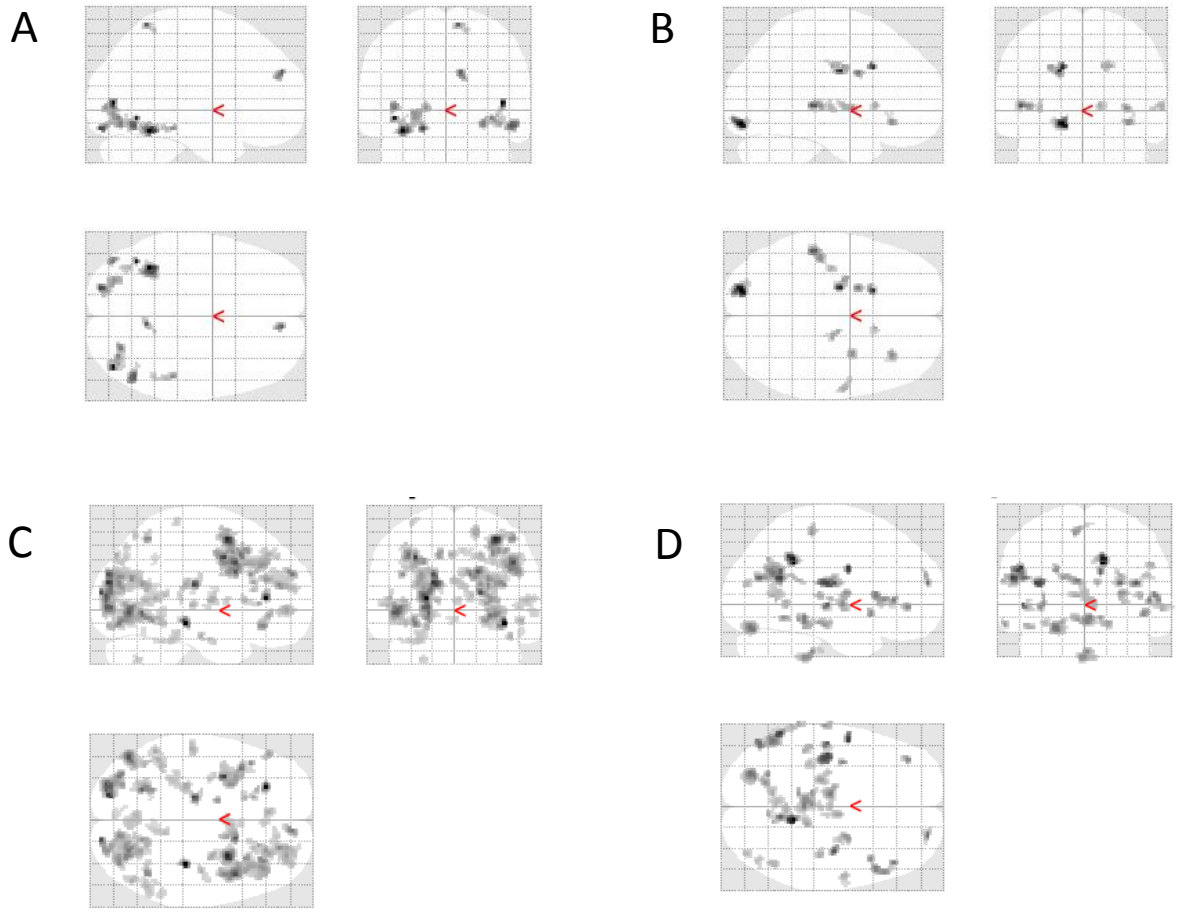

Fig. 4 Brain areas that showed time-dependent facilitation during sustained painful hot stimulation
(Magnitude of brain activity during the second half periods – that during the first half periods)

A: Controls; lip stimulation, B: Controls; palm stimulation,
C: BMS patients; lip stimulation, D: BMS patients; palm stimulation

	MNI (mm)				Brain Areas	Cluster-P FWE-corr	Expected voxel per cluster <K>	Cluster-K
	X	Y	Z					
BMS Palm	-26	-80	-20	Left	Cerebellum	0.073	11.408	110
	2	-38	-12	Right	Cerebellum	0.118		95
	-58	-56	20	Left	Supramarginal Gyrus	0.001		257
	-2	-36	-44	Median	Pons	0.08		107
BMS Lip	-18	-88	20	Left	V2	0	10.822	567
	30	4	54	Right	PMC	0		758
	-16	-22	18	Left	Thalamus	0.088		100
	-48	-72	-4	Left	V2	0		325
	-38	14	42	Left	dlPFC	0		323
	26	-72	-6	Right	V2	0.001		253
	26	34	22	Right	mPFC	0.002		215
Cont Palm	none						11.225	
Cont Lip	-36	-52	-18	Left	Fusiform Gyrus	0.019	8.703	124
	-20	-90	-16	Left	Cerebellum	0.019		124

