CURRENT SOURCE DENSITY ANALYSIS OF LASER HEAT-EVOKED INTRA-CORTICAL FIELD POTENTIALS IN THE PRIMARY SOMATOSENSORY CORTEX OF RATS

J. J. SUN, J. W. YANG AND B. C. SHYU*
Institute of Biomedical Sciences, Academia Sinica, Nan Kang, Taipei 11529, Taiwan, Republic of China

Abstract—The role of the primary somatosensory cortex in thermal pain perception has been established. However, the cortical circuitry that mediates the thermo-nociceptive information processing has not been elucidated. The aim of present study was to investigate the intracortical synaptic currents in primary somatosensory cortex evoked by short laser pulses and to determine their transmission pathway. Noxious CO2 laser pulse stimuli or innocuous electrical and mechanical stimuli were delivered to the hind paw of halothane-anesthetized rats. Multi-channel field potentials were recorded simultaneously in primary somatosensory cortex and laminar-specific transmembrane currents were analyzed using a current source density method. A distinct spatial–temporal pattern of intra-cortical sink source currents was evoked by laser pulse stimuli. The amplitude of the early component was graded by laser energy output and influenced by contralateral signals, whereas the late components were not intensity-dependent and exhibited bilateral excitation. Intra-cortical current flows revealed that synaptic activation occurred initially at layers IV and VI separately and then was relayed transynaptically to the more superficial and the deeper layers. Latency, amplitude and intracortical distributions of the activated intra-cortical currents evoked by noxious stimuli differed significantly from those evoked by innocuous stimuli. Conduction velocity data together with the results of tetrodotoxin, capsaicin and morphine treatments indicated that the early and late components were mediated separately by A-delta and C fibers. Our results suggest that large and small diameter thermal nociceptive afferents generated laminar-specific intracortical synaptic currents in primary somatosensory cortex and that these excitatory synaptic currents were conveyed separately by lateral and medial thalamic nuclei. © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: laser stimulation, capsaicin, cortical circuit, sink currents, thalamus, morphine.

Neuroimaging research in the last decade has identified multiple cortical and subcortical structures involved in pain processing. Data describing specific subfunctional processes, such as intensity encoding (Coghill et al., 1999; Timmermann et al., 2001), attentional (Peyron et al., 1999) and affective (Rainville et al., 1997) processes as well as processing and temporal dynamics (Porro et al., 1998; Casey et al., 2001) have been reported. Primary somatosensory cortex (S1) belongs to lateral pain system, receives information primarily from the ventral posterior lateral nucleus (VPL) of thalamus and is thought to mediate sensory-discrimination of stimulus localization and intensity (Talbot et al., 1991; Treede et al., 1999; Disbrow et al., 1998; Hofbauer et al., 2001). There is converging evidence implicating S1 involvement in pain processing from studies conducted in monkeys (Kenshalo et al., 1983), cats (Berkley and Parmer 1974), and rats (Lamour et al., 1983a).

The spatial–temporal characteristics of nociceptive processing in S1 have not been resolved. The dermal application of short laser pulses has been considered an ideal neurophysiological correlate of human pain (Bromm and Lorenz, 1998). Electrophysiological (Walker and Akhanjee, 1985; Kakigi et al., 1989; Ohara et al., 2004) and functional magnetic resonance imaging (Bingel et al., 2003) studies have shown functional activation in S1 in human subjects following laser stimulation on skin and laser-evoked potentials in S1 can be obtained in awake (Shaw et al., 1999) or anesthetized rats (Kalliomaki et al., 1993a). Laser-evoked cortical responses can be classified into two groups (Isseroff et al., 1982; Kalliomaki et al., 1993a; Danneman et al., 1994; Shaw et al., 1999). The first group mediated by A-delta fibers is more sensitive to pentobarbital anesthesia (Shaw et al., 2001) and laser pulse energy level (Kalliomaki et al., 1993a). The second group mediated by C-fibers is widespread across the cortical surface and can be diminished by topical morphine application onto the lumbar spinal cord and reversed by naloxone (Kalliomaki et al., 1993a, 1998). Early and C-fiber-related late evoked potentials following laser stimulation exhibited negative peaks in deep cortical layers (Isseroff et al., 1982; Kalliomaki et al., 1993a). Laser stimulation evoked higher multiunit activities within superficial and deep cortical layers in the 30–60 ms and 250–700 ms latency range respectively (Shaw et al., 1999).

The interpretation of previous electrophysiological studies regarding localization of previously identified intracortical components has not been straightforward. Highly variable latencies and amplitudes have been reported in studies recording evoked field potentials across cortical depths with a single electrode (Isseroff et al., 1982; Kalliomaki et al., 1993a). Laser-evoked intra-cortical synaptic activation sites cannot be determined precisely from the maximal negative potential alone. And although multiunit activities may reveal the location of excited cortical neurons, subthreshold synaptic events are not apparent. To
overcome these confounds, we used a microelectrode array with 16 recording channels dispersed among all cortical layers, a multichannel amplifier and a data acquisition system. This setup allows field potentials from all channels to be recorded simultaneously. Thus field potential variation between channels due to sampling bias can be avoided. We hypothesized that two major evoked sink currents in S1 corresponding to the previously described field potential components evoked by the laser stimuli would be observed. Short-pulses of CO\textsubscript{2} laser stimulation were applied to the hind paw to elicit thermal nociceptive signals. Extracellular field potentials across the S1 cortical layers were recorded simultaneously with silicon-based multichannel thin film microprobe. Current source density (CSD) data analyses were performed to reveal activated synaptic currents. The cortical layer distributions of these laser-evoked sinks and possible afferents and thalamic relay contributions to these evoked sink currents were investigated.

