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ORIGINAL PAPER

Dynamic Processing of Nociception in Cortical Network in Conscious Rats: A Laser-evoked Field Potential Study

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Abstract (1) Field potential study in conscious rats provides a convenient and effective animal model for pain mechanism and pharmacological research. However, the spatial-temporal character of nociception processing in cortex revealed by field potential technique in conscious rats remains unclear. (2) In the present study, multi-channel field potentials evoked by noxious laser stimulation applied to the hind paw of conscious rats were recorded through 12 chronically implanted skull electrodes. Independent component analysis (ICA) was used to remove possible artifacts and to extract the specific nociception-related component. (3) Two fast sharp responses and one slow blunt response were evoked by noxious laser stimulation. Systemic morphine (5 mg/kg, i.p.) preferentially attenuated the amplitude of the slow blunt response while had no significant effect on the first two sharp responses. ICA revealed that those responses came from activities of contralateral anterior parietal area, medial frontal area and posterior parietal area. A movement artifact was also detected in this study. Partial directed coherence (PDC) analysis showed that there were changes of information flows from medial frontal and posterior parietal area to anterior parietal area after noxious laser stimulation. (4) Characterization of the spatio-temporal responses to noxious laser stimulation may be a valuable model for the study of pain mechanisms and for the assessment of analgesia.

Keywords Laser stimulation · Nociception · Independent component analysis · Morphine · Conscious rat · Primary somatosensory cortex

Introduction

Accumulating evidence from neuroimaging, electrophysiological, and neuroanatomical studies in animals and humans has shown that the cerebral cortex participates in the

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perception of pain (Treede et al. 1999; Peyron et al. 2000; Price 2000, 2002). Multiple cortical areas were found to be activated by various painful stimulations including electrical (Inui et al. 2003; Disbrow et al. 1998), chemical (Iadarola et al. 1998; Porro et al. 2002), mechanical (Creac'h et al. 2000), and thermal (Valeriani et al. 2000; Casey et al. 2001) stimulation. These areas include primary and secondary somatosensory cortex (SI and SII), anterior and posterior cingulate cortex (ACC and PCC), insula, amygdala, posterior parietal cortex (PPC), and so on. Together with some subcortical structures, these areas form a network in the brain for pain processing. Currently, the research on how these cerebral areas participate in the dynamic processing of pain and what their functions are has become the focus of pain study.

The behaving animal model is very useful in providing the phenomenological description and functional aspect of nociception processing. Study on behaving animal model is crucial for the understanding of mechanisms underlying pain, because of the feasibility in performing various intervention experiments. Recently, single-unit recording technique has been used in study of nociception in behaving animals (Kuo and Yen 2005; Wang et al. 2003). Unit recording technique allows for simultaneously recording of spike activity of dozens of individual neurons. However, this technique only provides the observation of the activity of a small amount of neurons in a few interested cerebral areas, rather than the activity of the whole cortex. Multi-channel recording of evoked potential allows us to observe the response of the neural ensembles to peripheral stimulation, so that we may have better understanding on the dynamics of different cortical areas during the processing of nociception. Moreover, it also provides the possibility to investigate the temporal and functional relationships between different cortical areas.

Cortical response evoked by noxious laser stimulation has been demonstrated as an effective tool to study the processing of pain in central nervous system (Carmon et al. 1976; Bromm and Lorenz 1998; Kakigi et al. 2000). Cortical evoked potential studies carried out on behaving animals are few (Shaw et al. 1999, 2001). Shaw et al. (1999) observed the cortical response to noxious stimulation applied to the tail of behaving rats by multi-channel EEG recording. So far, the spatio-temporal characteristics of nociceptive processing in cortical network of behaving rats have not been well described. In the present study, we used 12 scattered electrodes chronically implanted in the skull to record the cortical responses evoked by noxious laser stimulation applied to the hind paw, and used the averaged potential as reference during the analysis of data, in an attempt to investigate the spatio-temporal features of laser-evoked potentials (LEPs) in behaving rats.

As we know, evoked potentials recorded from surface electrodes cannot be compartmentalized into functionally distinct components, because those signals are mixtures of signals coming from different brain generators overlapped in time courses and skull projections. However, independent component analysis (ICA) can effectively decompose multiple overlapping components from related evoked potentials. It has been proved that ICA is a powerful method for removing artifacts (Jung et al. 2000; Vigário 1997) and separating sources of the brain signals from EEG and event-related potentials recording (Makeig and Jung 1996a; Makeig et al. 1999; Zhukov et al. 2000). Therefore, ICA provides the possibility to explore the temporal relationship and evaluate the functional significance of those evoked responses. In the present study, we used an ICA to decompose the LEPs recorded from the 12 electrodes, attempting to explore the possible cortical generators of those responses. By performing further analysis of decomposed components using partial directed coherence (PDC) and cross correlation, as well as morphine treatment, we aimed to reveal the relationships between components and explore their functional significances.

Materials and Methods

A total of 19 adult male Sprague-Dawley rats weighing 300–350 g were used in this experiment. They were individually housed in cages under temperature- and humidity-controlled conditions with a reversed 12 h dark/light cycle (light on from 19:00 to 07:00 h). Food and water were available ad libitum. All experiments were carried out in accordance with the Institutional Animal Care and Use Committee of Peking University Health Science Center. All methods were taken to minimize animal suffering.

Implantation of Surface Electrodes

Rats were anesthetized initially with Ketamine (100 mg/kg, i.p.), and 1/3 of original doses were administrated whenever necessary to maintain proper anesthetic depth during surgery. The dorsal surface of the rat's head was shaved after anesthetized and fixed in a Kopf stereotaxic apparatus. A midline scalp incision was made and the skull surface was exposed. Twelve stainless steel screws (O.D. = 1 mm, impedance 300–350 Ω) used as recording electrodes were implanted symmetrically 3 mm apart from each other (see Fig. 1) following the surgical procedure described in details by Shaw et al. (Shaw et al. 1999). The zero point was at the bregma. The coordinates of channel PR1 were 1.5 mm posterior to and 1.5 mm lateral to bregma. The coordinates of channel RFR were 0 mm posterior to and 4.5 mm lateral to bregma. A reference and a ground electrode were positioned at the midline, 2 and 4 mm caudal to the lambda respectively. These electrodes were connected with a 2-array connector and fixed to the skull with dental cement. Animals were then injected with antibiotics (penicillin, 60,000 U, i.m.) and were housed individually in cages. A recovery period of 2 weeks was allowed before testing started.

