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Spatiotemporal Changes of Neuronal Responses in the Primary Somatosensory Cortex to Noxious Tail Stimulation in Awake and Pentobarbital-Anesthetized Rats

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Abstract

Primary somatosensory cortex (SI) is a key area in the processing of nociceptor inputs to our consciousness. To clarify the columnar and laminar organization of SI for pain processing, we compared spatiotemporal changes in neuronal activities of the primary sensorimotor cortex (SmI) of the rat in response to noxious laser heat stimulation applied to the mid-tail. Longitudinal and vertical array microelectrodes were chronically implanted in the cerebral cortex. Evoked neuronal activities, including intracortical local field potentials (LFP) and ensemble single-unit activity (SU) around SmI were simultaneously recorded. The effect of pentobarbital on the neuronal responses was evaluated in comparison with the neuronal responses in conscious animals to explore the potential substrate of nociceptive processing in the conscious state. The results from the experiment with longitudinal microelectrode arrays indicated that noxious stimulation induced a neuronal response which was spread widely around the SmI of the conscious rat, and the range of neuronal responses was limited to the tail region of the SmI under anesthesia. The results from the experiment with vertical microelectrode arrays showed the universal neuronal responses through all cortical layers of the SmI in conscious rats, and sodium pentobarbital suppressed these neuronal responses in the supragranular layers significantly relative to the deeper layers and basal activity. These results imply that a wider range of cortical activation, both in the horizontal or vertical dimension, might be important for nociceptive processing in the conscious state.

Key Words: ensemble single-unit activity, laser evoked potential, microelectrode array, nociception, pain, sodium pentobarbital

Introduction

Pain is a complex experience that alerts an organism about imminent danger and induces negative emotions including unpleasantness, anxiety and fear and, thus, motivates the subject to avoid further damage. A prevailing hypothesis suggests that pain has three components: sensory discrimination, affective

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motivation, and cognitive evaluation (23). The primary somatosensory cortex (SmI) is one of the cortical areas that process nociceptive information from the peripheral nervous system. Neurons in the SmI respond topographically to stimuli applied to specific parts of the body. The neuronal responses of the SmI correlated with fine characteristics of the stimulation and were associated with discriminative perceptions about the stimulation (12, 28). Even though the anesthetics block consciousness and perceptions, it has been indicated that neuronal activation was also induced by nociceptive stimuli in the SmI of anesthetized animals (13, 15, 20, 21, 33, 35). This activation represents that nociception from noxious stimuli might be still processed when the subject is under anesthetic condition. To explore the processing of nociceptive information in SmI, it is important to obtain a fine-grain map of the SmI in response to noxious stimulation. The evoked potential is a commonly utilized tool in neuroscience and clinical neurology for evaluating the function of the nervous system (25). Many studies have also indicated that potentials evoked by innocuous and noxious stimulations can be recorded in the SmI of anesthetized animals (2, 4, 13, 21). Evoked potentials on the cerebral cortex from the chronically implanted cortical surface electrodes induced by mechanical, electrical and laser heat stimuli have been mapped widely in conscious animals (27, 31, 33). The fast and slow components of laser-evoked potentials (LEPs) corresponded to Aδ and C fiber activation, respectively (31). The laser heat-evoked potentials were modified by analgesic and anesthetic drugs (11, 34, 37), indicating that these LEPs were nociception-related.

In addition, nociception-induced intracortical local field potential (LFP) and single unit responses in SmI have been investigated in anesthetized animals. The intracortical LFP data at different depths demonstrated a large but unclear somatotopical distribution of laser heat-eovked potential in SmI of halothane-nitrous oxide anesthetized rats (14). Multi-channel field potentials of layers in SmI were recorded simultaneously to analyze the laminar-specific transmembrane currents in halothane-anesthetized rats (35). Single cell recordings demonstrated that some SmI neurons responded to non-noxious and noxious stimulation in anesthetized animals (6, 15, 19, 20). Ensemble single-unit activity (SU) studies reported that SmI neurons responded to noxious stimulation in the conscious animals (17, 36, 37, 39). However, LFP and SU recordings in conscious animals had been studied at isolated points (16, 17, 37). A delineation of the precise spatiotemporal changes of neuronal responses in SmI to noxious stimulation is crucial to understand the processing of pain information in SmI.

The aim of the present study was to explore the potential substrate of nociceptive processing in the conscious state. The intracortical spatiotemporal maps of the SmI responding to laser heat stimulation on the tail were obtained in conscious behaving and anesthetized rats. By comparing the neural processing of nociceptive information in the conscious and anesthetized condition, critical components of nociceptive processing in the conscious state may be distinguished.

Materials and Methods

Animal Preparation

The animals were purchased from the National Laboratory Animal Center, Nankang, Taipei, Taiwan. All animals were housed 2-3 per cage in a temperature (21 ± 1°C)- and humidity (70 ± 5%)-controlled room, under a 12 h-12 h light-dark cycle (lights on at 7:00 a.m.) in the animal room of Department of Life Science, National Taiwan University. Animals had free access to food and water during the study. The animals were housed pairwise in type 3H cages filled with hardwood animal bedding and were allowed to acclimatize the breeding condition for at least 2 weeks after arrival. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee, National Taiwan University, in accordance with the Guide for the Care and Use of Laboratory Animals of the Agriculture Council of Taiwan. This study was conducted on 12 female adult Long-Evans rats, weighing 250-320 g. Longitudinal microelectrode arrays were implanted in 7 rats and vertical microelectrode arrays were implanted in 5 rats. The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) for implanting the microelectrode array. Ketamine hydrochloride (50 mg/kg, i.m.) was administered as necessary to maintain the animal areflexic throughout the surgical period. A small craniotomy was made over the target area for the implantation of microelectrodes. The longitudinal or vertical microelectrode array was implanted in the right SmI. Longitudinal and vertical microelectrode arrays were made using the stainless steel microwires insulated with Teflon (50 μm o.d.; California Fine Wires Company, Grover Beach, CA, USA). The methods on how to prepare the two types of microelectrode arrays have been described in the previous study (38). Briefly, the longitudinal microelectrode array was a linear array of 16 microelectrodes. Sixteen parallel microwires were arranged within 8 mm, and the microwires were soldered to 2 connectors (A11365-001, Omnetics Connector Corporations, Minneapolis, MN, USA). This type of microelectrode array was inserted approximately 0.4-0.9 mm deep into the brain. On the other hand, the vertical microelectrode array was made using

