Evoked Responses of the Anterior Cingulate Cortex to Stimulation of the Medial Thalamus

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Abstract

In the present study we characterized the field potentials in the anterior cingulate cortex (ACC) evoked by electrical stimulation of the medial thalamus (MT), and elucidated the synaptic organization of the ACC. Male Sprague Dawley rats were maintained in general anesthesia by α-chloralose (50mg/kg, i.v.). Tungsten micro-electrodes were used for electric stimulation and recordings. The field potentials and multiple unit activities in the ACC were evoked by electric stimulation of the MT where the nociceptive responses were identified. A MT-evoked positive-negative potential was recorded on the medial frontal surface. The polarity of the surface negative potential was reversed between 0.5 to 1.0 mm in the deep layer of the ACC. Maximum evoked negative potential appeared at about 4 mm anterior to the bregma and 1 mm lateral to the midline. The maximum evoked positive potential occurred at about 3 mm anterior to the bregma and 1 mm lateral to the midline. The evoked multiple unit activities coincided with the deep negative field potential at a latency between 16 ms and 24 ms at a depth between 0.5 mm and 1.5 mm in the ACC. These electrophysiological findings confirmed that nociceptive information in the MT is transmitted to the ACC and trans-synaptically activates deeper and more superficial layers of cortical neurons.

Key Words: evoked potentials, limbic cortex, intralaminar nuclei, synaptic activation, pain, nociception, rats

Introduction

The anterior cingulate cortex (ACC) lies ventral, rostral and dorsal to the corpus callosum, and receives diverse thalamic afferent inputs (5). The ACC is recognized as being involved in a wide range of functions including emotion (30, 36), motivation, attention (22, 37), learning and memory (29, 32), and regulation of skeletomotor and autonomic activity (18, 27, 31).

Several lines of evidence suggest that the ACC is also involved in responses to noxious stimuli both in human and animals. Recent functional neuroimaging studies of nociceptive responses in human have consistently shown that the ACC is activated during the application of acute, noxious stimuli to the body surface (21, 11, 26, 43) and during the application of painful electrical nerve stimulation (14). In awake patients, neurons in the ACC region respond to nociceptive stimuli (25). The neurosurgical operation cingulotomy, which disrupts tissues in or near area 24, can alleviate a patient's affective response to noxious stimuli, including those producing chronic, intractable pain (3). There is also evidence of altered pain and temperature discrimination in patients following cingulotomy (13), and a consistent finding in cingulectomized animals is a disruption of avoidance learning, i.e., learning that involved avoidance of noxious footshock (7, 19). Other studies involving experimental animals also support a functional role of the ACC in nociceptive responses.

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Electrical stimulation of the ACC in animal evokes a shrill vocalization (38), which might be associated with escape responses. Lidocaine reduced the pain reaction following formalin injections into rat's forepaw (44), and lesions in the ACC selectively attenuate hot plate responses (34). There is also a direct evidence that neurons in the rabbit cingulate cortex respond to noxious stimuli (42). These studies indicate that the cingulate cortex mediates behavioral responses to noxious stimuli..

Since rodents are widely used as experimental animals, their ACC organization and connections are well established, which may be relevant to interpreting human findings. The ACC of rats has been subdivided into a number of cytoarchitectonic areas including Fr2, Cg1, Cg2, Cg3, and IL (51, 52, 53). Each area can be differentiated according to its connectivity pattern. The main subcortical afferent inputs of the ACC are from the superior colliculus, nucleus of the solitary tract, parabrachial nucleus (50), mediodorsal thalamic nucleus (MD) (2, 21, 47, 48), anteromedial thalamic nucleus (50), midline and intralaminar thalamic nuclei (medial thalamus, MT) (4, 15, 41), and the basal ganglia (21, 50). A branch of the spinothalamic tract terminates in the thalamic midline and intralaminar nuclei (10, 20, 49) and the signal is relayed from there to the ACC (46). The MT plays a major role in the medial thalamic pain system (1), which is involved in the affective responses of pain perception. The nociceptive responses and affective responses of the ACC to noxious stimuli are similar to those in the medial and intralaminar nuclei (7, 19, 38, 44). Furthermore, injection of lidocaine into the medial thalamus of rats virtually abolishes the nociceptive-evoked activity of units in the ACC (42). Based on these electrophysiological and neuroanatomical data, it is assumed that the nociceptive responses of the ACC are probably mediated by specific midline and intralaminar thalamic nuclei.