**EXPERIMENTAL PROCEDURES**

**Preparation of animals**

Male Sprague–Dawley rats (300 – 400 g) were used in the present study. They were housed in an air-conditioned room (21–23 °C, humidity 50%, 12-h light/dark cycle with lights on at 08:00 h) with free access to food and water. All experiments were carried out in accordance with the guidelines of the Academia Sinica Institutional Animal Care and Utilization Committee, as well as the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80–23). All efforts were made to minimize the number of animals used and their suffering. Rats were initially anesthetized with 4% halothane (in 100% O\textsubscript{2}) in an acrylic box. A PE-240 tube was inserted via tracheotomy and was guided from the helium laser and produced a red light spot. The CO\textsubscript{2} laser beam was aligned from a distance of about 10 mm. The CO\textsubscript{2} laser beam was produced by a laser beam producing a round spot on the paw surface that covered an area of about 12.5 mm\textsuperscript{2}. Skin of the hind digits, from a distance of about 10 mm. The CO\textsubscript{2} laser beam was aligned from a distance of about 10 mm. The CO\textsubscript{2} laser beam was produced by a laser beam producing a round spot on the paw surface that covered an area of about 12.5 mm\textsuperscript{2}. Skin of the hind digits, paw and heel was stimulated in turn with a train of four consecu-
tive laser pulses at a frequency of 0.9 Hz, an intensity of 10 W and duration between 10 and 50 ms (corresponding to an output energy of 100–500 mJ). An averaged recording based on 40 stimulations was obtained and no visible damage to the skin was observed. A pause of at least 15 min was allowed before the same skin site was re-stimulated. The laser stimulation protocol was similar to that reported previously (Kalliomaki et al., 1998).

**Electrical stimulation**

Custom-made stainless steel wires were attached to the hind paw and used to deliver bipolar electrical stimulation (0.3–10 mA, 0.5 ms duration, 0.2 Hz) by an isolated pulse stimulator (Model 2100, A-M System Inc., USA). The anode electrode was placed about 1 cm distal to the cathode electrode. Minimal intensity for inducing the cortical evoked responses was regarded as the threshold. The intensity was delivered as a multiple of the threshold current value.

**Mechanical stimulation**

A 1.5 mm diameter stainless steel rod was attached to the voice coil of an 8 Ω, 15 W loudspeaker. The pressure exerted by the protrusion of the rod onto the paw surface was measured by a Transducer indicator (Model 1601C, IITC Inc., CA, USA). The exerted force was 1.2 g when a 10-V, 1-ms square wave of 10 V was applied to the coil. The tactile stimulus was triggered and controlled by a square wave pulse from a pulse generator (Model 2100, A-M Systems Inc.). The movement of the coil transmitted to the rod produced an outward excision of 5 mm.

**Recording of evoked multichannel field potentials on S1**

Extracellular field potentials evoked by an electrical pulse applied to the hind paw were first registered by a tungsten electrode and mapped in the S1 region. The maximal positive potential was located and was designated as the insertion point of the multichannel probe. The insertion angle was perpendicular to the cortical surface. Silicon-based multichannel thin-film microprobes, Michigan probe, with 16 contact points (150 µm interval spacing) was penetrated to a depth about 2.5 mm from the cortical surface and was used to record extracellular field potentials in the hind paw projection area of S1 (identified by electrophysiological mapping and approximately 1 mm posterior and 3 mm lateral to the bregma) and an Ag–AgCl reference electrode was placed in the nasal cavity. Analog signals were amplified by a 16-channel amplifier (Medusa Digital BioAmp, TDT Inc., USA) and passed through a 1.6 Hz to 7.5 kHz analog bandpass filter. The sampling rate of recording signals was 6 kHz and the data were processed in a multichannel data acquisition system (TDT Inc.) based on a PC system.

**CSD analysis**

The field potentials evoked by the peripheral stimulation were recorded for 1 s in each channel per trial. Twenty to 40 trials were averaged for each channel. To accurately locate the synaptic currents mediating the local extracellular potentials, the 16 channels of averaged field potentials were subjected to a one-dimensional CSD analysis formula (Freeman and Nicholson, 1975; Mitzdorf, 1985). With regard to the time span and the sampling variations in each recording session, we adopted a five-point formula (Freeman and Nicholson, 1975) to smooth the spatial sampling variability. The \( I_m \) was derived from the second spatial derivations of the extracellular field potentials, \( \Phi \) and was calculated with the finite difference formula

\[
I_m = -\left(\frac{V}{kh^2}\right) \sum_{m=-\infty}^{\infty} a_m \phi(x + mh)
\]
Where \( h \) is the distance between successive measuring points (150 \( \mu \text{m} \) in the present investigation), and \( x \) is the coordinate perpendicular to the cortical layer. The remaining constants are as follows: \( n=2, k=4, a 0=-2, a 1=0 \) and \( a \geq 1 = 1 \).