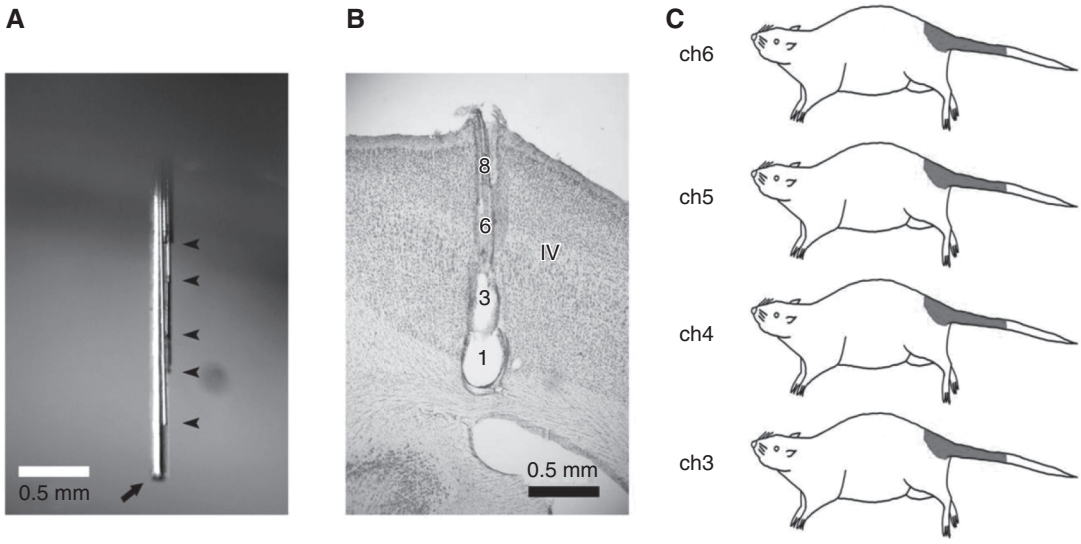

Fig. 1. Representative recording sites and their receptive fields of a vertical microelectrode array. A. An 8-channel vertical microelectrode array. The arrow indicates the deepest central microwire. The arrowheads indicate the tips of the other microwires. The other two wires were located at the backside and hidden from view. B. The histological section of lesion sites of this microelectrode array. The numbers in the picture indicate the channel numbers of the recording sites. C. The receptive fields (gray areas) of selected channels. Note the columnar organization of the receptive field located from the midtail to the base of the tail.

a bundle of 8 microwires. One thick microwire (150 μm o.d.; A-M systems; #793400; Sequim, WA, USA) was placed in the center, and seven fine microwires (50 μm o.d.; California Fine Wires Company) were arranged in sequence spirally (Fig. 1A). The tips of the microwires spanned 2 mm vertically. The 8 microwires were soldered to one connector. The vertical microelectrode array was inserted into the cortex, and the central microelectrode was at approximately 2 mm deep. The focus of the microelectrodes was the SmI tail region of the right hemisphere (2.5 mm posterior and 2.5 mm lateral to the bregma). The microelectrodes of the longitudinal array were located 1.5 mm rostral, 6.5 mm caudal and 2.5 mm lateral to the bregma. A previous study reported that the tail-evoked potentials could be recorded bilaterally over both sides of the SmI tail region (32). A stainless steel screw for recording superficial cortical potential was implanted on the left side of the skull over the SmI (2.5 mm posterior and 2.5 mm lateral to the bregma). Two other stainless steel screws were fixed on the frontal-parietal bone and mid-occipital bone for reference and ground electrodes, respectively. The microwires for the superficial cortical potential recording were soldered to the other connector. The implanted microelectrode array and the hole in the skull were sealed with Hygenic repair acrylic (the Hygenic Corporatio, Akron, OH, USA) and the connectors were secured on the skull. The receptive fields of the individual channels were identified when the rat was still under anesthesia to confirm the locations of microelectrodes (Fig. 1C). The rat was allowed to recover for at least 1 week after surgery.

Experimental Procedure

The experiments were performed in an isolation room with white light. Before the recording session, the rats were allowed to habituate the recording chamber in the testing room for 2 h a day for 5 subsequent days. The recording chamber was made of acrylic and its size was 60 cm in length, 45 cm in width and 45 cm in height. On the day of recording session, the rats were anesthetized with halothane (4% in 100% oxygen) temporally for connecting the headstages and recording cables to the connectors. The rats then stayed in the recording chamber for recovery from the halothane anesthesia at least 30 min before the experiment.

The laser stimulus was generated by a CO_2-laser and a concentric red light argon laser beam was used for indicating the stimulation site (medical surgical laser, 10.6 μm wavelength Tjing Ling #2, National Taiwan University) (41). The output energies were 105-120 mJ (output power: approximately 7-8 watts, pulse duration: 15 ms). The beam diameter was 3 mm (unfocused). A 1-cm length of local area was marked on the middle part of tail for the stimulation area. To minimize tissue damage, sensitization and habituation, the radiant heat pulses were applied manually and randomly within the stimulation area. The interstimulus interval was longer than 5 s. In each trial, 20

laser heat stimuli were applied, and the jerky movements of the tail (tail flick) were noted. The evoked neuronal responses to laser stimulation in SmI were also recorded (pre-control trial). After the pre-control trail, sodium pentobarbital (50 mg/kg, i.p.) was administered. The responses of evoked neural activities and tail flick were tested at 30, 60 and 120 min after the administration of sodium pentobarbital and on the next day (post-control trial).

After the experiment finished, the rats were anesthetized with a higher dose of sodium pentobarbital (80 mg/kg). Lesions were made at the tips of selected microwires with DC current (50 μA, 30 sec). The rats were perfused transcardically with saline followed by 10% formalin. The brains were removed and cut to 50 μm serial sections. The sections were stained with cresyl violet. The locations of the microelectrodes were determined under a microscope and photographed with a digital camera or plotted with Camera Lucida (Fig. 1B). For the longitudinal microelectrode array, the microelectrodes were usually distributed in the motor cortex, SmI hind paw and tail regions or in the occipital cortex. All of the channels were classified into 4 groups depending on the anatomical locations of microelectrodes or their physiological functions. For the vertical microelectrode array, the channels were categorized into three groups, including the supragranular layer group (layers I, II, III), granular layer group (layer IV) and infragranular layer group (layers V, VI), according to the anatomical positions of the microelectrode tips.

Data Acquisition and Analysis

Three types of neuronal activities, including the neuronal firing activity (single unit activities), intracortical LFP and superficial cortical potentials were acquired by a Multi-channel Neuronal Acquisition Processor system (MNAP, Plexon Inc., Dallas, TX, USA). The gains of signal amplification were 10,000X to 20,000X. The intracortical LFP and single unit activities were differentiated from signals of the same microelectrodes through the filters with different band passes. The filter bandpass for intracortical potential and skull cortical potential were 3-90 Hz and for the unit spike was 500-3K Hz. Real-time spike sorting was controlled by SortClient (Plexon Inc., Dallas, TX, USA), and the sampling rate of the individual channel was 40 kHz.