Recording of Laser-evoked Potentials

Laser-evoked potentials (LEPs) were recorded simultaneously over the 12 channels through a lightweight cable connected to a digital preamplifier. The impedance of all the electrodes was kept below 5 kΩ. Data were recorded using an EEG/ERP system (CogniTrace ERP, ANT Inc., the Netherlands) and sampled at a rate of 256 Hz together with the stimulus markers.

Experimental Procedure

Rats were put into a plastic chamber ($40 \times 40 \times 30$ cm^3) with an apertured bottom (5 mm diameter) and allowed to move freely during the experiment. The holes on the bottom of the chamber allowed the laser beam irradiates on the skin directly. For noxious stimulation, brief laser pulses (wavelength 10.6 μm, beam diameter 2.5 mm, pulse width 20 ms) were applied to the glabrous volar surface of the left hind paw delivered by a CO_2-laser stimulator (DIMEI-300, Changchun Optics Medical Apparatus Co. Ltd, China). The stimulation site could be visualized by a He–Ne laser beam. The inter-stimulus interval was no less than 20 s. The power of laser output increased stepwise from 1 W with a 1 W increment to the extent that obvious withdrawal of the hind paw could be elicited by each of the five consecutive stimulus, which was taken as the stimulation intensity (usually 5–7 W). In order to minimize the sensitization or habituation of nociceptors, the stimulation site was changed slightly after each stimulus. To avoid any type of conditioning, sham stimuli were randomly inserted throughout the entire experimental session (i.e., turned the laser on and off to mimic the real stimulation without focusing on the rat paw). Each rat received 80 noxious and sham stimuli, respectively. The LEPs were recorded during the entire stimulating course.

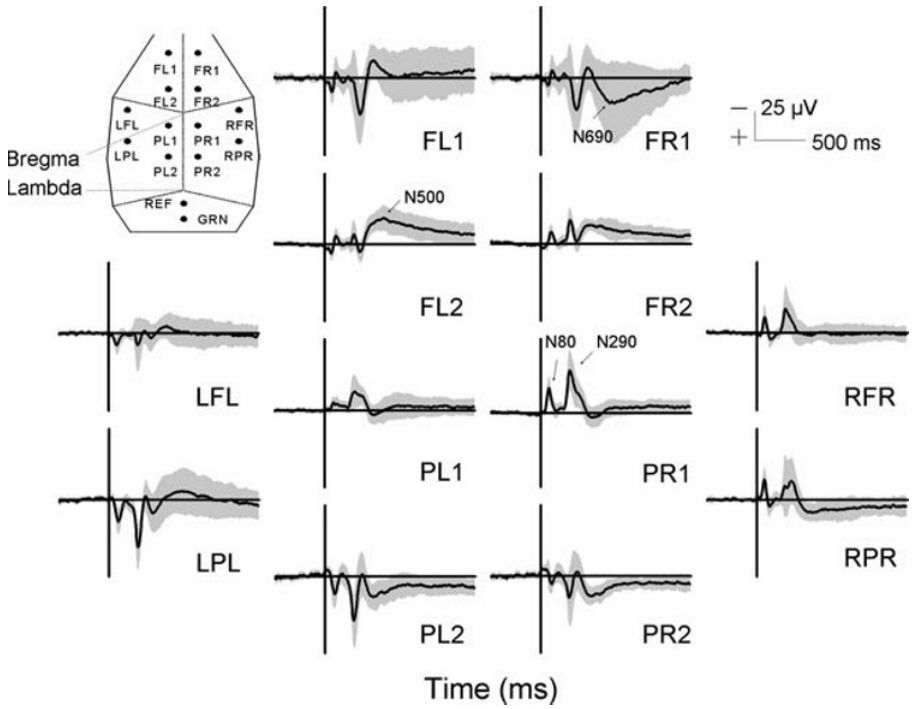


Fig. 1 Grand mean waveforms of LEPs in response to noxious CO_2 laser stimulation at the left hind paw. Locations of 12 electrodes were shown in the upper-left inset. The vertical line indicates the time of laser stimulation. Dark-shaded areas indicate standard deviation. The arrows show the four major responses (N80, N290, N500 and P690)

After the first recording session described above, morphine (5 mg/kg, provided by the Northeast Drug Company, Shenyang, China) or equivalent volume of saline was delivered intraperitoneally to 11 and 8 rats, respectively. The rats were then put back into the experimental chamber, and a similar recording session was carried out after 15 min.

Measurement of Pain Threshold

In order to evaluate the analgesic efficacy of morphine, tail flick latency (TFL) in response to a radiating thermal stimulus was assessed with a 12.5 W projector bulb, according to the method of D'Amour and Smith (1941). The temperature was adjusted to obtain a baseline of 4–5 s. The cut-off time was set at 15 s to avoid damage to the tail. For each rat, three tests were carried out before the recording of LEPs with an interval of 5 min. The results were averaged and taken as the baseline pain threshold. Fifteen minutes after morphine or saline injection, TFLs were again measured every 5 min during the recording of LEPs throughout the second recording session.

Data Analysis

Data were analyzed with free software EEGLAB (Delorme and Makeig 2004). Raw data were preprocessed before imported into EEGLAB, including rereferencing to average of all the recording channels, filtering with a band pass of 0–30 Hz and detrending. The data epoch for

calculating LEPs was 500 ms before and 1,500 ms after stimulus onset. Obvious contaminated evoked data trials were rejected before averaging.

