Available online at www.sciencedirect.com

SCIENCE DIRECT

Brain Research 1014 (2004) 197–208

BRAIN RESEARCH

www.elsevier.com/locate/brainres

Research report

Differential modulation of nociceptive neural responses in medial and lateral pain pathways by peripheral electrical stimulation: a multichannel recording study

Jin-Yan Wang[a], Han-Ti Zhang[a], Ji-Sheng Han[a], Jing-Yu Chang[b], Donald J. Woodward[b], Fei Luo[a,*]

[a] *Neuroscience Research Institute and Department of Neurobiology, Peking University Health Science Center, 38 Xue Yuan Road, Beijing 100083, PR China*
[b] *Department of Physiology and Pharmacology, Wake Forest University Health Science Center, NC 27157, USA*

Accepted 12 April 2004
Available online 1 June 2004

Abstract

It is well accepted that peripheral electrical stimulation (PES) can produce an analgesic effect in patients with acute and chronic pain. However, the neural basis underlying stimulation-induced analgesia remains unclear. In the present study, we examined the pain-related neural activity modified by peripheral stimulation in rats. The stimulation frequency of pulses applied to needle electrodes in the hindlimb was 2 Hz alternating with 100 Hz, with 0.6 ms pulse width for 2 Hz and 0.2 ms for 100 Hz. The intensity of the stimulation was increased stepwise from 1 to 3 mA with each 1-mA step lasting for 10 min. The nociceptive neural and behavioral responses were examined immediately after the termination of stimulation. Using a multiple-channel recording technique, we simultaneously recorded the activity of many single neurons located in the primary somatosensory and anterior cingulate cortex (ACC), as well as the ventral posterior and medial dorsal thalamus in behaving rats. Our results showed that peripheral electrical stimulation significantly reduced the nociceptive responses in ventroposterior thalamus and somatosensory cortex, indicating an inhibition of nociceptive processing. In contrast, the analgesic stimulation produced a significant increase in mediodorsal thalamus while a less significant decrease in cingulate cortex, reflecting a complicated effect associated with combined antinociceptive activation and nociceptive suppression. These results support the idea that peripheral electrical stimulation can ultimately alter the pain perception by specifically inhibiting the nociceptive transmission in the sensory pathway while mobilizing the antinociceptive action in the affective pathway, thus to produce pain relief.

Theme F: Sensory systems
Topic: Pain modulation: anatomy and physiology

Keywords: Antinociception; Descending inhibition; Microelectrode; Pain; Rat

1. Introduction

It has been clinically demonstrated that a variety of peripheral electrical stimulations (PES) such as transcutaneous electrical nerve stimulation, electroacupuncture (EA) and percutaneous electrical nerve stimulation can produce analgesic effect in patients with acute and chronic pain syndromes [10,14,16,19]. However, we have very limited understanding of the neural basis underlying PES-induced analgesia. It has been reported that PES in rats could increase the biosynthesis and release of opioid peptides in the spinal cord and supraspinal regions [12,18,52]. Blockade of opiate receptors by intraventricular injection of naloxone has been proved to decrease the analgesic effect of peripheral stimulation [65].

Many brain regions have been proposed to mediate PES analgesia. Wang et al. [57,58] reported that low frequency stimulation was selectively processed in arcuate nucleus of the hypothalamus while high frequency in parabrachial nucleus. The periaqueductal gray (PAG) is the common pathway for both stimulation modes [57,58]. Neuroimaging studies have demonstrated several areas related to EA stimulation including the primary somatosensory (SI) and

* Corresponding author. Tel./fax: +86-10-82801010.
E-mail address: fluo@bjmu.edu.cn (F. Luo).

0006-8993/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2004.04.029

anterior cingulate cortex (ACC), insula, as well as cerebellum [61]. Using functional magnetic resonance imaging, Zhang et al. [63,64] found that EA stimulation could specifically increase the pain-induced activation in bilateral secondary somatosensory and medial prefrontal cortex, as well as Broadmann area 32, while decrease the activation in contralateral SI, Broadmann area 7 and 24.

Extensive literature supports the idea that pain is processed by parallel ascending systems. The lateral pain system, including somatosensory cortices and lateral thalamus, is believed to process pain sensation [4,17,41,51], and the medial pain system, comprising limbic structures and medial thalamus, have been shown to code pain unpleasantness [43,44,49,55]. However, it remains unknown how PES modulates the two components that are associated with pain. In the present study, we make an initial attempt to evaluate the possible roles of medial and lateral pain systems in the mediating of PES analgesia by simultaneously recording the extracellular activity of many single neurons located in SI, ACC and ventral posterior and medial dorsal thalamus (VP and MD).The working hypothesis for the present study is that, if the two pathways are mainly involved in the transmission of nociceptive inputs, one would expect that the treatment with PES may produce analgesia by causing an overall decrease of the nociceptive responses in all recording areas.

2. Materials and methods

2.1. Animals and surgery

Experiments were performed on eight adult male Sprague–Dawley rats (300–350 g). Animals were housed under 12-h dark–light cycle for at least 1 week before surgery, with food and water available ad libitum. All experiments were carried out in accordance with the Institutional Animal Care and Use Committee of Peking University.

This study comprises the same animals that were used in a previous paper [59]. Animals were anesthetized with ketamine (100 mg/kg, i.p.), and then transferred to a Kopf stereotaxic apparatus. Supplementary doses (one-third of the original) of ketamine were given when necessary to maintain the anesthesia. Four small craniotomies were made for microelectrode array implantation. Table 1 shows the location of microelectrodes in each animal. Coordinates for the craniotomies were according to the atlas of Paxinos and Watson [39] as follows: (1) for SI, 1.0 mm posterior to bregma (−1.0 A), 2.0 mm lateral to midline (L) and 2.0 mm ventral to the skull surface (V); (2) for ACC, 3.2 A, 0.8 L and 2.8 V; (3) for MD thalamus, −2.3 A, 0.8 L and 5.5 V; (4) for VP thalamus, −3.0 A, 3.0 L and 6.0 V. Arrays of eight stainless steel Teflon-insulated microwires (50-μm diameter, Biographics, Winston-Salem, NC) were slowly lowered into the target areas. The microelectrode arrays were secured onto the cranium with dental cement using skull screws as anchors. Animals were administered penicillin (60,000 U, i.m.) before surgery to prevent infection and housed individually after surgery. All efforts were made to minimize animals' suffering.

Table 1
Summary of the location of microelectrodes

Rat no.	Target brain areas	Side of microelectrode arrays
1	SI, ACC, VP, MD	unilateral
2	SI, ACC, VP, MD	unilateral
3	SI, ACC, VP, MD	unilateral
4	SI, VP	bilateral
5	SI,VP	bilateral
6	SI,VP	bilateral
7	ACC, MD	bilateral
8	ACC, MD	bilateral

SI, the primary somatosensory cortex; ACC, the anterior cingulate cortex; VP, the ventroposterior thalamic nuclei; MD, the mediodorsal thalamic nuclei.

2.2. Experimental procedures

These experiments started 4 weeks after the previous research reported elsewhere [59]. Animals were placed in a plastic chamber ($44 \times 44 \times 44$ cm^3) and allowed to move freely during the entire recording session. Lightweight cables connected the detachable headset to a rotating commutator on the ceiling of the chamber to allow for the animal's free movements. Noxious radiant heat from a 12.5-W projector bulb was used as painful stimulation, which was applied to the plantar surface of the rats' bilateral hind-paws through a glass floor (1 mm thick). Nociceptive responses were identified by paw withdrawal reflex elicited by the radiant heat. Neuronal spike activities were recorded into a multichannel recording device (see below). The heat source was manually shut down at the occurrence of escape. A time stamp series (resolution, 1 ms) marking stimulus start and end was recorded and synchronized with the neural spike recordings. The interstimulus interval was no less than 20 s. Painful stimuli were delivered only when the animal was quiet and showed no voluntary motor activity. Sham stimuli were randomly inserted among real painful stimulation (i.e., turning the light on and off to mimic the real stimulation without focusing on the paw). The neural responses around sham stimulation were termed sham-control.

