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Nocifensive behavior-related laser heat-evoked component in the rostral agranular insular cortex revealed using morphine analgesia

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HIGHLIGHTS

- Morphine potentiate the long latency component of laser heat-evoked response in RAIC.
- RAIC potentiated component occurred specifically in paw lifting nocifensive behavior.
- RAIC, SmI and ACC process nociceptive inputs differentially.

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ABSTRACT

The rostral agranular insular cortex (RAIC), an opioid-responsive site, is essential for modulating nociception in rats. Our previous studies have shown that morphine suppressed long latency laser heat-evoked nociceptive responses in the primary somatosensory cortex (SmI). By contrast, morphine significantly attenuated both short and long latency responses in the anterior cingulate cortex (ACC). The present study assessed the effect of morphine on laser heat-evoked responses in the RAIC. Laser heat irradiation applied to the rat forepaws at graded levels was used as a specific noxious stimulus. In the RAIC, the first part of the long latency component (140–250 ms) of the laser heat-evoked response was enhanced by intraperitoneal morphine (5 mg/kg). When the laser heat-evoked cortical responses were examined for trials showing strong nocifensive movement (paw licking), moderate nocifensive movement (paw lifting), and no nocifensive movement, a 140–250 ms period enhancement was observed in the RAIC only for the paw lifting movement. This enhancement was absent in the SmI. Thus, our data suggest that the RAIC has a pain-related behavior-dependent neuronal component. Furthermore, the RAIC, ACC, and SmI are differentially modulated by morphine analgesia.

1. Introduction

Functional imaging studies in humans show that the rostral part of the insular cortex (InC) is often bilaterally activated during noxious somatosensory stimulation and has been suggested to play a crucial role in pain processing [1–4]. InC activation has been correlated with the intensity of noxious stimulation, indicating that the human InC may code process information on pain intensity [1,5]. Several clinical studies involving patients with InC damage support this notion [6,7]. The rostral agranular insular cortex (RAIC) is a distinct opioid-responsive area and contributes to opioid- and dopamine-related antinociception through a descending inhibitory mechanism [8–11]. Analgesia and hyperalgesia could be induced in the RAIC by the manipulation of γ-aminobutyric acid (GABA) neurotransmitter [11]. However, data on nociceptive circuitry around the RAIC are limited to anatomical and pharmacological results, and in vivo electrophysiology studies are lacking. On the assumption that the antinociceptive effect of RAIC is result from its excitatory projection to the brainstem, the RAIC neuronal activity under morphine treatment probably increased.

Our previous studies [12,13] have revealed that noxious laser heat irradiation applied to the tail or paws of rats evoked both short and long latency responses in the primary somatosensory cortex (SmI) and anterior cingulate cortex (ACC). Morphine administered intraperitoneally suppressed only the long latency responses in the SmI; however, both short and long latency responses were significantly attenuated in the ACC. To develop a more comprehensive understanding of how changes in the RAIC activity respond to noxious stimulation, electrophysiological recording was used to directly record RAIC neuron responses when laser heat stimulation was applied to the glabrous skin of the forepaw of conscious rats. The behavioral responses to graded intensities of noxious stimuli were used as an index to classify the experienced intensity of noxious stimulation. Furthermore, morphine was systemically

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injected to produce different nociceptive response and elucidate analgesic modulation in the RAIC.

2. Materials and methods

Experiments were conducted using adult female Long-Evans rats. The care of animals and entire experimental procedure were in accordance with the *Codes for Experimental Use of Animals* of the Council of Agriculture, Taiwan, based on the *Animal Protection Law*, Taiwan, and were approved by the Institutional Animal Care and Use Committee of National Taiwan University.

2.1. Animal preparation and surgical procedures

Experiments were performed using 11 adult female Long-Evans rats weighing 280 ± 20 g during electrode implantation. The rats were initially anesthetized with sodium pentobarbital (50 mg/kg, ip). Ketamine (50 mg/kg, im) was supplemented to maintain proper anesthetic depth so that the animals had no flexor reflexes throughout surgery. The core temperature of the rats was maintained at 37.5 °C by using a feedback-controlled heating pad. The rats were mounted on a stereotaxic apparatus. A midline incision was made over the skull. After retracting the skin and cleaning the soft tissue, small craniotomies were made for placing intracortical microelectrodes in the SmI and RAIC.

