行政院國家科學委員會補助專題研究計畫成果報告

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本成果報告包括以下應繳交之附件：

- □赴國外出差或研習心得報告一份
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- □國際合作研究計畫國外研究報告書一份

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Electroacupuncture produces intensity-dependent analgesia and suppress c-*fos* expression evoked by noxious somatic stimulation in the deeper layer of the spinal dorsal cord in the rat

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Key Words: Electroacupuncture, tail flick latency, formalin test, c-*fos* expression, Immunohistochemistry, Halothane

Abstract

Aim of Investigation: Unlike the paresthesia sensation caused by TENS, the induction of deep aching sensation (Der-Chi) is a traditional maneuver in acupuncture-induced analgesia. To examine whether stimulation-evoked nociceptive response is required for EA–induced analgesia, the present study compared tailflick latency and the spinal cord c-fos expression after various intensity of electric stimulation in rat. Inhalation anesthesia was used to reduce the escape behavior caused by intense electric current stimulation.

Methods: All SD rats were anesthetized with 1% of halothane in oxygen. Experimental rats received EA stimulation of 4 Hz at Zusanli (S36) in the right hindlimb for 30 min. The intensity was adjusted from basal intensity, i.e. local muscle twitch, to 10 or 20 times of basal intensity (BI), i.e. low intensity group and high intensity group respectively. Tail flick latency of all rats was measured at 10 min interval from 10 min before EA to 60 min after EA. Rat in morphine group received intraperitoneal morphine injection for comparison of analgesic effect. Maximal possible effect was calculated for statistical analysis.

Results: The control group showed constant tail-flick latency throughout the experiment without the influence of inhalation anesthesia. Both low intensity and high intensity groups illustrated gradually increasing analgesic effect after EA stimulation and the peak MPEs occurred at 20 and 30 min after the stop of stimulation (31.74 ± 8.44 % and 72.50 ± 16.87 % for low and high group, respectively). Low intensity group maintained the elevated latency for 20 min and then declined High intensity group exerted more inhibition effect on tail flick latency than control group and low intensity group (both statistically significant), and the analgesia was far outlasting the EA stimulation period. The analgesia of high intensity of EA stimulation is roughly equipotent to low dose of morphine (3 mg/kg).

Conclusion: Our result proved low frequency peripheral EA stimulation prolonged the tail flick latency in an intensity-dependent manner. This result implies that higher intense peripheral stimulation, which sufficiently activates the high-threshold primary afferents, is required to produce prolonged and stronger analgesic effect.

Results

1. Behavioral response

All rats during and after anesthesia were closely observed to evaluate their behavior response to the electrical stimulation. Corneal reflex and ear pinna reflex were preserved in most rats during anesthesia with 1 % halothane, and no obvious agitation or body movement occurred during electrical stimulation. No hematoma or local tissue swelling at needles insertion sites was observed. Rats could normally recover to consciousness within 5 min after cessation of anesthesia without hindlimb licking or walking handicap.

1.1 Tail-flick test

As illustrated in Fig 1 and 2, the control group kept considerably constant tail-flick latency throughout experiment without temporal influence of 2-hr inhalation anesthesia. Increase of tail-flick latency was shown after electrical stimulation of unilateral hindlimb at 4 Hz frequency for a duration of 30 min at low and high intensity groups comparing to that of control group. Nociception inhibited by low or high intensity EA was statistically significant since 50 and 20 min respectively after treatment (Fig. 1). In the 10T group, two peak MPEs appeared at 50 min (31.74 ± 8.4 %) and 70 min (28.58 ± 2.55 %), whereas, maximal MPEs of 20T group were at 60 min (72.50 ± 16.87

%) and 90 min (71.04 ± 15.25 %). The latter two values were located far beyond the EA treatment period. High inter-individual variations in MPE values could be observed in both EA groups.

