

## Research article

## Changes of gamma-band oscillatory activity to tonic muscle pain



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## HIGHLIGHTS

- Tonic muscle pain originating from deep tissue was proposed to better resemble the clinical pain.
- We observed the enhancement of gamma oscillations in frontal-central region during tonic muscle pain, as compared to control conditions.
- Positive relationship between the amplitude of gamma oscillations and pain intensity was also observed in frontal-central region.

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## ABSTRACT

It is well known that phasic pain could induce suppression of alpha oscillations and enhancement of gamma oscillations. However, the cortical responses to tonic pain, especially tonic pain originating from deep tissue, which was proposed to better resemble the clinical pain, are not well understood. Here we aimed to investigate electroencephalographic (EEG) responses to tonic muscle pain. EEG signals and pain perceptions of three order-counterbalanced conditions: innocuous condition (A, infusion of isotonic saline), noxious conditions with low (B) and medium (C) intensities (infusion of hypertonic saline) were recorded from 43 subjects. We observed the enhancement of gamma oscillations in frontal-central region in condition C, as compared to either condition A or B. Positive relationship between the amplitude of gamma oscillations and pain intensity was also observed in frontal-central region. Therefore, we provide novel evidence for the encoding of frontal-central gamma oscillations in tonic pain processing.

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## 1. Introduction

Pain related brain responses were characterized using numerous hemodynamic (e.g., functional magnetic resonance imaging; fMRI and positron emission tomography; PET) and electrophysiological (e.g., electroencephalography; EEG and magnetoencephalography; MEG) studies [1–3], which were primarily based on phasic cutaneous pain [4]. Phasic pain is too short to faithfully simulate clinical pain, which is rarely brief and exhibits an explicit onset and offset of pain perception. Besides, different from pain originating from deep tissue, cutaneous pain is not dominantly encountered

in clinical practice [5,6]. Currently, tonic cutaneous pain models (e.g., cold pain [7–12], tonic heat pain [13–17]) have been proposed to resemble clinical pain, and to investigate pain-related cortical features [5,18]. There is growing interest in elucidating the neural correlates of tonic deep pain using various physical stressors: muscular injections of capsaicin [19,20] and hypertonic saline [21]. Muscular injection of hypertonic saline could induce long-lasting cramp-like pain that closely mimicked the chronic pain in patients. Additionally, this pain has been considered to be more nociceptive-specific as compared to electrical stimulation, which acted on a much broader spectrum of receptors or afferents, e.g., A $\beta$  fibers [22,23].

Studies investigating tonic cutaneous pain reported inconsistent findings, most of them reported pain-induced suppression of alpha oscillations in frontal-central, temporal or parietal-occipital regions [12,13,24], while a few reported pain-induced enhancement of alpha oscillations [11,25] and gamma oscillations [14]. However, pain-related EEG changes to tonic deep pain were rarely

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reported, and the similarity and difference of cortical processing to tonic cutaneous pain and tonic deep pain remains unclear.

Here, by comparing EEG responses evoked by prolonged innocuous stimulation, noxious stimulation with low and medium intensities (muscular injection of hypertonic saline with different rates), we aimed to investigate the cortical processing to tonic muscular pain.

## 2. Methods

### 2.1. Subjects

The study included 43 right-handed, male subjects ( $22 \pm 3$  years). All subjects were nonsmokers with no personal history of any neurological or psychiatric disease. None of the subjects had any history of chronic or acute pain up to 4 weeks before and during the study period, and none of the subjects was on any medication. All subjects provided informed consent, and the Human Research Ethics Committee of the Shenzhen Institutes of Advanced Technology approved the experimental procedures.

