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Research Paper

Differential effects of opioid receptors in nucleus submedius and anterior pretectal nucleus in mediating electroacupuncture analgesia in the rat

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Abstract: Previous studies have indicated that the thalamic nucleus submedius (Sm) and the anterior pretectal nucleus (APtN) are involved in the descending modulation of nociception. The aim of the present study was to examine whether the opioid receptors in the Sm and APtN mediated the electroacupuncture (EA)-produced analgesia. The latency of tail flick (TF) reflex induced by radiant heat was used as an index of nociceptive response. The effects of microinjection of opioid receptor antagonist naloxone (1.0 μg, 0.5 μl) into Sm or APtN on the inhibition of the TF reflex induced by EA of "Zusanli" point (St. 36) with high- (5.0 mA) and low- (0.5 mA) intensity were examined in the lightly anesthetized rats. Sm microinjection of naloxone blocked the high- but not low-intensity EA-induced inhibition of the TF reflex. In contrast, naloxone applied to APtN blocked the low- but not high-intensity EA-induced inhibition. When naloxone applied to other brain regions adjacent to Sm or APtN, the EA-induced inhibition was not influenced under either high- or low-intensity condition. These results suggest that opioid receptors in Sm are involved in mediating the analgesia by high-intensity EA for exciting small (A-δ and C group) afferent fibers, while opioid receptors in APtN are involved in mediating the analgesia induced by low-intensity EA for only exciting large (A-β) afferent fibers.

Key words: opioid receptor; naloxone; nucleus submedius; anterior pretectal nucleus; electroacupuncture analgesia; tail flick reflex

阿片受体在大鼠丘脑中央下核和顶盖前区前核介导电针镇痛中的不同作用

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摘 要: 本文旨在研究阿片受体是否参与丘脑中央下核(nucleus submedius, Sm)和顶盖前区前核(anterior pretectal nucleus, APtN)所介导的不同强度电针的镇痛作用。以辐射热诱发甩尾(tail flick, TF)反射潜伏期为伤害性反应的指标, 观察了 Sm 和 APtN 微量注射阿片受体拮抗剂纳洛酮对不同强度电针"足三里"穴(St. 36)抑制大鼠 TF 反射的效应。结果表明, Sm 给予纳洛酮(1.0 μg, 0.5 μl)阻断强电针(5 mA)对 TF 反射的抑制效应，而对弱电针(0.5 mA)的效应无明显影响; 相反, APtN 给予纳洛酮阻断弱电针对 TF 反射的抑制效应，而对强电针的效应无明显影响；纳洛酮供给到 Sm 或 APtN 邻近其它脑区对强、弱电针的效应均无影响。这些结果提示, Sm 内的阿片受体参与介导强电针兴奋细传入纤维(A-δ 和 C 类)产生的镇痛，而 APtN 内的阿片受体则介导弱电针兴奋粗传入纤维(A-β 类)产生的镇痛。

关键词: 阿片受体; 纳洛酮; 丘脑中央下核; 顶盖前区前核; 电针镇痛; 甩尾反射
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Many studies have indicated that the thalamic nucleus submedius (Sm) and the anterior pretectal nucleus (APtN) are involved intensively in descending nociceptive modulation[1,2]. However, the mechanisms of the Sm and the APtN in modulating the nociceptive information are different and to some extent separate. Sm receives an excitatory input from high threshold afferents via the spinothalamic tract [3,4] and its descending modulatory action to spinal cord involves the Sm-ventrolateral orbital cortex (VLO)-periaqueductal gray (PAG) brainstem descending inhibi-

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tory system [1]. However, APtN mainly receives an excitatory input from low threshold afferents via the dorsal column pathway [5] and its descending modulatory action involves the lateral midbrain and lateral medulla including the deep mesencephalic nucleus, parabrachial nucleus and paragigantocellularis nucleus [6]. It has been suggested that the Sm and the APtN may mediate different analgesic effects by low and high threshold afferents respectively [7,8]. There is a line of evidence that opioid receptors are distributed in the Sm and the APtN[9], and that microinjection of morphine into Sm and APtN produces antinociception [10.11], suggesting that Sm and APtN may be involved in the opioid receptor-mediated antinociception. The aim of the present study was to examine whether the opioid receptors in Sm and APtN also participated in the analgesia induced by EA and to determine whether there was difference between them. The effects of microinjection of opioid receptor antagonist naloxone into the Sm and APtN on the inhibition of the TF reflex induced by different intensities of EA at the "Zusanli" point (St. 36) were observed in the lightly anesthetized rats.