The saved wavelet data of single unit activities were analyzed with principal component analysis (PCA) and re-sorted by the software Offline Sorter (Plexon Inc.) to isolate the spikes of different units. The firing rates of single-unit activities and amplitude of field potential signals were analyzed with Neuroexlorer (Nex Techologies, Littleton, MA, USA). Ensemble single-unit activity (SU) was assembled from the activities of single units from different microelectrodes within the same layer or area groups. The evoked responses of unit activities and field potentials (superficial cortical potential and intracortical LFP) were generated by peri-event analysis (bin width = 10 ms). Our previous study reported that laser stimulation induced fast and slow components of SmI neuronal responses (17, 31). The peak in the 30-150 ms duration after stimulation was identified as the fast component of response and the peak in the 200-500 ms duration after stimulation as the slow component of response. To identify the responsiveness of neuronal activities, the SU and field potentials were normalized to Z-scores as described in the previous study (37). Briefly, the mean and standard deviation of the basal activities in the 500 ms duration prior the application of laser heat (50 bins, bin = 10 ms) were calculated. Z value of each data point was calculated by subtracting the mean of basal activity from the value of each data point and then dividing by standard deviation of basal activity. The evoked responses of unit activities and field potentials to laser heat were transformed into Z-scale depending on the mean and standard deviation from the basal activities. The change of unit and field potential evoked responses over 99% confidence level with 3 consecutive bins were recognized as responsiveness. The neuronal responses of unit activities and field potentials were compared with one-way repeated measures ANOVA. The *post hoc* comparison was Dunnett's test to compare the data among different time courses. The data are shown as the mean ± S.E. unless otherwise specified.

Results

Longitudinal Distribution of the Neuronal Responses to Laser Irradiant Heat

In seven rats, intracortical LFP and ensemble SU were recorded with a longitudinal microelectrode array that was made with 16 microwires (channels). The tips of microelectrodes spanned from the motor cortex, SmI and occipital cortex. In the superficial cortical potential and intracortical LFP recording, two components of laser-evoked potentials were observed in freely moving rats. The short-latency (fast) component was LEP1 and the long-latency (slow) component was LEP2 (Fig. 2A). In the intracortical LFP recordings, LEPs appeared in approximately all channels within a large area around SmI. The LEPs were most prominent in SmI, including the tail region and hindpaw region, when the rat was conscious. After the application of sodium pentobarbital, the amplitudes of the LEPs were reduced, and the evoked potentials were limited in the channels of the

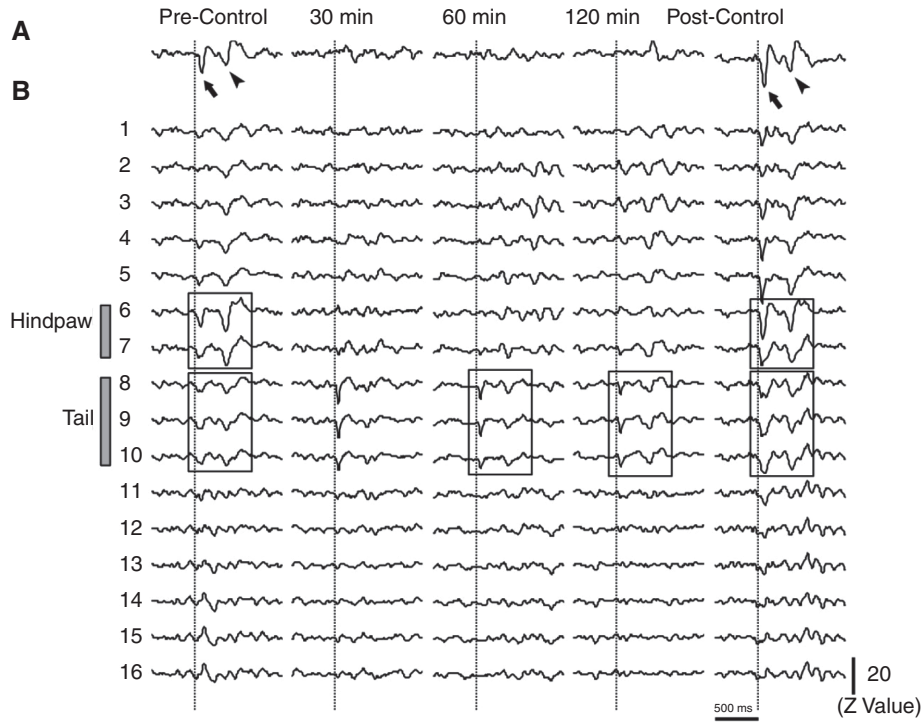

Fig. 2. A representative example of the laser heat evoked responses in superficial cortical potential and intracortical LFP recordings of a longitudinal microelectrode array before and after pentobarbital anesthesia. A. LEPs of the superficial cortical potential. The arrows indicate the LEP1, and the arrowheads indicate the LEP2. B. Intracortical LFP recordings. The dotted lines in the plots indicate the application of the laser heat irradiation stimulation.

SmI tail region (Fig. 2B).

The peak SU responses were also evoked in a wide range when the rat was conscious, especially in the SmI tail region and hindpaw region. In the tail region, both the fast and slow components of SU responses were over the 99% confidence level. In the hindpaw region, only the slow component response was significant in the Z-scale. In the other more caudal or rostral region, there was no significant response of SU. After the injection of sodium pentobarbital, short- and long-latency responses in the SmI tail region declined and were lower than the significance level at 60 min after pentobarbital administration. The inhibitory effect of sodium pentobarbital was also observed in the hindpaw regions of the SmI and the other two regions. Under anesthesia, the two-peak response only appeared in the tail region of the SmI. Those SUs of the 4 regions recovered on the next day (Fig. 3).

Laminar Analysis of the Neuronal Responses to Laser Heat

In five rats, vertical microelectrode arrays with eight channels were implanted in SmI to record the neuronal activities at the different depths of the cortex. The location of the vertical microelectrode array focused on the tail region of the SmI. The receptive fields of the units recorded from channels within a single vertical array were similar. For the field potentials, a representative example of superficial cortical potential and intracortical LFP from one rat is shown in Fig. 4. The responses of superficial cortical potential recording were consistent with those of the previous section (Fig. 4A). In the intracortical LFP recording, LEP1 and LEP2 were found in all channels within different SmI layers of the freely moving rats. After the application of sodium pentobarbital, the LEPs declined in the supragranular layers. Nevertheless, some LEPs remained in the deeper layer channels (Fig. 4B).

