Cortical circuitry of thermal nociception in the primary somatosensory cortex of rats

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Introduction
The role of the cerebral cortex in perceiving the thermal pain sensation has been well studied. However, the cortical circuitry that mediated the thermonociceptive information processing is not very clear. The Laser-evoked responses in different cortical layers could be recorded simultaneously in the primary somatosensory cortex (SI) using Michigan probe and laminar-specific transmembrane currents could be analyzed using current source density method.

The aim of present study is to investigate the intracortical synaptic currents evoked by laser pulses and to construct a putative intracortical circuitry that may mediate the thermal nociceptive signal transmission.

Materials and Methods

Preparation of Animals
The male Sprague Dawley rats (body weight 300–400 g) were initially anesthetized with 4% halothane (in 100% O2). After tracheal cannulation, the animals were anesthetized with 0.75–1.2% halothane.

Recording of Evoked field Potentials in SI
A Michigan probe with 16 contact points (150 μm interval spacing) was used to record extracellular field potentials in SI (P, L, I, L) and a reference electrode with an Ag-AgCl was placed in nasal cavity (P, L, I, L) and an Ag-AgCl ground electrode was placed in the connective tissues of the scalp. The sampling rate of recording was 20 kHz in a data acquisition system (TIDC Inc., USA) based on PC system. Field potential was determined by five-point formula of current source density (CSD) analysis method with Matlab program.

Electrical stimulation
Custom-made double wire stainless steel wires were used to deliver 20-40μA biphasic electrical stimulation (0.3-10V, 1μs duration, 0.2Hz) to hind foot by an isolated pulse stimulator (Model 210, A-M System Inc.). The anodal electrode was placed 1 cm distal to the cathodal electrode.

Laser stimulation
Each hind digit skin site was stimulated with four pulses, at a frequency of 0.9 Hz (10W, 30ms duration). An averaged recording based on 40 stimulations were obtained and no visible damage to the skin was observed. A series of 4-8 laser pulses was applied before the same skin site was stimulated again. The laser stimulation protocol was the same as Khalifam et al., 1998, and the "wind-up" phenomenon was produced by this stimulation protocol.

CSD Method
With regard to the time span and the sampling variations in each recording session, we adopted a five-point formula (Mitzdorf 1985;Freeman and Nicholson 1975) to smooth the spatial sampling variability. The CSD was derived from the second spatial derivations of the extracellular field potentials, and were calculated with the finite difference formula:

\[ \frac{d}{dx} = \frac{1}{h} \left( \frac{E(x + h) - E(x)}{2} - \frac{E(x - h) - E(x)}{2} \right) \]

Where h is the distance between successive measuring points (150 μm in the present investigation) and x is the coordinate perpendicular to the cortical layer. The remaining constants are as follows: m, 2, k1, k2, a1, a2, a3 and a4.

Results

Fig. 3. The CSD of evoked responses following electrical and laser stimulation in SI.

A. CSD profiles in SI following electrical and laser stimulation

Electrical evoked responses

Laser component 1

Laser component 2

A. The grand average of EMG and major sink traces of laser evoked response (n=5). B. After injection of 10% capsaicin triethiodide (50 μg, i.p.), a muscle relaxant, the movement effect disappeared, however the laser evoked responses still appeared. C. The superimposed figure from A and B. D. The result shows that the laser evoked responses still exist after injection of capsaicin triethiodide and don't have significant difference compared with control.

Fig. 4. The effects of muscle relaxant on laser evoked responses in SI.

A. The average of EMG and major sink traces of laser evoked response (n=5). B. After injection of 10% capsaicin triethiodide (50 μg, i.p.), a muscle relaxant, the movement effect disappeared, however the laser evoked responses still appeared. C. The superimposed figure from A and B. D. The result shows that the laser evoked responses still exist after injection of capsaicin triethiodide and don’t have significant difference compared with control.

Discussion
With the 2 × 1 Michigan probe, our results is the first to report the detailed temporal-spatial activation profiles in SI responding to laser stimulation. The laminar distribution of sink and source current of these two components were similar and were mainly located in layer IV and V. Sink current initially activated in the layer IV propagated to superficial and deep layer, whereas the sink current in layer V spread to layer VI. Inhibitory interneurons may exist in superficial layers which were coincided with the strong source current in layer IV and V.

Table 1. Latency and amplitude of sinks and sources of electrical and laser evoked responses.

<table>
<thead>
<tr>
<th>Component</th>
<th>Amplitude (μV)</th>
<th>Latency (ms)</th>
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<tbody>
<tr>
<td>Laser component 1</td>
<td>10.1 ± 1.4</td>
<td>0.0 ± 0.1</td>
</tr>
<tr>
<td>Laser component 2</td>
<td>8.3 ± 0.9</td>
<td>0.0 ± 0.1</td>
</tr>
</tbody>
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Conduction velocity is tested by stimulating two nearby sites on hind paw and the speed is calculated by difference in latency and distance. According to the conduction velocity, electrical stimulus might evoke the A-delta fiber (50 ± 1.5 m/sec) and laser and nerve responses are mediated by A-delta and C-fiber, respectively. The activation profiles evoked by A-delta and C-fiber are similar to that by A-beta.

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Acknowledgement
The microcircuit in SI following laser stimulation was constructed according to the data of CSD and histology.

A plastic chamber within cotton wool soaked in the 50 μl (100 μM) TTX solution was positioned around the sciatic nerve. After one hour, laser stimulation was performed. Our data showed that the early component was abolished completely after administration of TTX (15 μM) and late component was only partially diminished.

Fig. 7. The effects of TTX on laser evoked responses in SI.