The synaptic organization underlying nociceptive information processing in the anterior cingulate cortex in the rat has not been systemically studied. In a previous study, we used single unit recording technique to demonstrate the functional and synaptic connection of the MT and the ACC (24). In the present study, we employed extra-cellular field potential recording method to analyze the MT-evoked responses in the ACC to elucidate the cortical synaptic organization in terms of the laminar arrangements of afferent inputs.

Materials and Methods

Preparation of Animals

Twenty five male Sprague Dawley rats (body weight 250 to 340 g) were used. Rats were initially anesthetized with a mixture of ketamie and Rompun (ketamine, 35 mg/Kg and Rompun, 5 mg/Kg, i. p.). After tracheal and jugular vein catheterization, animals were anesthetized with α-chloralose (50 mg/kg, i. v.) and then mounted on a stereotaxic apparatus. Body temperature was maintained at 37°C via a homeothermo blanket system. The animals were paralyzed with gallamine triethiodide (Flaxedil, 40 mg/Kg, i. v.) and then artificially ventilated. Heart rate, respiratory rate and pCO_2 were monitored throughout the recording session. The end tidal CO_2 content was maintained at about 3 ~ 4%. Rats were allowed to periodically recover from the muscle paralysis to assess the arousal level of anesthesia by evaluating the corneal reflex or withdrawal reflex of the hind limb. Supplementary dose of α-chloralose (30 mg/kg, i.v.) was given if reflexes were present. A craniotomy was performed for ACC recording and MT stimulation. After slitting the dura, the cortex was immersed in a warmed-mineral oil pool.

Isolation of the Nociceptive Neurons in the MT

Tungsten electrodes were placed in the stereotaxic coordinates of the central lateral (CL) or parafasicular nucleus (Pf) (AP, -2.5 ~ -4 mm, ML, 0.5 ~ 1.5 mm, depth 5 ~ 7 mm) of the MT. The electrode was first connected to the amplifier. The Ag-AgCl ground electrode was placed in the connective tissues of the scalp. By varying the depth of the recording electrode, the nociceptive neuronal properties of the MT could be located by searching the evoked nociceptive responses to peripheral noxious stimulation of the body and extremities.

Noxious Stimuli

The noxious stimuli consisted of needling, mechanical pinching, squeezing and high intensity electric stimulation. To characterize the latency responses of MT nociceptive neurons, units were evoked by peripheral electrical stimulation (10 mA, 1 ms pulse duration, 333 Hz frequency, 10 ms train duration, needle electrodes inserted into the hind paw).

Electrical Stimuli in the Medial Thalamus

Once the nociceptive MT neurons were located, the electrode position was fixed and the connection of the electrode was switched to the stimulator. Constant current electrical stimuli were delivered to the MT using an isolated pulse stimulator (Model 2100, AM System Inc.). Electrical stimulation to evoke the neuronal activities in the ACC consisted of a single

pulse with pulse duration of 0.2 ms, or train at a frequency of 333 Hz and duration of 10 ms. An intensity of two to three times the threshold current was used in most of the experiments. The current intensity was limited to less than 500 μA.

Recording of Evoked Field Potentials and Multiple Units of ACC

Tungsten microelectrodes with a tip of 5-10 μm diameter and an impedance of 1-8 Mohm were used to record the evoked field potentials and extra-cellular units in the ACC. To localize the region of evoked activity and to analyze the components of evoked responses of the ACC, the evoked field potentials were systemically mapped by moving the recording electrode on the surface of the frontal cortex (AP, 0~5 mm; ML, 0~5 mm) during stimulation of the MT. The evoked depth field potentials and multiple unit activities were recorded by penetration of the electrode at a constant interval of depth in the medial frontal cortex in a systemic way. (AP, 0~5 mm; ML, 0~1.5 mm, depth, 0~5 mm).

Recording Devices, Data Acquisition and Analysis

The extracellular units and field potentials were amplified by a high input impedance amplifier (Axoclamp 2A, Axon Instrument Inc.). All analog signals were displayed either on an analog or digital storage oscilloscope (Model 450, Gould). The same analog signals were also sent to a PC-based data acquisition system for on-line A/D conversion (Sampling rate, 10k, Metrabyte DAS-16F AD/DA interface card) and digital analysis (with MicroSoft BASIC Language). The recorded spikes were converted to TTL pulses by a window discriminator (Model # 74-60-1, FHC) and then fed into the same data acquisition system via a counter/timer interface card (Metrabyte CTM-05) for on-line digital processing, as well as for constructing real-time frequency or peri-stimulus histograms. All signal types were stored in a hard disk for off-line processing in a PC-based data analysis system. Ten sweeps of evoked potentials were averaged digitally. The multiple unit potentials were fed to a low-pass filter and rectified and the area of the rectified potential was integrated. The maximum value of the integrated area in one series of recordings was taken as 100%. The remaining values were calculated as the percentage of this maximum value.

