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Background. The neural mechanism underlying the analgesic effect of acupuncture is largely unknown. We aimed at investigating the effect of abdominal acupuncture (AA) on the laser evoked potential (LEP) amplitude and laser-pain rating to stimulation of body parts either homotopic or heterotopic to the treated acupoint.

Methods. LEPs were recorded from 13 healthy subjects to stimulation of the right wrist (RW), left wrist (LW), and right foot (RF). LEPs were obtained before, during, and after the AA stimulation of an abdominal area corresponding to the representation of the right wrist. Subjective laser-pain rating was collected after each LEP recording.

Results. The amplitude of the N2/P2 LEP component was significantly reduced during AA and 15 minutes after needle removal to both RW ($F=4.14$, $p=0.02$) and LW ($F=5.48$, $p=0.008$) stimulation, while the N2/P2 amplitude to RF stimulation ($F=0.94$, $p=0.4$) remained unchanged. Laser-pain rating was reduced during AA and 15 minutes after needle removal only to RW stimulation ($F=5.67$, $p=0.007$).

Conclusion. Our findings showing an AA effect on LEP components to both the ipsilateral and contralateral region homotopic to the treated area, without any LEP change to stimulation of a heterotopic region, suggest that the AA analgesia is mediated by a segmental spinal mechanism.

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Original Article

Homotopic reduction of laser evoked potential amplitude and laser-pain rating by abdominal acupuncture

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Running Head: homotopic effect of abdominal acupuncture on LEPs

Significance: Although abdominal acupuncture has demonstrated to be effective in the reduction of laser evoked potential (LEP) amplitude and laser-pain rating, the exact mechanism of this analgesic effect is not known. In the current study, we found that treatment of an area in the "turtle representation" of the body led to a topographical pattern of LEP amplitude inhibition that can be mediated by a segmental spinal mechanism.

Abstract

Background. The neural mechanism underlying the analgesic effect of acupuncture is largely unknown. We aimed at investigating the effect of abdominal acupuncture (AA) on the laser evoked potential (LEP) amplitude and laser-pain rating to stimulation of body parts either homotopic or heterotopic to the treated acupoint.

Methods. LEPs were recorded from 13 healthy subjects to stimulation of the right wrist (RW), left wrist (LW), and right foot (RF). LEPs were obtained before, during, and after the AA stimulation of an abdominal area corresponding to the representation of the right wrist. Subjective laser-pain rating was collected after each LEP recording.

Results. The amplitude of the N2/P2 LEP component was significantly reduced during AA and 15 minutes after needle removal to both RW ($F=4.14$, $p=0.02$) and LW ($F=5.48$, $p=0.008$) stimulation, while the N2/P2 amplitude to RF stimulation ($F=0.94$, $p=0.4$) remained unchanged. Laser-pain rating was reduced during AA and 15 minutes after needle removal only to RW stimulation ($F=5.67$, $p=0.007$).

Conclusion. Our findings showing an AA effect on LEP components to both the ipsilateral and contralateral region homotopic to the treated area, without any LEP change to stimulation of a heterotopic region, suggest that the AA analgesia is mediated by a segmental spinal mechanism.

Keywords: Acupuncture, laser-evoked potentials, pain perception, spinal cord.