In this experiment, the neuronal firings in the different cortical layers were categorized separately. The six cortical layers were classified into supragranular (layer I, II, III), granular (layer IV) and infragranular (layer V, VI) layer groups. The effect of sodium pentobarbital on the original neuronal firings of SUs responding to laser heat averaged from five rats is shown in Fig. 5. The activities were assembled from all neurons recorded from microelectrodes in the tail region of the SmI. There were two peaks of responses with high basal activities when the rats were conscious. In the anesthetized state, the neuronal firing was strongly suppressed, including the evoked responses and basal activity but two evoked responses could

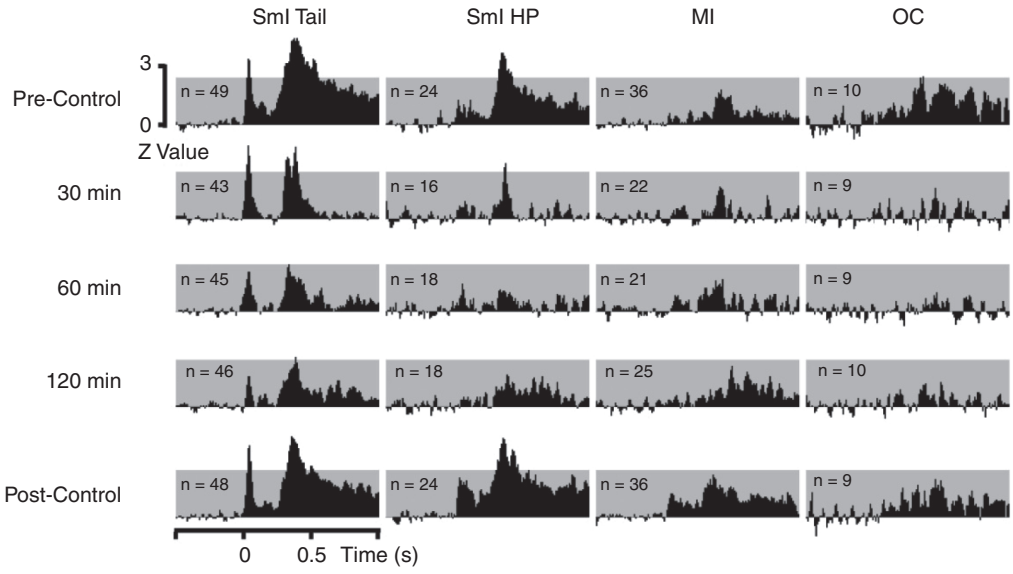

Fig. 3. Average and normalized responses of multiple single-unit activities from longitudinal microelectrode arrays before and after pentobarbital anesthesia from 7 rats. The unit activities recorded from microelectrodes in the 4 areas were assembled by averaging the normalized (Z value) responses of many single units. The n values represent the number of single units used. Silent units were not counted. The gray areas show the 99% confidence level. Laser stimulation was applied at time 0. SmI tail: tail region of the SmI, SmI HP: hind paw region of the SmI, MI: primary motor cortex, OC: occipital cortex. The numbers in each panel indicate the number of neurons assembled.

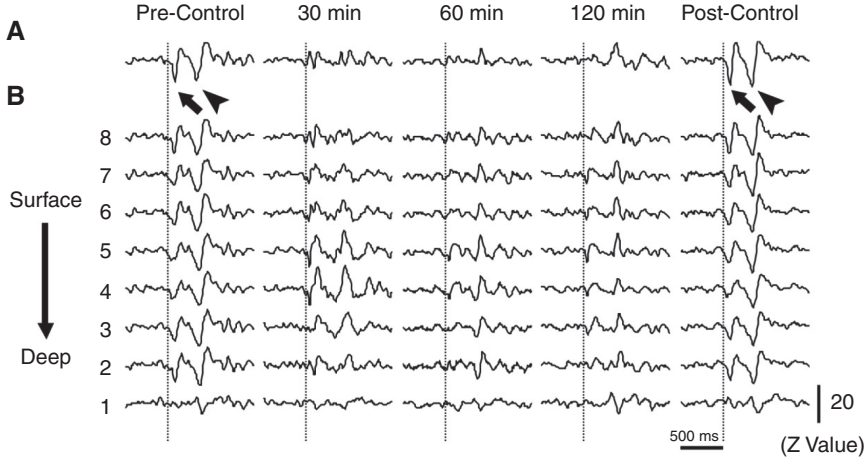

Fig. 4. A representative example of the evoked responses in superficial cortical potential and intracortical LFP recordings from a vertical microelectrode array under the pentobarbital anesthesia. A. superficial cortical potential recordings. The arrows and arrowheads indicate the LEP1 and LEP2, respectively. B. Intracortical LFP recordings. The dashed lines in the plots indicate the application of laser stimulation. Channel 1 was located in the white matter, and channel 8 was the most superficial recording electrode.

still be observed in the lower basal firing activity comparative to the higher basal firing activity (left panel in Fig. 5). For the SUs in the three layer groups, there were two firing peaks induced by laser heat in all of three layer groups when the rats were conscious. After the administration of sodium pentobarbital, either evoked responses or basal activities were reduced in the three layer groups. However, two evoked responses appeared in the granular and infragranular layers but not in the supragranular layer (3 right panels in Fig. 5).

The representative Z-transformation of SUs from one rat is shown in Fig. 6. Brisk short-latency responses and lasting long-latency responses evoked by laser radiant heat were observed in all layer groups of the conscious behaving animal. Under pentobarbital anesthesia, the SUs of the supragranular layer group were obviously decreased, especially for the slow component.