**Recording of hind limb muscle activity**

Teflon-coated stainless steel hook electrodes were inserted into the gluteus maximus and biceps femoris. Electromyograph (EMG) signals were obtained by passing the analog signals through a high-pass (200-10 kHz) filter and amplified by a Cyberamp 380 amplifier (Axon Inc., USA). Signals were digitized at a 5 kHz sampling rate by a 1202 analog/digital converter card (ICP DAS Inc., Taiwan). Data were processed by a custom-written Borland C++ Builder acquisition program. Summation of rectified EMG was performed in an off-line Matlab data processing program (The MathWorks, Massachusetts, USA).

**Drug administration**

Gallamine triethiodide (10%) (Sigma, USA), morphine (5 mg/kg) and naloxone (0.7 mg/kg) were administered by IV injection via a PE-50 catheter in the femoral vein.

**Capsaicin administration.** Capsaicin (Sigma) was dissolved in an emulsion of 10% Tween 80, 10% ethanol and 80% saline at a concentration of 1%. A small pledget of cotton-wool soaked in capsaicin solution was carefully placed around the sciatic nerve which was isolated in a paraffin pool. The vehicle solution was an emulsion of 10% Tween 80, 10% ethanol and 80% saline. Peripheral stimulation was administered 1 h after capsaicin or vehicle application.

**Tetrodotoxin (TTX) administration.** A plastic chamber containing cotton wool soaked with the TTX solution (100 \( \mu \text{M}, 50 \mu \text{l} \), Sigma) was positioned around the sciatic nerve. The nerve sheath around the nerve was carefully removed at the location of the TTX chamber. Peripheral stimulation was administered 1 h after TTX application.

**Reversible deactivation of lateral and medial thalamic nucleus**

The left lateral and medial thalamic nuclei were localized by recording the thalamic responses to laser pulse (10 W, 30 ms) stimulation of the right hind paw. A hybrid electrode combining a recording tungsten electrode and microinjection micro-tube (20 \( \mu \text{m} \) ID, 90 \( \mu \text{m} \) OD, MicroFil, WPI Inc., USA) was constructed. The micro-tube was filled with 2% lidocaine hydrochloride (Xylocaine, AstraZeneca) and connected via a PE10 catheter to a perfusion pump. This recording-microinjection electrode was inserted with a 30–40° angle of insertion relative to the right cortex and penetrated down to the left thalamic nucleus. The point of insertion was 2.5–3.0 mm posterior and 2.0–2.5 mm lateral (right) to the bregma. Thalamic activities were monitored and examined as the electrode advanced and laser pulse stimulation was applied to the right hind paw. The hybrid electrode was fixed at the location where the lateral or medial thalamic unit responses evoked by laser pulse stimuli were found. To deactivate the thalamic responses, 1 \( \mu \text{l} \) of 2% lidocaine was infused into the thalamus for 2 min. The inhibitory effect of the lidocaine and the recovery of the thalamic responses to the laser pulse stimulation were monitored continuously.

**Verification of electrode and injection sites**

In order to verify the corresponding cortical layers in each recording point, a small lesion was made by passing an anodal current (30 \( \mu \text{A} \) for 5 s) to the 16th (deepest) contact lead at the end of the experiment. Another lesion was made at the same lead after the Michigan probe withdrawal for 1000 \( \mu \text{m} \). In the selective thalamic deactivation experiment, an anodal current (30 \( \mu \text{A} \) for 10 s) was delivered to the recording-microinjection hybrid electrode to make a lesion in the recording site. The hybrid electrode was then withdrawn from the brain and the lidocaine in the micropipette was replaced with 2% Pontamine Sky Blue (Sigma). After re-insertion of the hybrid electrode into the previously recorded thalamic site, 1 \( \mu \text{l} \) of Sky Blue was infused to mark the injection site and for estimation of the approximate diffusion area of the lidocaine.

Rats were fixed by perfusion with normal saline followed by 10% formalin. The brains were cut in 60-\( \mu \text{m} \)-thick coronal sections using a cryostat and the sections were stained with Cresyl Violet. The rat atlas of Paxinos and Watson (1998) was used as reference when detailed cortical layer structures were estimated. The positions of multiple recording points in the cortex were estimated by determining their distance from the two lesions.

**Data analysis**

The peak latencies and amplitudes of sink currents evoked by electrical, mechanical and laser stimuli were determined from CSD data. Sink current changes were measured and compared before and after drug applications. The sink amplitudes between groups were analyzed with Student's t-test. One-way analysis of variance (ANOVA) was used to analyze the drug effects on the evoked sink currents. Two-way ANOVA was used to analyze drug treatment effects on the evoked cortical responses in different groups. Tukey’s post hoc test was used to detect the sources of significant difference between groups.

**RESULTS**

**CSD analysis of evoked responses in S1 following laser stimulation of the right hind paw**

A schematic diagram shows the position of the multichannel recording Michigan probe in S1 (Fig. 1a). Lesion markers (arrowheads) were used to identify the cortical layers in corresponding to recording channels. Averaged field potentials across the six layers in the left S1 in response to laser stimuli are shown in Fig. 1b. Two positive evoked potentials recorded in the superficial channels were similar in amplitude and latency to the “LEP1” and “LEP2” in previous reports (Shaw et al., 1999, 2001; Tsai et al., 2004). The polarity of these potentials was reversed below the layers II and III. The negative evoked potentials appeared through layer IV down to the layer VI. CSD traces calculated from these field potentials showed that the surface positive potentials were represented by strong source currents (upward directed). However the deep negativity potentials broke down into a series of sink (downward-directed and shaded) and source currents that were distributed across the deep cortical layers (Fig. 1c).