Verification of Electrodes Sites

A small lesion (50 μA for 30s, anodal DC current) was made by passing electric currents at the location of the tip of the recording and stimulation electrode. Rats were fixed by perfusion with saline followed by 4% paraformaldehyde (in 0.1M sodium phosphate-buffered, pH 7.4). The brains were cut using a freezing microtome at 50 μm thickness and the sections were stained with Cresyl violet (Sigma). Drawings were made of sections with clear electrode tracks and lesion markers that showed the structure of the ACC and MT, for reconstruction of the recording and stimulating sites. The rat atlas of Paxinos & Waston (1986) (35) was used as reference when detailed histological structures were examined. The position of some units were estimated by determining their distance from a marked unit location as measured by the microdrive.

Results

Location of Stimulation Sites in the MT

The field potentials were analyzed in 13 rats. After verification of the histological records of stimulating sites, cases in which the sites were not located in the MT were excluded. The distribution of MT stimulation sites included the CL, between the CL and paracentral (PC), the central medial (CM), the MD and Pf. (Fig.1A)

Characteristics of Nociceptive MT Units

The nociceptive MT units responded to peripheral innocuous stimuli and noxious stimulation applied to the ipsilateral and contralateral hind paw and lower back (Fig. 1B). The receptive field of MT nociceptive neurons covered a large body surface bilaterally. These units also responded to high intensity electrical stimulation in the receptive field (Fig.1C). The mean latency response of the MT was 49.5 ± 0.8 ms (mean ± SEM, n=56) and the amplitude was 0.18 ± 0.01 mV (mean ± SEM, n=56).

Evoked Field Potentials of ACC

The MT-evoked ACC surface field potential was investigated on the frontal cortical surface ranging from 0.5 to 5.0 mm anterior to the bregma and 0.5 - 4.0 mm lateral to the midline. A typical evoked-potential consisted of an initial positive potential and a negative potential. The mean latency and amplitude of these potentials are shown in Table 1. The latency for the negative components of the evoking ACC field potentials was shortened when the electric intensity in the MT was increased, and the potential's amplitudes reached plateau when eight times the threshold current was applied. Train pulses were more efficient than single pulses in evoking this component of the field