Each animal was exposed to three sessions in 3 consecutive weeks. In the PES session/week, rats were restricted in a special apparatus during times when stimulation was applied to the periphery (PES). Two stainless-steel needles, 0.2 mm in diameter, were inserted into each hind leg with one in the tibial anterior muscle region and the other in the posterior border of the tibia. Constant current PES of biphasic square waves (each pulse opposite to the previous one) generated from a Han's Acupoint Nerve Stimulator (HANS LH-402A, manufactured at Beijing University of

Aeronautics and Astronautics) was given via the needles for a total period of 30 min. The frequency of the square waves were '2/100 Hz' (2 Hz alternating with 100 Hz, each lasting for 3 s), with 0.6 ms pulse width for 2 Hz and 0.2 ms for 100 Hz. This kind of mode (i.e., mixed-frequency) has been selected because it is believed to be more effective than a fixed frequency in relieving pain [9,15]. The intensity of the stimulation was increased stepwise from 1 to 3 mA with each 1-mA step lasting for 10 min. Immediately after the termination of PES, rats were set free and the heat-elicited neuronal and behavioral responses were examined. Control sessions were conducted in the other 2 weeks before and after PES session, respectively. This helps to validate the stability of neuronal ensemble responses. Rats were restricted in the same apparatus without needle insertion (i.e., blank control). After a period of 30 min, the same length as PES, repeated pain test were performed to obtain data about the pain-related responses in a freely moving mode (see Fig. 1). Data from the last control session were analyzed, since no significant difference existed between the two. There were totally around 120 stimuli in each recording session (two-thirds for pain on either hindpaws and one-third for sham).

2.3. Behavioral and electrophysiological recording

Neuroelectric signals were obtained from the stainless steel microwires, and passed from the headset assemblies to a preamplifier via two light-weight cables and a commutator. The signals were then filtered (0.5 and 5 kHz, 6 dB cutoff) before sent to a multichannel spike-sorting device. Spike activities were monitored on a computer with a time resolution of 20 μs, picked up by setting proper high and low windows for amplitude and short and long windows for duration, and recorded into a database file with a PC-based software Magnet (Biographics). Data was then analyzed with a commercially available PC-based program (STRANGER, Biographics). The identity of clearly sorted single neurons was verified by graphical capture of waveform. The time stamps of these waveforms were then stored on a personal computer for off-line analysis. Single neuron recording was again validated by computation of interspike interval histograms to show that only one neuron was detected. In each animal, neurons were sorted 1 day before the recording session. When spike activity was recorded from the same microwire across different sessions, the determination that the same neuron was recorded was made in view of (i) constancy of the shape and polarity of the extracellular spike waveform and (ii) similarities in firing rate and pattern (e.g., interspike interval and autocorrelation histogram). However, since the three sessions were conducted in 3 weeks, it is often hard to track the same unit. Thus, we concentrated on the analysis of averaged responses of single units as well as those of neuronal ensembles, instead of comparing individual responses of each unit between two sessions. Animals' behavior during the whole session was videotaped with the time information synchronized to the neuronal recording. Frame by frame analysis was performed off-line for spontaneous paw movements with respect to concurrent spike activity.

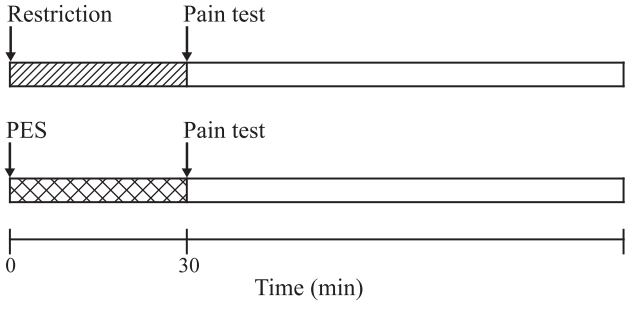

Fig. 1. Experimental procedures for the control (top) and PES (bottom) sessions.

2.4. Data analysis

Bin counts for each trial (0.1 s bin size) were calculated using the analysis program NeuroExplorer (Plexon, Dallas, TX). The results were exported to Matlab in spreadsheet form. Neural responses to pain stimulation were evaluated using a sliding window averaging technique [48], in which a 1-s time window was slid through the entire period of a trial at 0.1-s step. The bin counts of each window were compared with those of a preset 3-s control window 10 s before the stimulation event. The differences were considered significant when it reached a significance level of $p < 0.005$ in three consecutive steps, thus to achieve a global significance of $p < 0.05$ (as proposed by a Monte Carlo simulation with a program AlphaSim, see Ward [60]). Units that significantly increased their activities after painful stimuli were termed as excitatory responses; those that decreased their activities were considered inhibitory. A Student's *t*-test was employed for this comparison. Since there were as much as forty trials for each event, the difference between parametric and nonparametric calculation should be neglectable. Non-parametric chi-square tests were used to determine the significant differences of numbers of neurons across responses between PES vs. restriction sessions.

Cross-correlation histograms were created by the same computer analysis software. One neuron within a given region was selected as the reference neuron, and all neurons from another region were defined as partner neurons for the cross-correlograms. The time of occurrence of spikes from the reference neuron was set at 0 s and the partner neuron's firing 0.5 s before and after each reference neuron's spike was plotted using 5-ms bin size. The significance level of the cross-correlograms was tested using 95% confidence intervals. Data falling into the 20-s period (10 s before and 10 s after) around pain or sham stimulation were calculated separately.

To evaluate whether a neuron is possibly involved in pain coding or modulation, we also calculated the nonparametric (Spearman's, since the distribution of bin counts are not

Table 2
Number and percentage of excitatory and inhibitory neurons classified by different treatment in each recording area

	Restriction			PES		
	n	Excitatory	Inhibitory	*n*	Excitatory	Inhibitory
SI	148	85 (57.4%)	17 (11.5%)	146	56 (38.4%)**	6 (4.1%)*
VP	134	104 (77.6%)	5 (3.7%)	130	74 (56.9%)***	2 (1.5%)
ACC	116	44 (37.9%)	7 (6.0%)	124	31 (25.0%)*	4 (3.2%)
MD	112	31 (27.7%)	8 (7.1%)	112	40 (35.7%)	12 (10.7%)

Note that the numbers of excitatory and inhibitory neurons in the table are a sum of those responding to the noxious stimuli on either side. That is, if a neuron responds to both ipsi- and contralateral stimulation, it will be counted twice instead of once. Thus, *n* represents twice the number of units detected in each area to serve as the base for percentage calculation. Statistical significance of changes in percentage of excitatory or inhibitory neurons between PES vs. restriction-control is examined by chi-square test.

* $P<0.05$.

** $P<0.01$.

*** $P<0.001$.

necessarily *Gaussian*) correlation coefficients between individual neuronal firing rates and paw-withdrawal latencies. To verify whether the correlation coefficient (r) is statistically significant, a P-value was calculated for each of the correlation analysis testing the null hypothesis that r is really zero. Thus, a small P-value indicates that r is distinct from zero and hence the correlation is significant [38]. A similar sliding-window method was employed to determine correlations at each time point as a continuous function. Chi-square tests were used to detect the difference of percentage (excitatory or inhibitory neurons) between PES vs. restriction sessions.

The information theory concept *surprise* was used to evaluate the average response intensity [2]. *Surprise* is defined as the negative natural logarithm of the probability, i.e., $-\ln P$. This logarithmic transformation serves to expand the scale in the interesting region in which the probability density has low values. Moreover, it allows a more sensible continuous comparison of different 'P-values' of improbable (hence surprising) conditions. The 'P-values' were also produced by the sliding-window method.

2.5. Histology

After the termination of the experiment, rats received an overdose of ketamine. Recording sites were marked by