Distinct patterns of sink source currents were evoked by laser stimuli (10 W, 30 ms) delivered to the hind paw on contralateral and ipsilateral sides of different rats. Each recording trace was assigned to the correct cortical layer according to histological verification of electrode track. Grand averages of realigned traces across cortical layers in all rats showed a consistent sink source profile (Fig. 2a, \( n=14 \)). For contralateral laser stimuli, two prominent components, I and II, at different latencies were found. In each component, superficial (in layers II/III and IV) and deep (in layers V and VI) sink currents with minimal latencies could
be identified as Ia, IIa and Ib, respectively. In contrast, only the second component was activated following the ipsilateral laser stimuli (Fig. 2a, n=12). The amplitudes of laser evoked components were graded with laser intensity ranging from 10 to 50 ms at a 10 W output. The amplitude of laser evoked component sinks Ia and Ib increased with laser intensity from 10 to 30 ms (10 W) and reached maximal amplitude at 40 ms (Fig. 2b). Component II amplitude evoked by contralateral or ipsilateral stimulation however remained constant in intensity range from 20 to 50 ms (Fig. 2b).

CSD analysis of mechanic and electric evoked responses in S1

Two prominent short latency sinks, sink 1 and sink 2, were evoked in layers IV and VI, respectively, by mechanical or electrical (5× thresholds) stimuli (Fig. 3a and b, n=12). The amplitudes of the sinks and sources were higher by one order of magnitude than that evoked by laser stimuli. Even with high intensity electrical stimulation (>50× thresholds, n=12), the evoked CSD profiles differed from that evoked by laser stimuli and no late component at the approximate latency of the laser-evoked component II could be observed (Fig. 3c). Table 1 summarizes the amplitude and latency values of the major sink currents evoked by electrical, mechanical and laser stimuli.

Depth profile and temporal sequences of evoked intracortical sink currents

Amplitude of major sinks in cortical depth distribution and sequential changes of each sink following stimuli are plotted in Fig. 4a and b. Electrically evoked sink 1 peaked mainly at mid-layer IV. The early laser-evoked superficial...
Ia sink current spanned the entire layer IV and peaked at the border between layers III and IV. The late superficial sink IIa current was evenly spread throughout layer IV and had a broader distribution across the superficial layers than that evoked by electrical stimulation. Significant upward sweeping toward layer II/III was evident in the electrically evoked sink I and the early laser evoked sink Ia, but not sink IIa (Fig. 4b). The deep current evoked by electrical and laser stimuli was mainly distributed from the border of layer V and VI and within layer VI. The electrical stimulus evoked deep sink currents tended to sweep toward layer VI. Similarly, the laser evoked deep sink Ib and IIb currents swept deeper into layer VI.

Conduction velocities

Conduction velocity was calculated by dividing the distance between the two stimulation sites (hind paw heel vs. digits) by the difference of the latencies of evoked sink currents. The conduction velocities of the peripheral afferent fibers mediating the sink currents in two components were measured. The calculated conduction velocities of sink Ia, sink IIa and sink 1 were 9.9 ± 2.94 m/s (n=8), 1.8 ± 0.9 m/s (n=6) and 52.1 ± 10.7 m/s (n=3), respectively.
The influence of leg reflex on laser-evoked-potential
Leg withdrawal was induced by laser stimuli. The mean latency of the leg withdrawal (282.80±9.19 ms, n=5) was calculated from the onset of the evoked EMG responses. This latency is in the same range as the latency of the second component (294.27±10.20 ms) of laser-evoked cortical response. Component II of the laser-evoked cortical sink current remained after the rat was paralyzed. Two-way ANOVA showed that the amplitudes of the major sinks (sink Ia, Ib, Ila and Iib) were not affected by the application of gallamine ($F_{3,24} = 0.792, P=0.510$).

Fig. 3. Superimposed sink/source sweeps and depth profiles of evoked sink source currents induced by mechanical and electrical stimulation. The gray traces represent the averaged evoked responses from an individual animal. The thick black traces represent the grand average results (n=12 for mechanical stimulation, a, n=12 for electrical stimulation, b, 5–10× threshold and c, 50× threshold). Note that the short latency sink currents were evoked in layer IV (sink 1) and layer VI (sink 2). Scales in voltage and time were enlarged in c to illustrate the late response profile evoked by electrical stimulation.
Effects of capsaicin

To assess the contribution of peripheral afferents to the laser-evoked cortical responses, the effect of capsaicin on the withdrawal movement induced by the laser pulse was evaluated first (Fig. 5). Normally there are significant increases of the second and third EMG responses compared with the initially evoked EMG responses. These normal increments in EMG response to the four consecutive laser stimuli (Fig. 5a) were abolished by application of capsaicin (Fig. 5b and 5c) \( F_{3, 35}=0.313, P=0.816 \). Likewise, the evoked consecutive EMG signals differed before and after the treatment of the capsaicin \( F_{3, 72}=13.711, P<0.001 \).