Independent Component Analysis

The LEPs we obtained from each recording electrode were mixed signals from independent sources (brain activities), and the generated LEPs typically overlap both in time and space, which could be defined as:

$$x(t) = \mathrm{A}s(t),$$

where $x(t)$ represents the n observed signals over t time-points, $s(t)$ represents the n source signals, and A is the 'mixing matrix', that must be resolved for source separation. The goal of independent component analysis (ICA) is to find a linearly inverse matrix W, which can restore independent sources signals $s(t)$ from observed signals $x(t)$:

$$s(\mathrm{t}) = \mathrm{W}x(\mathrm{t}).$$

ICA was originally proposed to solve the 'blind source separation (BSS)' problem. However, it has been used widely in bio-medical signal analysis now (Jung et al. 2001). ICA can effectively decompose multiple overlapping components from selected sets of related averages (Makeig et al. 1999). ICA algorithms find a coordinate frame onto which the projection of the data has minimal temporal overlap. The core mathematical concept of this algorithm is to minimize mutual information among the data projections. By applying ICA, the location and the activities of these brain processes could be recovered, and further analysis of these signals becomes possible.

In order to compare the independent components (ICs) before and after morphine/saline treatment, we used an ICA-based subspace projection algorithm proposed in a recent paper on Brain-Computer Interface (Xu et al. 2004). First, we decompose the multichannel ERP data before treatment into ICs by ICA, then use the weight matrix produced in this procedure as a temporal-spatial filter to make temporal and spatial manipulation of the ERP data after treatment, and finally project the ICs created by this way back to the skull surface. In addition, ICA has been demonstrated as an effective method to segregate obvious artifactual EEG components such as eye movements and muscle noise from other sources (Makeig et al. 1996b; Vigário 1997). In order to obtain pure signals, we used ICA to remove the possible artifact embedded in the data before further analysis.

Partial Directed Coherence

Partial directed coherence (PDC) is a new frequency-domain approach to describe the direction of information flow among the multivariate time series based on the decomposition of multivariate partial coherences computed from multivariate autoregressive models. It has been described in detail elsewhere (Sameshima and Baccalá 1999; Baccalá and Sameshima 2001; Fanselow et al. 2001). Central to the method is its use of multivariate time series modeling in conjunction with the concept of Granger causality (Granger 1969). It has been used to describe neuronal ensemble interactions (Sameshima and Baccalá 1999). In the present study we used PDC to describe the direction of information flow between ICs, so that the relationship between cortical areas where those ICs originated from could be understood further. The PDC between each pair of ICs was calculated from 500 ms pre-stimulus to 1,500 ms post-stimulus. The mean PDC values were then normalized to the baseline level (pre-stimulus) as Z-score (for

detail see Yang et al. 2005). In order to show the patterns of information flow under stimulation, the normalized PDC between every two ICs were then summed along the whole frequency range.

Cross-correlation Analysis

In order to confirm the relationship demonstrated by PDC, cross-correlation analysis between ICs along the whole time range (from 500 ms pre-stimulus to 1,500 ms post-stimulus) was performed. Cross correlation is a standard method of estimating the degree to which two series are correlated. It was calculated by a program based on MATLAB. The data epoch was then normalized to Z value (i.e., subtracting the mean value and dividing by the standard deviation). The cross correlation could reflect the degree of correlation and time delay between the two ICs.

Data are presented as the mean ± SD where not specified. Paired Student's t-test was used for statistical analysis (sham vs. noxious stimulation or before vs. after morphine/saline treatment). Results were considered significant when $P < 0.05$.

Results

Nociceptive Behaviors

All rats showed obvious behaviors related to nociception after noxious laser stimulus, including immediate withdrawal of the stimulated hind paw followed by aversive behavior, such as licking accompanied occasionally by gentle biting the stimulated hind paw. No nociceptive behavior was observed after sham stimulation.

The Waveforms of LEPs

The averaged 12-channel LEPs (averaged of responses from 19 rats) after noxious laser stimulation applied to left hind paw are shown in Fig. 1. Each channel exhibited a different response pattern. In brief, there were four major responses: N/P80, N/P290, N/P500, and P690. The maximal N80 and N290 responses appeared at anterior parietal channel PR1. The average peak latencies and amplitudes of these two signals at PR1 are 78 ± 6 ms, –20.7 ± 12.3 μV and 289 ± 22 ms, –39.6 ± 14.9 μV, respectively. The two earlier negative waves were sharp and brief in morphology. However, the following responses were broad and long-lasting, which presented at channels FR1, FL2, FR2, PL2 and PR2. The maximal negative amplitude of N/P500 was reliably presented at channel FL2 in the anterior midline region, and P690 had its largest amplitude at channel FR1. The mean values of the onset- and peak-latency and the amplitude of these responses with long latencies at these five channels are shown in Table 1. Furthermore, a small negative component earlier than N/P80 with a latency of about 20 ms (N20) could also be identified at channel PL2 and PR2. Interestingly, the N20 response also could be observed at PL2 and PR2 even after sham stimulation (Fig. 2). Except for this potential, sham stimulus could not elicit any significant response.

The Spatial Distribution of LEPs

The grand mean topographies (averaged of responses from 19 rats) showed that there were four times of obvious potential changes following noxious stimulation which occurred around the peak time of N/P80, N/P290, N/P500, and P690, respectively (Fig. 3a). Grand mean of the

Table 1 The onset- and peak-latency as well as peak amplitudes of the late evoked responses

Channel	Onset latency (ms)	Peak latency (ms)	Peak amplitude (μV)
FR1	491 ± 69 (10/19)	680 ± 62	55.3 ± 27.9
FL2	387 ± 31 (19/19)	562 ± 69	–25 ± 10.7
FR2	380 ± 29 (17/19)	612 ± 114	–20.1 ± 5.1
PL2	375 ± 71 (14/19)	559 ± 104	26.8 ± 10.6
PR2	389 ± 54 (16/19)	539 ± 90	23.1 ± 7.3