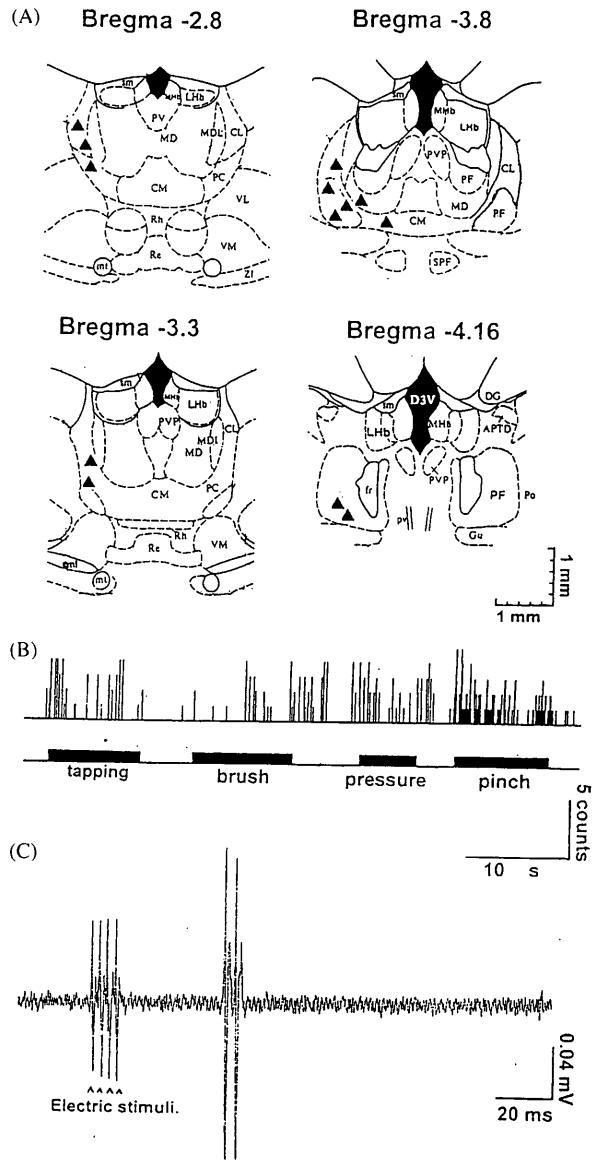


Fig. 1. (A) The distribution of stimulating sites in the MT of 13 rats for field potentials study. The nociceptive responses of these sites in the medial thalamus have been verified. Solid triangles indicate the stimulating sites after histological verification in the medial thalamus. Diagrams are taken from a stereotaxic atlas (35). Abbreviations: PV, paraventricular nucleus; MD, mediodorsal nucleus; CM, central medial nucleus; VM, ventromedial nucleus; Re, reuniens nucleus; PC, paracentral nucleus; VL, ventrolateral nucleus; Rh, rhomboid nucleus; Pf, parafasicular nucleus; MHb, medial habenular nucleus; LHb, lateral habenular nucleus; Sm, stria medullaris nucleus; Po, posterior nucleus group; D3V, 3rd ventricle. (B) An example of activities of medial thalamic nociceptive units evoked by peripheral stimulation. The frequency histrogram shows the evoked activities of nociceptive thalamic units responding to peripheral innocuous (tapping, brush, and pressure) and noxious (pinch) stimulation. Vertical bar indicates the calibrated counts of the total number of action potentials in a bin width of 1 s. The horizontal bar indicates the time scale. (C) The nociceptive thalamic units responded to high intensity (10 mA, train pulses with 1ms duration) electrical stimulation applied to the hind paw.

potential, as they required less intensity to produce maximal amplitude of negative field potentials than did the single pulse.

Two Dimensional Mapping and Isopotential Profile

The MT-evoked ACC potential was surveyed in the parasagittal plane, 1 to 5 mm anterior to the bregma and 0.5 mm lateral to the midline. A two dimensional potential profile was mapped and is shown in Figure 2. Train pulses were used to stimulate the MT. Negative potentials were recorded throughout the surface of the medial frontal cortex. The negative potentials were reversed to positive potentials at about 0.5 mm to 1 mm below the surface. The positive potentials became dominant deep in the medial frontal cortex. To further illustrate the distribution of the prominent surface negative potentials and deep positive potentials in the frontal cortex, evoked-field potentials were systemically mapped with 0.5 mm spatial sampling interval in both the sagittal and transverse planes and isopotential contour profiles were constructed. A series of isopotential profiles of the sagittal plane are shown in Figure 3A, each separated by 0.5 mm. Another series of isopotential profiles of the transverse plane are shown in Figure 3B, each separated by 1 mm. The time point of 21.1 ms, at the maximum negative potential of this series of recordings, was chosen. The spatial distribution of the iso-positive and -negative potential was plotted as equi-potential curves. The solid lines represent the positive potentials and the dashed lines indicate the negative potentials. The zero line is the de-marcation of the reversal of negative-positive potentials. Maximum negativity appeared at about 4 mm anterior to the bregma and 1 mm lateral to the midline. Maximum positivity occurred at about 3 mm anterior to the bregma and 1 mm lateral to the midline.

Multiple Unit Activities

MT-evoked multiple unit activities were recorded simultaneously with evoked field potentials and were investigated in 5 rats. Unit activities corresponded approximately in time to the negative field potentials in the deep layer (Fig. 4A). The evoked multiple unit activities occurred at a latency between 16 ms and 24 ms of negative potentials at a depth between 0.5 to 1.5 mm in the ACC. The amount of each evoked multiple unit activity was calculated and represented as the magnitude of the integrated area of the rectified multi-unit potentials. In Figure 4B, the distribution of the multiple unit activities is presented in the parasagittal plane with dots whose size are proportional to the percentage of the maximum magnitude.

Table 1. The Latency and Amplitude of MT-Evoked Surface ACC Potentials

	Latency (ms)		Amplitude (mV)	
	Mean±SEM	Range	Mean±SEM	Range
Positive-potential (n=13)	6.3±1.16	3.1 ~ 8.7	0.2±0.09	0.07 ~ 0.37
Negative-potential (n=13)	18.7±2.92	11.2 ~ 26.7	2.3±0.47	0.73 ~ 3.9