The effects of capsaicin application on the amplitudes of superficial (Ia and IIa) and deep (lb and llb) evoked sink components are shown in Fig. 6. Typical examples of capsaicin and vehicle effects were shown in the upper panel of Fig. 6a and b. Results from five rats were summarized in the lower panel of Fig. 6a. Capsaicin did not significantly alter the amplitudes of sink Ia (from \(-1.88 \pm 1.12 \) to \(-1.05 \pm 0.44 \) mV/mm², \( P=0.52 \)) or sink Ib (from \(-0.65 \pm 0.20 \) to \(-0.19 \pm 0.09 \) mV/mm², \( P=0.18 \)). However, capsaicin reduced the amplitude of sink Ia to 13.37% of the control value (from \(-1.66 \pm 0.25 \) to \(-0.22 \pm 0.26 \) mV/mm², \( P<0.004 \)) and the amplitude of sink Ib to 30.06% of the control value (from \(-1.34 \pm 0.21 \) to \(-0.4 \pm 0.11 \) mV/mm², \( P<0.004 \)). A control experiment in a separate group of rats (n=4) demonstrated that vehicle alone did not affect the evoked components \( F_{3, 24}=1.084, P=0.375 \).

Effects of TTX

Application of TTX on the peripheral nerve gradually diminished the early component of electrical-evoked response until it finally completely vanished by 1 h post-treatment (data not shown, n=3). TTX diminished both the early and, to a lesser extent, the late components of laser-evoked response. Typical examples of the TTX effect on laser-evoked sink currents in superficial and deep layers were shown in Fig. 7a. Results from five rats were summarized in Fig. 7b. TTX reduced the amplitude of sink Ia to 27.92% of the control value (from \(-1.54 \pm 0.21 \) to \(-0.43 \pm 0.07 \) mV/mm², \( P<0.01 \)) and the amplitude of sink Ib to 34.69% of the control value (from \(-0.49 \pm 0.04 \) to \(-0.17 \pm 0.08 \) mV/mm², \( P<0.01 \)). TTX reduced the amplitude of sink IIa to 60.15% of the control value (from \(-1.33 \pm 0.33 \) to \(-0.8 \pm 0.17 \) mV/mm², \( P<0.05 \)) and the amplitude of sink IIb to 38.94% of the control value (from \(-1.13 \pm 0.28 \) to \(-0.44 \pm 0.12 \) mV/mm², \( P<0.01 \)).

Effects of morphine and naltroxone

As shown in a typical example and statistical results in Fig. 8a and b, administration of morphine (5 mg/kg IV; n=5) did not alter the amplitudes of sink Ia and Ib, but did reduce the amplitude of sink IIa to 44.72% of the control value (from \(-1.61 \pm 0.31 \) reduced to \(-0.72 \pm 0.17 \) mV/mm², \( P<0.05 \)), and the amplitude of sink IIb to 56.79% of the control value (from \(-0.81 \pm 0.25 \) reduced to \(-0.46 \pm 0.08 \) mV/mm², \( P<0.05 \)). The depressive effect on sinks IIa and IIb was reversed by naltroxone (0.7 mg/kg, IV).

Effect of lateral and medial thalamic deactivation

To evaluate the relative contribution of the lateral and medial thalamus in relaying nociceptive inputs to the S1, the respective thalamic nuclei were deactivated temporarily by microinjection of lidocaine. The sites of deactivation and typical sweeps of the peri-stimulus histogram of laser-evoked thalamic unit activities recorded in lateral thalamus (VPL nucleus, n=8) and medial thalamic nuclei (n=4) are illustrated in Fig. 9a and d respectively. The electrical stimulus-evoked sinks 1 and 2 were blocked temporarily by VPL thalamic deactivation (from \(12.96 \pm 1.50 \) to \(-2.80 \pm 0.49 \) mV/mm² and from \(5.49 \pm 1.02 \) to \(-0.86 \pm 0.29 \) mV/mm², respectively, \( P<0.001 \)); recovery of the evoked sink currents was evident by 30 min post-injection. There was a differential effect of the VPL deactivation on laser-evoked component I and II sink currents as shown in a typical example in Fig. 9b. Laser-evoked sinks Ia and Ib were suppressed (from \(2.76 \pm 0.37 \) to \(-0.67 \pm 0.15 \) mV/mm², \( P<0.001 \), and from \(0.69 \pm 0.17 \) to \(-0.27 \pm 0.08 \) mV/mm², \( P<0.05 \), respectively); complete recovery of Ia and partial recovery of Ib was observed. The laser-evoked Ia and IIb components were not affected by the VPL deactivation (Fig. 9c). Medial thalamic deactivation clearly diminished the evoked components Ia and IIb (from \(1.55 \pm 0.35 \) to \(-0.62 \pm 0.05 \) mV/mm², \( P<0.05 \), and from \(1.12 \pm 0.43 \) to \(-0.11 \) mV/mm², \( P<0.05 \), respectively) as shown in a typical result in Fig. 9e and statistical results in Fig. 9f; complete recovery of these effects was realized within 30 min. Neither the electrical nor the laser-evoked component I sink currents were affected by medial thalamic deactivation (Fig. 9f).