1 MATERIALS AND METHODS

1.1 Animals. Experiments were carried out on 40 male Sprague-Dawley rats weighing 270~300 g provided by the Medical Experimental Animal Center of Shaanxi Province, China. Animals were initially anesthetized with sodium pentobarbital (50 mg/kg, i.p.) for surgery. After a tracheotomy and cannulation of a jugular vein were performed, the rat's head was positioned in a stereotaxic frame. A small craniotomy was performed over the thalamus or midbrain, and a guide cannula placed at a position 2 mm dorsal to the Sm or APtN for inserting an injection needle to Sm or APtN. The animal was maintained in a lightly anesthetized state by an intravenous infusion of sodium pentobarbital at a constant rate (4~5 mg/kg·h^{-1}) which was sufficient to prevent spontaneous movements and yet allowed the tail flick (TF) to be elicited. The rectal temperature was monitored and kept between 37~38ºC by a thermostatically regulated heating pad to prevent body temperature changes elicited by anesthesia and drug injections.

1.2 Tail flick test. Radiant heat from a projector lamp was applied to the blackened ventral surface of the rat tail at a distance of 5~6 cm from its tip to evoke the TF reflex. The TF latency (TFL) was measured manually with a stopwatch. The heat lamp intensity was adjusted in order that the baseline TFL was in the range of 3.0~3.5 s. The TFL was used as an index of the nociceptive response and examined repeatedly with an interval of 5 min between applications of the noxious heating. Before EA stimulation and intracerebral injection (see below), at least three baseline TFLs were measured. During EA stimulation, if the TF reflex was significantly suppressed, a cut-off time of 7 s was adopted to minimize damage to the skin.

1.3 Electroacupuncture stimulation. A pair of stainless steel acupuncture needles (shank diameter 150 μm, length 2.0 cm) separated by 5 mm was inserted with a depth of 5 mm into a unilateral "Zusanli" point (St. 36) that is located in musculus tibialis anterior innervated by the deep peroneal nerve. After three baseline TFLs were recorded, a series of constant-current square wave pulses (0.3 ms) were delivered by an electrical stimulator for a period of 15 min at an intensity of 0.5 mA with a frequency of 50 Hz or 5.0 mA with a frequency of 5.0 Hz for the low and high intensity stimulation respectively [12]. During and after EA stimulation the TFLs were recorded repeatedly to observe the EA stimulation-produced antinociceptive effects.

1.4 Intracerebral microinjection. To determine if opioid receptors in Sm and APtN mediate the EA stimulation-produced analgesia, the effects of microinjection of opioid receptor antagonist naloxone into Sm and APtN on the TF reflex inhibition induced by EA stimulation were examined. A needle attached to a 1.0 μl Hamilton syringe was inserted via the guide cannula and protruded the guide cannula 2 mm to approach the Sm (2.3~2.8 mm posterior to Bregma, 0.5~0.9 mm lateral, 6.0~7.0 mm from the cortical surface) or the APtN (4.3~5.3 mm posterior to Bregma, 1.5~2.5 mm lateral, 5.5~6.5 mm from the cortical surface) contralateral to EA stimulation [13]. After three baseline TFLs were measured, naloxone hydrochloride (1.0 μg, in 0.5 μl, Sigma) was slowly injected into Sm or APtN over 2 min. The EA stimulation commenced at the termination of injection and lasted for 15 min, and the measuring of the TFLs was repeated at 5 min intervals throughout the 30 min observation period. The same volume of 0.9% saline was injected into Sm or APtN in control experiments. A period of at least 1.5 h elapsed before testing the same animals again to assure the drug effect elimination and the TFL return to the baseline level.

1.5 Histology. At the end of the experiment, the drug injection sites were marked by injection of Pontamine

Sky Blue dye (0.5 μl, 2% in 0.5 mol/L sodium acetate solution). Under deep anesthesia, the animal was perfused transcardially with 0.9% saline followed by 10% formalin. The brain was then removed and fixed in fresh formalin for 3~7 d. One hundred micron sections were cut with a freezing microtome and mounted and stained with cresyl violet. The injection sites were plotted on coronal sections modified from the Paxinos and Watson's atlas[13].