Comparing two EA-treated groups, 20T produced higher analgesia than 10T did for 30 min after cessation of electrical stimulation It was important to note that the analgesia produced by 20T group did not decay throughout time, instead, steady increasing of analgesia was present till the end of study. The maximal inhibition of tail flick reaction of high intensity group was comparable to that of morphine-treated group (MPE= 66.29 ± 7.63 %, dose: 2.5 mg/kg), but peak effect of morphine appeared earlier. (Fig. 2)

1.2 Formalin test

The typical biphasic response was illustrated in 3 groups receiving formalin injection (figure 3). Intense mechanical stimulation caused a barrage of primary afferents activation leading to an early phase for a period of 5 min, and followed by another 40-min of late phase representing ongoing inflammation of peripheral nociceptors and increased afferent activities. In EF and MF groups, similarly analgesia at late phase was evident when comparing with control group illustrated by figure 3 and 4 ($p<0.05$), but no statistical difference could be detected between EF and MF groups. We can conclude that the analgesic potency of 20T EA was approximately equal to the strength of 2.5 mg/kg morphine in formalin-induced pain model. Besides, analysis of two summed scores at biphasic time courses, analgesia appeared only at late phase rather than early phase (figure 3, 4), indicating that EA fails to inhibit the brief, intense mechanical pain produced by mechanical injection. Since EA exerted a prolonged after-effect that outlasted its treatment period for over 60 min in tail-flick model, we believe that the decreased pain scores at late phase of EF group was the analgesic effect produced by EA treatment.

2. Fos-like immunoreactivity

C-*fos* immunoreactivity was very slightly detected in all laminae of spinal dorsal horn of rats in both control group and EC group (figure 5). Analyzing individual lamina of two sides of dorsal horn in figure 5, there is no significant difference in numbers of FLI cells can be demonstrated between side-to-side comparison in the same group or group-to-group comparison in the same side ($p<0.05$). Our results revealed that high voltage peripheral electrostimulation did not activate the significant expression

of nociceptive neurons specifically localized at spiral lamina I, II, and V, though it was presumed that EA intensity to the extent of noxious level was required to obtain a analgesic effect. Further, typical induction of c-*fos* expression is predominantly shown at left dorsal horn of either FC group after 1 % formalin injection (figure 6), especially at superficial and deep laminae, but much less shown at right dorsal horn. Noxious response of formalin irritation, however, was significantly suppressed by either pretreatment with high intensity of EA or 2.5 mg/kg morphine for the consequence of decrease in total Fos-LI cells in all laminae of dorsal horn to the extent of 25 % and 20 % in group EF and MF respectively ($p < 0.05$, three columns of T at left panel of figure 6). More specifically, the decrease was mainly manifested at the location of deep layers but not superficial layer or nucleus proprius (figure 6). Similarly, no significant difference in FLI neurons is seen between EA and morphine treatment groups at right side dorsal horn.

