Modulation of pain signal processing by electric acupoint stimulation: an electroencephalogram study

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KEY WORDS Electroencephalogram; Power spectrum; theta rhythm; beta rhythm; Acupuncture

SUMMARY **Objective:** To investigate the analgesia-related modulation of electroencephalographic activities by transcutaneous electric acupoint stimulation (EAS). **Methods:** In 15 healthy human beings, 64-channel electroencephalogram was recorded and power spectrum analysis was employed before, during and after EAS. Non-acupoint electric stimulation was used as control. All subjects were asked to rate their sensation to painful stimulations before and after treatment. **Results:** The relative theta power near contra-lateral centro-parietal area during EAS was negatively correlated with the pain score after EAS. Similarly, the beta activity during EAS near contra-lateral prefrontal cortex, ipsi-lateral inferior frontal and temporal lobe, and ipsi-lateral occipito-parietal cortex, were all negatively correlated with pain score after EAS. **Conclusion:** These changes might reflect a modulation of brain activity by EAS in specific areas, which were in turn involved in modulation of certain aspects of pain-signal processing.

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Acupuncture and other acupoint stimulation techniques have been used for the treatment of painful disorders for a long time. Great progresses have been made in the past decades to elucidate the underlying mechanisms of acupuncture-induced analgesia[1-3], especially in the field of neurotransmitter release[2] in relevant brain nuclei[4]. Endogenous opioid peptides, such as beta-endorphin and enkephalins[5], were be-

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leved to play an important role in acupuncture-induced analgesia. However, there were two main methodological limitations in the previous research of acupuncture analgesia. One is that most of the invasive techniques applied in animals could not be repeated in human beings; the other is that there is still yet no universal control setting for acupuncture, which might be one of the reasons that different groups reported different, sometimes paradoxical, results. Therefore, the mechanisms of acupoint stimulation-induced analgesia in human beings are still unclear.

In the past decade, electroencephalogram (EEG) was used extensively to explore brain functions for its non-invasive property and high temporal resolution. Several studies had addressed the mechanism of acupuncture-induced analgesia with EEG method[6-8]. However, these previous EEG studies were not properly controlled (no control at all[8] or only compared with analgesics[7]), and it was not clear whether the acupuncture-related changes they demonstrated were anyhow related with the effect of analgesia. Thus, their results were very difficult to be properly interpreted. In the present study, a non-acupoint stimulation session was employed as control condition during EEG recording. The results of power spectrum analysis were also correlated with the effect of analgesia. Our purpose are (1) to find out if there is any difference between the effect of acupoint and non-acupoint stimulation; and (2) to verify if the acupuncture-induced changes of EEG activity are correlated with the effect of analgesia.

1 Materials and Methods

Fifteen healthy and right-handed male volunteers (aged 20 – 28 years, $\bar{x} \pm s$: 23.8 ± 2.8) were enrolled in this study. The experimental procedures were approved by the local ethical committee. Transcutaneous electric acupoint stimulation (EAS) was used to replace conventional acupuncture. The electrical parameters (frequency, pulse width, intensity, *etc*.) have been optimized to produce analgesic effect similar as manual acupuncture. A pair of skin electrodes were placed on the selected acupoints (SP6 or Sanyinjiao and ST36 or Zusanli) on the right leg through which to deliver stimulation. The sham EAS was set at non-acupoints (4 cm medial to ST36 and 3 cm anterior to SP6, respectively, both located on top of the tibia where none of the classical acupoints exists) as the control condition. Each subject underwent both real and sham EAS (the order is balanced across the subjects) separated by an interval of at least 24 hours. The intensity of stimulation varied from subject to subject, ranged from 5 to 15 mA ($\bar{x} \pm s$: 9.79 ± 3.57, $n = 15$), with a subjective criterion that the intensity should be just below the threshold to induce pain or other uncomfortable sensation. The frequency of stimulation was 2/100 Hz (dense-and-disperse square waves, with 2 Hz for 3 seconds and 100 Hz for another 3 seconds in a 6-second cycle). The width of the square wave was 0.6 ms for 2 Hz and 0.2 ms for 100 Hz. Each EAS treatment lasted 15 minutes.