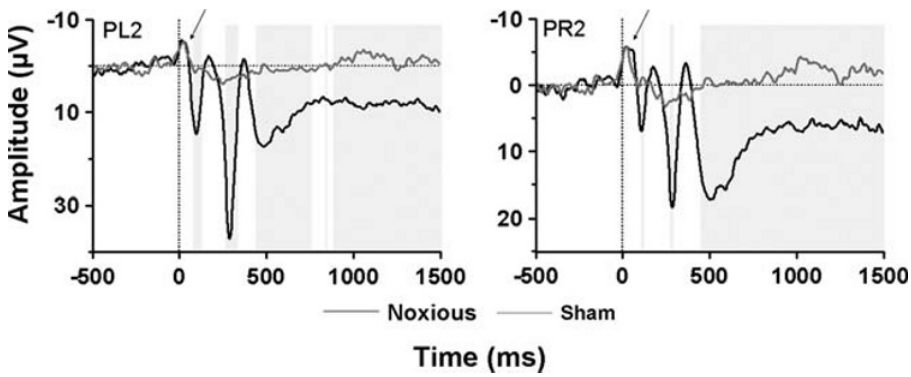


Fig. 2 Grand averages of LEPs recorded at channel PL2 and PR2 following noxious and sham laser stimulation. Shaded areas represent statistically significant differences between responses in the two conditions ($P < 0.05$). Arrows indicate the responses presented in both conditions

topography at 78 ms showed a negative potential distribution over the contralateral anterior parietal region whose underneath cortical structure is SI. The topography of N290 revealed a larger and stronger potential distribution than that of N80. Topographies of complex N/P80 and N/P290 also showed a widespread positive potential distribution at left occipital regions. The following response N/P500 showed a novel distribution from that of N/P80 and N/P290. A negative potential distribution in the medial frontal region was found at 460 ms. The last potential changes occurred around 690 ms after stimulus, and its topographical maps showed an obvious positive potential activity in far-frontal region contralateral to the stimulated hind paw combined with a left bias medial frontal negative potential distribution. Between the two sharp potentials, some weak potentials could also be observed (Fig. 3b).

The Independent Components of LEPs

Average LEPs of each rat were decomposed into independent components (ICs) by applying ICA method. Four major ICs were distinguished (Fig. 4) in this study. The mean peak latencies of the four ICs are shown in Table 2. The first IC (IC1) presented in all 19 rats and showed two peaks (Fig. 4b). The component topography showed an anterior parietal distribution contralateral to the stimulated hind paw (Fig. 4a). IC2 was a medial frontal component with a slight left bias. Its waveform contained two sharp waves followed by a large, broad wave. Posterior parietal component IC3 also contained several peaks. Interestingly, a significant very early response (46 ± 20 ms) was observed from IC3 in some rats (7/19). In most of the rats (13/19), there was also an independent component IC4 which displayed a distribution in the

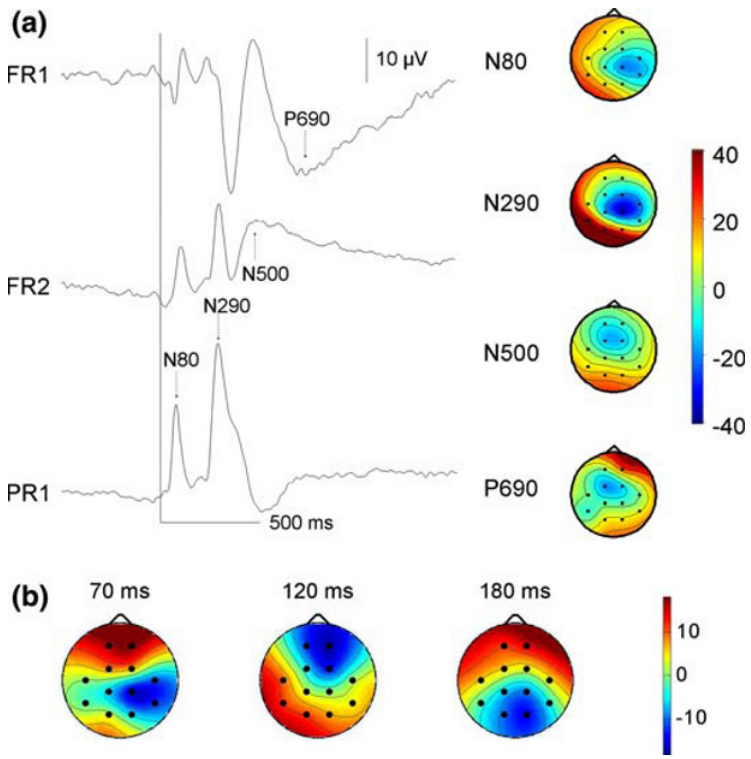


Fig. 3 Spatio-temporal features of the major responses. (**a**) Waveforms (left) and topographical maps (right) of grand averaged N/P80, N/P290, N/P500, and P690. The color bar shows the amplitude and polarity of evoked potentials. (**b**) An example of weak potentials before the strong potential N290

contralateral far-frontal region. It was characterized by a late long-lasting wave, which peaked at 695 ± 125 ms.

To determine a component is brain-related component or a muscle movement artificial component, the component activity image and power spectrum should also be considered. The component images of all four ICs showed a regular plots (meaning that the component does not account for activity occurring in only a few trials) (Fig. 4c). The component activity power spectrum of the former three ICs had a strong alpha band peak at about 7 Hz, while IC4 did not show such a power spectrum activity (Fig. 4d).

Changes of Information Flow and Cross-correlation Between ICs

The changes of the information flow between cortical areas were revealed by PDC of the four ICs. As shown in Fig. 5a, information flow between each two ICs changed after noxious laser stimulation except between IC2 and IC3. Red color represents the increase, while the blue color represents the decrease of information flow. The most prominent changes were the two transiently increased information flows from IC2, IC3, and IC4 to IC1 (Fig. 5a, the first row. Peak latency: 147 and 442 ms). Persistently increased information flows from IC1, IC2, and IC3 to IC4 (starting at 480, 284 and 225 ms, respectively) also can be observed (Fig. 5b, the fourth row). However, the pattern of information flow between IC1 and IC4 was not inversed as expected. Complete different pattern was shown between them. Moreover, there was no significant change of information flow between IC2 and IC3.