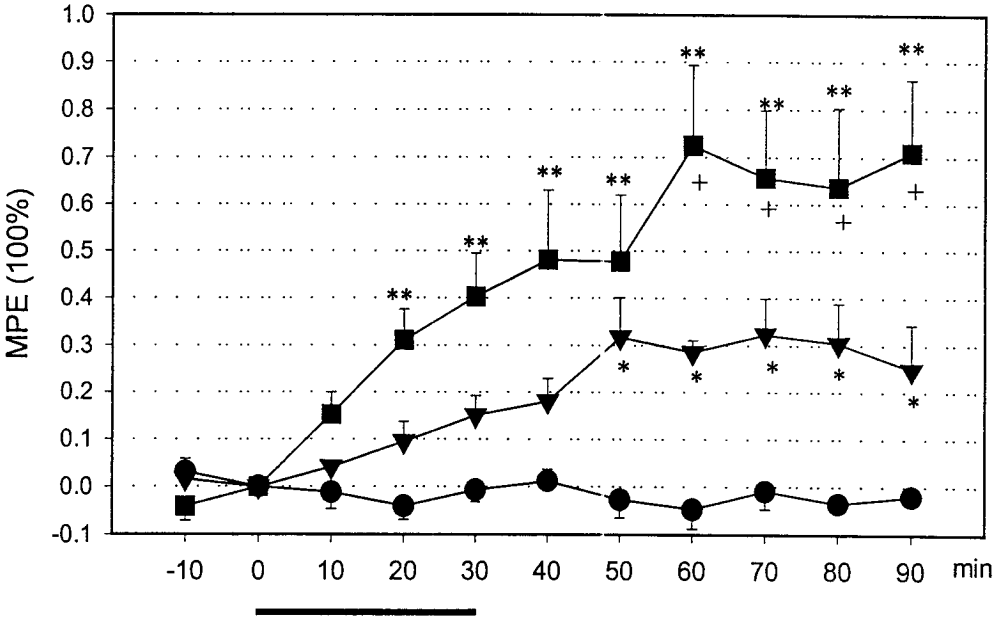


Fig.1. Changes of tail flick latency by electroacupuncture of different stimulation intensities. Tail flick latency is expressed as MPE. Horizontal thick bar indicate electroacupuncture stimulation. Comparison of two group over the whole 90 min test period was analyzed by one way ANOVA with Dunnett's post-hoc test. ■, 20T; ▼,10T; ●, control group. *$P < 0.05$ for 10T group vs. control group, ** $P < 0.05$ for 20T group vs. control group, and + $P < 0.05$ for 20T group vs. 10T group.

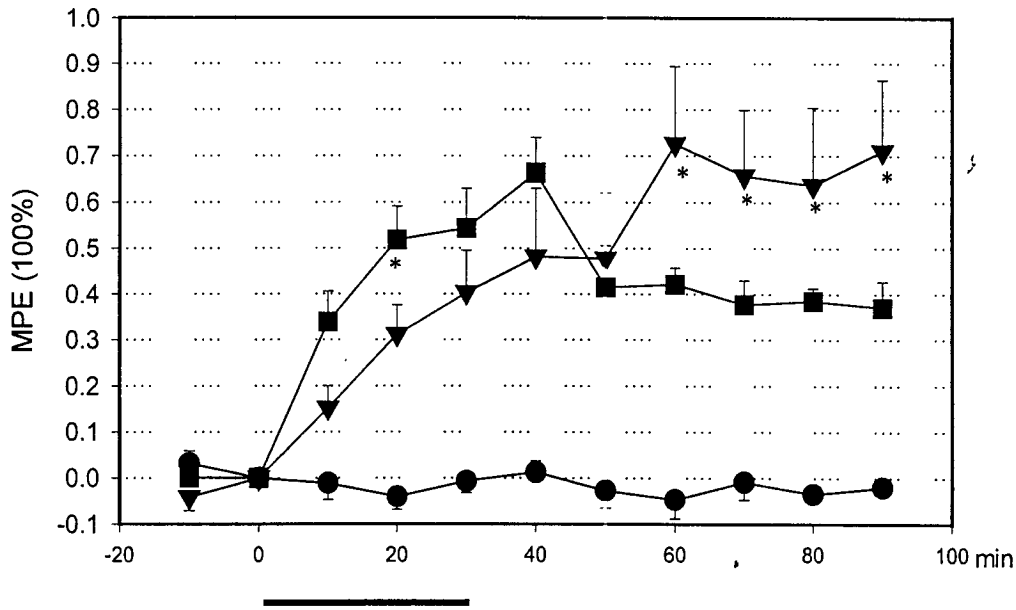
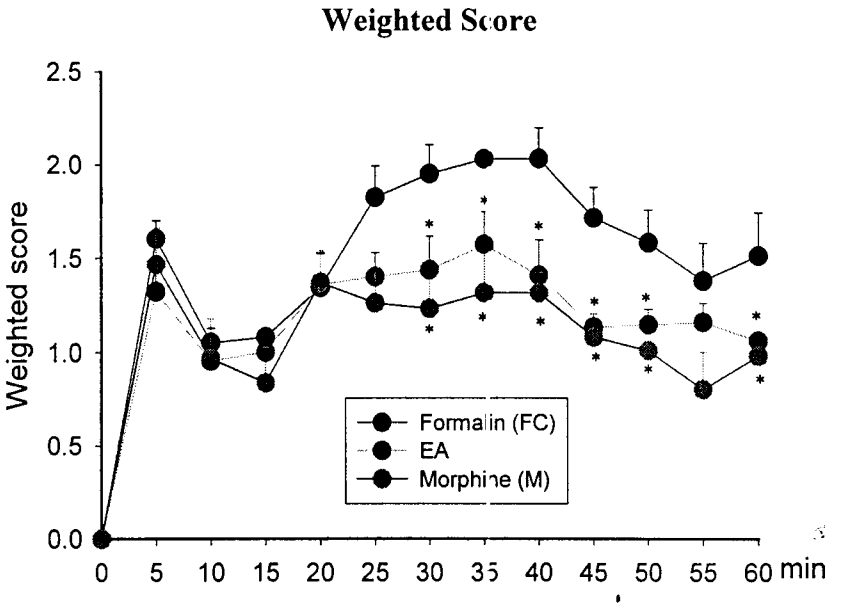