### 2.2. Experimental design

Experiments were conducted in a silent and separate room. The experiment consisted of three order-counterbalanced 15.5-min conditions (Fig. 1, top panel), i.e., innocuous condition (A), noxious conditions with low (B) and medium intensities (C). Each condition consisted of 2-min baseline and following prolonged stimulation. An acoustical signal (beep) reminded subjects to rate the level of perceived pain on a numerical rating scale (NRS; 0: no pain; 10: the worst imaginable pain) at the beginning of each 15-s period, using a small numeric keypad with their right hands.

A three-way stopcock was connected with the two automated syringe infusion pumps through two disposable extension tubes and with one disposable vein detained needle (24-gauge). The needle was inserted in left masseter muscle to a depth of 1 cm approximately. Innocuous stimulation was introduced by infusion of isotonic saline (0.9% NaCl) at a constant speed of  $75 \mu\text{l}/\text{min}$ . Noxious stimulation was introduced by infusion of hypertonic saline (5% NaCl). Noxious conditions included a bolus infusion (B: 0.15 ml; C: 0.25 ml) over 15 s at the beginning and subsequent continuous infusion at variable speed, which was adjusted using a computer-controlled closed-loop system based on the real-time feedback of ratings to ensure perceived pain intensity maintained at an approximate preset NRS levels (B:3; C:5) [26] to avoid habituation [24]. The generated muscle pain disappeared 5–10 min after the completion of infusion [27], and consecutive conditions were separated by a break for at least 10 min.

Subjects were instructed to fill out the Positive and Negative Affective Scale (PANAS) [28], McGill Pain Questionnaire (SF-MPQ) [29] after each condition. The Chinese version of these questionnaires had acceptable reliability and validity [30,31].

### 2.3. Behavioral data analysis

The stimulation intensities of two noxious conditions were compared using a paired-sample *t*-test. The average rating of pain perception across all rating points (once every 15 s) was calculated for each subject for each condition. The rating of pain perception, MPQ sensory and affective scores, positive and negative affect ratings were compared across all three conditions using a one-way repeated-measures analysis of variance (RM-ANOVA), with “pain” as within-subject factor (three levels). Post-hoc tests were performed when the main effect was significant.

### 2.4. EEG recording and preprocessing

The EEG data were recorded using a 64-channel Neuroscan system (pass-band: 0.01–100 Hz, sampling rate: 1000 Hz) using a standard cap based on the extended international 10–20 system [32]. The reference channel was located at the vertex, and all channel impedances were kept lower than  $10 \text{ k}\Omega$ . To monitor ocular movements and eye blinks, electro-oculographic signals were simultaneously recorded from four surface electrodes, one pair placed over the higher and lower eyelid, the other pair placed 1 cm lateral to the outer corner of the left and right orbit.

EEG data were analyzed using EEGLAB [33]. Continuous EEG data for each condition were down-sampled to 500 Hz and band-pass filtered (1–100 Hz). EEG data were corrected using independent component analysis (ICA) algorithm [33–36]. ICA components that were considered as purely or predominantly driven by artifacts (such as ocular artifacts, myogenic artifacts and other low variance noises) were discarded based on visual inspection of power spectrum, time course and topography. The remaining components were projected back into the original sensor space [37]. The denoised EEG data were re-referenced to a common average reference. EEG data were segmented into epochs of 1 s. Epochs (30 s) at the beginning and ending of each condition and epochs involved the rating of pain perception were discarded. Furthermore, epochs contaminated by residual muscle artifacts were manually rejected by visual inspection. Remaining epochs (8 min with relatively stable pain perception and 1.5-min baseline) were selected for the following spectral analysis.