To keep the eye-blink contamination as little as possible, eye-closed EEG was recorded for 3 minutes before and after treatment. On-going EEG was also recorded during the 15-minute EAS treatment with subjects' eyes closed. In addition, fifty painful electric stimulations were delivered to the middle finger of the right hand before and after treatment. All subjects were asked to rate pain sensation with an 11-point (0-10) numerical rating scale for each trial of stimulation[9]. Before the formal recording, subjects were acquainted with this pain rating scale, and the intensities of the stimulation applied on their fingers were individually adjusted to induce the sensation of moderate pain (a score of 6). The subjective pain scores were compared with two-way ANOVA (real EAS *vs* sham EAS and before *vs* after treatment).

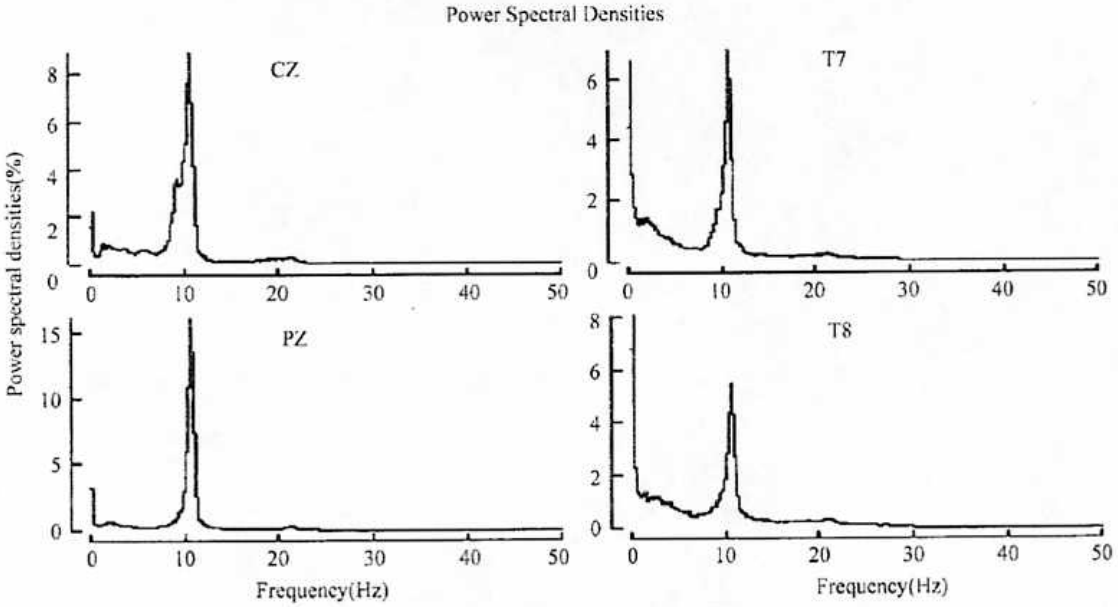
Sixty-four channel EEG recordings, including two pairs of bipolar electro-oculogram (EOG) channels, were continuously performed by the ANT EEG system (Enschede, Netherlands), with the electrode montage following the international 10-20 system (Electro-Cap International, Inc., Dayton, OH, USA). The impedance of all channels was set below 10 kΩ. The signals in all channels were referred to one earlobe, but will be re-referred to the averaged signal across all channels on the scalp in the off-line analysis. EEG data were sampled at 256 Hz, with a gain of 20 and a band-pass of 1 – 30 Hz (digital filtering off-line).

After rejection of EOG contamination, filtering, and re-referring, each set of EEG data were subjected to power spectrum analysis with commercially available software NeuroExplorer (Nex Technologies, Littleton MA, USA). Then the result is sent to Matlab® to summarize the EEG power at each electrode in the following bands: delta (1 – 3.9 Hz), theta (4 – 7.4 Hz), alpha1 (7.5 – 8.9 Hz), alpha2 (9 – 13.4 Hz), beta1 (13.5 – 24.4 Hz), and beta2 (24.5 – 30 Hz). The relative EEG power (%) in three conditions (before, during, and after treatment) was compared with one-way analysis of variance (ANOVA) band-by-band and channel-by-channel. Furthermore, noticing that there is a difference between the analgesic effect of EAS, we divided the subjects into two groups: responders (those who showed decreased pain score after EAS treatment) and non-responders (those who showed increased pain score after EAS treatment). The number of responders for EAS and control stimulation is 9 and 8 subjects, respectively, out of the overall 15 subjects. The comparison between responders and non-responders and that between real and sham EAS were also done by two-way ANOVA in some representative channels of each lobe (FZ, CZ, T7, T8, P1, PZ, P2, OZ). Finally, we found the difference of EEG power spectrum between EAS responders and non-responders (see results) in some selective channels. Hence, correlation analysis was performed between