Introduction

Though largely used in the clinical practice, the analgesic effect acupuncture is still debated (Frass et al., 2012). The mechanisms at the base of the acupuncture analgesic action are unknown, even if there is evidence that acupuncture can lead to changes in several brain areas included in the so-called "pain matrix" (Treede et al., 1999; Zhao, 2008). Functional magnetic resonance imaging (fMRI) studies showed the key role played by the hypothalamus-limbic system in acupuncture analgesia (Hui et al., 2000, 2005; Zhang et al., 2003a, 2003b). This is supported also by positron emission tomography (PET) studies (Biella et al., 2001; Hsieh et al., 2001) suggesting that both hypothalamus and insula are activated by acupuncture. Cai et al. (Cai et al., 2018) reviewed all the fMRI studies investigating the functional connectivity during acupuncture. They showed that acupuncture increases the connectivity of both default mode network and sensorimotor network with pain-, affective- and
memory-related brain areas. Moreover, also the connectivity between the periaqueductal gray, anterior cingulate cortex, left posterior cingulate cortex, right anterior insula, limbic/paralimbic and precuneus is increased by acupuncture. It is to be underlined that all the aforementioned areas have been claimed to be involved in the processing of the nociceptive input (Bastuji et al., 2016). In 2015, we showed that in healthy volunteers real "abdominal" acupuncture (AA) was able to reduce the laser evoked potential (LEP) amplitude and laser-pain rating, as compared to sham AA. A reduction of LEP amplitude during AA was found also in patients with fibromyalgia (de Tommaso et al., 2009). The effect of acupuncture on LEP amplitude represents a solid neurophysiological evidence of interaction between acupuncture stimuli and the nociceptive pathway. Indeed, LEP recording represents the most reliable laboratory tool for assessing nociceptive pathway function (Haanpää et al., 2011; Valeriani et al., 2012). The afferent volley generating the scalp LEP components is conducted along the small myelinated (Aδ) primary sensory neurons and the spino-thalamic pathway (Bromm and Treede, 1991).

Although modifications of several brain areas have been demonstrated during and immediately after acupuncture, one may wonder whether acupuncture initially acts at a more peripheral level. The introduction of the needle in the skin stimulates the peripheral nervous fibers, including the nociceptive Aδ and C afferents. This activation can trigger a conditioning pain modulation (CPM) mechanism, which could be responsible of the analgesic effect of acupuncture (Bing et al., 1990; Fields et al., 2005; Villanueva et al., 1986). However,

whether such a CPM mechanism were involved, the needle insertion in any skin area, independently of the stimulation of a specific acupoint, should produce analgesia. On the contrary, the segmental specificity of the analgesic effect would suggest that the nociceptive input inhibition by acupuncture occurs at spinal level.

Here, we aimed at investigating the site of the AA analgesic action. "Abdominal" acupuncture is a particular technique based on the stimulation of abdominal points, according to a "turtle representation" of the somatosensory areas (Fig. 1). As compared to somatic acupuncture, AA is not based on differentiation among syndromes, but only on the symptom location, thus allowing the treatment to be more standardized than in somatic acupuncture. The present experimental design aimed at investigating whether the AA analgesic effect takes place at spinal or cortical level. Therefore, LEPs were recorded to stimulation of three skin areas, differently located in respect of the AA stimulated body region in the "turtle representation": 1) homotopic ipsilateral, 2) homotopic contralateral, and 3) heterotopic.

Materials and Methods

The study protocol was approved by the local Ethics Committee. Thirteen healthy subjects (6 males and 7 females, mean age 35 years, age range 27-50 years) were recruited. All of them signed an informed consent form to participate to the study. Subjects have never experienced any type of acupuncture. Exclusion criteria were: symptoms and/or signs of focal upper limb entrapment, cervicobrachialgia or polyneuropathy in personal history. No subject was aware of the aim of the study. The experiment included three times: 1) baseline, in which LEPs were recorded before AA; 2) acupuncture (AA), in which LEPs were recorded during AA; 3) post, in which LEPs were recorded 15 minutes after needle removal.

Laser stimulation and LEP recording

Laser pulses (wavelength, 1.34 μm) were delivered by a YAP Stimul 1340 (Electronic Engineering, Florence, Italy). LEPs were recorded to stimulation of on three skin areas: 1) the right wrist dorsum (RW), 2) the left wrist dorsum (LW), and 3) the right foot dorsum (RF). The order of the stimulated areas was counterbalanced across subjects. Laser stimulus intensity was fixed at 38 mJ/mm2, which was perceived by all subjects as a painful pinprick (Cruccu et al., 2003; Valeriani et al., 2002). Before LEP recording to stimulation of each site (RW, LW, and RF), subjects were asked to judge this laser pulse intensity according to a 11-point numerical rating scale (NRS), where 0 corresponded to no pain and 10 to the worst imaginable pain. The average ratings were 3.8±1.1, 3.8±0.7, and 3±0.8 for RW, LW, and RF,

respectively. The interstimulus interval varied randomly between 9 and 11 sec. In order to avoid nociceptor fatigue, laser spot was slightly moved from one stimulus to another.