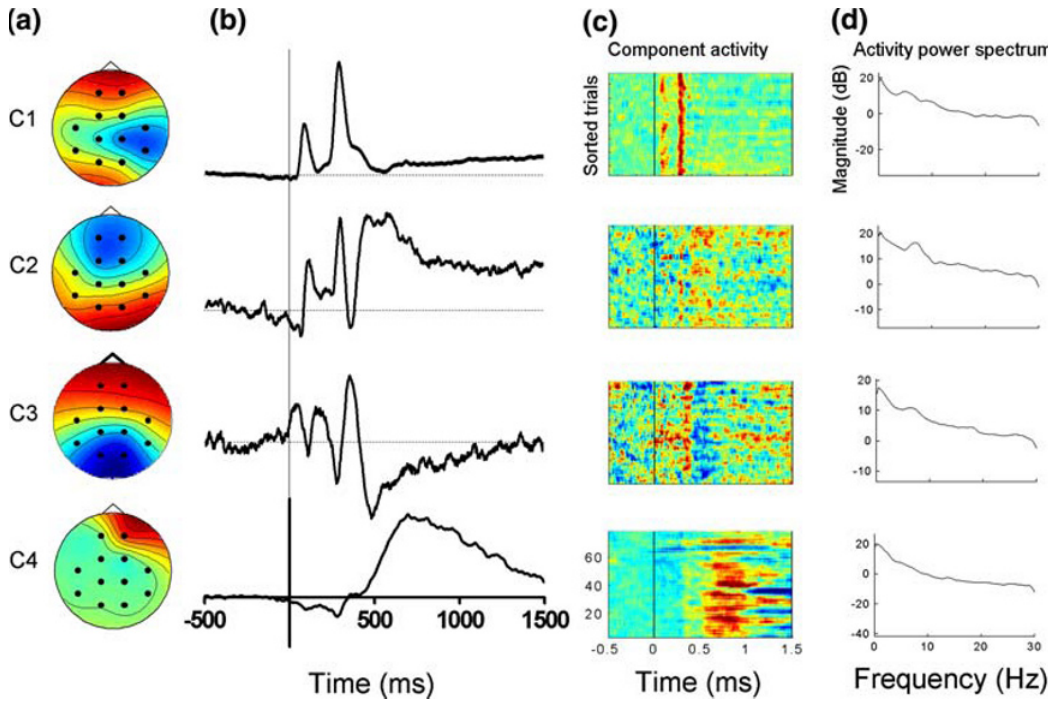


Fig. 4 The four major ICs after noxious laser stimulation. (**a**) The component topographical maps of the four ICs. (**b**) The corresponding waveforms of each IC. The vertical line indicates the onset of laser stimulation. (**c**) Component activity image. The vertical line indicates the onset of laser stimulation. (**d**) Activity power spectrum of the four ICs. Note the change of energy at 6–10 Hz frequency band