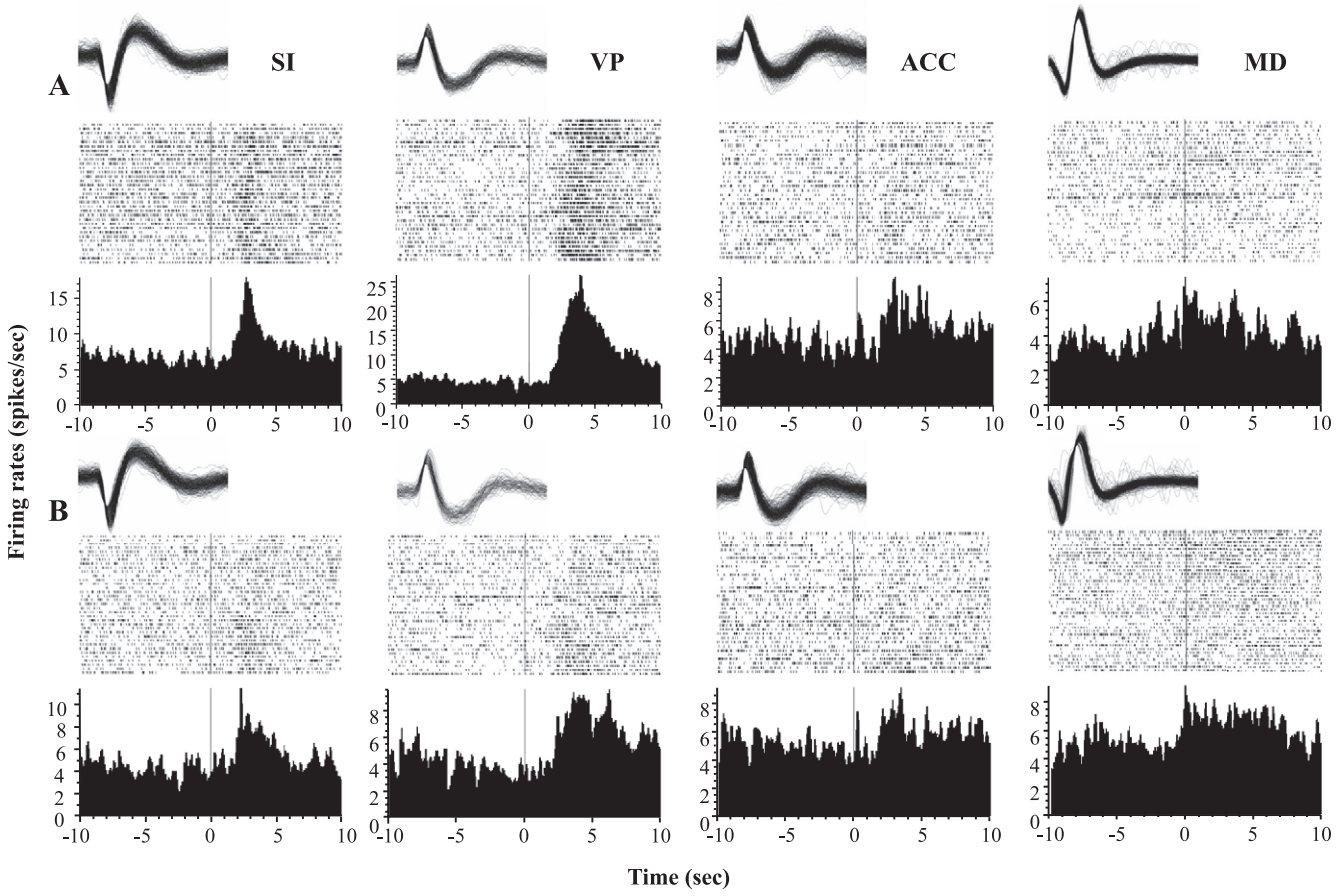

Fig. 2. Raster and perievent histograms showing the typical nociceptive responses during restriction and PES sessions. Four neurons recorded simultaneously from SI (the first column from left), VP (the second), ACC (the third) and MD (the fourth). Neuronal spike activities are displayed 10 s before and 10 s after the painful stimuli, which occur at time zero (vertical line). Insets show spike waveforms from each recording, ensuring that the same single unit was measured for each session. (A) Typical pain responses in the restriction session. (B) Modified pain responses by PES.

electrophoretically deposited iron (10–20 μA DC current, 10–20 s duration, anode at the electrode) at the tips of selected wires. Animals were then perfused with 4% paraformaldehyde. The brains were post-fixed in a solution of 5% potassium ferricyanide/4% paraformaldehyde for several days. Coronal sections (40 μm) were cut through the SI, ACC and thalamus. Recording sites were determined under a light microscope. The iron deposits were easily identified as blue dots. The histological results have been reported elsewhere [59].

3. Results

3.1. Behavioral responses

During the restriction sessions, when noxious radiant heat was applied to the hind paws, rats usually promptly lifted their feet with an average latency of 2.9 ± 0.1 s (mean ± S.E.). No difference exists between the two restriction sessions. PES administration significantly increased paw withdrawal latency (PWL) to 3.6 ± 0.2 s (mean ± S.E., $P < 0.01$, paired t-test). In contrast, sham stimulation did not give rise to any escaping reflexes.

3.2. Pain-related neural activity during restriction-control session

A total of 255 neurons were recorded during the last restriction session (74 SI, 58 ACC, 67 VP and 56 MD). Noxious stimuli induced significant neural responses in each recording area. These responses are predominantly excitatory (57.4%, 77.6%, 37.9% and 27.7% for neurons in SI, VP, ACC and MD, respectively), although inhibitory responses were occasionally encountered (11.5%, 3.7%, 6.0% and 7.1%, respectively), as shown in Table 2. The perievent histograms used to quantify the pain-evoked responses in Fig. 2A show typical nociceptive responses within each area. Noxious stimulation usually elicited a strong and sharp response in SI and VP while caused moderate and longer-lasting increase in ACC and MD. These characteristic responses may be consistent with their distinct roles in the perception of pain. In contrast, sham stimulation never induced any significant changes in the

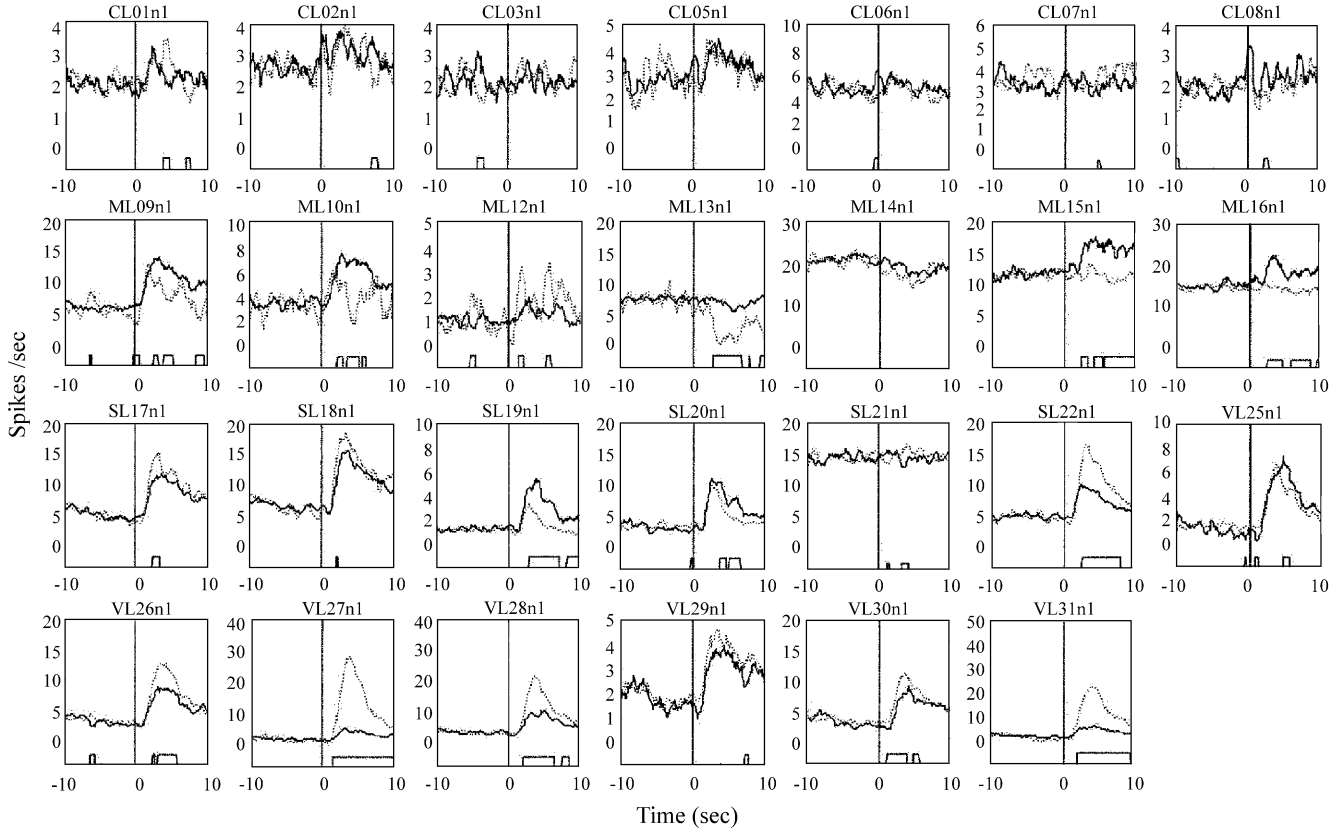

Fig. 3. Comparison of firing rates between different recording sessions using sliding window analysis. Twenty-seven neurons were recorded simultaneously from the four brain regions (C for ACC, M for MD, S for SI and V for VP). Painful stimuli were delivered at time = 0 s. The baseline firing rates (10 s before pain stimuli) of PES and restriction are aligned in order to better evaluate the alterations of neural activity. The trapezoid marker along the x-axis indicates the statistically significant difference between the neuronal firing rates during PES (solid line) and restriction (dashed line) session (Student's t-test, $P < 0.05$). Note the difference among the different regions. The pain-evoked response in SI, VP and ACC were generally reduced while those in MD were enhanced by PES as compared with restriction.

neuronal activity. We also examined whether neurons were responsive to spontaneous paw movements. Video analysis did not reveal significant spike activity within recording regions. Since no significant difference exists between the averaged neuronal responses of the two restriction sessions (data not shown), the last restriction session was used in further analysis.