**DISCUSSION**

CSD analysis of the simultaneously recorded cortical responses revealed that distinct spatial–temporal pattern of
intra-cortical sink source currents in the S1 were evoked by peripheral noxious laser heat stimuli. The sink source currents are essentially caused by excitatory synaptic activations that resulted in ensemble neuronal activity (Mitzdorf, 1985). Intracortical current flows revealed that early and late synaptic activations occurred initially in layers IV and VI, separately, and were relayed transynaptically to both more superficial and deeper layers. The sweeping of sink currents across different cortical depths is a product of a laminar arrangement of the excitatory synapses. The spatial extent of each sink current and its corresponding source current implies that the cortical regions over which the excited neurons extend their process are involved in the successive intra-cortical excitatory networks. The sink source currents underlying somatosensory-evoked potentials by electrical stimulation of the forepaw were recently described (Jellema et al., 2004). Our results showed that although innocuous inputs produced by electrical or me-
channical stimuli in the hind paw also evoke distinct pattern of intracortical sink source currents in the S1, the latency, amplitude and intracortical distributions of the currents were significantly different from that evoked by thermal nociceptive afferents.

Sink source currents of the laser-evoked components I and II

The early component of laser-evoked responses observed in the present study was consistent with the previously...
reported “LEP” findings (Shaw et al., 1999, 2001; Tsai et al., 2004). The late component in the present study is closely related to C-fiber-mediated cortical responses evoked by laser pulses in anesthetized (Kalliomaki et al., 1993a, 1998; Shaw et al., 2001) and awake rats (Shaw et al., 1999). The laser-evoked negativity reversal in layers III/IV and layers V/VI in previous reports revealed indirectly the intracortical current sources that generate a closed field (Isseroff et al., 1982; Kalliomaki et al., 1993a). And although the multiunit activity with short and long latencies evoked by laser pulses in a previous study revealed the excitatory properties of intra-cortical synaptic activation, the laminar profiles of the subthreshold intra-cortical process could not be localized (Shaw et al., 1999).

Two major early sink currents, Ia and Ib, were activated by laser stimuli and they are located in layers IV and V–VI, respectively. The afferent volleys of the thalamo-cortical fibers are reflected by the early part of the Ia and Ib sinks.

Fig. 7. Effects of TTX on laser-evoked cortical sink currents (n=5). (a) Typical example of TTX treatment-induced attenuation of laser-evoked layer IV and layer VI sink currents. (b) The amplitudes of the representative sink currents were calculated from five rats and comparisons were made before and after the treatment of the TTX. There were significant decreases in the sink currents of both components. Data are expressed as means±SEM. * P<0.05 and ** P<0.01.
at the shortest latency induced by laser stimuli (Vanegas et al., 1979; Mitzdorf, 1985). Previous neuroanatomical tracing studies support the view that there is a separate thalamic projection that terminated in layer IV and layer V/VI (Jones 1981; White and Keller, 1989; Lu and Lin, 1993). Thus dual thalamocortical projections may give rise to parallel cortical synaptic activation.

In layer IV, specific thalamic afferents terminate mainly onto dendrites of pyramidal and granular cells (White and Keller, 1989). The depth location and the initiation timing of sink Ia suggest that this sink current is derived from a depolarization of the dendrites of cortical neurons that receive inputs from specific thalamic afferents. Sinks located in layers III and II have successively longer latencies than that of sink Ia. These sinks likely represent the intracortical synaptic current flows sweeping upward from layer IV to layer II. Source in the superficial layers complements these sink currents and contributes to the small but con-
Fig. 9. Effect of lateral and medial thalamic deactivation on the electrical- and laser-evoked cortical sink currents. (a) Schematic diagram of the placement of recording-microinjection hybrid electrode, Michigan probe and the anatomical location of thalamic nuclei. Encircled solid line represents the lidocaine spread area in a typical experiment. The gray area in VPL nucleus represents the overlapped area studied over eight experiments. A sweep of the peri-stimulus histogram of laser-evoked lateral thalamic unit activities is shown in the upper panel. (b) Typical example of intra-VPL lidocaine inhibition of laser-evoked component I response, but not component II response. Representative sink currents were chosen from cortical layer IV and VI respectively. (c) The electrical-evoked responses of sink 1 and sink 2 were reduced significantly after lidocaine deactivation. The laser-evoked responses of component I (sinks Ia and Ib), but not component II (sinks IIa and IIb), were inhibited by VPL inhibition. (d) The lidocaine spread throughout the medial thalamus (n=4). Encircled solid line represents the lidocaine spread area in a typical experiment. The gray area in medial thalamus nucleus represents the overlapped area studied over four experiments. A sweep of peri-stimulus histogram of laser-evoked medial thalamic unit activities is shown in the upper panel. (e) Typical example of intra-medial thalamus lidocaine selective inhibition of component II. Representative sink currents were chosen from cortical layers IV and VI respectively. (f) Overall, the electrical-evoked responses were not affected by lidocaine treatment. The lidocaine treatment inhibited laser-evoked component II (sinks IIa and IIb), but not component I (sinks Ia and Ib), responses. Data are expressed as means±SEM. * P<0.05 and *** P<0.001.
siderable positive potential in surface recording (Bode-Greuel et al., 1987; Jellema et al., 2004). The pairing of sink/source distributions in the supragranular layers suggests that layer III pyramidal cells are involved. The basilar dendrites in layer III are activated trans-synaptically either by thalamic afferents or by the outputs of excited granule cells in the layer IV. These active sink currents can draw passive source current from distal apical dendrites of these pyramidal cells.