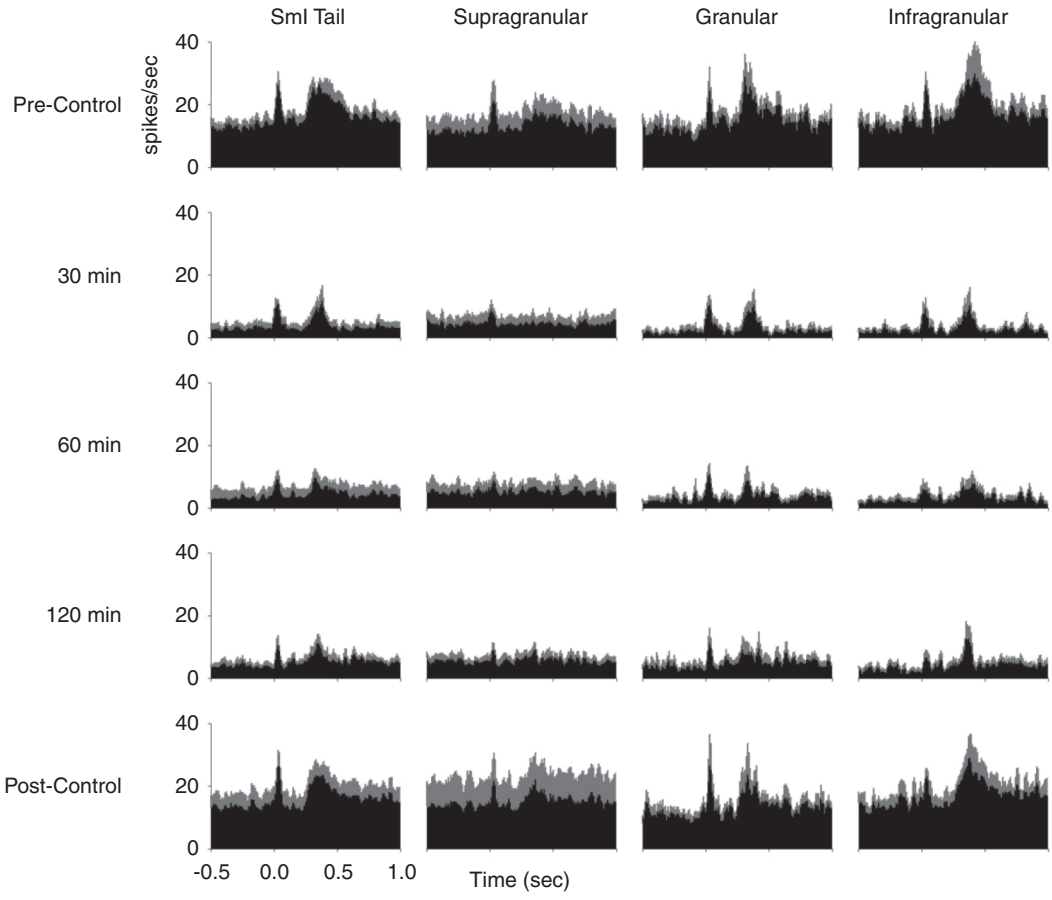

Fig. 5. Average responses of original ensemble unit activities from 5 rats with a vertical microelectrode array under pentobarbital anesthesia. The SUs were assembled from the units of all layers and units in each layer groups. The solid areas indicate the average of the original firing rates, and the gray areas represent the standard error of each bin (bin = 10 ms). SmI tail: all layers in tail region of the SmI; supragranular: layers I, II and III; granular: layer IV; infragranular: layers V and VI.

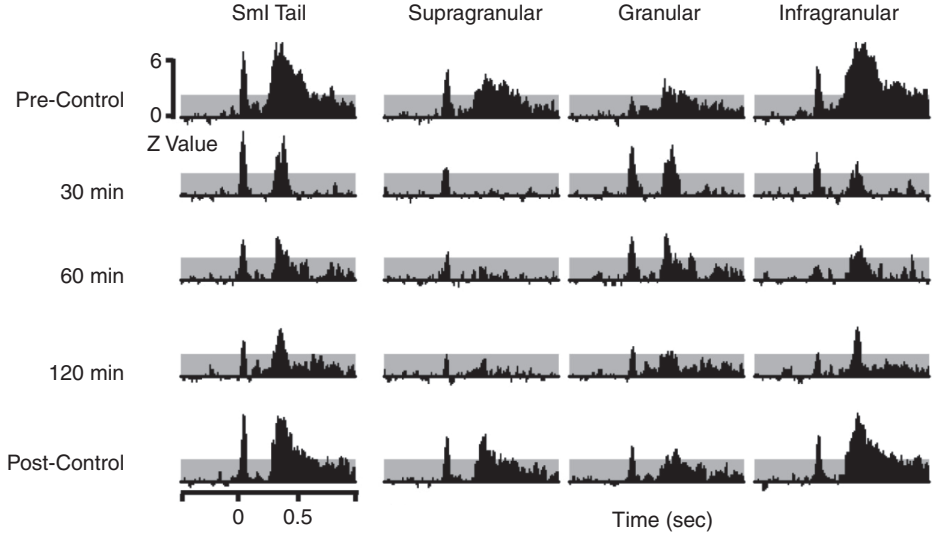

Fig. 6. The effect of sodium pentobarbital on the SU responses from a vertical microelectrode array in Z-scale. The SUs were assembled from the units recorded from all layers and units in each layer groups. The gray areas indicate the 99% confidence level. Laser stimulation was applied at time 0. SmI tail: all layer in tail region of the SmI; supragranular: layers I, II and III; granular: layer IV; infragranular: layers V and VI.