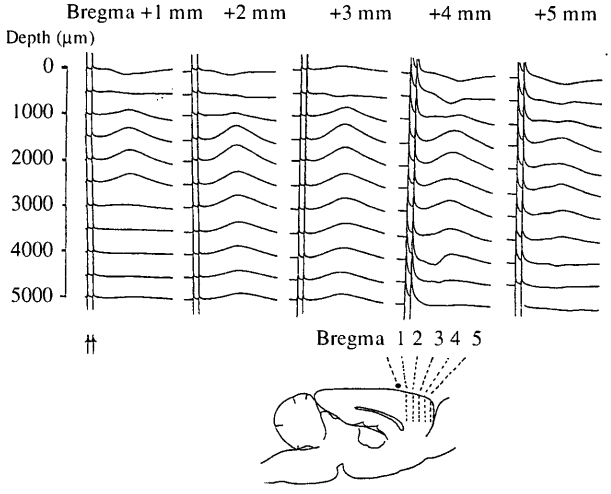


Fig. 2. The profile of depth recordings of evoked field potentials of the ACC with electrical stimulation of the MT. On the cortex surface, an evoked negative potential of 24 ms latency was recorded. The polarity of the negative potential was reversed between 0.5 to 1.0 mm below the cortex surface.

Depth Potential Analysis

A detailed profile of depth recording of evoked field potentials is shown in Figure 5. An early positive potential and negative potential was recorded on the surface 3.5 mm anterior to the bregma and 0.5 mm lateral to the midline. Serial recordings were carried out in the direction perpendicular to the surface. Each recording was separated by a distance of 150 μm. Two vertical dashed lines mark the peak latencies of the positive and negative potentials respectively. The early surface positive potential was reversed at about 750 μm, as indicated by the arrow. The surface negative potential was reversed at a more superficial layer of about 450 μm. A short latency negative potential at a depth of 1350 μm occurred at about 3 ms. This potential was not usually recorded and could not be observed at the surface.

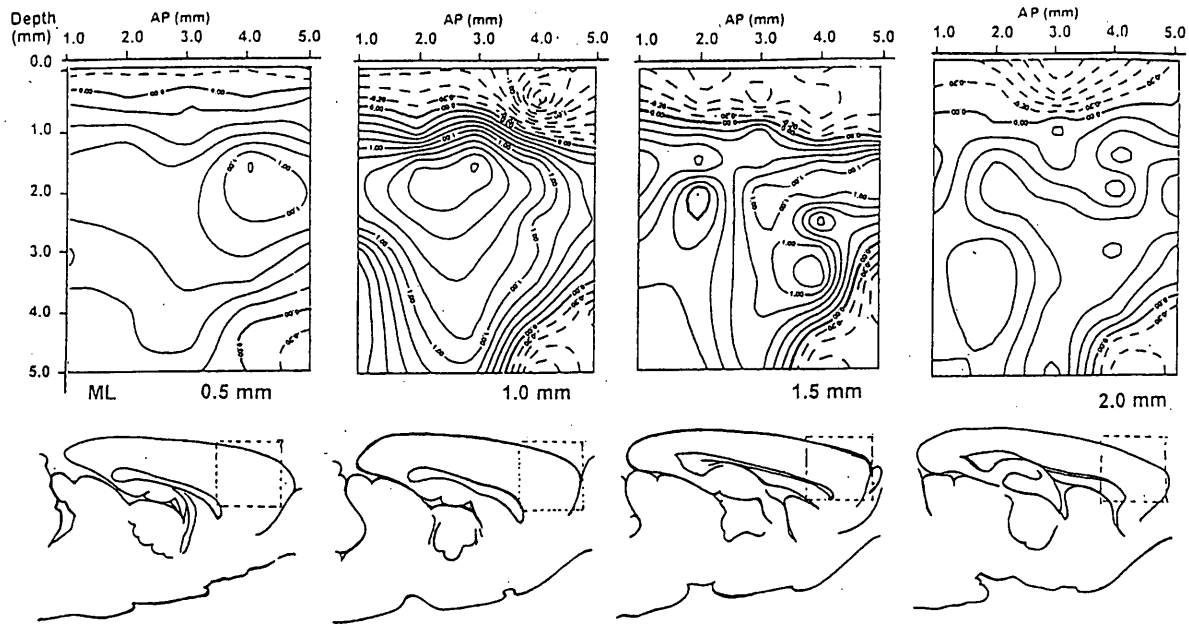
Discussion

In the present study, our electrophysiological findings in field potentials and multi-units confirmed the synaptic projections from the MT to the ACC. We also demonstrated the localization of synaptic activation in the ACC. Since the field potentials and multiple units occurred in the ACC as a result of electrical stimulation of the MT where nociceptive inputs were identified, we speculate that specific afferent inputs projecting to this cortical region may constitute a part of the medial pain system (45).