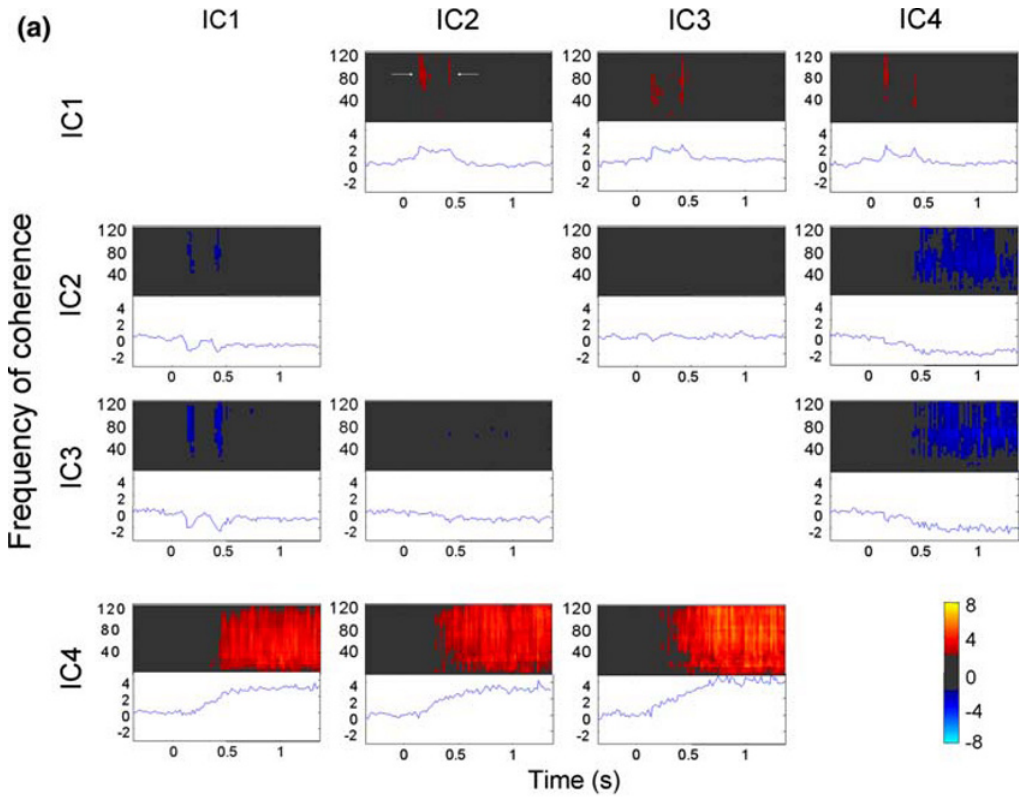
Table 2 Peak latencies of the four ICs

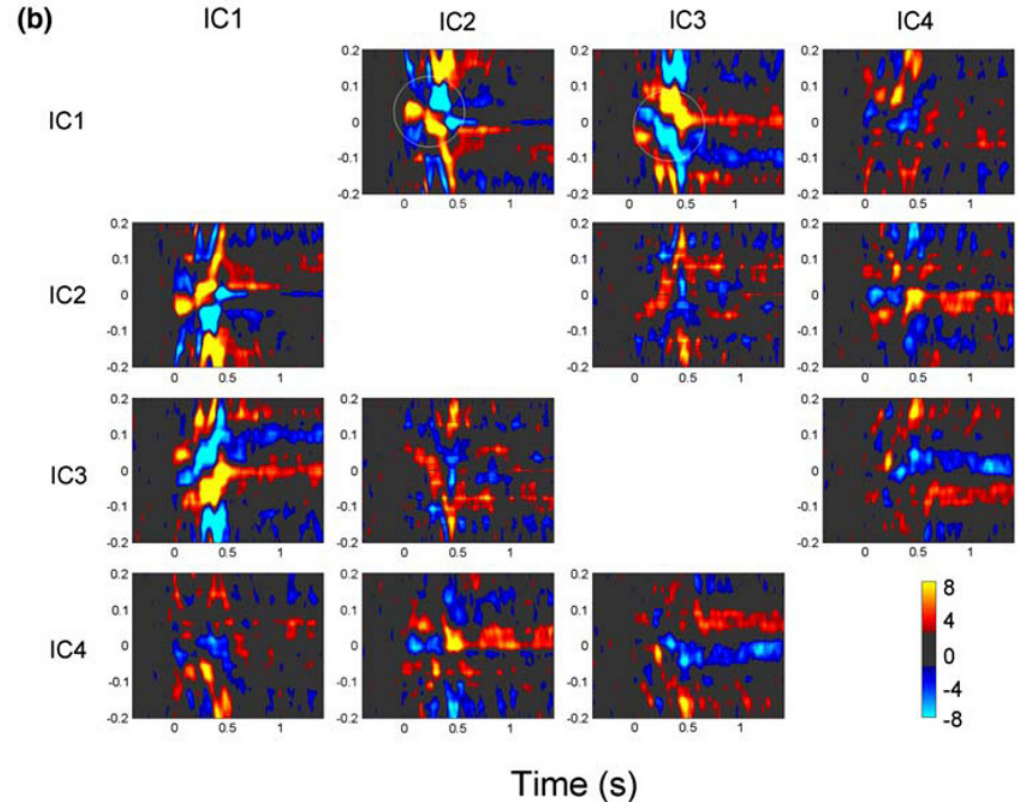
Peaks	IC1	IC2	IC3	IC4
P1	–	–	46 ± 20 (7/19)	–
P2	88 ± 14 (19/19)	101 ± 19 (6/19)	98 ± 10 (5/19)	129 (1/19)
P3	290 ± 15 (19/19)	288 ± 18 (14/19)	287 ± 14 (7/19)	297 ± 43 (10/19)
P4	–	–	352 ± 26 (8/19)	–
P5	469 ± 68 (6/19)	512 ± 87 (16/19)	501 ± 102 (10/19)	695 ± 125 (13/19)

Cross correlation analyses between the four ICs were also performed (Fig. 5b). The evident correlations were presented among IC1 and IC2, IC3 before 500 ms post-stimulus, which was in accord with the results of PDC. Unmatched with the results of PDC, there was no strong correlation between the other ICs and IC4, especially between IC1 and IC4. As expected, there was no strong correlation between IC2 and IC3.

The Effect of Morphine on Cortical Response to Noxious Laser Stimulation

Intraperitoneal injection of 5 mg/kg morphine ($n = 11$) significantly prolonged the tail flick latency (TFL) to radiant heat stimulation (5.1 ± 1.57 s vs. 14.76 ± 1.01 s, $P < 0.001$) while saline injection had no effect on TFL (4.72 ± 0.98 s vs. 4.41 ± 1.19 s, $P > 0.05$). However,


(a)
IC1
IC2
IC3
IC4
Frequency of coherence
Time (s)


(b)
IC1
IC2
IC3
IC4
Time (s)

◀**Fig. 5** The relationship between independent components (ICs) shown by partial directed coherence (PDC) and cross-correlation analyses. (**a**) Grand mean of normalized PDC between the four ICs –0.5–1.5 s around laser stimulation. In each panel, the upper part shows the increase (red and yellow) and decrease (blue) of information flow for each frequency of coherence and time-point, while the lower part shows the mean PDC across all frequencies at each time-point, The direction of information flow is from the component in column to the one in row, for example, the sub-figure in row 2 and column 1 indicate that the information flows from IC1 to IC2. The white arrows show the information flow between IC2 to IC1. (**b**) Cross-correlogram between the four ICs along the time. White circles show strong correlation between IC1 and IC2, IC3 before 500 ms post-stimulus

the effect of 5 mg/kg morphine on nociceptive behaviors after noxious laser stimulation was not consistent with that on TFL. The hind paw withdrew after about half of the stimulus, but the accompanied aversive behaviors such as licking and biting of the hind paw were reduced significantly after morphine injection.

LEPs before and after morphine or saline injection were compared (Fig. 6). Morphine selectively attenuated the N/P500 at channel FR2, FL2, PR2, and PL2 ($P < 0.05$). In contrast, saline had no significant effect on the LEPs. Similarly, except IC1, morphine inhibited the activation of all components (Fig. 7), especially during the late response of IC2 and IC4 and the first peak of IC3 ($P < 0.05$). As expected, saline had no significant effect on any of the ICs.

Discussion

In the present study, spatio-temporal characteristic of evoked responses by noxious laser stimulation applied to the hind paw of behaving rats was described. Systemic morphine had different effect on those evoked responses. Independent component analysis revealed that multiple cortical areas were involved in the processing of nociception. Moreover, the results of PDC and cross-correlation analysis showed the interesting relationships between those areas.

The Temporal-spatial Character of Laser-evoked Potentials

Inconsistent with previous findings (Shaw et al. 1999, 2001), two types of responses were evoked by laser stimulation in the present study. One kind of response is the two sharp negative waves presented in channel PR1 above the anterior parietal area. The topographical maps of the two responses also showed negative current activity in anterior parietal region. The characters of these two responses on location (contralateral anterior parietal area) and waveform (sharp) imply that they may be related to the sensory-discrimination of noxious laser stimulation. The peak latencies of the two responses are similar to those reported previously (Shaw et al. 1999, 2001; Tsai et al. 2004). Another type of response which has not been reported in previous studies is the late and long-lasting blunt wave (channel FL2, FR2). The topographical map of this long-lasting late potential showed the activation of medial frontal region. This type of response possibly reflects the affective-cognitive component of nociception. The difference between Shaw's results and our results is probably due to the different reference electrode selected in data presenting. They used the common lambda reference while we performed rereferencing in data processing (i.e., rereference to average of the 12 electrodes).