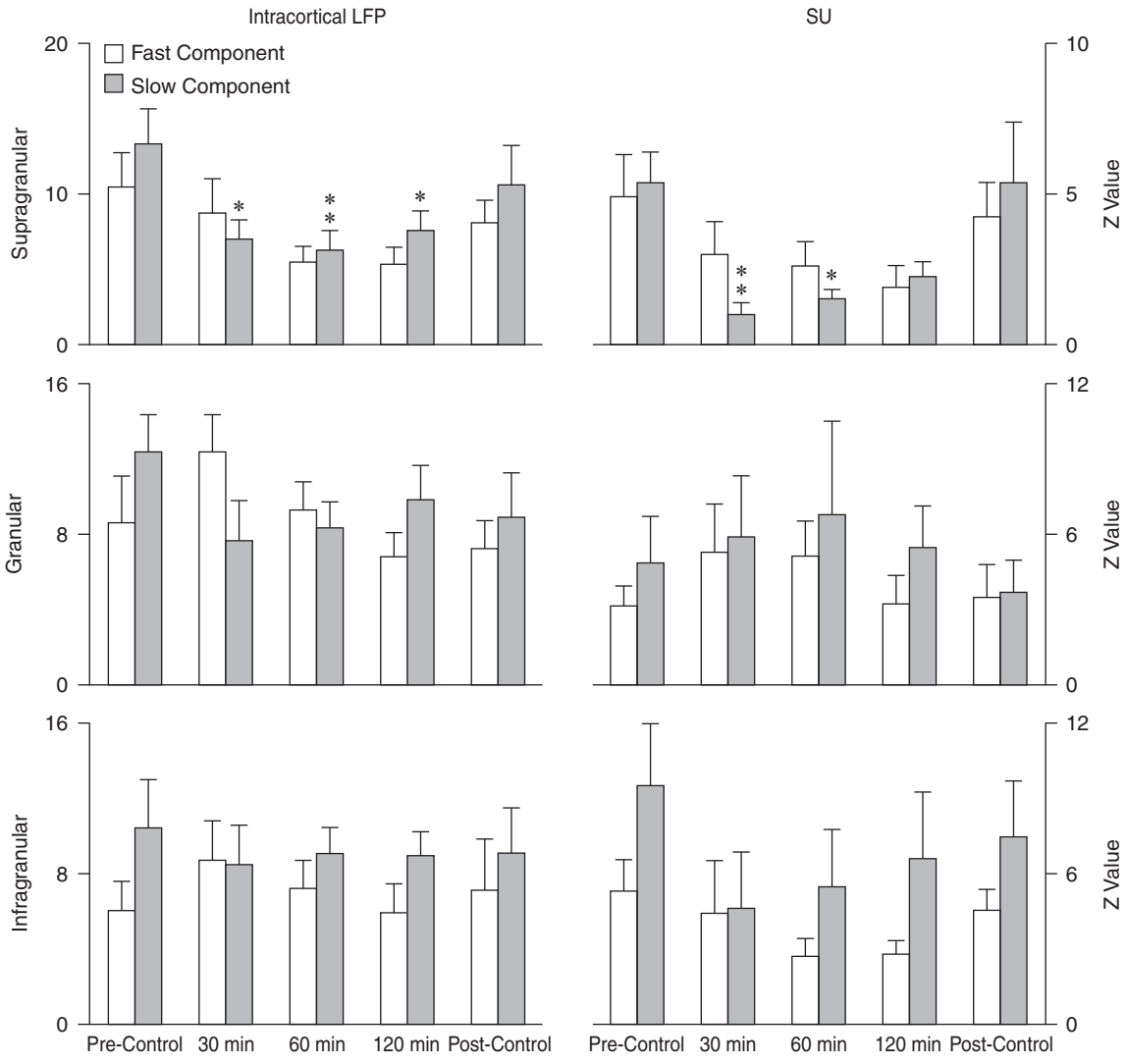

Fig. 7. The average changes of the fast and slow components of intracortical LFP and SU responses in Z-scale of 3 cortical layer groups under pentobarbital anesthesia (n = 5). *$P < 0.05$, **$P < 0.01$, compared to the pre-control trial.

In the granular and infragranular layer groups, the two components of the responses were also affected. The lasting pattern of the slow component was suppressed to a burst pattern after the administration of sodium pentobarbital. The Z-transformed responses of intracortical LFP and SU in different groups to sodium pentobarbital were compared. For the responses of the intracortical LFP, the results indicated that LEP2 in supra granular groups was decreased significantly ($F(4,16) = 5.52$, $P < 0.01$) during 30-120 min after the application of sodium pentobarbital ($P < 0.01$ or $P < 0.05$) but the change of LEP1 in the supragranular layers was not statistically significant. The LEPs of the granular and infragranular groups were not affected significantly by sodium pentobarbital (Fig. 7, left column). Similar results are shown for the responses of the SUs in Z-scale. The slow components in the supragranular layer group were suppressed significantly ($F(4,16) = 6.813$, $P < 0.01$) at 30 and 60 min after pentobarbital administration ($P < 0.01$ or $P < 0.05$). However, the effect of sodium pentobarbital on the SUs of the granular and infragranular groups was not significant (Fig. 7, right column).

Discussion

In the present study, the spatiotemporal pattern of neuronal activities responding to noxious laser heat stimuli applied to the mid-tail were recorded in two dimensions with longitudinally- and vertically-arranged microelectrode arrays. Laser heat induced wide-spread neuronal responses in a diffuse cortical area of the conscious rat, especially in the SmI. The range of neuronal responses was reduced and limited in the tail region of the SmI under pentobarbital anes-

thesia. The neuronal responses to laser stimuli were observed in all cortical layers of the SmI in conscious rats and were suppressed by sodium pentobarbital more seriously in the supragranular layers relative to the deeper layers and the basal activity.

LEPs have been observed over a wide range of the cerebral cortex in conscious rats (27, 31). In this study, the neuronal responses to noxious stimulus around the SmI were studied at the cellular level with neuronal recording techniques. The LFP and SU responses to the short-duration laser heat stimulation were surveyed with a 16-channel longitudinal microelectrode array which spanned from the frontal, parietal and occipital cortices. Our result showed the obvious fast and slow components of responses in the tail and the hindpaw regions of the SmI in the intracortical LFP recording. In the more rostral or caudal area, the neuronal responses were obscure (Fig. 2). In the SU responses, the fast component was only significant in the SmI tail region of conscious rats, whereas the slow component was significant in a larger area, including the tail and rostral hindpaw region of the SmI (Fig. 3). The topographic distribution analysis of the LEPs calculated from 12 EEG recordings over the cerebral cortex suggested the involvement of a localized component around the SmI, followed by a widespread distributed component involving the rostral part of the SmI after laser heat stimulation (31). A previous study reported that the LEPs from superficial cortical potential recordings were total suppressed under pentobarbital anesthesia and were recovered afterwards (33). Our results also demonstrated an inhibitory effect caused by sodium pentobarbital on the responses of intracortical LFP and SU. Under anesthesia, the responses of the intracortical LFP and SU were limited in the tail region of the SmI.

In addition, the processing of acute pain is necessary for animals to react to potentially life-threatening situations. Interruptive function of pain has been proposed that painful stimuli demanded attention to achieve salience in complex environments with several competing sensory stimuli and then ongoing processes were interfered (8). fMRI studies have revealed that pain modulates visual object processing in lateral occipital complex and the source of this modulation was from the anterior cingulate cortex (5). It has been recognized that parallel lateral and medial pain systems processed sensory-discriminating and affective-motivational components of pain (1, 23). The features of pain were thought to be transmitted to lateral thalamus and SmI and the affective-motivational aspects of pain were mediated by medial thalamus and cingulate cortex. In this study, some evoked neuronal response was observed in the cortical area more caudal than SmI. The neuronal response in the occipital area might be related to the interruptive function of pain.

In this study, shrinkage of the cortical response area was observed under pentobarbital anesthesia. One possible reason is that the excitation spreading in the cortex was limited by pentobarbital. An *in vivo* study reported that layer 4 stimulation in the barrel cortex induced excitation spreading horizontally in layer 2/3, and this horizontal spreading of excitation markedly enhanced in layer 2/3 in the presence of bicuculline (29). The density of GABAergic neurons has also been reported to exhibit peaks in the upper third of layer 2/3 and layer 5 (24). The nociceptive input from the thalamus may spread to a wide cortical area through horizontal communication between cortical neurons, especially in layer 2/3 when the animal is conscious. Under pentobarbital anesthesia, the spread of information in the cortex was suppressed, limiting the cortical response region. Our results implied that the GABA receptor is involved in the regulation of cortical activation. In addition, the suppression of sodium pentobarbital administered systemically on the sensory afferents originating in the spinal cord could not be ruled out. The ascending arousal signals from the reticular activating system might involve in the cortical responses of conscious animals and the suppression on cortical responses by sodium pentobarbital might be augment by reducing the ascending arousal signals (3, 7). The decrease of basal firing activities after administration of sodium pentobarbital observed in the original ensemble unit activities (Fig. 5) might also reflect the reduction of general arousal from the reticular activating system.