Sink Ib, located in the layer V/VI border, has a slightly longer latency than that of sink Ia and it likely reflects the activation by infragranular thalamocortical afferent projections. Sink Ib is complemented by sources in the upper part of layers V and VI. This source/sink/source configuration and the onset latencies of the corresponding currents suggest that layer V pyramidal cells may be involved in the initiation of the sink source activation pattern. Noicceptive-specific neurons in S1 activated by laser pulses or noxious stimuli have been found in layers V and VI (Lamour et al., 1983b; Tsai et al., 2004). The excitatory synaptic actions of specific thalamic afferents at the proximal basilar dendrites of layer V pyramidal neurons reflect the active sink Ib current and simultaneous complementary source currents transmit outward passively at the distal basilar and proximal apical dendrites.

Sinks Ila and Ib of component II appear in layers IV and V following laser stimulation. The sink source activation patterns of component II were similar to that in the early component. Thus the excitatory synaptic events followed a similar intracortical layer-specific pathway. The onset latencies of sink Ila and the sink currents lying above were very similar (Fig. 4b), suggesting that serial action potentials are initiated by C-fibers arriving non-synchronously along axons of stellate cells and axon-collaterals of pyramidal cells that ascend to form cartridge synapses on the apical dendrites of layer II and III pyramidal cells.

Separation of two laser-evoked components

Concern about the coincidence of the initiation of the hind leg withdrawal movement and the late laser-evoked responses has been raised previously (Shaw et al., 2001). In this regard the advantages of the present CSD analysis of evoked field potentials and the technique of conducting the experiment while rats were paralyzed were two-fold. Firstly, the possibility for EMG and far field potential contamination was excluded by verifying the intra-cortical origin of the laser-evoked surface late responses. In addition our methods also enabled the contribution of sensory feedback inputs immediately following the motor withdrawal response to laser stimuli to be ruled out.

The peripheral fiber contributions and the nature of the cortical inputs (serial or parallel) of the early and late cortical responses have not been resolved. The present findings strongly support the view that laser-evoked component I and II sink currents are mediated separately by A-delta and C fibers. If these two components were conducted in same peripheral fiber system, then the time for conducting these two components would be the same. However we found that components I and II had different conduction velocities, which were in the conduction ranges of A-delta and C fibers, respectively. Although it has been estimated that there is a 30–60 ms skin surface to receptor thermal conduction time with laser pulses (Bromm and Treede, 1984; Danneman et al., 1994), the two-point measurement of conduction velocity method excludes the activation time (Shaw et al., 1999).

Furthermore, the two laser-evoked components were separable in their occurrence in S1. Only component II was elicited by the ipsilateral laser stimuli, which indicates that these two components are conveyed by separate peripheral transmission pathways: laser-excited A-delta fibers projecting mostly to the contralateral S1 and laser-excited C fibers projecting to bilateral S1. The present data provide the first clear demonstration of this side-specific property. There are three likely reasons why this effect has not been described previously. Firstly, left and right sides are not easily distinguished with tail stimulation (Shaw et al., 1999, 2001). Secondly, A-delta fibers mediating laser-evoked responses are highly variable and sensitive to anesthesia (Kalliomidou et al., 1993a; Shaw et al., 2001). And finally a high energy level is required to obtain stable signals (Isseroff et al., 1982).

Our observation that component II, but not component I, was significantly affected by capsaicin treatment provides additional evidence in support of the view that laser-evoked component I and II sink currents are mediated by separate fiber systems. Acute block of the C compound action potential but not the A potentials by local application of capsaicin on the peripheral nerve has been reported previously (Wall and Fitzgerald, 1981; Petsche et al., 1983). In our study, evidence of selective suppression of C fibers by capsaicin treatment can be found in the elimination of windup augmenting responses in EMG responses, which were a frequency-dependent increase in the excitability of spinal neurons elicited by the excitation of unmyelinated afferent C-fibers (Mendell and Wall, 1965; You et al., 2003). Likewise, the notably stronger effects of TTX on component I than component II suggest that they are activated separately. Greater TTX blocking of A fibers relative to C-fibers has been seen previously (Schomburg et al., 2000; Akopian et al., 1999). Thus it is likely that component II was conducted by C fibers with TTX resistant sodium channels (Brock et al., 1998; Strassman and Raymond, 1999).

The differential effects of morphine

Our CSD analysis of the differential effects of systemic morphine on the late activated synaptic currents confirmed the preferential effect of morphine on the long latency S1 nociceptive neurons (Matsumoto et al., 1987; Tsai et al., 2004). The analgesic effect of morphine is thought to be due to both spinal and supraspinal mechanisms. Morphine has been shown to preferentially affect C-fiber-evoked neural responses in the spinal cord (Carstens et al., 1979; Jurna and Heinz, 1979; Johnson and Duggan, 1981), medulla (Bing et al., 1989) and thalamus (Abdulla and Aneja, 1993). In the present study we found that the cortical nociceptive responses were elicited first by activation of
the peripheral nociceptors and subsequently by complex processes in subcortical structures. The suppression of intracortical evoked synaptic responses observed in the present study was likely the result of a collective action of morphine on multiple levels of the ascending nociceptive pathway (Kalliomaki et al., 1998).