3.3. Effect of PES on pain-related neural activity

In the PES session, a total of 256 single units were recorded (73 SI, 62 ACC, 65 VP and 56 MD). As in the restriction-control session, pain-related activation continues to be a prominent feature following application of peripheral electrical simulation. However, the pain-related responses in SI, VP and ACC were globally decreased although those in MD were somewhat increased (Figs. 2B and 3), as compared with the restriction session (when the animal was restricted without insertion of simulation needles). We do not make direct comparisons of average peak frequency between PES and restriction-control because (i) the pain response was comprised of both excitatory and inhibitory categories and (ii) the baseline firing rates were exclusively decreased following PES (see below). Therefore, we use *surprise* analysis to assess the effect of PES continuously over time, since *surprise* values can directly reflect the evolving statistical significance of the response as the logarithmic transformation of *P*-values computed at each time point (Fig. 4).

3.3.1. Changes in SI activity

Pain-related activity within SI was markedly reduced following PES treatment (Figs. 3 and 4). Direct comparison of PES and restriction in Table 2 confirmed the significant decrease in proportions of SI neurons with both excitatory and inhibitory response. *Surprise* plots further revealed the overall inhibition in SI by illustrating the significant reduction of average *surprise* values in the peak response (Fig. 4). The level of significance could be seen to vary over time as a variable *surprise*.

3.3.2. Changes in VP activity

Painful stimuli always evoked firing of VP neurons. Moreover, PES treatment can substantially reduce pain-associated activity within VP (Figs. 3 and 4). As compared with SI, numbers in Table 2 show a greater reduction in the proportion of excitatory neurons of VP, although there is no significant decrease in the fraction of inhibitory units.

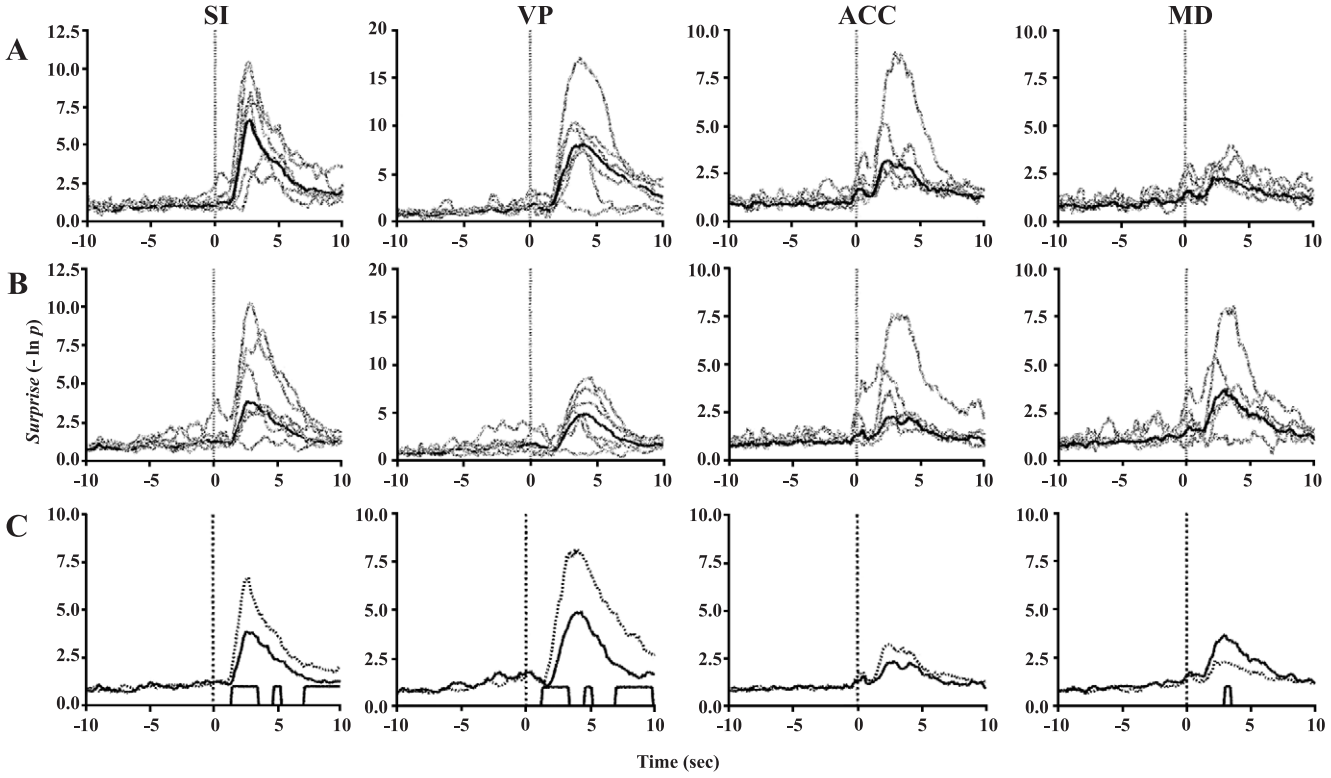

Fig. 4. Neuronal response magnitude in the four recording areas measured by *surprise* analysis. Individual *surprise* results (gray lines) (SI and VP from six rats, ACC and MD from five rats, respectively) from restriction session (A) and PES session (B) are shown here. Although the diversity of response magnitude existed in each animal studied, the average results (black lines) represented the tendency in each individual case. (C) Significant differences (markers along the *x*-axis) between the average response magnitude of restriction (dashed lines) and PES (solid lines) are observed in three recording regions including SI, VP and MD. There seems to be no significant difference for ACC neurons.

Surprise results also demonstrate notable suppression of the level of significance of the pain response in VP following PES (Fig. 4), which is similar to that in SI.

3.3.3. Changes in ACC activity

Noxious heat stimuli induced, in most cases, less activation of ACC neurons. PES suppressed the activation by reducing the fraction of responding neurons for both the excitatory and inhibitory categories (Table 2). Note that there is a significant reduction in proportion of excitatory neurons, although no significant decrease in that of inhibitory units. The *surprise* plot shows no significant reduction of peak values after PES (Fig. 4).

3.3.4. Changes in MD activity

MD exhibited completely different changes from any other recorded area. As can be seen in Table 2, PES yielded a trend toward an increase in the ratios of both excitatory and inhibitory neurons within MD. Consistent with this, *surprise* plots show significant increases in the peak response of MD neurons (Fig. 4). This statistical increase reflects the enhancement of the response intensity following PES. The results suggest that the modulation strategy of PES in MD was fundamentally different from that in SI and VP.

Table 3
Comparison of correlated neuronal activity between different sessions and events

	Sham	Pain	Total pairs of neurons
SI-VP correlations			
Restriction	39 (7.3%)	144 (25.2%)	532
PES	35 (6.9%)	96 (18.8%)	510
P-value	0.8100	0.0020	–
ACC-MD correlations			
Restriction	15 (3.2%)	29 (6.1%)	472
PES	11 (2.2%)	18 (3.6%)	498
P-value	0.4726	0.0734	–

Number and percentage of correlated neuron pairs in the medial and lateral pain pathways are shown here. Statistical difference between PES and restriction-control is examined by chi-square test. Significant decrease of SI-VP correlations was found during the pain-stimuli episode. In contrast, the decrease of ACC-MD correlations was not significant.