Multiple Cortical Areas are Involved in Nociception Processing

In our study, from either LEP or component (IC1) topographical maps, a current activity in contralateral anterior parietal region has been observed after noxious laser stimulation, which

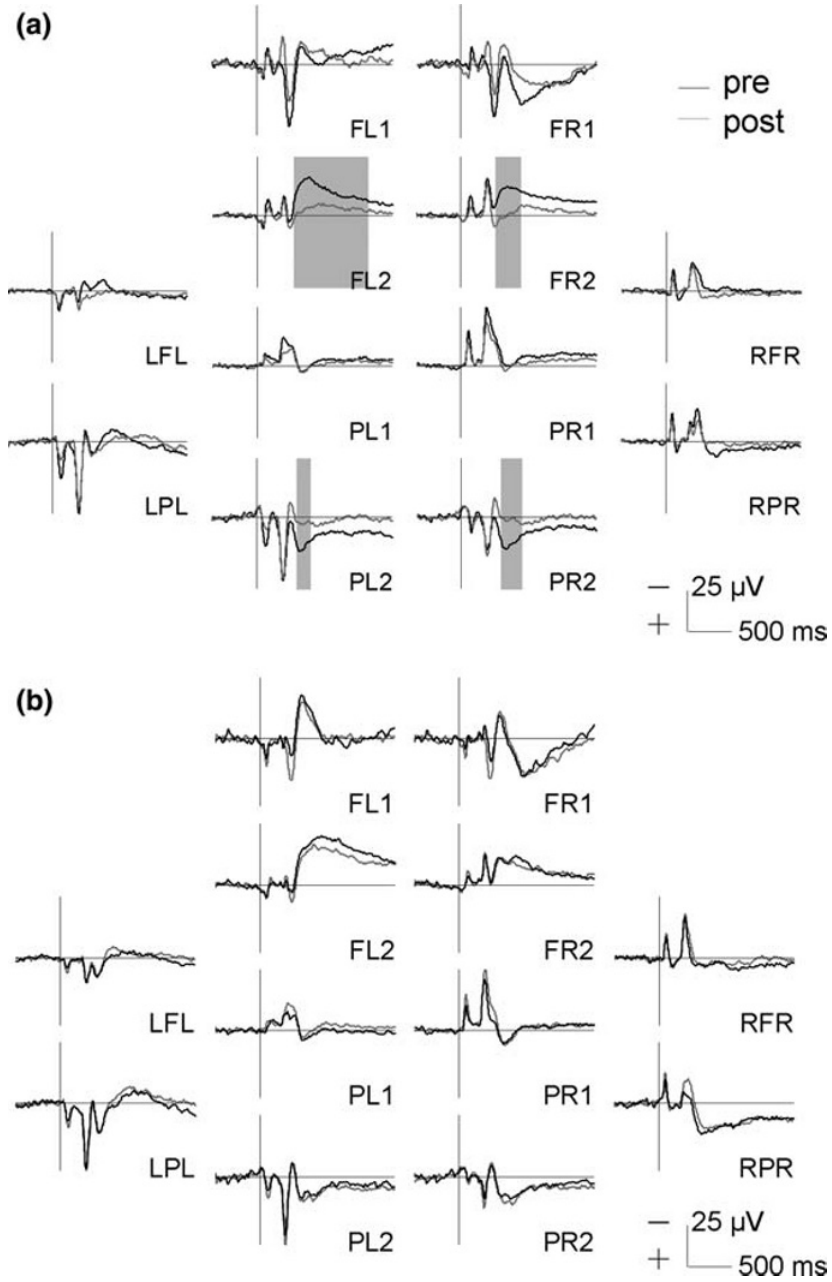


Fig. 6 Comparison of LEPs before (black lines) and after (grey lines) morphine (**a**, $n = 11$) and saline (**b**, $n = 8$) treatment. Shaded areas indicate the significant difference between pre- and post-treatment (two-tailed t-test, $P < 0.05$). Systemic administration of morphine mainly decreased LEPs in channel FL2, FR2, PL2, and PR2, while saline injection have no effect on LEPs

is above the SI cortex. The involvement of SI in pain perception has long been in dispute. By using functional magnetic resonance imaging (fMRI), some authors reported that SI was activated by pain stimulus (Kanda et al. 2000; Ploner et al. 1999, 2000). However, there are also many other studies which did not find significant activation of SI (Bromm and Chen 1995;

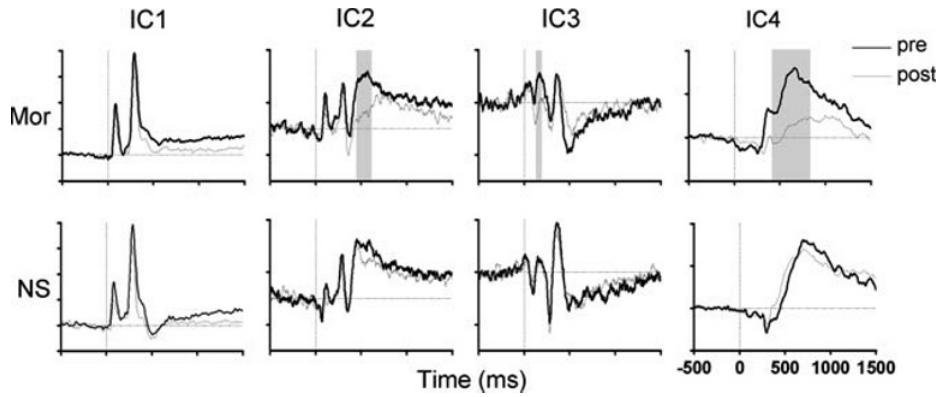


Fig. 7 Comparison of independent component activities before (black lines) and after (grey lines) morphine or saline injection. The grey shaded areas show the significant differences between them (paired *t*-test, $P < 0.05$). Compared to saline, system morphine mainly decreased the late activity of both IC2 and IC4

Valeriani et al. 2000). Although the role of SI in pain processing is still in debate, there is converging evidence suggesting that SI participates in the pain perception (Bushnell et al. 1999). Recently, evidence from multiple single-unit recording in conscious rats showed that SI neurons exhibited excitatory type responses to noxious laser stimulation applied to the tail (Kuo and Yen 2005). Previous work in our laboratory also supported the role of SI in processing of noxious radiate heat stimulation delivered to the hind paw of the rat (Wang et al. 2003, 2004). The findings in this study also provide evidence for the role of SI in pain processing.