With a vertical microelectrode array, our results demonstrated that LEPs were induced in all layers of the SmI in conscious rats. In the somatosensory cortex, layer 4 neurons are organized into identifiable clusters of neurons and have vertical and column-restricted axonal arbors (22, 40). Intracortical LEPs in all layers of the SmI have been reported to be simultaneously recorded by multichannel recording in halothane-anesthetized rats. With current source density analysis (CSD), two current sinks induced by laser heat stimulation were found in layer 4 and layer 6 (35). In this study, the microelectrode array used in this study was hand-made, so the spacing between each channel was imperfect and the channel number was limited. CSD was not applicable to determine the precise foci of the current source. However, our ensemble single unit data could partially compensate for the deficit. Our SU data indicated that general neuronal activation was induced by laser heat stimulation in all SmI layers of conscious rats. Under pentobarbital anesthesia, significant evoked responses relative to basal activity were presented in the granular and infragranular layers (Fig. 6). In addition, upon a comparison the responses under conscious and anes-

thetized conditions, the evoked response of the supragranular layer was found to be more severely suppressed than those of deeper layers. Layer 4 neurons receive excitation relayed from the thalamus and then spreads the signal vertically to the pyramidal cells in layer 2/3 (9, 18, 26) and layer 5A (10, 30). In a brain slice study of the barrel cortex, each connected L4 spiny neuron was found to produce a weak but reliable EPSP in the pyramidal cell (9). GABAergic interneurons are found in all neocortical areas and layers, and GABA receptors are also distributed widely in the cortex (24). The comprehensive mapping of interneurons in the cortical columns of the rat somatosensory cortex has been performed, and the ratio of interneuron to neurons is much higher in layers 1 and 2 (24). The major reduction in the neuronal response in the supragranular layers might be correlated with the high fraction of inhibitory neurons in that region.

Both analgesics and anesthetics modulate pain perception of a subject. Under morphine analgesia, a subject's perception is not abolished, and only the sensation of pain is relieved. The differential effects of morphine were shown on short- and long-latency laser-evoked responses of the SmI in conscious rats, and little reduction was observed for the basal activity of SmI neurons after systemic morphine application (17, 37). However, the systemic application of pentobarbital intensely diminished neuronal activities (17). In this study, both the basal activity and evoked responses were reduced, not totally abolished, by pentobarbital in three layer groups (Fig. 5). The pentobarbital suppressed not only pain perception but also consciousness, hence, observed differences in the neuronal responses between awake and anesthetized conditions may be complicated by consciousness. In this study, the evoked responses were emphasized by Z transformation which was corresponding to the variant of basal activity. By performing a comparison of neuronal responses between the conscious and anesthetized states, changes in the spatiotemporal pattern of cortical neuronal responses to noxious heat after the application of sodium pentobarbital were observed and may help to elucidate the potential substrates of nociceptive processing in the conscious state. These results suggested that widespread activation either in the horizontal or vertical dimension of the cortex was the potential substrate of pain-related functions under conscious condition.

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References

1. Albe-Fessar, D., Berkley, K.J., Kruger, L., Ralston, H.J., 3rd and Willis, W.D., Jr. Diencephalic mechanisms of pain sensation. *Brain Res. Rev.* 356: 217-296, 1985.
2. Allison, T. and Hume, A.L. A comparative analysis of short-latency somatosensory evoked potentials in man, monkey, cat, and rat. *Exp. Neurol.* 72: 592-611, 1981.
3. Antognini, J.F. The relationship among brain, spinal cord and anesthetic requirements. *Med. Hypotheses* 48: 83-87, 1997.
4. Arezzo, J.C., Vaughan, H.G., Jr. and Legatt, A.D. Topography and intracranial sources of somatosensory evoked potentials in the monkey. II. Cortical components. *Electroencephalogr. Clin. Neurophysiol.* 51: 1-18, 1981.
5. Bingel, U., Rose, M., Glascher, J. and Buchel, C. fMRI reveals how pain modulates visual object processing in the ventral visual stream. *Neuron* 55: 157-167, 2007.
6. Chudler, E.H., Anton, F., Dubner, R. and Kenshalo, D.R., Jr. Responses of nociceptive SI neurons in monkeys and pain sensation in humans elicited by noxious thermal stimulation: effect of interstimulus interval. *J. Neurophysiol.* 63: 559-569, 1990.
7. Dong, Y.F., Tang, J.S., Yuan, B. and Jia, H. Morphine applied to the thalamic nucleus submedius produces a naloxone reversible antinociceptive effect in the rat. *Neurosci. Lett.* 271: 17-20, 1999.
8. Eccleston, C. and Crombez, G. Pain demands attention: a cognitive-affective model of the interruptive function of pain. *Psychol. Bull.* 125: 356-366, 1999.
9. Feldmeyer, D., Lubke, J., Silver, R.A. and Sakmann, B. Synaptic connections between layer 4 spiny neurone-layer 2/3 pyramidal cell pairs in juvenile rat barrel cortex: physiology and anatomy of interlaminar signalling within a cortical column. *J. Physiol.* 538: 803-822, 2002.
10. Feldmeyer, D., Roth, A. and Sakmann, B. Monosynaptic connections between pairs of spiny stellate cells in layer 4 and pyramidal cells in layer 5A indicate that lemniscal and paralemniscal afferent pathways converge in the infragranular somatosensory cortex. *J. Neurosci.* 25: 3423-3431, 2005.
11. Granmo, M., Jensen, T. and Schouenborg, J. Nociceptive transmission to rat primary somatosensory cortex--comparison of sedative and analgesic effects. *PLoS One* 8: e53966, 2013.
12. Hofbauer, R.K., Rainville, P., Duncan, G.H. and Bushnell, M.C. Cortical representation of the sensory dimension of pain. *J. Neurophysiol.* 86: 402-411, 2001.
13. Kalliomaki, J., Weng, H.R., Nilsson, H.J. and Schouenborg, J. Nociceptive C fibre input to the primary somatosensory cortex (SI). A field potential study in the rat. *Brain Res.* 622: 262-270, 1993.
14. Kalliomaki, J., Weng, H.R., Nilsson, H.J., Yu, Y.B. and Schouenborg, J. Multiple spinal pathways mediate cutaneous nociceptive C fibre input to the primary somatosensory cortex (SI) in the rat. *Brain Res.* 622: 271-279, 1993.
15. Kenshalo, D.R., Jr. and Isensee, O. Responses of primate SI cortical neurons to noxious stimuli. *J. Neurophysiol.* 50: 1479-1496, 1983.
16. Kuo, C.C., Chiou, R.J., Liang, K.C. and Yen, C.T. Differential involvement of the anterior cingulate and primary sensorimotor cortices in sensory and affective functions of pain. *J. Neurophysiol.* 101: 1201-1210, 2009.
17. Kuo, C.C. and Yen, C.T. Comparison of anterior cingulate and primary somatosensory neuronal responses to noxious laser-heat stimuli in conscious, behaving rats. *J. Neurophysiol.* 94: 1825-1836, 2005.
18. Laaris, N., Carlson, G.C. and Keller, A. Thalamic-evoked synaptic interactions in barrel cortex revealed by optical imaging. *J. Neurosci.* 20: 1529-1537, 2000.
19. Lamour, Y., Guilbaud, G. and Willer, J.C. Rat somatosensory (SmI) cortex: II. Laminar and columnar organization of noxious and non-noxious inputs. *Exp. Brain Res.* 49: 46-54, 1983.