Table 1 MR data of brain areas that showed time-dependent facilitation

MNI: Montreal Neurological Institute, FWE-corr: family-wise error corrected, Cluster-K: extent threshold, V2: secondary visual cortex, dlPFC: dorsolateral prefrontal cortex, mPFC: medial prefrontal cortex

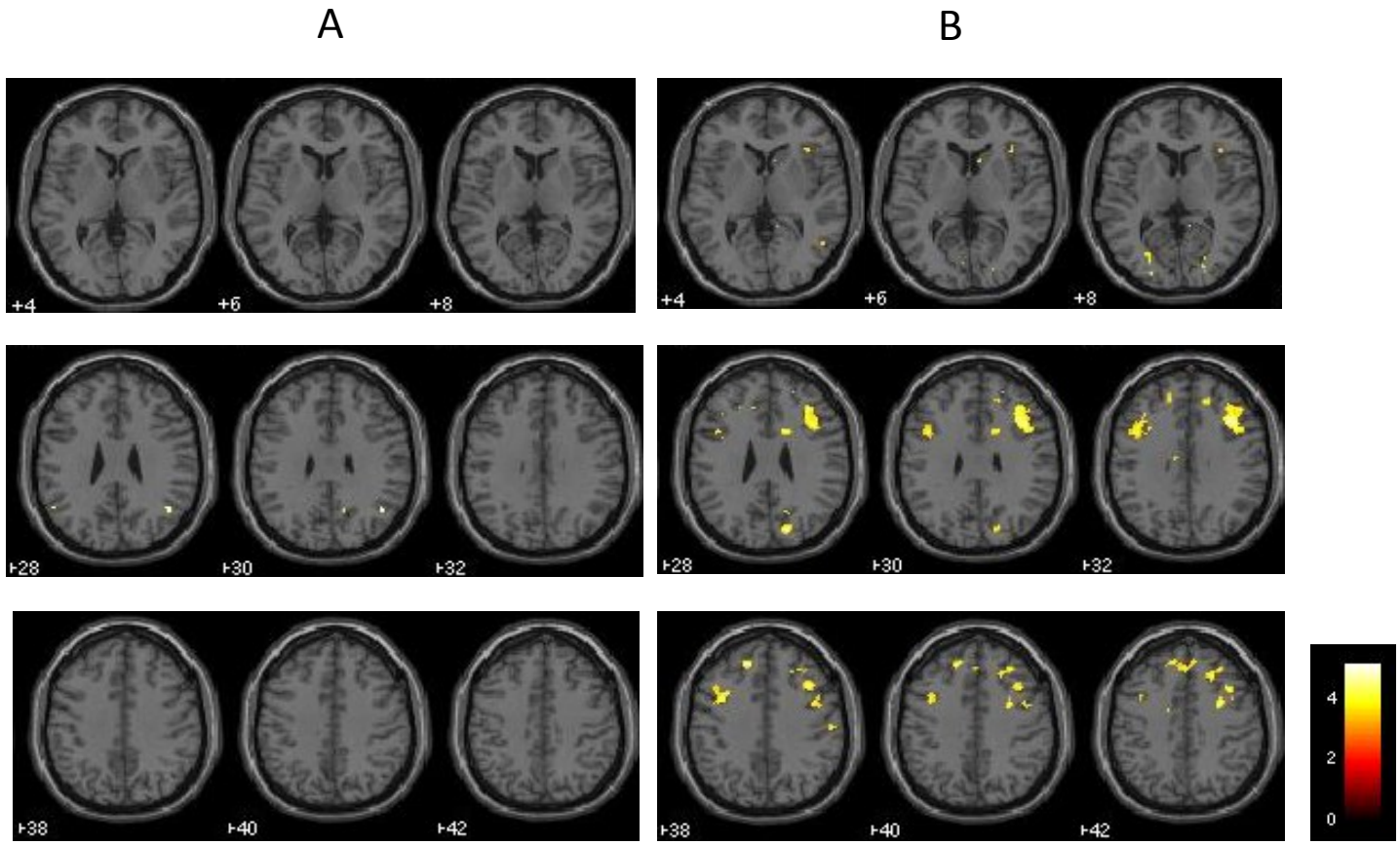

Fig. 5 BMS-specific time dependent facilitation in brain activity

A: BMS-specific time dependent brain activation during palm stimulation
B: BMS-specific time dependent brain activation during lip stimulation
Time dependent facilitation was significant in M1, PMC, IC, PFC and ACC in BMS patients as compared to controls.
Color bar indicates Z score.