LEPs were recorded from 32 EEG scalp electrodes, placed according to the 10-20 International System. The reference electrode was at the nose, and the ground on the forehead (Fpz). An electrode located above the right eyebrow recorded the electrooculogram (EOG). Signals were amplified and filtered (bandpass 0.3-70 Hz). The analysis time was 1000 ms with a bin width of 2 ms. The pre-stimulus 50-ms interval was set as baseline. Averages of 30 trials were recorded for each stimulation site. In order to ensure that the attention level of our subjects did not change across the whole experiment, they were asked to count the number of the received laser stimuli silently. Averages with a percentage of mistakes higher than 10% were discarded. Since all our subjects kept their attention focused on the laser stimuli throughout the whole experiment, no LEP trial was discarded.

After each LEP recording, all subjects were asked to rate laser-pain intensity by using a 100 mm visual analog scale (VAS), in which "0" corresponds to no pain and "100" to the worst conceivable pain.

Abdominal acupuncture protocol

Acupuncture needles were inserted by the same acupuncturist (SL). The needles were fixed in the areas of the "turtle representation" of the body corresponding to the right wrist dorsum (Fig. 1). Acupoints were selected on meridians crossing the abdomen: 1) the Conception Vessel (CV) meridian (also known as Ren meridian), 2) the Kidney (K) meridian, and 3) the Stomach (ST) meridian (Fig. 1). Distances were calculated according to the traditional Chinese medicine system of *cun*, 1 *cun* corresponding to the width of the thumb of the individual subject. On CV meridian, the treated acupoints were: 1) the 12 CV located 4 cun above the umbilicus, 2) the 10 CV located 2 cun above the umbilicus, 3) the 6 CV located 1.5 cun below the umbilicus, 4) 4 CV located 3 cun below the umbilicus. On K meridian, the contralateral 17 K acupoint, located 2 cun above the umbilicus and 0.5 cun lateral to the 10 CV (2 cun above the umbilicus), was treated. On ST meridian, the bilateral 24 ST acupoint, located 1 cun above the umbilicus and 2 cun lateral to the 9 CV (1 cun above the umbilicus), was treated. Moreover, two extra-acupoints were treated: a) the ipsilateral AB1 located ½ cun above the 9 CV and ½ cun laterally to the 24 ST, and b) the ipsilateral AB2 located 1 cun laterally to the 24 ST.

LEP analysis and statistics

LEP components were identified according to their polarity and distribution. In particular, we identified a temporal lateralised component (N1), with an almost simultaneous frontal

positive potential (P1). These were followed by a larger vertex biphasic potential reaching its maximal amplitude on the Cz vertex (N2/P2). Intracerebral recording studies and dipolar modeling studies agree in suggesting that the N1 and P1 potentials are probably generated in the opercular (SII/insula) area (Frot et al., 2008; Valeriani et al., 1996, 2000). The N2 and P2 are probably originated from different sources including midcingulate cortex and insula (Dowman et al., 2007; Garcia-Larrea et al., 2003).

N2 and P2 peak latencies were measured at Cz electrode. The peak-to-peak N2/P2 amplitude was calculated at F3, Fz, F4, Fc1, Fc2, C3, Cz, C4, Cp1, Cp2, P3, Pz, P4 electrodes. The N1 latency was measured on the temporal trace contralateral to the stimulation, and the N1 amplitude was calculated by referring the temporal electrode contralateral to the stimulation (T3/T4) to the Fz lead off-line (Kunde and Treede).

In order to investigate the effect of the laser stimulation site, LEP latencies underwent two-way ANOVAs by considering stimulation site (RW, LW, and RF) and time (baseline, AA, post) as variables.