3.4. Cross-correlation of neurons in the medial and lateral pain pathways

Pairwise cross-correlations were calculated for simultaneously recorded neurons (see Fig. 5 for examples). Correlated activity was found between neuronal pairs in both pain

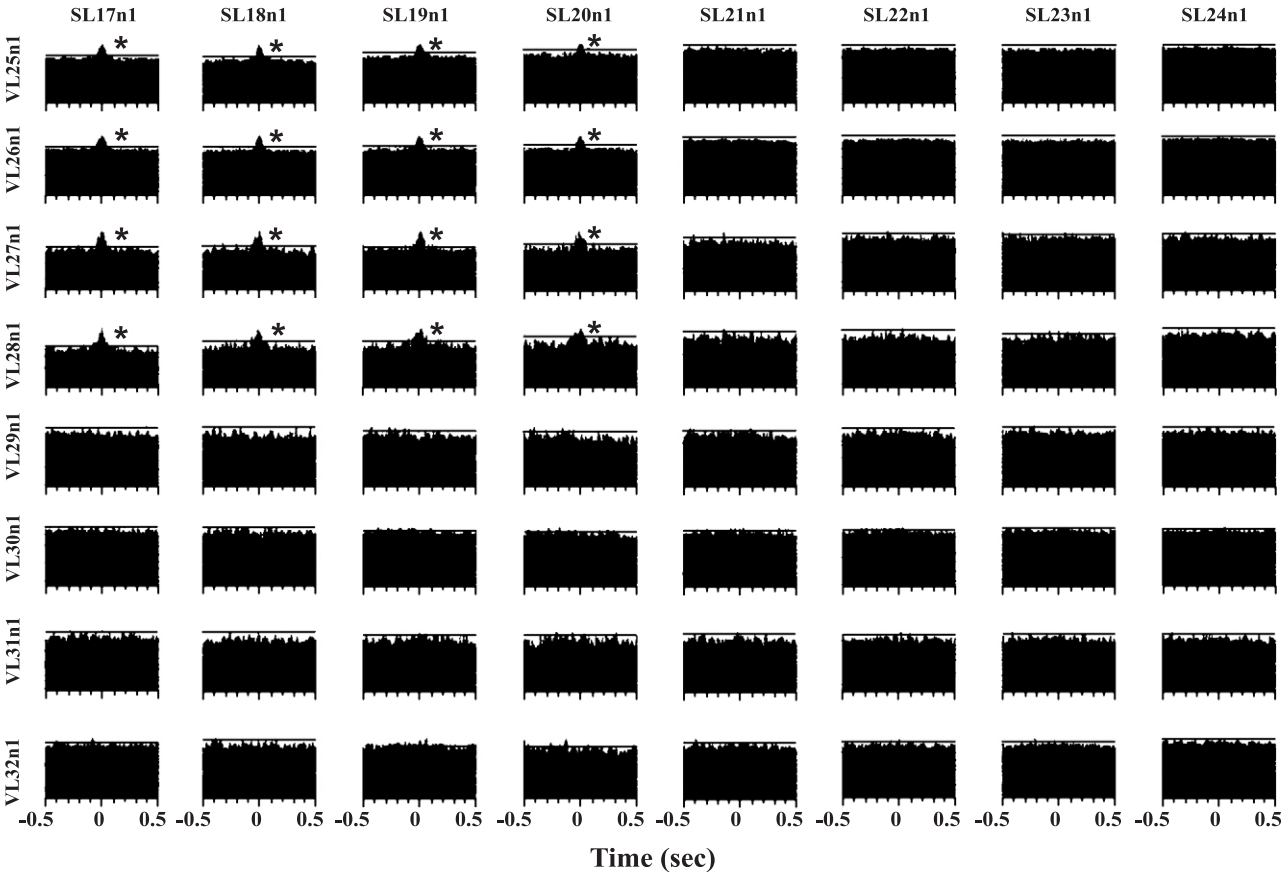

Fig. 5. Cross-correlogram plot of 16 neurons recorded simultaneously from the SI and VP during restriction session (8 SI and 8 VP). The number of pair combinations was therefore 64. Among them, 16 significantly correlated units were found (indicated by asterisks).

pathways. The correlated activity around painful stimulation in the lateral pathway (between neurons within SI and VP) was significantly higher than that in the medial pathway (between neurons within ACC and MD) (25.2% vs. 6.1%, $P<0.0001$, chi-square test; see Table 3). On the other hand, the peri-pain correlations within both pathways were decreased following PES as compared to that of restriction: the correlated activity between SI and VP was significantly decreased (restriction, $n=144$ pairs, 25.2% of total; PES, $n=96$, 18.8%; $P=0.002$, chi-square test; see Table 3); in contrast, the correlation between ACC and MD was also reduced, but the difference did not reach a significant level (restriction, $n=29$, 6.1%; PES, $n=18$, 3.6%, $P=0.0734$, chi-square test; see Table 3). In addition, correlations in sham epochs were significantly less than that in pain (restriction session, $P<0.001$ for SI-VP and $P=0.0436$ for ACC-MD), and PES did not produce further significant reduction (for SI-VP, $p=0.8100$; for ACC-MD, $p=0.4726$; see Table 3).

Table 4
Numbers of neurons showing correlation between firing rates and paw withdrawal latency

Brain areas	Ipsilateral stimulation		Contralateral stimulation	
	Restriction	PES	Restriction	PES
SI	6 (8.1%)	2 (2.7%)	6 (8.1%)	6 (8.2%)
VP	10 (14.9%)	5 (7.7%)	7 (10.4%)	8 (12.3%)
ACC	3 (5.2%)	5 (8.1%)	5 (8.6%)	8 (12.9%)
MD	5 (8.9%)	16 (28.6%)**	3 (5.4%)	16 (28.6%)**

Statistical significance of changes in percentage of PWL-correlated neurons between PES and restriction-control is examined by chi-square test.
** $P<0.01$.

3.5. Correlations between neuronal activity and PWL following PES

The PWL is an index for the evaluation of the analgesic effect of PES. The correlation between neuronal firing rates and PWL indicates the relationship between neural activities

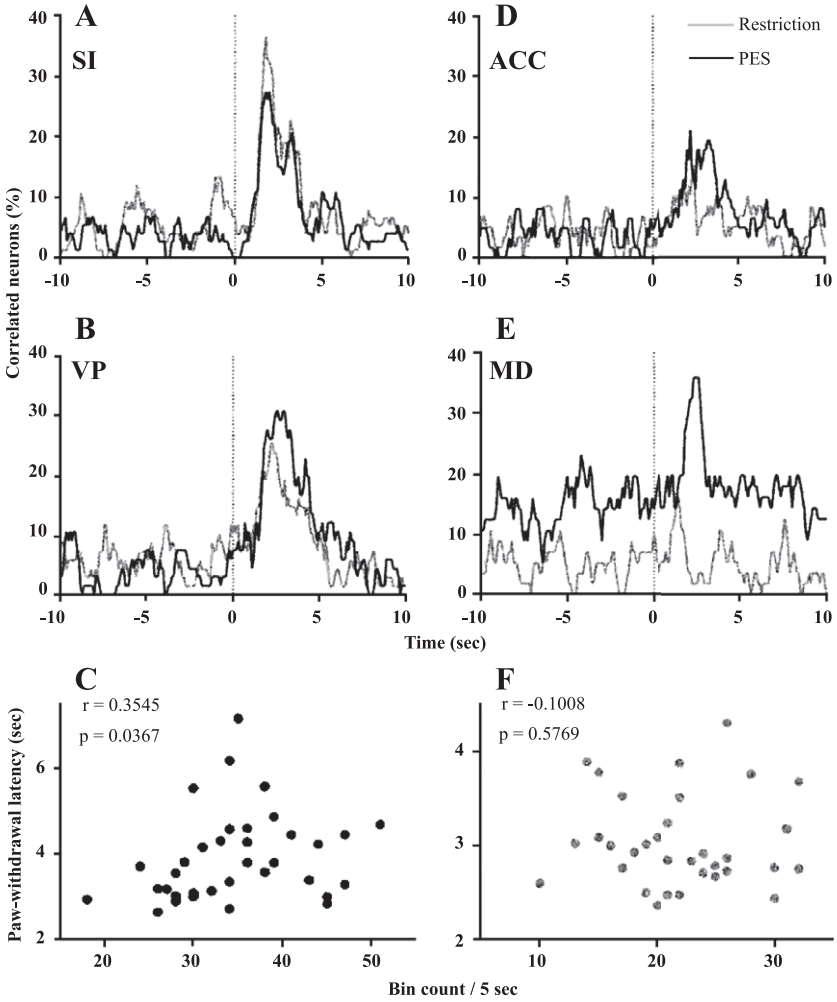

Fig. 6. Correlations between neuronal firing rates and paw-withdrawal latencies. Percentage of neurons showing correlations is plotted along the time dimension in SI (A), VP (B), ACC (D) and MD (E). Marked correlations are observed in SI and VP, and to a less extent, ACC during pain perception. Importantly, in PES session, about 20% of MD neurons exhibit constantly significant correlation. Scatter plot of a sample MD neuron is used to illustrate the significant correlation in PES session (C) but not restriction (F).