Fig.2. Changes of tail flick latency by high intensity electroacupuncture and morphine. Tail flick latency is expressed as MPE. The thick horizontal bar indicates electroacupuncture stimulation. Comparison between 20T group and morphine group during the whole 90 min test period was analyzed by one way ANOVA with Dunnett's post-hoc test. ■, morphine; ▼,20T; ●, control group. * $P < 0.05$.

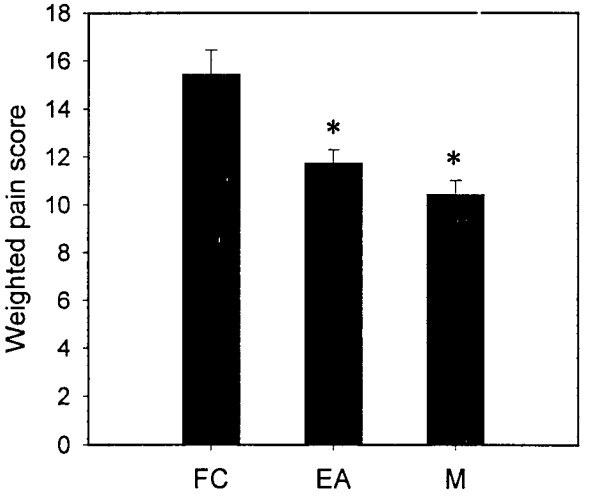


EA and morphine could significantly attenuate the wetighted pain score of formalin injection at contralateral paw