The N1 amplitude was analysed by two-way ANOVA with laser stimulation site and time as variables. For each laser stimulation site, possible AA-related modifications in the topography of the N2/P2 amplitude were checked by two-way ANOVAs by considering the recording electrodes and the time as variables. No significant interaction electrode X time was found ($p>0.05$), thus suggesting that the N2/P2 topography did not change across the experiment. Therefore, we submitted the N2/P2 amplitude measured at Cz to two-way ANOVA by considering stimulation site and time as variables. Moreover, in order to reduce the effect of the N1 and N2/P2 amplitude interindividual variability, we calculated the N1 and N2/P2 amplitudes recorded during AA and post as percentages of the corresponding amplitudes obtained at the baseline. These normalized values underwent two-way ANOVA by considering stimulation site and time as variables. Post-hoc analysis was performed by paired Student's t-test with Bonferroni correction for multiple comparisons.

VAS values underwent two-way ANOVA by considering stimulation site and time as variables. Post-hoc analysis was performed by paired Student's t-test with Bonferroni correction for multiple comparisons.

Statistical significance was set at $p<0.05$.

Results

All LEP latencies (Table 1) did not change across the experiment ($p>0.05$).

Neither the absolute ($F_{(2,13)}$=0.05, p=0.8) nor the normalized ($F_{(2,13)}$=0.24, p=0.6) N1 amplitudes were significantly modified during and after AA (Fig. 2).

As for the absolute N2/P2 amplitudes we found a significant effect of time ($F_{(2,13)}$=10.59, p<0.001). Moreover, when we considered the normalized N2/P2 amplitude values (Table 2), a significant interaction stimulation site X time was found ($F_{(2,13)}$=3.18, p=0.04), suggesting a different effect of time on LEPs recorded to the different stimulation sites (Figures 2 and 3). Therefore, we investigated the AA effect on the N2/P2 amplitude recorded to stimulation of each site (RW, LW, and RF) by one-way ANOVAs, considering the time as the variable. A significant effect of time was found for RW ($F_{(2,13)}$=4.14, p=0.02) and LW ($F_{(2,13)}$=5.48, p=0.008) stimulation, while no significance was found for the RF stimulation ($F_{(2,13)}$=0.94, p=0.4). Post-hoc analysis showed that for both RW and LW stimulation the N2/P2 amplitudes at AA and post times were significantly lower than that at the baseline (p<0.001) (Fig. 4). No difference was found between the N2/P2 amplitude recorded during AA and 15 minutes after needle removal (p>0.05).

When we examined the laser-pain rating, a significant effect of time was found ($F_{(2,13)}$=3.99, p=0.02). AA effect for each stimulation site was investigated by one-way ANOVA which was significant only for the RW stimulation ($F_{(2,13)}$=5.67, p=0.007), but not for both LW ($F_{(2,13)}$=0.15, p=0.9) and RF ($F_{(2,13)}$=1, p=0.4) stimulation. Post-hoc analysis showed that VAS values obtained to RW stimulation at both AA and post were significantly lower than those at baseline (p<0.001) (Fig. 4).

Discussion

Our study showed that AA reduces the N2/P2 LEP amplitude evoked by stimulation of both the ipsilateral and contralateral region homotopic to the treated area in the "turtle representation" of the body. Moreover, the subjective rating of laser-pain is reduced only to stimulation of the ipsilateral homotopic area. Both N2/P2 amplitude reduction and laser-pain rating kept being reduced even 15 minutes after needle removal.

AA effect on LEP amplitudes

Two previous studies demonstrated that AA exerts an inhibitory action on both LEP amplitude and laser-pain rating. De Tommaso and colleagues (de Tommaso et al., 2014) showed that AA reduced the N2/P2 amplitude to stimulation of the chest tender points in 10 patients with fibromyalgia. Interestingly, when laser pulses were delivered on the abdomen, closely to the treated acupoints, the N2/P2 amplitude was increased. According to the authors, this finding suggests that the AA analgesic effect is possibly mediated by a complex

interaction, occurring within the brain, between the nociceptive input triggered by needle insertion and that originated from a different body region. Our group showed that in 10 healthy subjects AA was able to reduce both N1 and N2/P2 amplitudes to stimulation of the region corresponding to the treated area in the "turtle representation" of the body (Pazzaglia et al., 2015). This inhibition persisted even 15 minutes after needle removal. Both studies had a crossover design, meaning that the same patient/healthy subject was treated also with sham AA, that did not have any inhibitory effect on both LEP amplitude and laser-pain rating. De Tommaso et al. considered the needle insertion in points that should not be effective as sham AA, although acupoint specificity is still a debated issue (Choi et al., 2012). In our study, the subject was cheated, since the needle did not penetrate the skin during sham AA, but it was inserted in a plastic tuboguide with a closed sharp base just applied on the abdomen (Juel et al., 2016; de Tommaso et al., 2014).

