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

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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 CO_2 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.

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

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Abbreviations: ANOVA, analysis of variance; CSD, current source density; EMG, electromyograph; S1, primary somatosensory cortex; TTX, tetrodotoxin; VPL, ventral posterior lateral nucleus.

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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_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_2) in an acrylic box. A PE-240 tube was inserted via tracheotomy and EMLA® Cream (lidocaine 2.5% and prilocaine 2.5% cream, AstraZeneca, Södertälje, Sweden) was smeared over the wound. Rats were placed in a stereotaxic apparatus and maintained with 2% halothane in 70%/30% nitrous oxide/oxygen during surgery. Body temperature was maintained at ~36.5 °C via a homeothermic blanket system (Model 50–7079, Harvard Apparatus, USA). After craniotomy was performed over the hind limb projection area in S1 (centered at 1 mm posterior and 3 mm lateral to the bregma), a small part of the dura over S1 was carefully removed and warm paraffin was applied to keep the cortical surface moist. Heart rate was monitored by electrocardiography. In some experiments, a femoral venous catheter (PE-50 tube) was prepared for drug infusion. To prevent halothane-induced suppression of responses, animals were anesthetized with 0.75–1.5% halothane in a mixture of nitrous oxide and oxygen. Some animals were also paralyzed with 10% gallamine triethiodide (50 mg/kg, Sigma, IV). Depth of anesthesia was checked and maintained periodically by pinching the tail so that no overt body reflexive movement or, in case of muscle paralysis, acceleration of the heart rates was observed.

Laser stimulation

An infrared laser pulse of adjustable intensity (20 W maximum) was generated from a surgical CO_2 laser (Model 20 CH, Direct Energy Inc., CA, USA). The laser beam was projected to the paw from a distance of about 10 mm. The CO_2 laser beam was aligned with a visible helium laser beam, and the laser beam projection was guided from the helium laser and produced a red light spot. The CO_2 laser beam produced a round spot on the paw surface that covered an area of about 12.5 mm^2. Skin of the hind digits, paw and heel was stimulated in turn with a train of four consecutive 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 excursion 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, Φ and was calculated with the finite difference formula

$$I_m = -\left(1/kh^2\right) \sum_{m=-n}^{n} \alpha_m \phi\left(x+mh\right)$$