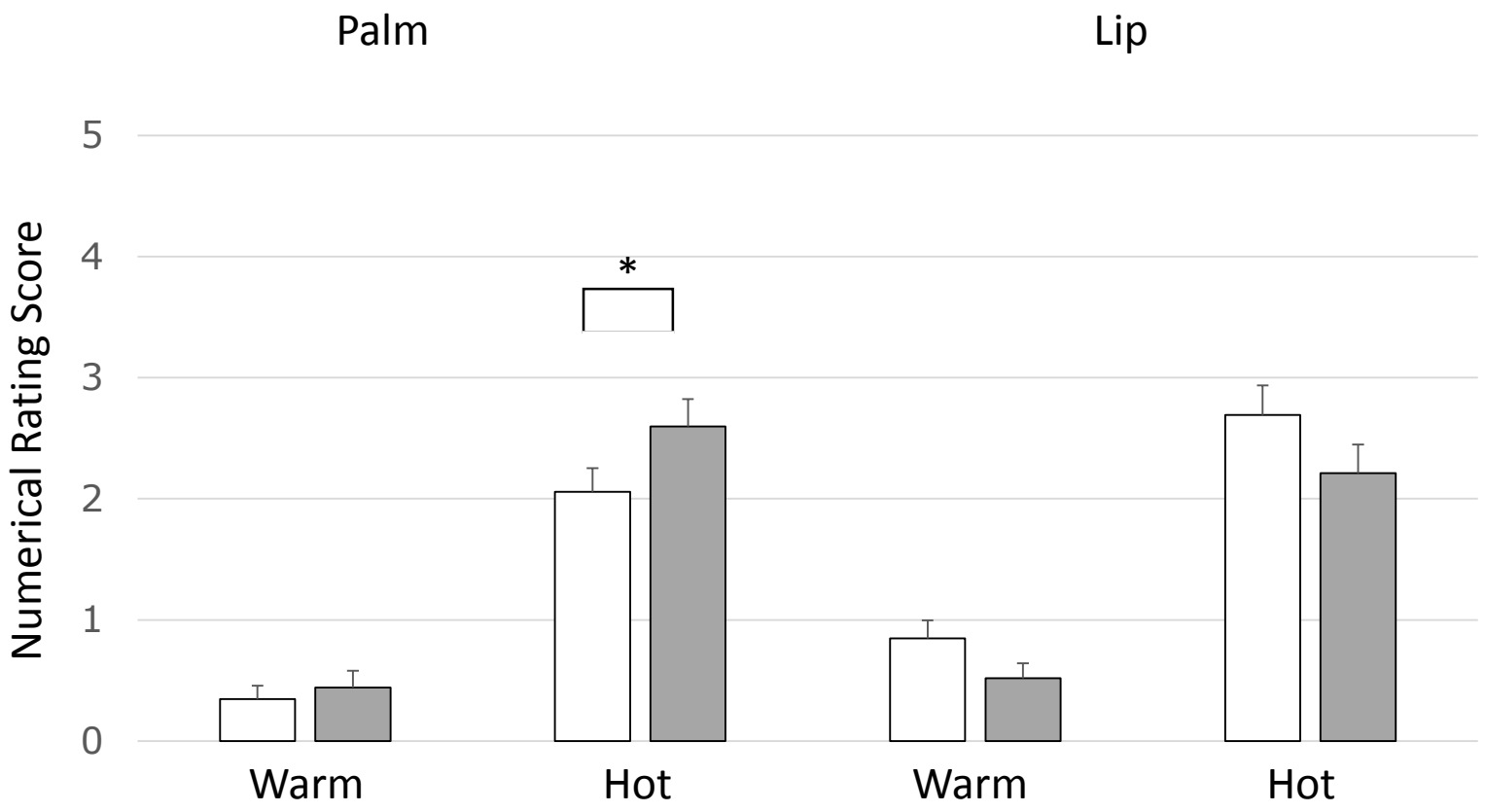

Fig. 6 Perceived pain intensity during sustained thermal stimuli in healthy volunteers

First half period Second half period * $P < 0.05$