### 2.5. EEG spectral analysis

For each subject and each condition, EEG epochs were transformed to the frequency domain using a discrete Fourier transform, to yield amplitude spectra (in  $\mu\text{V}$ ) ranging from 1 to 100 Hz. Amplitudes of EEG oscillations in theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz), and gamma (50–90 Hz) bands were calculated for each condition and electrode. The obtained single-epoch amplitude spectra for each condition and electrode were averaged across epochs to enhance the signal-to-noise ratio. Baseline correction was performed by subtracting the mean spectra of baseline from the mean spectra of responses for each condition. The baseline corrected spectra were normalized across conditions for each frequency band, and expressed as *z* values (subtracting the mean and dividing by the standard deviation of the spectra of three experimental conditions). Such normalization was applied to ensure equal contributions from each subject for the following comparisons [14,38]. Normalized spectra were compared across three conditions using a point-by-point one-way RM-ANOVA, with “pain” as within-subject factor. For conditions B and C, we applied a linear mixed model (LMM) to investigate the relationship between pain intensity (response variable) and brain activity (predictor) for each electrode and frequency band [15,39]. The brain responses (spectra within each 15-s period), used in LMM analysis, were obtained by calculating the average of single-epoch spectra within the same period.

A permutation test with 5000 iterations was used to construct the null distribution of the max statistic across electrodes to control for multiple comparisons. We identified the statistic that corresponded to the 5% most extreme parts of the maximal *F* distribution. We thresholded original statistical maps at that 5% level from the maximal distribution [40]. Compared with this statistic, higher statistical value represented significant result after correction.

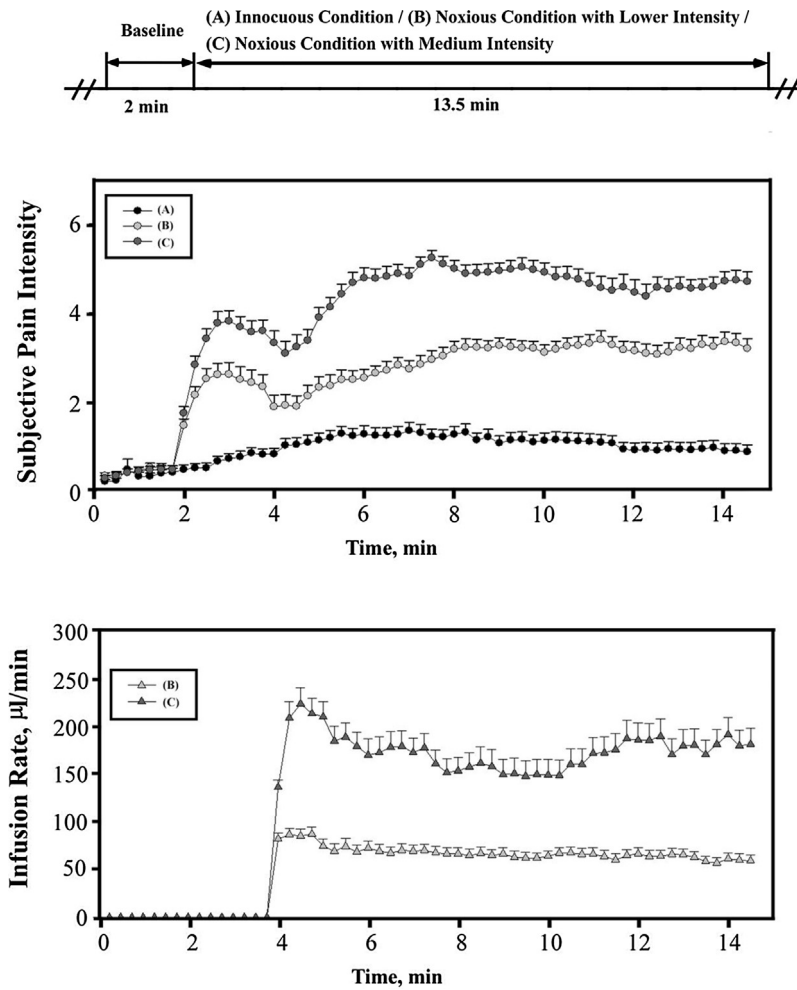


**Fig. 1.** Experimental design and subjective pain intensity.

Top panel: The experiment consisted of three order-counterbalanced conditions: innocuous condition (A), noxious conditions with low (B) and medium (C) intensities. Middle panel: The subjective pain intensity (mean  $\pm$  SEM) was collected every 15 s. Bottom panel: infusion rate (mean  $\pm$  SEM) of prolonged hypertonic saline infusion after bolus infusion in conditions B and C.