Two eight-channel microwire array electrodes were implanted in each rat, one in the SmI and the second in the RAIC (Fig. 1). The array electrodes were built in house. The details on the array electrode fabrication procedure are provided in a previous report [14]. In brief, eight stainless steel wires individually insulated with Teflon (50 μm in diameter; M219810, California Fine Wire Co., USA) were aligned linearly with an equal interelectrode distance and a total width of 2.5 mm. Small longitudinal holes were opened in the frontoparietal bone above the SmI and RAIC. The coordinates of the SmI were 2 mm anterior to 1 posterior and 3–5 mm lateral to the bregma and at a depth of approximately 1.5 mm in the cortex. The SmI contralateral forepaw region was identified by brisk responses restricted to the forepaw observed when the rat was under anesthesia during implantation. The coordinates of the RAIC were 1.5–3.5 mm anterior and 3–5 mm lateral to the bregma and at a depth of 4.5–5 mm from the surface of the cortex.

A pair of stainless steel screws (1 mm OD) was placed in the skull over parietal lobe, 2.5 mm posterior and 2.5 mm lateral to the bregma, for electroencephalography (EEG). The ground electrode was a stainless steel screw located in the mid-occipital bone over the cerebellum. In addition, five stainless steel screws were placed in the frontal and parietal bones for anchoring. A pair of seven-stranded stainless steel wires (793200, A-M systems) was inserted into the neck muscles for electromyography (EMG). After implantation, the holes in the skull and the implanted electrodes were sealed and secured with dental cement.

After completion of the experiment, each rat was deeply anesthetized. The positions of the electrodes were marked by applying a 5-μA positive current for 30 s to deposit iron ions. The rats were perfused with 10% formalin containing 1% potassium ferrocyanide to observe iron deposition. The brains were removed, placed in the same perfusion fluid for 7 days, and serially sectioned in 100-μm thicknesses by using a sliding microtome. The sections were stained with crystal violet. The positions of the electrode tips were ascertained using camera lucida drawings under a stereomicroscope.

2.2. Experiment protocol

After a 1-week recovery period, the rats were transferred to a transparent plastic chamber (20 × 26 × 36 cm in width, depth, and height, respectively, with an open top and bottom). To habituate the rats to the experimental chamber, each rat was placed in the same chamber three times (1 h/day) before the experiment. A video camera was placed in front of the chamber. Spikes, EEG and EMG activity were transmitted

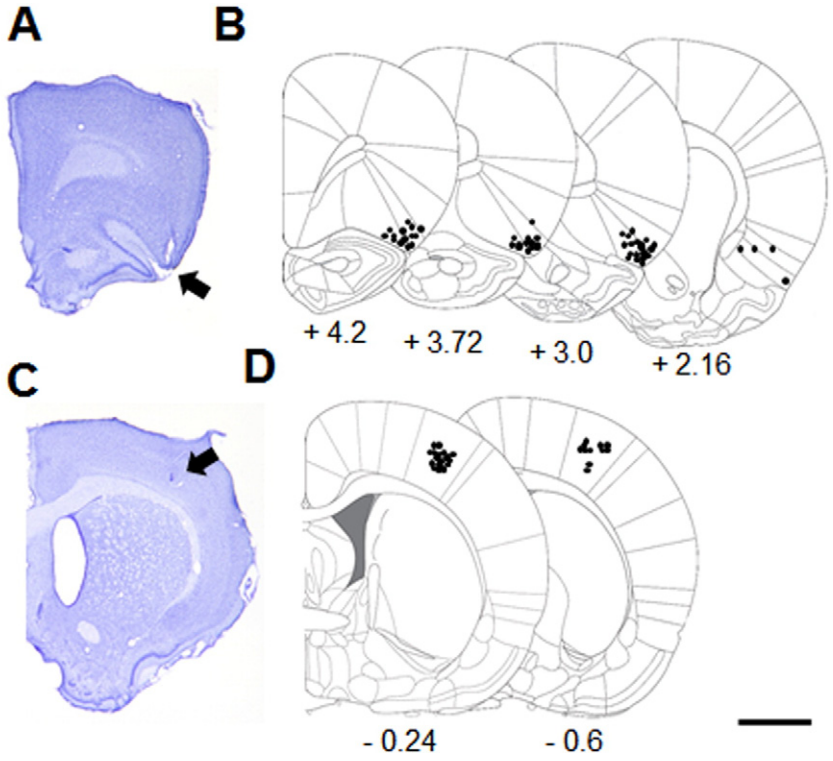

Fig. 1. (A and C) The photomicrographs show coronal sections of the brain that contain marking iron depositions (arrows) in RAIC (A) and SmI (C), respectively. Scale bar = 2 mm. Composite recording sites for all RAIC (B) and SmI (D) recording electrodes in 11 rats. Numerical values at bottom are AP value of the coronal sections from a standard atlas [30] in mms rostral to the bregma.