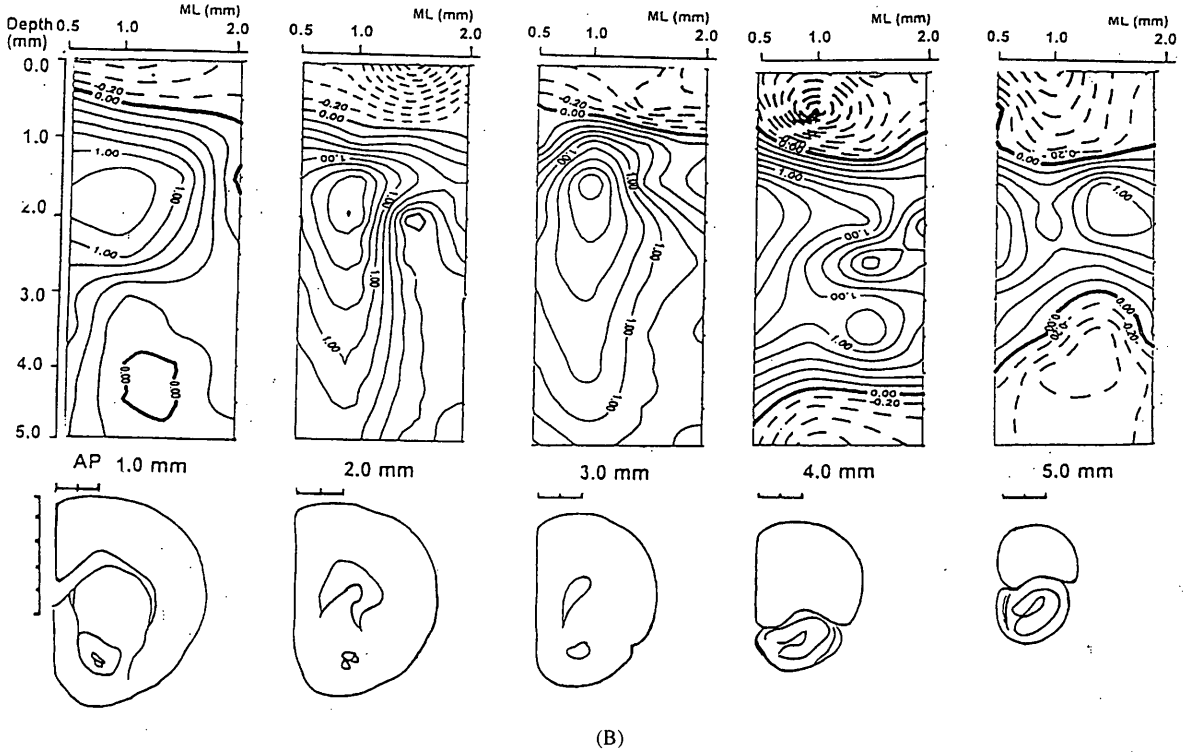
The nociceptive neurons in the MT selected for stimulation in the present study were located at the boundaries of CL/PC/CM, MD/MDL, or in the CL, PC, CM and Pf. The existence of these nociceptive neurons in CL, PC and CM is consistent with previous findings (17, 23). The responses of the MD to peripheral noxious stimuli were also consistent with the finding of spino-thalamic inputs to these regions (12). All the nociceptive neurons in the present study had large, bilateral receptive fields. These characteristics are similar to those of nociceptive neurons in a variety of species (16, 9). The properties of these neurons are consistent with the possible involvement of these nuclei in mediating the affective-motivational aspects of pain.

In the present study, nociceptive neurons of the MT-evoked positive-negative surface potentials distributed in large areas of the ACC. The extracellular recording on the cortical surface sometimes picked up grossly the field potential adjacent to the recording sites, making it difficult to detect the specific cortical areas responding to the stimuli. The systemic deep field potential and iso-potential profile were more accurate means for determining the cortical regions actually responding to the stimulation of medial thalamic nuclei. This characteristic laminar profile, e.g. the reversal of the polarity, of the MT-evoked potentials could be used as a measure to identify approximately the synaptic activation sites.

Based on the analysis of the polarity reversal of the surface negative potentials in deep sbucortical areas in the ACC (such as Fr2 and Cg1-Cg3), the activated synaptic connections were generated between 0.5 to 1.5 mm below the surface of the ACC. However, the iso-potential contour two-dimensional map showed that there was a regional difference in the response profiles within the cingulate cortex, which probably indicates an uneven distribution of afferent terminals within these cytoarchitectonic



(A)



(B)

Fig. 3. The iso-potential contour profiles of MT-evoked ACC potentials. (A) 4 parasagittal planes, each separated by 0.5 mm and extending from 0.5 mm to 2.0 mm, lateral to the midline. (B) 5 transverse planes, each separated by 1 mm, and extending from 1.0 mm to 5 mm, anterior to the bregma. The solid lines indicate equi-positive potentials and the dashed lines indicate the equi-negative potentials. The blackened line is the zero line, a demarcation of the reversal of negative to positive potentials. The time point of 21.1 ms, at the peak of the negative potential, was chosen.

boundaries.