In this study, another area can be activated by noxious laser stimulation is medial frontal cortex (MFC). MFC is a cortical area including ACC and prelimbic-infralimbic cortex (PL-IL). MFC lesions attenuated the hot plate response, which suggest that the medial frontal cortex of the rat mediates certain types of supraspinally organized responses to noxious heat pain (Pastoriza et al. 1996). Among those areas, ACC is the most mentioned cortical areas that could be activated by noxious stimulation. It has been demonstrated that neurons in ACC could be activated by noxious laser stimulation applied to the tail of conscious rat (Kuo and Yen 2005). There were also short- and long-latency responses of numerous ACC neurons. Most of those neurons mainly exhibited long-latency responses peaked at 395 ± 23 ms, but there were also some neurons showed a short-latency response meanwhile (95 ± 11 ms). In this study, topography of IC2 showed a current activity distributed in MFC. The waveforms of IC2 included a late, long-lasting wave. Combined with the fact that this component has no side-specific phenomenon, all these evidences implied that this potential maybe related to the affective aspect of pain. We, therefore, predict that the most possible region contributing to the activity in MFC is bilateral ACC.

Except for the two areas mentioned above, we also found an activity in posterior parietal region (Fig. 3b, 180 ms; Fig. 4, IC3). PPC is well known as an epicenter within a neuro-cognitive network of spatial attention (Mesulam 1999). Previous studies in humans have demonstrated that PPC can be activated by painful stimulation (Apkarian et al. 1999). However, the role of PPC in pain perception remained unclear. The possible role of PPC in pain is that it participates in the orientation and spatial attention to painful stimuli and the preparation of purposeful motor acts that would be needed to reduce or prevent the pain (Davis et al. 2002; Witting et al. 2001). Our findings showed that in the four ICs, the earliest response is exhibited in IC3 (46 ± 20 ms), which is earlier than the first response of IC1 (88 ± 14 ms).

Our result is consistent with previous studies (Forss et al. 2005; Waberski et al. 2002). Since, PPC is the earliest source activity within the network involved in the temporal and spatial attention to a task, Waberski et al. (2002) proposed that PPC seems to play a priming role in this network.

Unexplainably, our results of PDC showed that there was no information flow between IC2 and IC3. The possible explanation is that there is no change of information flow between MFC and PPC during noxious laser stimulation. However, we cannot exclude the possibility that PDC method cannot calculate the change of information flow between IC2 and IC3 in situation that those two components are both central components.

In study of nociception related evoked potential in behaving animals, a big problem that may contaminate the result is the movement artifact. In the present study, by using ICA, we have detected an artifact-like activity located in the contralateral rostral frontal region which peaks at 695 ± 125 ms and lasts a very long time (about 1 s) (Fig. 4, IC4). The smoothly decreasing power spectrum implied that IC4 is not a cognitive related component. Moreover, the results of PDC (different pattern of information flow between IC1 to IC4 and IC4 to IC1) and cross correlation (no significant correlation between IC1 and IC4) also indicate that IC4 seems like a nocifensive behavior related response other than a brain-related component. When the rats were restricted with a suspended bag, noxious laser stimulation cannot evoke a component like this (data not shown). It has been reported that the mean latency of the leg withdrawal was 282.80 ± 9.19 ms when calculated from the onset of the evoked electromyography (EMG) responses (Sun et al. 2006). Tsai et al. (2004) also reported laser stimulation applied to the rat tail evoked tail flick latency at 350 ± 9 ms. Conscious, behaving animals is very useful in the study of pain. However, the coincidence of evoked cortical responses and the secondary responses to leg withdrawal movement as well as other nocifensive behaviors result in the contamination of evoked potentials. Relative pure evoked response could be obtained by subtract artifact-like activity. The advantage of ICA in processing of nociception related evoked potential data in animals is very prominent in removing movement-like independent component.

Information Flow Among the Cortical Regions

Another important result in this experiment is that we provide the first description of information flow between the cortical regions participating in nociception processing based on EP data. Our results showed that information flow from IC2, IC3 to IC1 changed twice after noxious laser stimulation. The first change presented between the two peaks of IC1 and the second one occurred after the second peak of IC1. That is, after the first activation of SI, there was information flowing from MFC to it. The change of information flow occurred again after the second activation of SI. Based on the theory of medial and lateral pain pathway, SI and MFC belong to lateral and medial pain pathway respectively (Albe-Fessard et al. 1985; Fields 1988; Schnitzler and Ploner 2000; Vogt and Sikes 2000). Our results suggested that, dynamic interaction of those areas involved in different aspects of pain occurred on cortical level during the experience of pain. Further studies are needed to explain the physiological significance of these information flows.

Effect of Morphine on Nociceptive Related Cortical Responses

Previous studies demonstrated that low dose of systemic morphine preferentially attenuates the second pain mediated by unmyelinated C fiber, whereas the first pain sensation mediated by myelinated Aδ nociceptive afferents are not highly sensitive to modulation by systemic

morphine (Cooper et al. 1986). Our results showed that morphine significantly attenuate the amplitude of N/P500 and the later part of IC2. This result implies that N/P500 and the later response of IC2 are related to the excitation of C fibers. A recent study on laser-evoked intracortical field potentials in SI of rats revealed that intravenous morphine (5 mg/kg) reduced amplitude of the second response of SI (Sun et al. 2006). Laser-evoked long latency cortical potential was also found to be blocked by intrathecal morphine in halothane-anesthetized rats in another study (Kalliomaki et al. 1998). However, in another study, intraperitoneal 5-mg/kg morphine had no effect on both short- and long-latency LEPs, while 10-mg/kg morphine suppressed the long-latency LEP significantly (Tsai et al. 2004). In this study, we did not detect significant changes of the second peak of LEP at channel PR1 and IC1 after morphine injection. It seems like intraperitoneal 5-mg/kg morphine is not a reliable dose to produce obvious effect on sensation of nociception.

In summary, our current results found that two fast sharp responses and one slow blunt response can be elicited by noxious laser stimulation, which may be related to the sensitive-discriminative and affective-motivational dimension of pain respectively. Systemic morphine preferentially attenuates the slow blunt response. Those responses attribute to activities of several cortical regions including SI, MFC and PPC. There are information flows from MFC and PPC to SI, which implies the interaction of those cortical areas involved in different dimension of pain on cortical level.

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