In the present study, our healthy subjects did not undergo sham AA, since here we aimed at investigating the AA effect on LEPs also to stimulation of regions different from that corresponding to the treated one in the "turtle representation" of the body. We confirmed that AA is able to reduce the N2/P2 amplitude and laser-pain rating to stimulation of the ipsilateral body region homotopic to the treated area. Moreover, we originally showed that the N2/P2 amplitude was significantly reduced during AA and 15 minutes after needle removal also to stimulation of a body part (LW) homotopic but contralateral to the treated area. No AA effect on LEPs was found to stimulation of a heterotopic region, such as the right foot. As compared to our previous results (Pazzaglia et al., 2015), here we did not find a significant reduction of the N1 amplitude during AA. We think that this could be due to the large interindividual variability of the N1 amplitude which may prevent slight modifications to be detected. Indeed, although a decrease of the N1 mean amplitude during AA was observed to both RW and LW stimulation, it did not reach the statistical significance.

Presumed sites for the AA analgesic effect

The present results allow us to speculate about the AA site of action. Indeed, AA could act at level of the brain trough two different general mechanisms. First, a general inhibition of the pain perception and the nociceptive brain responses may be due to the divergence of the subject's attention far from the painful stimuli. In other words, it is possible that AA represented a distractor for the subject who addressed a lower attention amount towards the laser pulses. Several studies showed that distraction from the painful laser pulses affects the LEP, especially N2/P2, amplitude largely (Lorenz and Garcia-Larrea, 2003). Second, the N2P2 amplitude and laser-pain rating reduction in our experiment might be explained by the

phenomenon of the habituation. This consists in a reduction of long latency evoked potential, including LEP (Valeriani et al., 2003), amplitudes after repetitive stimulation. The design of the present study, in which LEPs were recorded at three different times (baseline, AA, and post), could make habituation bias our results. However, both distraction from the painful laser pulses and habituation should involve also LEPs to stimulation of the foot. The unchanged LEP amplitude and laser-pain rating to RF stimulation are strongly against both hypotheses.

N2/P2 and laser-pain inhibition during and after AA could take place at spinal level. This effect may occur through an activation of the descending inhibitory control. Some cerebral areas generating LEPs, such as insula and anterior cingulate cortex, belong to the brain opioid system (Petrovic et al., 2002), thus AA could trigger a descending inhibition mediated by endogenous opioids. This mechanism of action is supported by the close relationship between the analgesic effects of acupuncture and opioids (Harris et al., 2009; Pomeranz and Chiu, 1976; Zhao, 2008). Spinal dorsal horn is particularly rich of opioid receptors, especially μ-opioid receptors (Mansour et al., 1995). They are localized on both the terminal of primary afferent neurons and cell bodies, so that their stimulation by μ-opioid receptor agonists, including endogenous opioids, leads to presynaptic inhibition of neurotransmitter release (Mense, 1983) and postsynaptic hyperpolarization of the excitatory second-order neurons (Mizoguchi et al., 2014), respectively. The hypothesis can be made that AA, stimulating the nociceptive fibers, triggers a CPM, which hampers both LEP amplitudes and laser-pain, maybe through endogenous opioids. CPM represents the human behavioral correlate of diffuse noxious inhibitory control (DNIC), described in rats by Le Bars and colleagues (Le Bars et al., 1979). CPM is usually evaluated as the analgesic effect of a painful conditioning stimulus on a distant painful test stimulus, which can be concomitant to or slightly following the conditioning stimulation (Kennedy et al., 2016). However, if it were the CPM to inhibit LEPs in our study, we should expect that the N2/P2 amplitude and the subjective rating of laser-pain were reduced also to RF stimulation.