### 3. Results

#### 3.1. Psychophysical results

The pain intensity and infusion rate was shown in Fig. 1. The pain intensity during innocuous stimulation increased slightly with increased stimulus duration and this may be caused by the needle effect, which has been reported in previous studies using tonic muscle pain model [41]. The total infusion volume of innocuous condition was the same for all subjects (1012.5  $\mu$ l in total). The total infusion volume was significantly different between two noxious conditions (B:  $725 \pm 339 \mu$ l in total; C:  $1862 \pm 826 \mu$ l in total;  $t(42) = -11.134$ ,  $p < 0.001$ ).

The pain intensity (A:  $1.09 \pm 1.00$ ; B:  $3.17 \pm 0.82$ ; C:  $4.77 \pm 0.95$ ;  $F(2, 84) = 242.404$ ,  $p = 0.000$ ), MPQ sensory (A:  $2.12 \pm 2.51$ ; B:  $5.86 \pm 2.53$ ; C:  $8.91 \pm 3.60$ ;  $F(2, 84) = 76.570$ ,  $p = 0.000$ ) and affective ratings (A:  $0.47 \pm 0.77$ ; B:  $1.60 \pm 1.71$ ; C:  $2.28 \pm 1.86$ ;  $F(2, 84) = 25.225$ ,  $p = 0.000$ ), as well as PANAS negative ratings (A:  $11.65 \pm 2.51$ ; B:  $12.74 \pm 3.16$ ; C:  $14.05 \pm 4.54$ ;  $F(2, 84) = 10.272$ ,  $p = 0.000$ ) were significantly different among three conditions. All post-hoc pair-wise comparisons were significant. However, PANAS positive ratings were not significantly different among three conditions (A:  $16.84 \pm 6.40$ ; B:  $16.74 \pm 5.78$ ; C:  $17.16 \pm 6.79$ ;  $F(2, 84) = 0.354$ ,  $p = 0.703$ ).

#### 3.2. Electrophysiological results

Significant differences of spectra were dominantly observed at frontal-central electrodes (FC1 and C1) within gamma band (Fig. 2, top left panel), and no significant difference was observed within other frequency bands (Supplementary Fig. 1). Supplementary Fig. 2 shows the group level scalp topography of gamma oscillations in condition C. Top middle panel of Fig. 2 displays the normalized average spectra of three stimulation conditions. The summarized amplitude within gamma band at frontal-central electrodes is displayed in top right panel of Fig. 2. One-way RM-ANOVA revealed that the summarized amplitude within gamma band was significantly different among three conditions ( $F(2, 84) = 6.445$ ,  $p = 0.002$ ). Post-hoc comparisons revealed that the summarized amplitude was significantly higher in condition C than those in conditions A and B (A vs. C:  $p = 0.013$ ; B vs. C:  $p = 0.006$ ).

As revealed by LMM analysis, positive relationship between the amplitude of gamma oscillations and pain intensity was observed at electrode FCz (Fig. 2, bottom left panel). No significant relationship was observed at other frequency bands (Supplementary Fig. 3). As shown in bottom right panel of Fig. 2, the positive relationship was maximal around 66 Hz.