the relative EEG power of the on-going EEG during the EAS treatment and the averaged pain score after treatment across the fifteen subjects. For clearer presentation of the results, the P values of the correlation analysis in each channel were logarithmically transformed and projected to the scalp with commercially available software ASA (ANT, Enschede, Netherlands).

2 Results

There was no significant difference between the pain scores obtained before and after either kind of treatment (real or sham EAS) (data not shown, $P>0.05$). Two-way ANOVA showed that inter-subject variation explained 86% of the total variation ($P<0.000\ 1$). EEG during either real or sham EAS in most of the subjects was alpha-dominated (Fig. 1). There was no significant difference on the relative EEG power of different time and different treatment (ANOVA, data not shown). However, after dividing the subjects into responders and non-responders, we found theta rhythm of responders during real EAS at PZ channel ($11.04\pm1.16, \bar{x}\pm s_{\bar{x}}, n=9$) was significantly higher than those of non-responders (6.23 ± 0.76, $\bar{x}\pm s_{\bar{x}}, n=6$) ($P<0.01$). Similarly, beta1 rhythm at T8 channel of responders (14.79 ± 1.32, $\bar{x}\pm s_{\bar{x}}, n=9$) was significantly higher than that of non-responders (7.63 ± 1.23, $\bar{x}\pm s_{\bar{x}}, n=6$) ($P<0.01$). No significant difference was detected between responders and non-responders of sham EAS.



Note that alpha rhythm (around 10 Hz) was dominated in all four channels representing different brain areas

Figure 1 Example of power spectrum analysis of EEG during real or sham EAS

More detailed information about the relationship between the theta and beta rhythm and analgesic effect of EAS was found in the correlation analysis. It was found that the theta rhythms (4 – 7.4 Hz) in channels of CPZ, CP1, CP3, PZ, P1 and P3 during EAS were negatively correlated with pain score after EAS (for a sample correlation see Fig. 2), i. e. the lower the pain score in a subject (meaning a better analgesic effect), the higher ratio the theta rhythms are in overall EEG power. In the topographic representation (Fig. 3), a brighter color in the image showed more significant correlation. As shown in the first row of Fig. 3, the most prominent area with theta activity highly correlated with the analgesic effect of EAS was left parietal cortices including primary somatosensory area (SI), which is contralateral to the site of stimulation. Similarly, we found that beta1 and beta2 rhythms (13.5 – 30 Hz) in four channel-clusters, including F3, F5, F7, FC3, FC5 and FT7 (projecting to contralateral prefrontal cortex); F6, F8, FT8, C6 and T8 (projecting ipsilateral inferior frontal cortex); CP6, TP8, P8 and PO8 (projecting to ipsilateral inferior temporal cortex); as well as PZ, P2, P4, P6, PO4, OZ and O2 (projecting to ipsilateral occipito-parietal cortex) during EAS were all negatively correlated with pain score after EAS (see the second and third rows of Fig. 3). No such clustered correlation could be detected in other broad band in real EAS session, and no correlation could be found in sham EAS, either (see right column of Fig. 3).