Our data showing a reduction of the N2/P2 amplitude to stimulation of both the ipsilateral and contralateral region corresponding to the AA treated area in the “turtle representation” of the body strongly suggest a segmental inhibition at level of the spinal cord. Gjerstad and colleagues (Gjerstad et al., 2000) showed that intra muscular-injected capsaicin inhibits the contralateral dorsal horn neurons, demonstrating a connection between bilateral spino-thalamic neurons of the same spinal segment. Therefore, a segmental spinal event is likely to involve neurons of both sides. That the nociceptive input coming from a heterotopic zone,

such as the right foot, is not dampened by AA further supports the hypothesis of a segmental spinal inhibition. Our data agree with the findings of Zhang et al. suggesting a direct spinal action of acupuncture on spinal calcium-dependent protein kinase II, linked to analgesia (Zhang et al., 2018).

In our subjects, laser-pain rating was reduced during and after AA only to stimulation of the ipsilateral homotopic region (RW). This is not surprising, since it is possible that the contralateral AA effect is weaker, thus evident only at level of the LEP amplitude.

Conclusions

Our study showed that AA is able to dampen the LEP amplitude to stimulation of the bilateral region homotopic to the treated area. The lack of any change of LEP amplitude to stimulation of a heterotopic body part supports the hypothesis of a segmental analgesic effect at spinal cord level. Interestingly, laser-pain rating was reduced only to stimulation of the region corresponding to the treated area (right wrist), thus suggesting that the AA analgesic effect in a determined body region can be obtained only by stimulating specific acupoint(s). Since LEP amplitude reduction to stimulation of the contralateral homotopic region (left wrist) did not have a psychophysical counterpart, it is possible that the contralateral AA effect is weaker, thus evident only at level of the neurophysiological measurement.

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Table 1 - Mean values (± SD) of VAS, N1 latency and amplitude, N2 and P2 latency, and N2/P2 amplitude

	VAS			**N1 latency (ms)**			**N1 amplitude (μV)**			**N2 latency (ms)**			**P2 latency (ms)**			**N2/P2 amplitude (μV)**		
Laser stimulation	*Baseline*	*AA*	*Post*	*Baseline*	*AA*	*Post*	*Baseline*	*AA*	*Post*	*Baseline*	*AA*	*Post*	*Baseline*	*AA*	*Post*	*Baseline*	*AA*	*Post*
RW	47.3 (±15.9)	33.1 (±13.9)	28.8 (±14)	195.6 (±54.5)	195 (±47.7)	208.1 (±23)	6.9 (±6.2)	3.8 (±2.7)	5.8 (±3.5)	234.6 (±24)	221.7 (±24.3)	231.7 (±29.2)	347.7 (±26.5)	340.3 (±42.6)	343.1 (±39.6)	29.8 (±17.9)	17.9 (±9.4)	20 (±11.2)
LW	41.7 (±15.5)	38.6 (±20.3)	38.1 (±18.3)	211.5 (±26.1)	193.1 (±42.5)	200.9 (±58.9)	7.6 (±6.1)	5.2 (±4.5)	6 (±4.3)	226.8 (±16)	237 (±13)	237.5 (±22.2)	342.8 (±30.8)	325.5 (±24.7)	333.1 (±24)	30.3 (±11.7)	16.8 (±9)	20.9 (±11.3)
RF	52.5 (±22.6)	42.7 (±19.5)	41.9 (±21.8)	246.4 (±50.3)	241.9 (±53.6)	238.2 (±58.9)	6 (±4.9)	4.5 (±3.6)	5.2 (±3.8)	263.7 (±23)	278.4 (±30.7)	263 (±28.2)	375.5 (±24.6)	389.5 (±43.5)	371.5 (±31.4)	25.2 (±11.4)	22.5 (±9.8)	19.6 (±10)