1.6 Data analysis. All average values were expressed as mean ± SEM. The effects of EA of acupoint on the TF reflex were expressed by percentage changes in the TFL compared with the baseline TFL (a mean TFL value of 3 trials before electrical stimulation). Data were analyzed for statistical significance (P<0.05) by two-way analysis of variance (ANOVA) with a post hoc multiple comparison.

2 RESULTS

2.1 Inhibitory effects of EA stimulation of "Zusanli" point on TF reflex

As reported previously, the TFL could stably maintained for over 4~6 h and intracerebral microinjection of 0.9% normal saline (0.5 μl) did not influence the TFL in lightly anesthetized rats. EA stimulation delivered to unilateral "Zusanli" point (St. 36) significantly depressed the TF reflex under either low- or high-intensity condition. As shown in Fig.1, the difference among the control group and two intensity EA groups was statistically significant ($F_{(2,162)}$=200.82, P<0.001). The mean TFLs during 15 min period of EA stimulation increased by (31.5±1.5)% (n=25, for low-intensity) and (63.0±2.9)% (n=25, for high-intensity) above the baseline value, respectively, which were significantly larger than those in the control group (0.6±0.9)%, (n=7, t=9.03, P<0.001 and t=18.2, P<0.001, respectively). The inhibitory effect induced by high-intensity EA stimulation was significantly larger than that induced by the low-intensity stimulation (t=13.90, P<0.001).

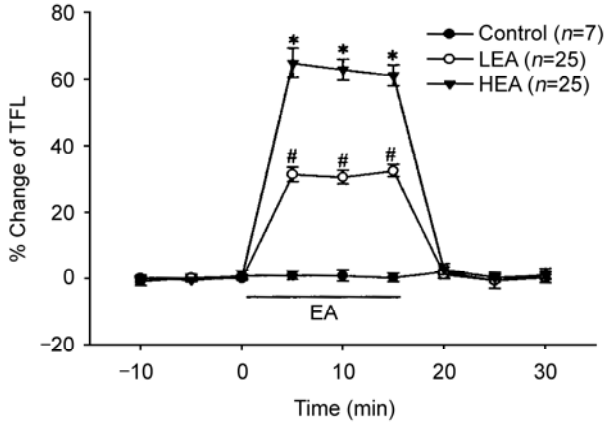

Fig. 1. Effects of EA stimulation of the "Zusanli" point (St. 36) with low- and high-intensities (LEA and HEA) on the TF reflex. *P<0.05 compared with low-intensity EA stimulation, #P<0.05 compared with the control experiment.

2.2 Effects of microinjection of naloxone into Sm on TF reflex inhibition induced by EA stimulation

Microinjection of opioid receptor antagonist naloxone into Sm contralateral to EA stimulation significantly blocked the high-intensity EA-induced inhibition of TF reflex, but did not influence that induced by low-intensity EA. As shown in Fig.2*A*, the difference among the 4 treated groups was statistically significant ($F_{(3,156)}$=190.49, P<0.001). After Sm naloxone injection, the mean TFL changes [(2.6 ± 1.2)%, n=*14*] during high-intensity EA maintained at the baseline level which was significantly smaller than that [(68.3±2.2)%, n=14]of the saline injection group (t=23.77, P<0.001). However, the TFL changes [(29.3±2.4)%, n=14] in the naloxone with low-intensity stimulation group was not different from that [(33.7±2.1)%, n=14] of the saline with low-intensity stimulation group (t=1.60, P=0.67).

Naloxone microinjected into the adjacent thalamic regions more than 0.5 mm dorsal (n=3), lateral (n=4), and ventral (n=4) to the Sm did not influence the TF inhibition induced by EA stimulation under either the high- or low-intensity condition. As shown in Fig.2*B*, the difference among the 4 treated groups was statistically significant ($F_{(3,120)}$=18.90, P<0.001). Except that there is significant difference between high- and low-intensity EA groups [(58.0±4.0)% *vs* (28.7±2.8)%, n=11, t=6.03, P<0.001], no significant difference was found between the naloxone and saline groups either with high-intensity or low-intensity [(55.4±5.0)% and (33.6±2.7)%, n=11, P=1.0, respectively] .