Thalamic inputs synapsed to cortical pyramidal cells generated extra-cellular currents, resulting in a potential field is oriented vertically to cortical layers. Excitatory and inhibitory synapses serve as active sink and source for the currents, respectively. The negative field potentials recorded in certain laminae are largely attributable to EPSPs generated in the

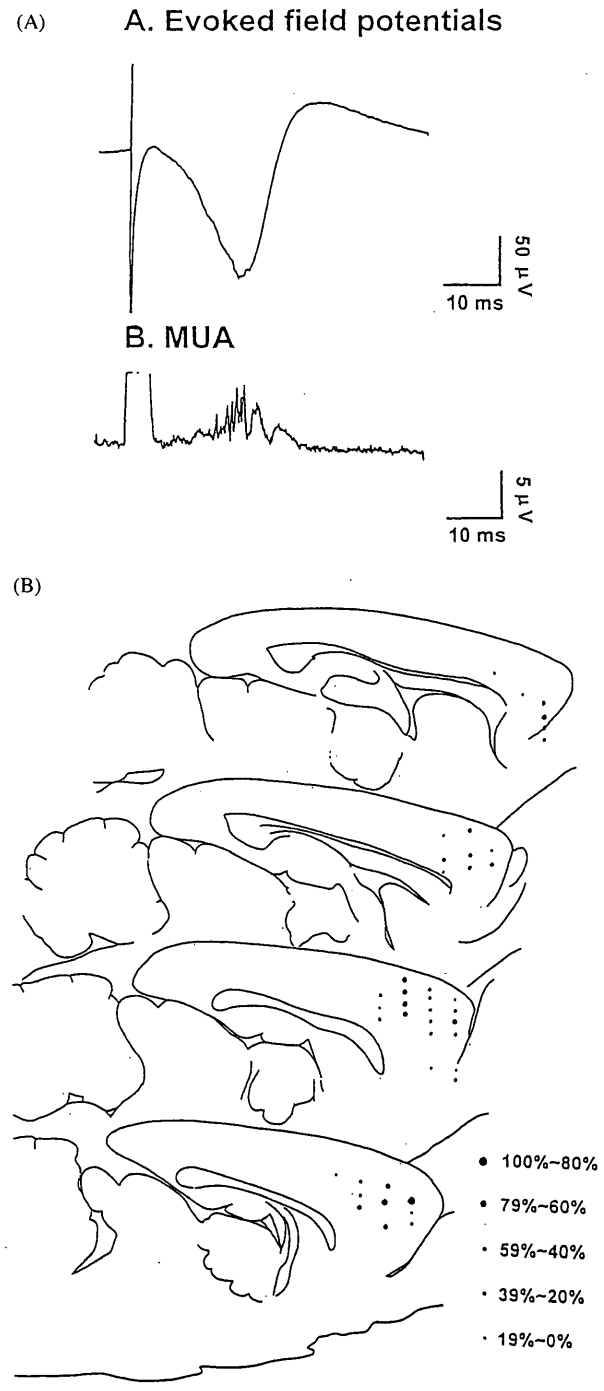


Fig. 4. The multiple unit activities in the ACC evoked by electrical stimulation of the MT. (A) The simultaneous recording of the negative potentials (upper seep) and the filtered, rectified multiple unit activities (lower sweep). (B) The distribution of multiple unit activities in 4 parasagittal planes in the frontal cortex. The size of each dot is proportional to the percentage of the maximum value.

vicinity. In the present study, MT stimulation activated initial positive and negative potentials that differed in amplitude and laminar distribution. This difference likely reflected different excitatory inputs with