RW=right wrist, LW=left wrist, RF=right foot, AA=acupuncture, P=post

Table 2 - Mean values (± SD) of normalized* N1 and N2/P2 amplitudes

	N1 amplitude (%)		**N2/P2 amplitude (%)**	
Laser stimulation	*AA*	*Post*	*AA*	*Post*
RW	63.7 (±31.4)	82 (±34.9)	59.5 (±15.9)	66.4 (±21.8)
LW	75.6 (±51.2)	87.9 (±56.7)	54.2 (±18.1)	66.5 (±25)
RF	95.1 (±77.8)	82.6 (±59)	96.9 (±30.4)	80.1 (±18.8)

*Values obtained in AA and Post are expressed as percentages of those recorded at baseline

RW=right wrist, LW=left wrist, RF=right foot, AA=acupuncture

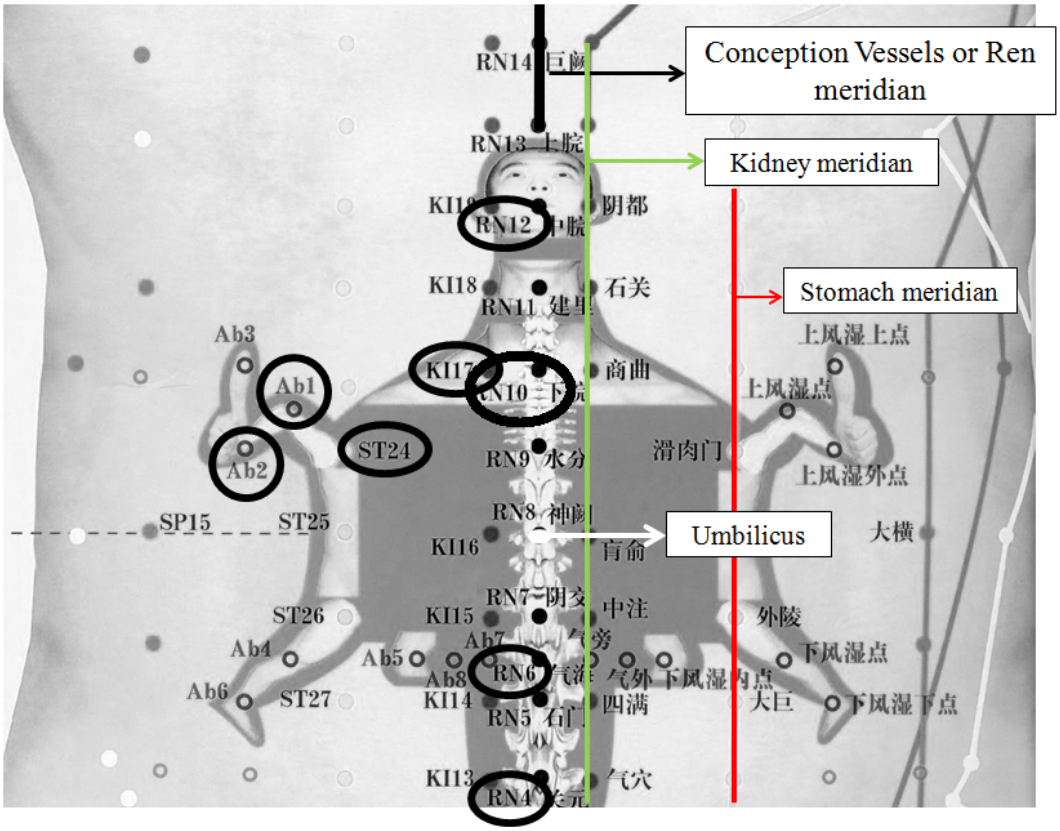
Legends

Figure 1. Localization of acupoints around the umbilicus. They are distributed according to a so – called "turtle representation of somatosensory areas". The acupoints selected in our protocol are included in circled boxes.

Figure 2. Grand-average waveforms recorded from the contralateral temporal electrode referred to Fz (Tc-Fz) and Cz lead. Traces recorded at baseline (blue), AA (orange), and post (gray) times are superimposed. Notice the N2/P2 amplitude reduction to both RW and LW stimulation in both the AA and post times, as compared to baseline.

Figure 3. Cz traces recorded from all our subjects at baseline (left), AA (middle), and post (right) times are superimposed. LEPs recorded to RW, LW, and RF stimulation are shown in the upper, middle, and lower raw, respectively. Notice the N2/P2 amplitude reduction to both RW and LW stimulation in both the AA and post times, as compared to baseline.