2.3 Effects of microinjection of naloxone into APtN on TF reflex inhibition induced by EA stimulation

Microinjection of the naloxone into the APtN blocked the inhibitory effect on the TF reflex induced by low-intensity EA of the "Zusanli" point (St.36), but did not significantly influence the high-intensity EA-induced inhibition. As shown in Fig.3, the difference among the 4 treated groups was statistically significant ($F_{(3,126)}$=136.50, P < 0.001). After APtN naloxone injections, the mean TFL change [(2.7±0.9)%] induced by low-intensity EA was significantly smaller than that [(28.7±1.8)%] of the saline with low-intensity EA group (t=8.83, P<0.001, n=11), while the mean TFL change [(50.6±3.1)%] induced by high-

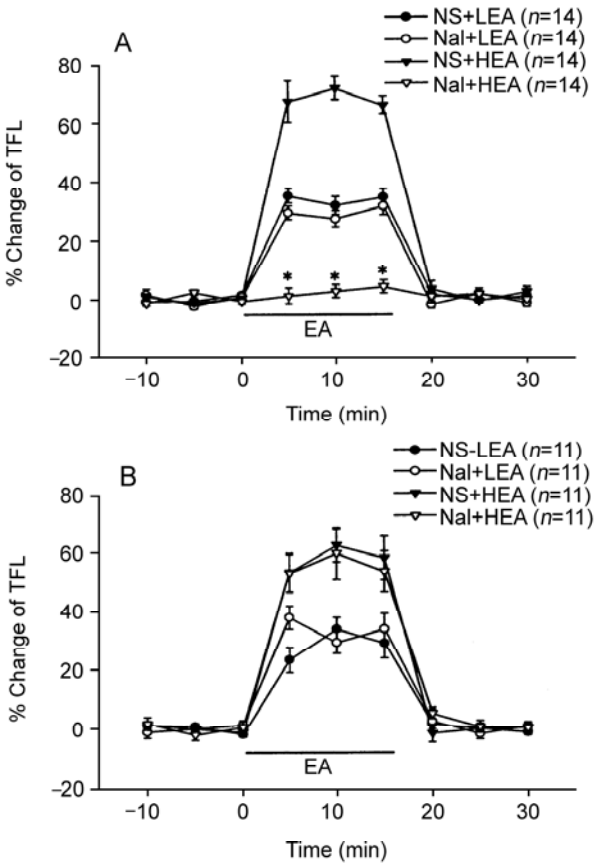

Fig. 2. Effects of opioid receptor antagonist naloxone (Nal) microinjected into Sm (*A*) and its adjacent regions (*B*) on the TF reflex inhibition induced by low- and high-intensity EA (LEA and HEA) of the "Zusanli" point (St.36). $^{*}P<0.05$ compared with saline associated with high-intensity EA group (NS+HEA).

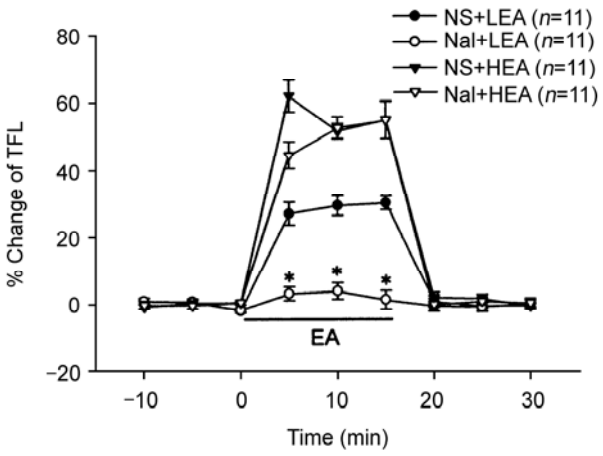

Fig. 3. Effects of opioid receptor antagonist naloxone microinjected into APtN on the TF reflex inhibition induced by the low- and high-intensity EA of the "Zusanli" point (St.36). $^{*}P<0.05$ compared with APtN saline associated with low-intensity EA group (NS+LEA).

intensity EA was not significantly different from that [(56.2±3.3)%] of the saline with high-intensity EA group (t=1.88, P=0.38, n=11).