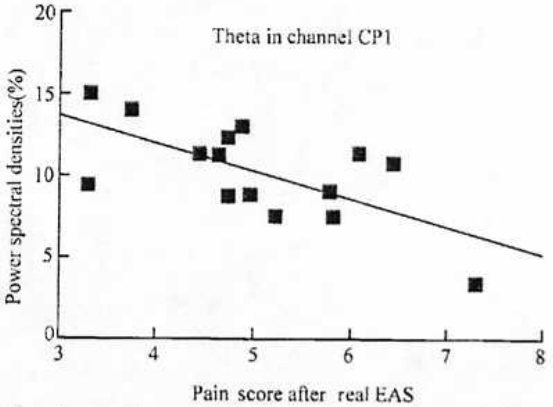
3 Discussion

One reason for the lack of global analgesic effect of EAS could probably be attributed to the moderate intensity of painful stimulation employed in the current study. Another reason was probably the well-known individual variation of acupuncture-induced analgesia[10]. Both in experiments with rats and clinic practice with human beings, diversified responses to acupuncture analgesia were observed. However, there is no acceptable criterion to differentiate high respon-

ders from low-responders. The non-invasive EEG made it possible to find some mechanism related with this phenomena in human beings.

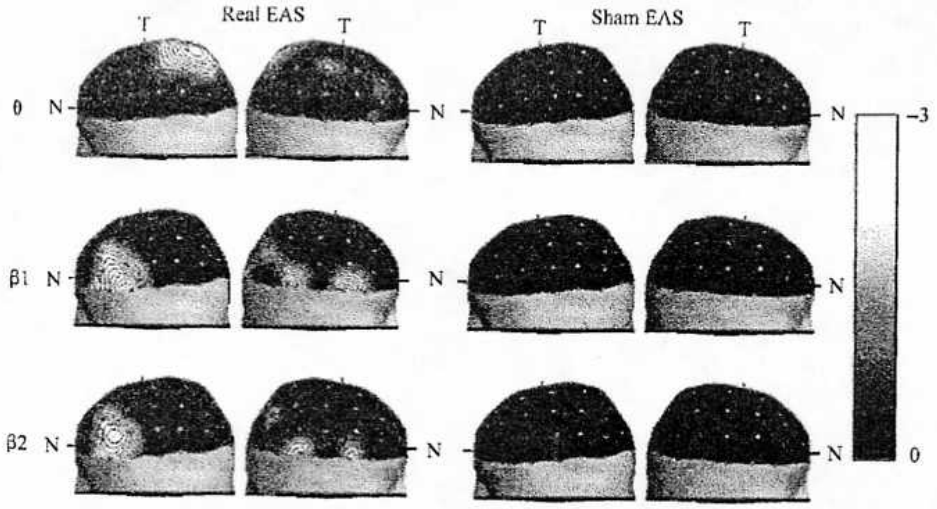
In the current study, we did not find any significant change in the relative EEG power at each electrode, either during or after real or sham EAS, compared with the baseline. It seemed, at first glance, that there was no difference between the real and sham EAS. However, since there were very big variance for both the relative EEG power and the analgesic effect among the subjects, it became interesting to test whether there might be correlation between the relative EEG power and the analgesic effect across subjects.

Our results showed that acupoint stimulation could specifically induce some changes of EEG activity that correlated with the post-stimulation pain score (reflecting analgesic effect); while non-acupoint sham EAS could not. Thus, this might be the first report of EEG study concerning acupoint specificity (no literature was found in PubMed with key words of "EEG acupoint non-acupoint"). Negative correlation was found between the post-stimulation pain scores and the relative power density of theta during the stimulation. In a placebo-controlled double-blinded cross-over study, Bromm *et al*.[11] found that the opioid analgesics like meperidine could increased theta activity of spontaneous EEG. Hence in the present study, the better analgesic effect in some of the subjects might due to the more profound release of the endogenous opioid peptides induced by EAS[2,3], therefore the more abundent theta activity in those subjects.



Relative theta activity was represented as the percentage of the overall EEG power. $r = -0.652\,6$, $P = 0.008\,4$.

Figure 2 Correlation analysis between theta activity during EAS in channel CP1 and pain score after EAS treatment($n = 15$)



P values were first transformed into its negative logarithm based on 10, then assigned the same sign as the correlation coefficients. Note that all correlation represented in this graph is negative so that the grayscale is from −3 to 0. "T" means top and "N" means nose.