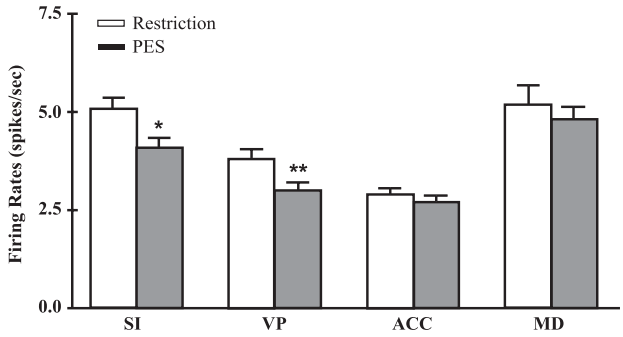

Fig. 7. Influence of PES on basal firing rates. For SI and VP, the baseline firing levels are significantly reduced following PES. In contrast, the basal levels in ACC and MD remain constant. *$P<0.05$, **$P<0.01$.

and behavioral reflexes associated with pain. Fig. 6 illustrates the proportion of neurons exhibiting correlations over time. It is not surprising that the correlation between peak firing rates (i.e., pain-related response) and PWL is always high for each recording area in both PES and restriction sessions. Such a correlation between behavior and neural activity could be interpreted as coding of pain intensity perceived.

Of note, we found a significantly increased percentage of MD neurons whose basal firing rates are correlated with PWL after PES application (Fig. 6, Table 4). In contrast, similar correlation analysis did not detect obvious difference in any other brain areas. These results suggest the uniqueness of MD in mediating PES-induced analgesia among the recording areas.

3.6. Effects of PES on basal firing rates

To assess the effects of PES on the basal neuronal activity within each area, the baseline levels of firing rates recorded during PES session were compared with those during restriction-control. We calculated average firing rates for all trials in the session over a period of 4–10 s before the application of painful stimulus. The analysis revealed that the background firing rates were influenced by PES in varying degrees for different recording regions. For SI and VP, the prestimulus firing levels were significantly reduced following PES (Fig. 7), whereas for ACC and MD the basal firing rates remain constant after PES.

4. Discussion

The present study was designed to investigate the central neural mechanisms of PES-induced analgesia in the cortex and thalamic nuclei in awake behaving rats. The study for the first time demonstrated that the electrical stimulation could modulate the pain-evoked neuronal responses in both lateral and medial pain pathways. First, PES could significantly inhibit the nociceptive activation of VP and SI neurons. Second, PES induced a significant increase in the pain-related activation of MD neurons while causing a less significant decrease in that of ACC. These results indicate that the electrical stimulation could change the neural activity within pain-processing networks in a more complex way than we originally hypothesized.

The distinct roles of pain-related brain regions in the sensory (e.g., pain location) and affective (e.g., pain unpleasantness) dimensions lead to the concept of the lateral and medial pain systems [3,23,35,47]. The somatosensory cortex receives nociceptive information mainly from the VP thalamus [13,17]; these two areas have been thought to mediate the sensory-discriminative aspects of pain perception. ACC receives nociceptive inputs mainly from the medial thalamus and they both participate in the processing of pain affect [49,56]. This and our previous studies [59] have confirmed that SI and VP neurons have very similar response profiles to noxious stimuli, and such is the case for ACC and MD. These responses are much in accord with their postulated distinct roles in the perception of pain, i.e., the processing of pain sensation and pain unpleasantness, respectively [59].

With a dense-and-disperse stimulation mode (2 Hz alternating with 100 Hz), we investigated the central mechanisms for PES analgesia. Our experiment demonstrated that the PES with mixed frequency can modify the pain-related response in the dual pain pathways. We propose that inhibition in the lateral pain pathway might be explained as the suppression of the nociceptive afferents. Since PES can mobilize the release of opioid peptides in the spinal cord [12,20], there is little doubt that the analgesic effect produced by PES results in part from direct suppression of nociceptive activity at the spinal cord level. On the other hand, the analgesic effect produced by electrical stimulation might be partly attributed to a modulation of sensory transmission, as formulated for the gate control theory [36]. The gate control theory champions the idea that stimulation of low threshold somatosensory pathways inhibits the afferent pain signals. Since the afferent impulses induced by EA stimulation have been characterized to be transmitted by Aβ and Aδ fibers [24,30], it is reasonable to propose that direct excitation of large-diameter afferents by electrical stimulation could produce pain relief at lower levels. Another possibility is that much of the inhibition may happen at higher levels as expressed by the phenomena revealed in this study.

Both inhibition and activation can be observed in the medial pathway, which suggests that even more complicated mechanisms may exist. Neurochemical and brain imaging studies revealed marked differences in the distribution of in vivo opioid receptor binding between the two pain pathways. Positron emission topography (PET) and immunohistochemical studies in primates have identified the opioid receptors mainly in the structures within medial pain system such as the prefrontal and ACC, the medial and intralaminar nuclei of the thalamus and the superficial dorsal horn [5,27,40]. In contrast, the lateral system has a relatively

sparse distribution of opioid receptors density [27]. Previous work has demonstrated that opioid peptides have an important role in mediating PES analgesia [12,18,52]. Given the unequal distribution of opioid receptors, it is not surprising that the influences of PES on the medial pain pathway are not identical to that in lateral pathway.

In our study, the increased neuronal activity in MD may represent the antinociceptive activation produced by PES. Correlation analysis supports this idea by showing that the basal firing rates of some MD neurons become more correlated with PWL after PES application. This suggests that these neurons can be mobilized by PES and as a result participate in pain modulation. Early studies also indicate that the medial thalamus is involved in activation of descending pain suppression mechanisms [28,37,45]. Projections from the intralaminar thalamic nuclei to the PAG have been described [33], and stimulation of the parafascicular nucleus causes a predominantly excitatory response of the PAG neurons [45]. It is well known that PAG has descending fibers that have inhibitory influences on spinal nociceptive neurons by acting on opioid or non-opioid receptors [6,34,42]. On the other hand, midline thalamic nuclei have been demonstrated to have efferents to mesolimbic structures (e.g., amygdaloid complex and nucleus accumbens) through means of multiple anterograde or retrograde tracing [50,53]. The mesolimbic system plays an important role in drug addiction as well as pain modulation [11,46]. Therefore, another possibility is that the role of MD in antinociception is mediated by mesolimbic reward circuits. At the same time, MD does have a critical role in signaling nociceptive information. One might imagine that PES ought to suppress the pain response in MD if activity in the region plays a direct role to process pain. The finding of the opposite result indicates a more complex role in pain regulation, including both mediation and suppression.

The now experimental observation shows a reduction, though less significant, of activity in ACC, a phenomena similar to SI and VP but opposite to the increase in MD. One possibility is that ACC receives dual effects of pain suppression similar to SI and VP and antinociceptive activation similar to MD. ACC has been shown to comprise different subareas with different functions (e.g., motor, cognitive and affective). In our study, the recording electrodes were histologically confirmed to be located rostrally within ACC, where nociceptive neurons are mostly found [26,62] and we may have sampled with a bias toward pain perception. However, accumulating evidence supports ACC as also having an important role in pain modulation. Anatomical studies demonstrate that ACC has direct projections to the PAG [32,54]. A high density of opioid receptors and activation induced by fentanyl within ACC strongly support the participation of it in the down-regulation of pain perception [1,8,27]. Moreover, it has been argued that ACC exhibits a biphasic regulation of the nociceptive activity in the dorsal horn [66]. Calejesan et al. [7] reported that electrical stimulation of ACC did not activate endogenous analgesic systems; instead, activation of the ACC could enhance animal's response to painful stimuli. Thus, ACC seems to be involved in both the inhibition and facilitation of pain transmission. Further study needs to be conducted to investigate the complicated role for ACC in pain modulation.

In the present study, correlated neuronal activity has been found within the medial and lateral pain pathways (mainly in the pain epochs), suggesting that the processing of pain is definitely a result of coherent activity within different neuronal circuits. We found that the functional link between SI and VP neurons was much stronger than that between ACC and MD. This may be because the lateral thalamus has a more restricted cortical projection than the medial. It is noteworthy that a decreased neuronal correlation within each pain pathway was observed following PES (again only around painful stimulation). One can presume that PES may disturb the functional connection within the pain-signaling pathways, thereby preventing the transmission of nociceptive information.

