The Current Source Density Analysis of the Paired-Pulse Facilitation in the Anterior Cingulate Cortex in vivo

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Introduction
- Recent functional neuroimaging, MRI and PET studies of nociceptive responses in humans have consistently shown that the anterior cingulate cortex (ACC) is activated during the application of painful electrical nerve stimulation.
- The ACC is recognized as being involved in a wide range of functions including emotional and cognitive regulation of somatosensory and autonomic activity. The ACC has been subdivided into a number of cytoarchitectonic areas including Fr2 (motor cortex, area 2), Cg1 (cingulate cortex area 1), Cg2 (cingulate cortex area 2), Cg3 (cingulate cortex area 3), and IL (lateral prefrontal cortex).
- The medial thalamus (MT) plays a major role in the mediodorsal thalamic pain system which involves in the effective responses of pain perception, and it is assumed that the nociceptive responses of the ACC are probably mediated by specific thalamo-cortical inputs.
- Paired-pulse facilitation (PPF) is an example of frequency-dependent short-term plasticity of synapses, and it is an ideal preparation for investigating synaptic changes and its underlying mechanisms in vivo.

Materials and Methods
- Preparation of Animals
  - Sprague-Dawley rats (body weight 250-350 g) were initially anesthetized with 4% halothane (in 100% O2). After tracheal cannulation, the rats were paralyzed with gallamine triethiodide and artificially ventilated.
- Electrical Stimulation in the Medial Thalamus
  - Mono-polar tungsten electrode was placed in the MT (AP, 2.5 mm; ML, 1.0 mm; depth 5 mm). Electrical paired-pulse stimulation (0.1 ms, 100-300 μA; inter-pulse interval, 100 ms) were delivered to the MT.
- Recording of Evoked field Potentials in the ACC
  - 16-channel electrode (150 micrometer interval) was used to record the evoked field potentials in the ACC (AP, 2.5 mm; ML, 2.0 mm; angle 45 degree and depth, 0-4 mm).

Results

Fig.1. Michigan electrode.
- Current Source Density method (CSD)
  - Field potentials were collected along the track vertical with an oblique angle of 45° to the ACC layers with an interval of 150 μm down to 4000 μm. We adopted a five-point formula to smooth the spatial sampling variability. The kernel was derived from the second spatial derivatives of the extracellular field potential, $\hat{u} \cdot$, and was calculated with a finite difference formula:
    $$h_{m} = \{2 - 2(\hat{u} \cdot \delta(0)) + 3(\hat{u} \cdot \delta(1)) + 2(\hat{u} \cdot \delta(2))\}$$
  - Where $h$ is the distance between successive measuring points and $s$ is the coordinate perpendicular to the cortical layer. The remaining constants are as follows: $k = 100$, $a_{n-10}$, $a_{n-8}$, $a_{n-7}$, $a_{n-6}$ and $a_{n-4}$.

Fig.2. Experimental set up and histological verification

A. Electrophysiologically set up: the measurement of MT-evoked ACC extracellular field potentials. B. anatomical location of the recording (Cg1) and stimulation site (mediodorsal thalamus).

Fig.3. PPF and depth potential profiles
- A. The PPF of the MT-evoked ACC field potentials. The inter-pulse interval is 100 ms. B. Depth potential profiles at 0.19 ms (comp 1) and 14.75 ms (comp 2). The ratio at maximal response (arrow) was 1.54.

Fig.4. The CSD profiles of the PPF
- A and B. The localization of sources and sinks in ACC using CSD analysis method. The CSD analysis showed that the sink 1 and sink 2 located in layer 2/3 and layer 5, and the sources located in layer 2/3. C and D, the color maps were transferred from A and B clearly showing PPF, sinks and sources.

Fig.5. Response ratio and IPI test of PPF
- A. Intensity responses of ratio of the sink 1, sink 2 and source 1. The intensity test shows that the stronger intensity the higher ratio of comp 2 vs. comp 1 in sink 1, 2 and source 1. B. The IPI test shows that higher ratio was obtained in sink 1 and source 1 at 100 ms but not sink 2.

Discussion
- The shortest sink current was activated at a latency of 7.5 ms in layer 2/3 of the ACC. The layer 2/3 sink current evoked by the second pulse from the MT significantly facilitated and the amplitude ratio of the 2nd and 1st sink current was 2.0. The maximal potentiation occurred at the pulse interval of 100 ms. The CSD analysis in PPF and high frequency stimulus (100 Hz) showed the similar pattern in channels especially in sink 1. Morphine and halothane decrease the sink 1 and source 1 and effects of morphine was reserved by naloxone.
- These results suggest that a basic neuronal circuit in the ACC consisting of sequential activation of layer 2/3 and layer 5/8 pyramidal neurons causes the extracellular field potential evoked by MT stimulation. The current source density analysis reveals the synaptic facilitation in the layer 2/3 of the ACC by the paired-pulse stimulation of the MT. The short-term plasticity may provide a neuronal mechanism for the conditioned learning and working memory in the ACC.

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