Thalamic transmission relay

Electrophysiological studies have demonstrated that neurons in the medial dorsal and central lateral nuclei in the medial thalamus and in the ventrobasal nucleus respond to nociceptive stimuli (Guilbaud et al., 1986; Dostrovsky and Guilbaud, 1990; Shyu et al., 1992; Berkley et al., 1995). Anatomical tracing data support the notion that ventrobasal and medial thalamic nuclei are the two major thalamocortical pathways that relay nociceptive inputs to S1 (Gingold et al., 1991). Our results suggest that thermal nociceptive responses in S1 were conveyed by A-delta and C fiber nociceptive afferents that are transmitted primarily via lateral and medial thalamic nuclei respectively. In contrast to the proposal that there are dual spinothalamocortical projections from cutaneous C fibers to rostral S1 and that these two, medial and lateral, thalamic inputs project to superficial and deep cortical layers separately (Kalliomaki et al., 1993a), we found that the A-delta and C fibers mediating cortical activations were conveyed by different thalamocortical projections. The selective thalamic deactivation experiments showed that the A-delta fiber-mediated early component (I) was conducted by the same pathway as the innocuous inputs via the VPL thalamic nucleus, whereas the C fiber-mediated component (II) was conducted by the medial thalamic nuclei. Our findings confirmed that the VPL is composed of segregated nociceptive and non-nociceptive networks (Apkarian et al., 2000). Anatomical evidence of corticopetal projections of the spinothalamic terminals conveying noxious and thermal cutaneous signals is consistent with the view that A-delta fiber-mediated cortical responses may be conveyed via nociceptive inputs from the ventrobasal thalamic nucleus (Gingold et al., 1991). Medial thalamic inputs to the cortex have been reported previously and it was found that there are deep and superficial sink currents in cortex evoked by electrical stimulation of the intralaminar nucleus via distinct projections to areas 5 and 7 (Rydahg et al., 1986; Shyu et al., 1989; Olausson et al., 1989). An intracellular study suggested that there is a monosynaptic projection from the CL to the deep layers of area 5 (Olausson et al., 1990). Although direct evidence of intralaminar thalamic projections to cortex conveying nociceptive C-fiber information is lacking, results from a double labeling experiment suggested that distinct spinothalamic pathways that terminate within the CL nucleus are capable of relaying nociceptive information to the S1 (Gingold et al., 1991). Other studies examining S1 cortical inputs from the medial thalamus found that afferents from intralaminar thalamic nuclei terminated in cortical layers 5 and 6 (Herkenham, 1980; Berendse and Groenewegen, 1991). The presence of two distinct ascending thalamocortical pathways suggests that thermal nociceptive information to S1 may be transmitted by separate parallel systems that mediate different nociceptive functions.

Functional implications

The present findings are consistent with the view that S1 may have an essential role in the sensory-discriminative aspects of pain. First, results from our CSD analysis indicated that the A-delta-mediated early intra-cortical synaptic currents, Ia and Ib, are intensity-dependent. Second, the evoked synaptic currents were contralaterally biased and conveyed via the lateral thalamus. Previous electrophysiological studies in animals have demonstrated that the nociceptive S1 neurons have restricted receptive fields (Kenshalo and Isensee, 1983; Lamour et al., 1983a; Kenshalo et al., 1988; Chudler et al., 1990), and encode stimulus intensity and location (Kenshalo and Isensee, 1983; Lamour et al., 1983a). Our findings were also consistent with the findings of a human study that used a noxious laser stimulus and found significant contralateral preference in S1 (Bingel et al., 2003). Lateral thalamus appears to be functionally linked with S1 in the processing of nociceptive signals as nociceptive inputs projected to the VPL were very likely to be relayed to S1 (Gingold et al., 1991). The laser-evoked early component may be involved in coding the short-term temporal element of pain which is characterized by a short duration, with a sharp and precise location and may be related closely with the sensation of “first pain” (Ploner et al., 2002).

In contrast to the A-delta-mediated early component, our CSD analysis showed that the C-fiber-mediated intracortical synaptic currents were less intensity-dependent, activated bilaterally during laser stimulation and that these currents received inputs from medial thalamic nuclei. The ascending spinal pathways of the C-fiber transmission appear to involve bilateral pathways in lateral funiculi and dorsal funiculi (Kalliomaki et al., 1993b). The downward sweeps of synaptic currents in the deep layers indicate that the cortical signals may be relayed to the contralateral side via commissural connections. Both of the spinal and cortical pathways make it possible for S1 to integrate information of the nociceptive C fiber inputs from both contralateral and ipsilateral hind-paws. A recent human study using laser stimuli to induce first and second pain sensation showed that second pain was closely related to anterior cingulate cortex activation (Ploner et al., 2002) and psychophysical and behavioral studies found that that morphine preferentially attenuates input from unmyelinated nociceptors and reduces second pain (Cooper et al., 1986; Yeomans et al., 1996). Thus the bilateral excitation and the more diffused cortical and subcortical activation of the nociceptive C fiber inputs may subserve the role of forwarding the nociceptive signals to other areas such as insula and anterior cingulated cortex for further nociceptive information processing. The nociceptive C fiber-evoked responses in S1 may be the basis for the experience of a radiating burning sensation and may underlie indirectly the sensation of second pain.
References


Lu S-M, Lin R-S (1993) Thalamic afferents of the rat barrel cortex: a light and electron-microscopic study using Phaseolus vulgaris leu-