Our data also revealed that the baseline level of neuronal firing rates was significantly changed within SI and VP following PES, while those in ACC and MD remained unaffected. Since PES per se can be considered as a source of vibratory modality input, and the lateral pathway is characteristic of sensory transmission, it is conceivable that the alterations of baseline level in the sensory pathway may reflect mainly the specific processing of PES signals rather than a specific action on nociceptive information.

A properly set control condition is essential for neuronal recording. Ideally the control condition should be arranged in the same session to make sure the same groups of neurons are recorded. However, since we have to deliver around 40 nociceptive stimuli to get sufficient trials for data analysis under one condition, a pre-PES control section would mean another 40 nociceptive stimulation. That will be totally 80 stimuli within 2 h. This may cause damage to local skin as well as generate central sensitization, thus bias the whole observation [25]. Therefore, the pre-PENS control is inapplicable. On the other hand, it is well known that PES will cause tolerance if given once a day [21,22] and cumulate effect if given two to three times a day [29,31]. These indicate that PES may produce central plasticity within 1–4 days and makes a control session in this range improper. Hence, we employed a separate control session a week away from the PES session, which is safe for both reason mentioned above. The only limitation of this kind of control is that it can not take the advantage of recording the same single units under different conditions. We compensate this by analyzing the mean ensemble responses instead of comparing changes of individual single unit response. To validate the stability of ensemble neuronal behavior, we recorded another control session a week before the PES session and found no significant difference between these two control sessions in term of average ensemble responses (data not shown). We put the last control session into

analysis in the hope that it was under a condition more closely to the PES session.

Taken together, our results suggest that the mechanisms mediating PES analgesia involve (i) the activation of descending pain suppression in the medial pathway, especially MD thalamus, and (ii) the inhibition of nociceptive processing in the lateral pathway.

Acknowledgements

This study was supported by National Natural Science Foundation of China (30170307 and 30370461), a grant from the 211 project and a startup grant for young scientists from the 985 project of Peking University to F.L., NIH grants EB002092 and AA10980 to D.J.W., and NS-43441 and TW06144 to J.-Y.C.

References

[1] L.J. Adler, F.E. Gyulai, D.J. Diehl, M.A. Mintun, P.M. Winter, L.L. Firestone, Regional brain activity changes associated with fentanyl analgesia elucidated by positron emission tomography, Anesth. Analg. 84 (1997) 120–126.

[2] A.M. Aertsen, G.L. Gerstein, M.K. Habib, G. Palm, Dynamics of neuronal firing correlation: modulation of "effective connectivity", J. Neurophysiol. 61 (1989) 900–917.

[3] D. Albe-Fessard, K.J. Berkley, L. Kruger, H.J. Ralston, W.D. Willis Jr., Diencephalic mechanisms of pain sensation, Brain Res. 356 (1985) 217–296.

[4] J.L. Andersson, A. Lilja, P. Hartvig, B. Langstrom, T. Gordh, H. Handwerker, E. Torebjork, Somatotopic organization along the central sulcus, for pain localization in humans, as revealed by positron emission tomography, Exp. Brain Res. 117 (1997) 192–199.

[5] S.F. Atweh, M.J. Kuhar, Distribution and physiological significance of opioid receptors in the brain, Br. Med. Bull. 39 (1983) 47–52.

[6] D. Budai, H.L. Fields, Endogenous opioid peptides acting at mu-opioid receptors in the dorsal horn contribute to midbrain modulation of spinal nociceptive neurons, J. Neurophysiol. 79 (1998) 677–687.

[7] A.A. Calejesan, S.J. Kim, M. Zhuo, Descending facilitatory modulation of a behavioral nociceptive response by stimulation in the adult rat anterior cingulate cortex, Eur. J. Pain 4 (2000) 83–96.

[8] K.L. Casey, P. Svensson, T.J. Morrow, J. Raz, C. Jone, S. Minoshima, Selective opiate modulation of nociceptive processing in the human brain, J. Neurophysiol. 84 (2000) 525–533.

[9] X.H. Chen, S.F. Guo, C.G. Chang, J.S. Han, Optimal conditions for eliciting maximal electroacupuncture analgesia with dense-and-disperse mode stimulation, Amer. J. Acupunct. 22 (1994) 47–53.

[10] L. Chen, J. Tang, P.F. White, A. Sloninsky, R.H. Wender, R. Naruse, R. Kariger, The effect of location of transcutaneous electrical nerve stimulation on postoperative opioid analgesic requirement: acupoint versus nonacupoint stimulation, Anesth. Analg. 87 (1998) 1129–1134.

[11] T.M. Costa Gomez, M.M. Behbehani, An electrophysiological characterization of the projection from the central nucleus of the amygdala to the periaqueductal gray of the rat: the role of opioid receptors, Brain Res. 689 (1995) 21–31.

[12] H. Fei, G.X. Xie, J.S. Han, Low and high frequency electroacupuncture stimulation release [Met5]enkephalin and dynorphin A in rat spinal cord, Sci. Bull. China 32 (1987) 1496–1501.

[13] D.P. Friedman, E.A. Murray, Thalamic connectivity of the second somatosensory area and neighboring somatosensory fields of the lateral sulcus of the macaque, J. Comp. Neurol. 252 (1986) 348–373.

[14] E.A. Ghoname, W.F. Craig, P.F. White, Use of percutaneous electrical nerve stimulation (PENS) for treating ECT-induced headaches, Headache 39 (1999) 502–505.

[15] E.A. Ghoname, W.F. Craig, P.F. White, H.E. Ahmed, M.A. Hamza, N.M. Gajraj, A.S. Vakharia, C.E. Noe, The effect of stimulus frequency on the analgesic response to percutaneous electrical nerve stimulation in patients with chronic low back pain, Anesth. Analg. 88 (1999) 841–846.

[16] E.A. Ghoname, W.F. Craig, P.F. White, H.E. Ahmed, M.A. Hamza, B.N. Henderson, N.M. Gajraj, P.J. Huber, R.J. Gatchel, Percutaneous electrical nerve stimulation for low back pain: a randomized crossover study, JAMA 281 (1999) 818–823.

[17] S.I. Gingold, J.D. Greenspan, A.V. Apkarian, Anatomic evidence of nociceptive inputs to primary somatosensory cortex: relationship between spinothalamic terminals and thalamocortical cells in squirrel monkeys, J. Comp. Neurol. 308 (1991) 467–490.

[18] H.F. Guo, J. Tian, X. Wang, Y. Fang, Y. Hou, J. Han, Brain substrates activated by electroacupuncture (EA) of different frequencies (II): role of Fos/Jun proteins in EA-induced transcription of preproenkephalin and preprodynorphin genes, Mol. Brain Res. 43 (1996) 167–173.

[19] M.A. Hamza, P.F. White, H.E. Ahmed, E.A. Ghoname, Effect of the frequency of transcutaneous electrical nerve stimulation on the postoperative opioid analgesic requirement and recovery profile, Anesthesiology 91 (1999) 1232–1238.

[20] J.S. Han, Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies, Trends Neurosci. 26 (2003) 17–22.

[21] J.S. Han, S.J. Li, J. Tang, Tolerance to acupuncture and its cross-tolerance to morphine, Neuropharmacology 20 (1981) 593–596.

[22] C. Huang, H. Long, J.S. Han, Y. Wan, Nocistatin potentiates electroacupuncture antinociceptive effects and reverses chronic tolerance to electroacupuncture in mice, Neurosci. Lett. 350 (2003) 93–96.

[23] A.J. Hudson, Pain perception and response: central nervous system mechanisms, Can. J. Neurol. Sci. 27 (2000) 2–16.

[24] R.R. Ji, X.M. Wang, J.S. Han, Induction of Fos-like protein in the rat spinal cord following electroacupuncture stimulation, Chin. J. physiol. 245 (1992) 606–612.

[25] R.R. Ji, T. Kohno, K.A. Moore, C.J. Woolf, Central sensitization and LTP: do pain and memory share similar mechanisms, Trends Neurosci. 26 (2003) 696–705.

[26] J.P. Johansen, H.L. Fields, B.H. Manning, The affective component of pain in rodents: direct evidence for a contribution of the anterior cingulate cortex, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 8077–8082.

[27] A.K. Jones, L.Y. Qi, T. Fujirawa, S.K. Luthra, J. Ashburner, P. Bloomfield, V.J. Cunningham, M. Itoh, H. Fukuda, T. Jones, In vivo distribution of opioid receptors in man in relation to the cortical projections of the medial and lateral pain systems measured with positron emission tomography, Neurosci. Lett. 126 (1991) 25–28.