* P< 0.05 for FC group *vs.* EA and M group

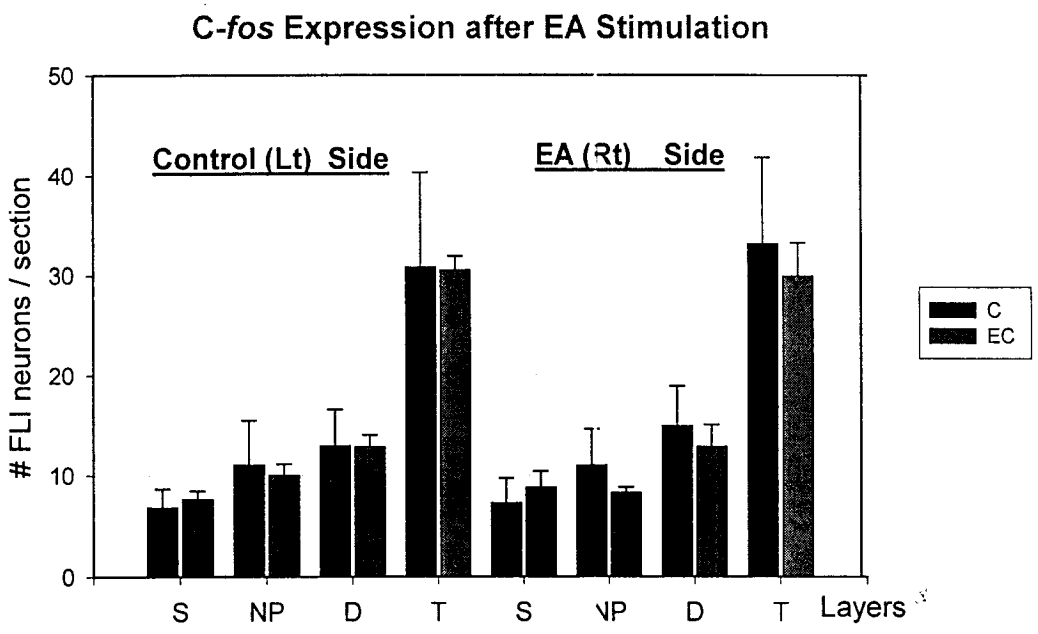
Figure 3

Comparison of Nociceptive Score at late phase



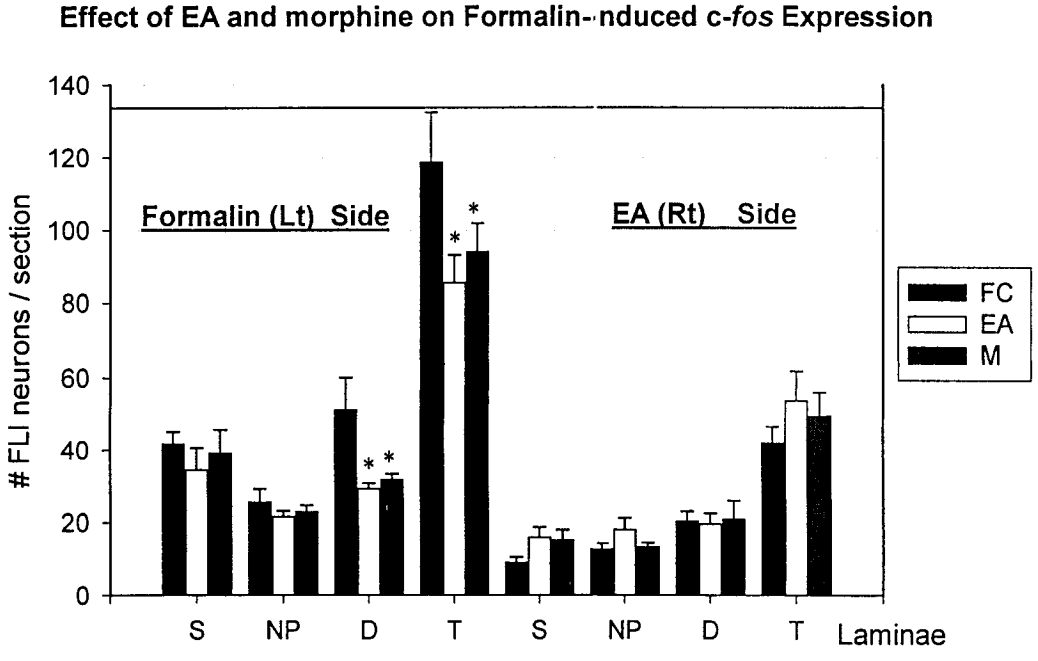
* p<0.05 comparison with Formalin control group using one-way ANOVA with Dunnett's post-hoc test

Figure 4



No difference in c-*fos* expression between the EC group and Control group as well as EA stimulus side and non-stimulus side

Figure 5



Both EA and morphine treatment could significantly attenuate the c-*fos* expression in deep layer of dorsal horn but have no effect on the superficial layer (* $p < 0.05$)

Figure. 6