Figure 3 Topographic presentations of the *P* values of the correlationanalysis in each channel

It should be noted that correlation between theta rhythm and the pain score is the strongest in contralateral centro-parietal area, including the primary somatosensory cortex (SI). Single cell recordings in rats[12] and monkeys[13] had proved there were nociceptive neurons in SI that responded to noxious stimuli and the activity of these neurons correlated with duration and intensity of the stimulus. In human beings, the same conclusion that SI was involved in the coding of pain intensity was drown via imaging studies[14,15]. Therefore, the correlation between the analgesic effect and theta power in the area near SI in the present study indicated that the activation of the endogenous opioidergic transmission induced by EAS treatment might be able to modulate the processing of the sensory and discriminative dimension of pain signal.

However, theta rhythm is not the sole band of EEG that related with analgesia, as revealed by our present study and the work of Watson *et al*[16]. Watson *et al* found that beta rhythm was correlated positively with pain threshold, which is in accordance with what we found in the current study that higher relative power in beta bands correlate with lower pain score (i. e., better analgesic effect). It should be noted that the involved areas of beta-correlation with analgesic effect were more extensive than that of theta rhythm. Also it tended to involve brain areas of both

side. The involved brain areas were contralateral prefrontal cortex, ipsilateral inferior frontal and temporal lobe, and ipsilateral occipito-parietal cortex. All these areas have been repeatedly mentioned to be involved in the affective and cognitive dimension of pain[7,17,18]. Thus, electric acupoint stimulation might exert its analgesic effect by modulating the activity of these areas and therefore change the processing of the affective and cognitive dimension of pain signal.

In conclusion, our results suggested that there was a difference between acupoint and non-acupoint stimulation from the EEG examination. Changes of theta and beta rhythm in some specific brain areas, during transcutaneous electric acupoint stimulation, are important for the effectiveness of EAS-induced analgesia.

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·短篇报道·

盐酸万乃洛韦缓释胶囊的动物药代动力学及相对生物利用度研究

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盐酸万乃洛韦(valaciclovir hydrochloride)是阿昔洛韦的前体药,即阿昔洛韦的L-缬氨酸酯盐酸盐。其对单纯疱疹病毒1型(HSV-1)和2型(HSV-2)的抑制作用强,对水痘疱疹病毒、EB病毒以及巨细胞病毒的抑制作用弱。

盐酸万乃洛韦口服吸收后分布广泛,可分布到14种组织中,其中胃、小肠、肾、肝、淋巴结和皮肤组织中浓度最高,脑组织中的浓度最低。盐酸万乃洛韦口服后被迅速吸收并转化为阿昔洛韦,达峰时间为0.88~1.75 h,消除为单相,半衰期为(2.86±0.39) h,口服生物利用度为(67±13)%,是阿昔洛韦的3~5倍。其常见不良反应为恶心、头痛、呕吐、腹泻、便秘、眩晕及食欲不振等,因此,为减少给药次数,使血药浓度平稳及降低副作用,本研究制备了盐酸万乃洛韦缓释胶囊,探讨了缓释胶囊多剂量口服达稳态过程和稳态药代动力学特征,并将其与普通片剂进行生物利用度和生物等效性评价比较,为该缓释制剂的临床合理用药提供了实验依据。作者采用高效液相色谱法测定盐酸万乃洛韦的体内转化物阿昔洛韦的血药浓度,考察了盐酸万乃洛韦缓释胶囊和普通片剂多剂量口服达稳态过程和稳态药代动力学特征,研究盐酸万乃洛韦缓释胶囊的动物药代动力学和相对生物利用度。结果表明,连续口服盐酸万乃洛韦900 mg缓释胶囊3 d后,其代谢物阿昔洛韦的血药浓度基本达到稳态水平。缓释胶囊和普通片剂的稳态药物动力学参数T_{max}分别为(1.50±0.45) h和(0.79±0.19) h,C_{max}分别为(7.27±1.20) mg·L^{-1}和(8.07±1.53) mg·L^{-1},C_{min}分别为(3.66±1.36) mg·L^{-1}和(2.48±0.93) mg·L^{-1},波动度DF分别为(80.0±51.6)%和(206.1±78.3)%,以上均提示该缓释胶囊具有缓释特征。缓释胶囊的相对生物利用度为(114.0±14.8)%,经3p97程序生物等效计算,两制剂生物等效。

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