Figure 4. The histograms show the N2/P2 amplitude (upper), N1 amplitude (middle), and laser-pain rating (lower) modifications at the baseline (black), and during the AA (red) and post (green) times. Asterisks show the significant differences.



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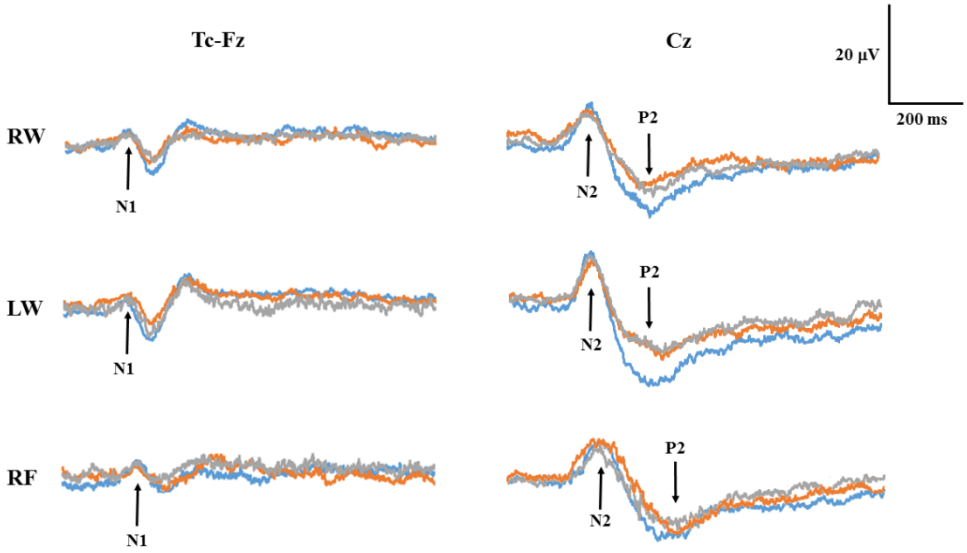

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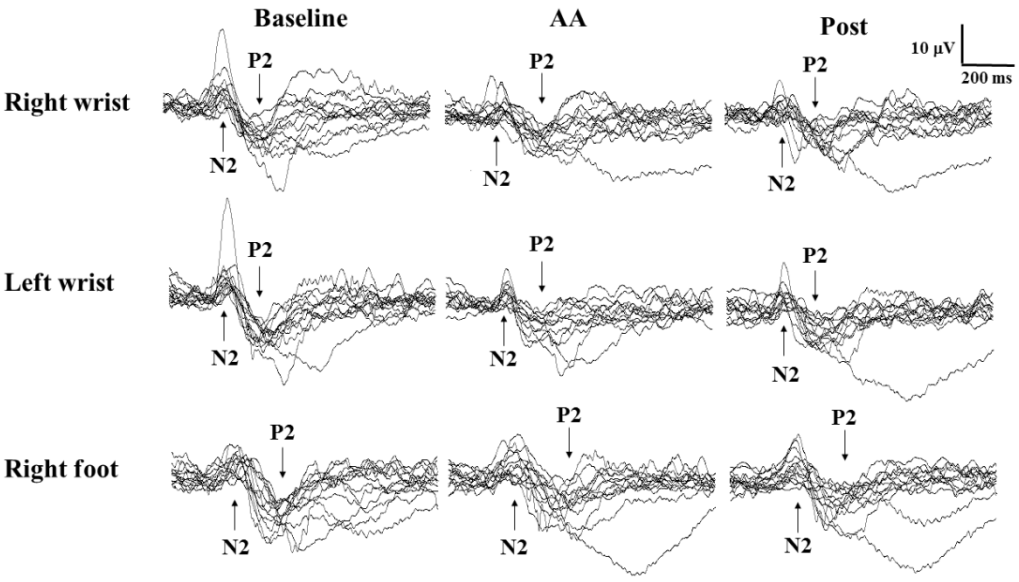

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