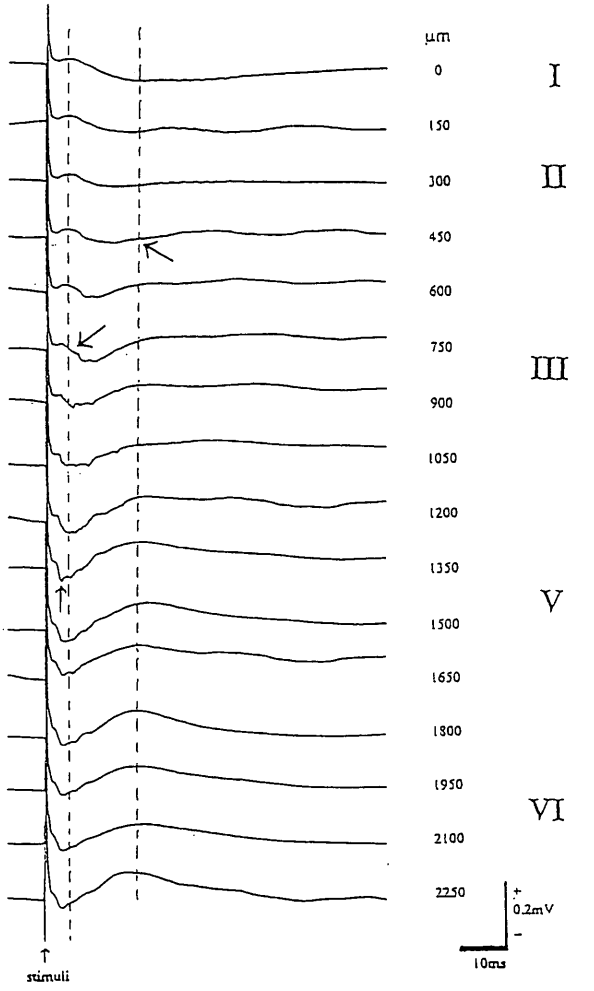


Fig. 5. The depth field potentials profiles of MT-evoked ACC potentials. The two vertical dashed lines mark the peak latencies of the surface positive and negative potentials, respectively. The two upper arrows indicate the reversal of the positive and negative potential in the deep layers. The corresponding cortical layers in different depths are indicated in the right column.

different laminar distribution along the dendrite shafts of pyramidal cells. Depth laminar field potential recordings revealed deep positive potentials which may be the counterpart of the surface negative potentials, acting as a passive current source in the deep laminae (40).

The laminar potential distribution of the MT-evoked potentials suggested that the responses constitute different stages of signaling processing. The superficial positive potential was reversed to a negative potential at the depth of 750 μm. Negative potentials in the region of polarity reversal generally reflect excitatory inputs to pyramidal cells, thus the first surface positive potential, which was negative at depths below 750 μm, could be ascribed to EPSPs generated at a deeper layer (layer III) in the cingulate cortex. The potential profile of the second surface

potential, which was reversed at more superficial layers (450 μm), suggested that the response could be due to excitatory inputs distributed mainly at layer I and II. These two stages of neuronal responses may be depicted as two different processes. One is a serial, sequential excitation of the deeper laminar cells to superficial laminar cells. The other process may be mediated by parallel pathways, in which cortical cells of each layers are excited by different medial thalamic afferent inputs. We assumed that the difference in latency reflected different MT inputs projected to different laminas. This is in good agreement with previous anatomical observations, which indicated that the projection of MT to the cerebral cortex has a high degree of topographical ordering (6) and laminar distribution (28). This is also consistent with the electrophysiological findings that there is a topographical relationship between the medial thalamic nuclei and isocortex (33).

A deep negative potential with much shorter latency was observed in the deeper layer (V). The latency was too short to consider it as mono-synaptic excitation. One possibility of the arrangement was antidromic excitation of terminals of the deep layer pyramidal cells projecting to the medial thalamus. Electrophysiological evidence for this possibility was provided in our previous single unit recording experiments (24).

The correlation between the multiple unit activities and negative field potentials often occurred deep in the ACC. The multiple unit activities data indicated that nociceptive information from the MT might be transmitted differentially to different layers in the ACC. The cortical layer distribution of rodent intralaminar nuclei is not yet completely understood. Only the Pf projection to layer V & VI in cingulate areas of rats has been investigated (28). With respect to the laminar organization and synaptic activation of nociceptive MT projections to the ACC, a current source density analysis of the evoked field potentials together with the intracellular recording of neurons in the ACC might reveal more detailed information.

Based on our analysis of the evoked field potentials, we propose that the medial thalamus receives nociceptive inputs, provides excitatory inputs for pyramidal cells at a short latency at deeper layers, and at longer latency at more superficial layers. Although the delay of the onset of the second negative potential could be an intra-cortical synaptic relay, the relevant neuro-anatomical evidence suggests that it is more likely that the inputs to the superficial cingulate cortical layer are conveyed by slow-conducting medial thalamic fibers (39). The arrival of impulses to the cortex might be delayed if they are conveyed through an indirect medial thalamic-cingulate pathway. One of the possible routes is by way of the amygdala. Further lesion experiments may be able to elucidate this possibility.

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