20. Lamour, Y., Willer, J.C. and Guilbaud, G. Rat somatosensory (SmI) cortex: I. Characteristics of neuronal responses to noxious stimulation and comparison with responses to non-noxious stimulation. *Exp. Brain Res.* 49: 35-45, 1983.
21. Lee, J.C. and Yen, C.T. Frequency coding ability of the somatosensory thalamocortical system and its modulation by anesthesia depth. *Chinese J. Physiol.* 44: 89-95, 2001.
22. Lubke, J., Roth, A., Feldmeyer, D. and Sakmann, B. Morphometric analysis of the columnar innervation domain of neurons connecting layer 4 and layer 2/3 of juvenile rat barrel cortex. *Cereb. Cortex* 13: 1051-1063, 2003.
23. Melzack, R. and Casey, K.L. Sensory, motivational and central control determinants of pain: a new conceptual model. In: *The Skin Senses*, edited by Kenshalo, D.R. Springfield, IL, USA: Thomas, C.C., 1968, pp. 423-443.
24. Meyer, H.S., Schwarz, D., Wimmer, V.C., Schmitt, A.C., Kerr, J.N., Sakmann, B. and Helmstaedter, M. Inhibitory interneurons in a cortical column form hot zones of inhibition in layers 2 and 5A. *Proc. Natl. Acad. Sci. USA* 108: 16807-16812, 2011.
25. Nuwer, M.R. Fundamentals of evoked potentials and common clinical applications today. *Electroencephalogr. Clin. Neurophysiol.* 106: 142-148, 1998.
26. Petersen, C.C., Hahn, T.T., Mehta, M., Grinvald, A. and Sakmann, B. Interaction of sensory responses with spontaneous depolarization in layer 2/3 barrel cortex. *Proc. Natl. Acad. Sci. USA* 100: 13638-13643, 2003.
27. Qiao, Z.M., Wang, J.Y., Han, J.S. and Luo, F. Dynamic processing of nociception in cortical network in conscious rats: a laser-evoked field potential study. *Cell. Mol. Neurobiol.* 28: 671-687, 2008.
28. Rainville, P., Carrier, B., Hofbauer, R.K., Bushnell, M.C. and Duncan, G.H. Dissociation of sensory and affective dimensions of pain using hypnotic modulation. *Pain* 82: 159-171, 1999.
29. Sato, H., Shimanuki, Y., Saito, M., Toyoda, H., Nokubi, T., Maeda, Y., Yamamoto, T. and Kang, Y. Differential columnar processing in local circuits of barrel and insular cortices. *J. Neurosci.* 28: 3076-3089, 2008.
30. Schubert, D., Kotter, R., Luhmann, H.J. and Staiger, J.F. Morphology, electrophysiology and functional input connectivity of pyramidal neurons characterizes a genuine layer Va in the primary somatosensory cortex. *Cereb. Cortex* 16: 223-236, 2006.
31. Shaw, F.Z., Chen, R.F., Tsao, H.W. and Yen, C.T. Comparison of touch- and laser heat-evoked cortical field potentials in conscious rats. *Brain Res.* 824: 183-196, 1999.
32. Shaw, F.Z., Chen, R.F., Tsao, H.W. and Yen, C.T. A multichannel system for recording and analysis of cortical field potentials in freely moving rats. *J. Neurosci. Methods* 88: 33-43, 1999.
33. Shaw, F.Z., Chen, R.F. and Yen, C.T. Dynamic changes of touch- and laser heat-evoked field potentials of primary somatosensory cortex in awake and pentobarbital-anesthetized rats. *Brain Res.* 911: 105-115, 2001.
34. Shaw, F.Z., Kuo, C.C., Tsai, M.L. and Yen, C.T. Cerebral cortical responses to nociceptive laser heat stimulation in conscious behaving rats. In: *Nocrveptive and Neuropathic Pain: Mechanisms and Treatments*, edited by Shyu, B.C. and Chien, C.C. Kerala, India: Research Signpost, 2009, pp. 105-119.
35. Sun, J.J., Yang, J.W. and Shyu, B.C. Current source density analysis of laser heat-evoked intra-cortical field potentials in the primary somatosensory cortex of rats. *Neuroscience* 140: 1321-1336, 2006.
36. Toda, T. and Taoka, M. Hierarchical somesthetic processing of tongue inputs in the postcentral somatosensory cortex of conscious macaque monkeys. *Exp. Brain Res.* 147: 243-251, 2002.
37. Tsai, M.L., Kuo, C.C., Sun, W.Z. and Yen, C.T. Differential morphine effects on short- and long-latency laser-evoked cortical responses in the rat. *Pain* 110: 665-674, 2004.
38. Tsai, M.L. and Yen, C.T. A simple method for fabricating horizontal and vertical microwire arrays. *J. Neurosci. Methods* 131: 107-110, 2003.
39. Wang, N., Zhang, Y., Wang, J.Y., Gao, G. and Luo, F. Effects of pentobarbital anesthesia on nociceptive processing in the medial and lateral pain pathways in rats. *Neurosci. Bull.* 26: 188-196, 2010.
40. Woolsey, T.A. and Van der Loos, H. The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. *Brain Res.* 17: 205-242, 1970.
41. Yen, C.T., Huang, C.H. and Fu, S.E. Surface temperature change, cortical evoked potential and pain behavior elicited by CO_2 lasers. *Chinese J. Physiol.* 37: 193-199, 1994.