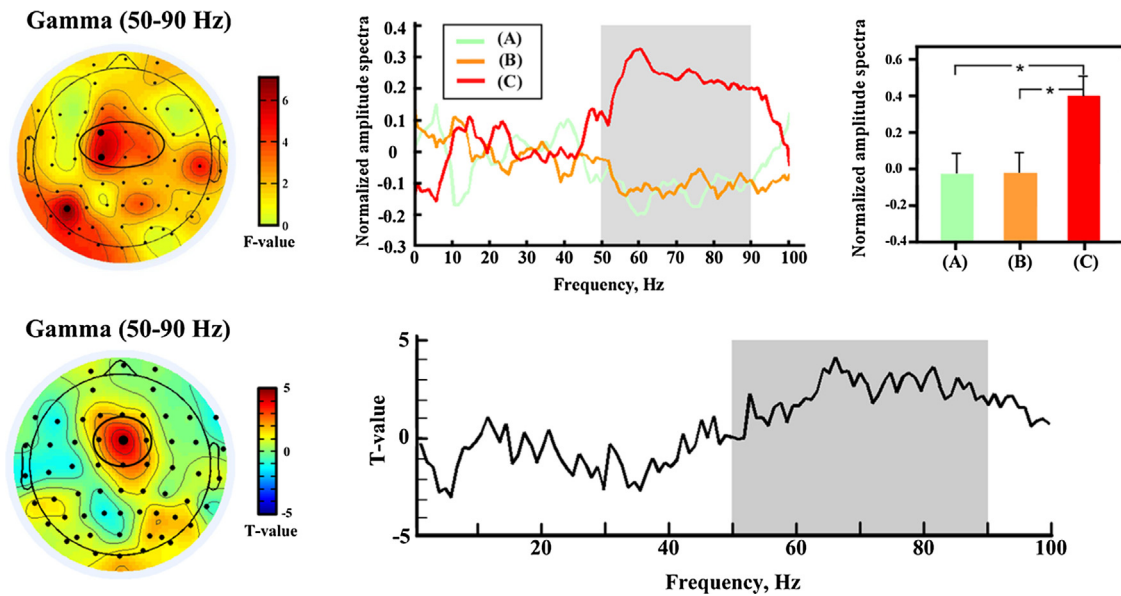


**Fig. 2.** Comparison of normalized amplitude spectra among three conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Top left panel: significant difference was dominantly observed at frontal-central region within gamma band. Electrodes with a significant difference after multiple comparison correction are marked by bold black dots. Top middle panel: normalized amplitude spectra of gamma oscillations, measured at frontal-central electrodes were respectively displayed in green, orange, red for conditions A, B and C. Top right panel: the summarized amplitude spectra of gamma oscillations, measured at frontal-central electrodes were respectively marked in green, orange and red. Error bars represent, for each condition,  $\pm$  SEM across subjects. Asterisk \* indicates a significant difference ( $P < 0.05$ , Tukey's post-hoc comparisons). Bottom left panel: topography of the relationship between the amplitude of gamma oscillations and pain intensity and as assessed by LMM analysis. Positive and negative relationships are depicted by warm and cold colors, respectively. Electrode with a significant relationship after multiple comparison correction is marked by bold black dots. Bottom right panel: frequency course of the relationship between the amplitude of gamma oscillations and pain intensity at electrode FCz.

#### 4. Discussion

Gamma oscillations have been suggested to be related to cortical activities subserving phasic laser pain perception [1,42]. Another study observed increases in gamma oscillations during tonic pain, but it was only demonstrated between noxious condition and no-task resting condition [14]. Variability both between and within subjects in vigilance, attention, arousal, etc. during the no-task resting condition could result in large error variance, which would make it much more difficult to identify and isolate the pain-related EEG features. When innocuous stimulation condition was used as control [21,25], these results cannot unequivocally be attributed to pain as a distinct perceptual modality compared to non-painful sensations because the two qualitatively different perceptual conditions were regularly confounded with substantial difference in stimulus intensity. Here we observed tonic muscle pain induced significant frontal-central gamma enhancement when compared with both innocuous condition and noxious condition with low stimulus intensity. We also observed positive relationship between gamma oscillations and subjective pain intensity at frontal-central electrode FCz. This is in consistent with one recently published study which reported that the subjective pain intensity was encoded by prefrontal gamma oscillations, but stimulus intensity was not [15].