2.4 Histology profile

The locations of naloxone injection sites in the Sm and APtN regions and their effectiveness in blocking the high- or low-intensity EA-induced inhibition of the TF reflex were shown in Fig.4*A* and *B*, respectively. All injection sites within Sm were effective in blocking the high-intensity but not low-intensity EA-induced inhibition of the TF reflex, and all injection sites within APtN and three sites dorsal closely to APtN were effective in blocking the low-intensity but not high-intensity EA-induced inhibition of the TF reflex. But all of the injection sites more than 0.5 mm dorsal, lateral and ventral to Sm were ineffective in blocking either the high- or low-intensity EA-induced inhibition. Three sites located in the lateral posterior thalamic nucleus (pulvinar) more than 0.5 mm lateral to APtN were also ineffective.

3 DISCUSSION

Electroacupuncture stimulation-produced analgesia has been widely used in clinical practices or in experimental studies, and its effectiveness is not only dependent on stimulation intensity but also dependent on its frequency. Studies in our laboratory [12] have demonstrated that, due to different excitation threshold and refractory period of different types of afferent fibers, high intensity (5.0 mA) with low frequency (5.0 Hz) EA for excitation of the small (A-δ and C-group) fiber afferent can produce best analgesic effect, while only excitation of the large (A-β group) fiber afferent with low intensity (0.5 mA) and high frequency (50 Hz) EA also can produce better analgesic effect. In the present study, using both such parameters, the analgesic effects can be effectively produced, but lack of a markedly induction period and an after-effect that is different from previous studies. This may be due to different EA parameter, animal anesthesia state and the time interval of pain measurement.

Results of this study showed that microinjection of the opioid receptor antagonist naloxone into the Sm blocked the high-intensity EA-evoked inhibition of the TF reflex, but did not influence that of low-intensity EA, suggesting that the opioid receptors in Sm mediate the high-intensity but not low-intensity EA-evoked antinociception. This result is closely correlated to previous investigations that the Sm neurons primarily receive the direct projection from dorsal horn lamina I (in cat) and deep lamina (in rat) neu-