[28] K.E. Krout, A.D. Loewy, Periaqueductal gray matter projections to midline and intralaminar thalamic nuclei of the rat, J. Comp. Neurol. 424 (2000) 111–141.

[29] H.X. Liu, Y.H. Jiang, L. Xiong, F. Luo, J.S. Han, Investigation of the proper parameters of transcutaneous electric nerve stimulation in the treatment of chronic inflammatory pain: I. Comparison of the effect of different frequencies, Chin. J. Acupunct. 20 (2000) 41–46.

[30] G.W. Lu, Characteristics of afferent fiber innervation on acupuncture points zusanli, Am. J. Physiol. 245 (1983) 606–612.

[31] F. Luo, A study on the cumulative effect of repeated electroacupuncture on chronic pain, Prog. Physiol. Sci. 27 (1996) 241–244.

[32] P.W. Mantyh, Forebrain projections to the periaqueductal gray in the monkey, with observations in the cat and rat, J. Comp. Neurol. 206 (1982) 146–158.

[33] J.E. Marchand, N. Hagino, Afferents to the periaqueductal gray in the rat. A horseradish peroxidase study, Neuroscience 9 (1983) 95–106.

[34] P. Marek, J.S. Mogil, W.F. Sternberg, I. Panocka, J.C. Liebeskind, *N*-Methyl-D-aspartic acid (NMDA) receptor antagonist MK-801 blocks

non-opioid stress-induced analgesia: II. Comparison across three swim-stress paradigms in selectively bred mice, Brain Res. 578 (1992) 197–203.
[35] R. Melzack, K.L. Casey, Sensory, motivational and central control determinants of pain: a new conceptual model, in: D.R. Kenshalo (Ed.), The Skin Senses, Thomas, Springfield, 1968, pp. 423–443.
[36] R. Melzack, P.D. Wall, Pain mechanisms: a new theory, Science 150 (1965) 971–979.
[37] C.M. Montagnese, S.E. Mezey, A. Csillag, Efferent connections of the dorsomedial thalamic nuclei of the domestic chick (*Gallus domesticus*), J. Comp. Neurol. 459 (2003) 301–326.
[38] H. Motulsky, Prism 4 Statistics Guide—Statistical analyses for laboratory and clinical researchers, GraphPad Software, San Diego, 2003.
[39] G. Paxinos, C. Watson, The Rat Brain In Stereotaxic Coordinates, Academic Press, San Diego, 1998.
[40] A. Pfeiffer, A. Pasi, P. Mehraein, A. Herz, Opiate receptor binding sites in human brain, Brain Res. 248 (1982) 87–96.
[41] M. Ploner, H.J. Freund, A. Schnitzler, Pain affect without pain sensation in a patient with a postcentral lesion, Pain 81 (1999) 211–214.
[42] H.K. Proudfit, Pharmacologic evidence for the modulation of nociception by noradrenergic neurons, Prog. Brain Res. 77 (1988) 357–370.
[43] P. Rainville, Brain mechanisms of pain affect and pain modulation, Curr. Opin. Neurobiol. 12 (2002) 195–204.
[44] P. Rainville, G.H. Duncan, D.D. Price, B. Carrier, M.C. Bushnell, Pain affect encoded in human anterior cingulate but not somatosensory cortex, Science 277 (1997) 968–971.
[45] S. Sakata, F. Shima, M. Kato, M. Fukui, Effects of thalamic parafascicular stimulation on the periaqueductal gray and adjacent reticular formation neurons. A possible contribution to pain control mechanisms, Brain Res. 451 (1988) 85–96.
[46] B.L. Schmidt, C.H. Tambeli, R.W. Gear, J.D. Levine, Nicotine withdrawal hyperalgesia and opioid-mediated analgesia depend on nicotine receptors in nucleus accumbens, Neuroscience 106 (2001) 129–136.
[47] A. Schnitzler, M. Ploner, Neurophysiology and functional neuroanatomy of pain perception, J. Clin. Neurophysiol. 17 (2000) 592–603.
[48] W. Schultz, R. Romo, Role of primate basal ganglia and frontal cortex in the internal generation of movements: I. Preparatory activity in the anterior striatum, Exp. Brain Res. 91 (1992) 363–384.
[49] R.W. Sikes, B.A. Vogt, Nociceptive neurons in area 24 of rabbit cingulate cortex, J. Neurophysiol. 68 (1992) 1720–1732.
[50] H.S. Su, M. Bentivoglio, Thalamic midline cell populations projecting to the nucleus accumbens, amygdala, and hippocampus in the rat, J. Comp. Neurol. 297 (1990) 582–593.
[51] J.D. Talbot, S. Marrett, A.C. Evans, E. Meyer, M.C. Bushnell, G.H. Duncan, Multiple representations of pain in human cerebral cortex, Science 251 (1991) 1355–1358.
[52] J. Tang, J.S. Han, Changes in opioid activity in brain and pituitary during electroacupuncture analgesia in the rat, J. Beijing Med. Coll. 3 (1978) 150–152.
[53] E.H. Van Vulpen, R.W. Verwer, Organization of projections from the mediodorsal nucleus of the thalamus to the basolateral complex of the amygdala in the rat, Brain Res. 500 (1989) 389–394.
[54] B.A. Vogt, The Cingulate cortex, in: E.G. Jones, A. Peters (Eds.), The Cerebral Cortex, Plenum, New York, 1985, pp. 89–149.
[55] B.A. Vogt, R.W. Sikes, The medial pain system, cingulate cortex, and parallel processing of nociceptive information, Prog. Brain Res. 122 (2000) 223–235.
[56] B.A. Vogt, D.N. Pandya, D.L. Rosene, Cingulate cortex of the rhesus monkey: I. Cytoarchitecture and thalamic afferents, J. Comp. Neurol. 262 (1987) 256–270.
[57] Q. Wang, L.M. Mao, J.S. Han, Diencephalon as a cardinal neural structure for mediating 2 Hz- but not 100 Hz-electroacupuncture-induced tail flick reflex suppression, Behav. Brain Res. 37 (1990) 149–156.
[58] Q. Wang, L.M. Mao, J.S. Han, The role of periaqueductal gray in mediation of analgesia produced by different frequencies electroacupuncture stimulation in rats, Int. J. Neurosci. 53 (1990) 167–172.
[59] J.Y. Wang, F. Luo, J.Y. Chang, D.J. Woodward, J.S. Han, Parallel pain processing in freely moving rats revealed by distributed neuron recording, Brain Res. 992 (2003) 263–271.
[60] D.B. Ward, AFNI program: AlphaSim [Online]. Medical College of Wisconsin. http://afni.nimh.nih.gov/afni/AFNI_Help/AlphaSim.html, 2002.
[61] M.T. Wu, J.M. Sheen, K.H. Chuang, P. Yang, S.L. Chin, C.Y. Tsai, C.J. Chen, J.R. Liao, P.H. Lai, K.A. Chu, H.B. Pan, C.F. Yang, Neuronal specificity of acupuncture response: a fMRI study with electroacupuncture, NeuroImage 16 (2002) 1028–1037.
[62] H. Yamamura, K. Iwata, Y. Tsuboi, K. Toda, K. Kitajima, N. Shimizu, H. Nomura, J. Hibiya, S. Fujita, R. Sumino, Morphological and electrophysiological properties of ACCx nociceptive neurons in rats, Brain Res. 735 (1996) 83–92.
[63] W.T. Zhang, Z. Jin, G.H. Cui, K.L. Zhang, L. Zhang, Y.W. Zeng, F. Luo, A. Chen, J.S. Han, Relations between brain network activation and analgesic effect induced by low vs. high frequency electrical acupoint stimulation in different subjects: a functional magnetic resonance imaging study, Brain Res. 982 (2003) 168–178.
[64] W.T. Zhang, Z. Jin, J. Huang, L. Zhang, Y.W. Zeng, F. Luo, A. Chen, J.S. Han, Modulation of cold pain in human brain by electric acupoint stimulation: evidence from fMRI, NeuroReport 14 (2003) 1591–1596.
[65] Z.F. Zhou, M.Y. Du, W.Y. Wu, Y. Jiang, J.S. Han, Effect of intracerebral microinjection of naloxong on acupuncture- and morphine-analgesia in the rabbit, Sci. Sin. 24 (1981) 1166–1178.
[66] M. Zhuo, D.E. Lee, P. Li, A.A. Calejesan, The cingulate cortex biphasically regulats spinal nociception, Soc. Neurosci. Abstr. 24 (1998) 1135.