Phasic pain induced suppression of alpha oscillations mainly located in sensorimotor and occipital scalp regions [43,44]. Tonic pain also induced suppressed alpha oscillations over the parietal-occipital part of the brain [7,9,13,14,21]. The relation between the changes in alpha oscillations and pain perception was questioned, because decreases in alpha oscillations also occurred in response to non-painful stimulations. The decreases in alpha oscillations were observed when the tonic non-painful heat or the tonic painful heat was compared with baseline, but no significant difference was obtained when comparing these two conditions with tonic thermal stimulation [13]. No suppression of alpha oscillations was

observed in the present study and there might be certain explanations for this result. First, baseline correction was performed before comparison of amplitude spectra among three conditions. During baseline and prolonged stimulation, subjects were asked to focus on pain and rate its intensity. Alpha oscillations were involved in the mechanisms of top-down modulation, attention, and consciousness, and baseline and stimulation period might share same top-down modulation processes [45]. Secondly, alpha suppression has been observed from the comparison of noxious attended condition with innocuous distracted condition [14]. The attention modulation could affect alpha oscillations significantly and tonic pain induced suppression in alpha band might largely result from the attention shift to the somatosensory stimuli instead of directly reflecting the stimulus-related processing [14]. In this study, both innocuous and noxious conditions were attended. Therefore, we could not observe significant difference of alpha oscillations among three conditions after baseline correction.

Cutaneous pain differs from muscle pain in quality and affective dimension, and previous studies have made effort to capture differences between EEG response to cutaneous pain and muscle pain. EEG response induced by infusion of capsaicin in skin and muscle has been compared, the results showed that muscle pain induced significantly increased beta oscillations (25–35 Hz) in extensive frontal, parietal and occipital areas compared to skin pain [20]. Here we demonstrated that frontal-central gamma oscillations showed more specific to tonic muscle pain perception, future study should include same stimulation paradigm for cutaneous pain and muscle pain, to make direct comparison of EEG topographic patterns.

Continuous EEG recordings are possible to be contaminated by muscle activities (e.g., EMG signals originating from facial or masticatory muscles) [46–48]. Two published studies interpreted the pain induced gamma enhancement as related to muscle artifacts [12,41]. The intramuscular infusion could lead to an increase in baseline EMG activity. Yilmaz et al. investigated the possible interference of the muscle activities around the head on EEG records and

their results confirmed that the contamination was most prominent on the side ipsilateral to the intramuscular stimulation and also notable in other electrodes away from the stimulation site [46]. Strongest muscle activities happened around lateral electrodes, but the central electrodes exhibited relative weaker contamination [47,49]. In this study, continuous EEG data was corrected using a validated ICA algorithm [33] and EEG segments contaminated by residual muscle artifacts were manually rejected. And the group level scalp topography of gamma oscillations during noxious stimulation (Supplementary Fig. 2) exhibited a completely different distribution compared with the topographic result of amplitude comparison among three conditions (Fig. 2, top right panel). Therefore, increased gamma activity at frontal-central electrodes could be mainly explained by the brain responses to pain.

## 5. Conclusion

Tonic muscle pain induced enhancement of gamma oscillations in frontal-central scalp region compared to both innocuous condition and noxious condition with low intensity. Furthermore, we observed positive intra-individual relationship between the amplitude of gamma oscillations and pain intensity. These observations provide novel evidence for the encoding of frontal-central gamma oscillations in tonic pain processing.

## Conflict of interest

None.

## Author contributions

LL performed data analysis, drafting and revising of the manuscript. X.L., C.C., Y.Y. and D.L. made substantial contributions to the data collection. L.X., D.X., L.H. and Y.Q. contributed in study design and manuscript revision.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neulet.2016.05.067>.

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