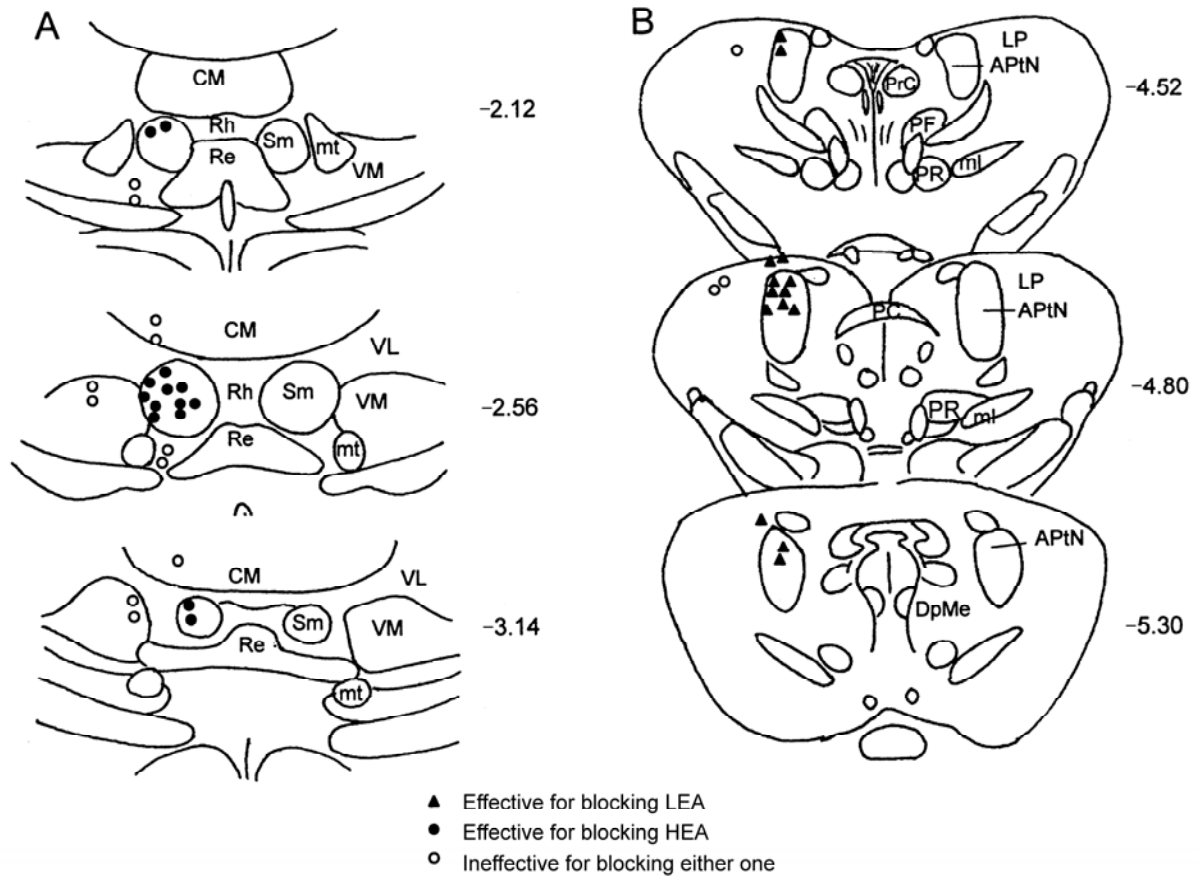

Fig. 4. Locations of naloxone injection sites in the Sm (*A*) and APtN (*B*) regions and whether they produced a blocking effect on the high-intensity EA-induced antinociception in Sm (*A*) and on the low-intensity EA stimulation-induced antinociception in APtN (*B*). Numbers at the right side each diagram showing the section corresponding to Bregma. CM, central medial thalamic nucleus; mt, mammillothalamic tact; Re, reuniens thalamic nucleus; Rh, rhomboid thalamic nucleus; Sm, thalamic nucleus submedius; VL, ventrolateral thalamic nucleus; VM, ventromedial thalamic nucleus; APtN, anterior pretectal nucleus; DpMe, deep mesencephalic nucleus; LP, lateral posterior thalamic nucleus (pulvinar); ml, medial lemniscus; PC, paracentral thalamic nucleus; PF, parafaciscular thalamic nucleus; PR, prerubral field; PrC, precommissural nucleus.

rons that contain nociceptive neurons [3,4], and only respond to peripheral noxious but not innocuous stimulation, and the electrical stimulation intensity for activating the Sm neurons is in the range of A-δ and C-fibers [14]. Since the Sm is involved in an endogenous analgesic system (a feedback loop) consisting of spinal cord-Sm-VLO-PAG-spinal cord [1,15] and since the axon terminals from enkephalinergic neurons in the deep dorsal horn project to Sm [16] and μ-opioid receptors are distributed in the Sm [9], it is reasonable to propose that the opioid receptors may be involved in the Sm-mediated antinociception. The results of the present research that Sm naloxone blocks the high- but not low-intensity EA-induced antinociception provide support for this hypothesis.

In contrast to above described results, opioid receptor antagonist naloxone microinjection into the APtN blocked the low- bot not high-intensity EA-induced inhibition of the TF reflex, suggesting that the opioid receptors in the APtN mediate the analgesia induced by low-intensity EA stimulation for exciting larger afferent fibers. Studies have demonstrated that somatosensory afferents ascend to the APtN via dorsal column pathway[5] and dorsal column stimulation at an intensity of only activates large-diameter afferents potently excites cell within the APtN and produces a long-lasting antinociception [2]. Since APtN has been demonstrated to be involved in descending nociceptive modulation via the polysynaptical lateral midbrain and lateral medulla pathway to spinal cord [8], and since there is evidence that the μ-opioid receptors are distributed in the APtN [9], it is possible that antinociceptive effects induced by low- but not high-intensity EA, except for the spinal segmental mechanisms, may be mediated superspinally by the opioid receptors in APtN. The results of this study provide evidence for this possibility.

In addition, results of this study indicated that microinjection of the naloxone into the other thalamic regions more than 0.5 mm lateral, dorsal and ventral to Sm and lateral to APtN were ineffective in blocking either the high- or low-intensity EA-induced inhibition of the TF reflex, suggesting that these regions may be not involved in the descending nociceptive modulation [17] although opioid receptors were distributed in these regions [9]. As far the blocking effects of three naloxone injection sites dorsal closely to APtN that were similar to those in APtN might be a result of drug diffusion to APtN [17].

In conclusion, results of this study further provide evidence for central mechanisms of analgesia induced by high-intensity EA exciting small fibers and by low-intensity EA exciting large fibers and suggest that opioid receptors in Sm and APtN are involved in mediating the differential analgesia produced by high-intensity and low-intensity EA, respectively.

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