The Effect of Acupuncture on Temporal Summation of Pain: A Randomised, Double-Blind, Sham-Controlled Study

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Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed.

Signature:

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Date: 25 August 2007

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Summary

BACKGROUND: The analgesic effects of manual acupuncture (MA) and electro-acupuncture (EA) have been studied in healthy humans and patients with pain. The advantage of studying pain in healthy humans is that the intensity of stimulation can be accurately controlled and thereby the analgesic effect can be quantitatively assessed. However, an important difference between experimentally induced pain and clinical pain is central sensitisation, that is, an enhanced activity of the central nervous system (CNS). Temporal summation (TS) of pain refers to pain induced by repeated stimulations at sub-threshold level. It is a central phenomenon that reflects the sensitivity of CNS. The electrical TS pain model has been validated and applied to quantitatively determine the levels of analgesia and the central inhibition effects of analgesic medications.

OBJECTIVES: The present study aimed to: 1. systematically review available randomised, controlled trials (RCTs) of acupuncture on experimentally induced pain in healthy humans; 2. conduct a RCT to assess the effect of MA and EA on TS of pain and the spatial characteristics of this effect (i.e. the same and different dermatome segments to the acupuncture point), and the temporal factors (i.e. immediately after and 24-hours after intervention).

METHODS: The systematic review was carried out in accordance with the requirements of a Cochrane Systematic Review. The methodological quality and quality of the acupuncture techniques of the included RCTs were assessed. The Review Management software (RevMan version 4.2, The Cochrane Library) was used for data extraction and data analysis.

For the present experiment, 27 healthy volunteers were recruited and randomly assigned to either EA, MA or sham-acupuncture (SA) group, with nine volunteers in each group. The acupuncture sites were ST36 and ST40 on the dominant leg. Both the volunteers and the assessor were blinded to the treatment allocation. A second researcher, who was blinded to the outcome assessment processes, delivered all the interventions. Each treatment lasted for 25 minutes. To test pain thresholds, transcutaneous electrical stimulation was delivered to three sites: 1. the tibia anterior muscle along the sural nerve path of the treatment leg and parallel to the mid-point between ST36 and ST40; 2. the same area on the other leg; and 3. the dorsum of the non-dominant forearm along the median nerve path and 3 to 4 cm above the wrist crease. Pain thresholds to single electrical stimulation (SPT) and to TS stimulation (TST) were assessed before, 30-minutes after and 24-hours after the intervention. Ratings to supra-threshold stimulation at intensities of 1.2 and 1.4 times the TST were assessed with a visual analogue scale. The level of anxiety was assessed before and after acupuncture.

Data on pain thresholds and anxiety scores were analysed with one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS, Version 13.0) to detect between treatment group differences. Significance for each of the ANOVAs was assessed at $\alpha = 0.05 / 3 = 0.0167$ (Bonferroni Correction). When a significant ANOVA was obtained, Bonferroni corrected post-hoc analyses were applied for multiple-comparisons. Equivalence of the groups on demographic variables was assessed by ANOVA and *chi*-square tests. Power analysis and sample size calculations were performed using MINITAB (Version 15.0).

RESULTS: The literature search identified 605 papers, however, only nine papers met the inclusion criteria and thus included in this review. The methodological quality and

quality of the acupuncture procedures were satisfactory. In these studies, the pain models and interventions varied substantially, therefore, meta-analysis was not practicable. Four studies employed both invasive and non-invasive controls with three of them reported that the invasive controls induced significantly stronger analgesia than the non-invasive controls. One study reported that there was no difference between EA and MA tested using a heat pain model.

In the present RCT, baseline values were comparable among the three groups with respect of SPT and TST. Overall, the baseline values of TST were lower than those of SPT. Within group comparison, the level of anxiety did not change significantly after the inventions. Between group comparisons, when sufficient statistical power was demonstrated, indicated that EA significantly increased SPT and TST on the treatment leg 24-hour after the treatment when compared with SA. In addition, the EA effect was not found on the non-treatment leg or the forearm. For the delivery of the supra-threshold stimulation, 1.2 and 1.4 times of the TST of each time point, instead of baseline TST, was mistakenly applied. Data from this component of the study were not analysed, however presented in the thesis for information.

As the first study in this field, the current findings provide the base for sample size calculation. For example, the sample sizes for EA and MA comparisons with 80% statistical power at a significance level of 0.05 will be 21 subjects in each group to detect the immediate effect of acupuncture on TST, ; and 11 to detect the effect of acupuncture after 24-hour.

CONCLUSIONS: The systematic review showed that there has been only a small number of experimental RCTs. Comparing acupuncture with non-invasive control,

significant acupuncture analgesia was reported. These studies also demonstrated that invasive controls produced analgesia. Thus, future studies should consider using non-invasive intervention as control. No conclusion could be drawn regarding the relative analgesic effect of EA versus MA.

Consistent with previous studies, TS of pain can be successfully elicited in healthy humans with electrical stimulation. This study on TS demonstrated that the effect of EA was stronger than SA. The fact that such an effect increased within 24 hours after acupuncture might indicate the potential role of neurohumoral mechanisms in acupuncture analgesia. The spatial effect of acupuncture tended to be localised at the needling site. It is important to note that acupuncture increased both SPT and TST, which may suggest that both peripheral and central nervous systems mechanisms are involved in acupuncture analgesia.

Chapter 1: Introduction

1.1 Studying acupuncture analgesia in healthy humans

Acupuncture is a stimulation-dependent intervention which has been widely used in clinical practice to handle a wide range of pain syndromes (1). For example, patients with low back pain (2; 3; 4); arthritis (5); headache (6); fibromyalgia (7), and other painful disorders (8; 9; 10).

Clinical pain is difficult to study and to compare between patients as many factors may affect the reporting of pain, such as the psychological state of patients and the degree of peripheral or central nervous system changes associated with diseases. In a clinical setting, these psychological and physiological changes are almost impossible to quantify (11; 12). Evoking and testing pain in healthy humans eliminates these factors, and has been proved to be a useful way to investigate the nociceptive functions of humans. Studying pain in healthy humans also has the advantages of precise control of experimental stimulations, the delivery of interventions and the utilisation of multiple outcome measurement tools. It allows researchers to control and quantify the intensity of stimulation, correlate the strength of stimuli with the rating of pain and directly compare pain within and among subjects or before and after an intervention (13). Studying pain in healthy humans has improved our understanding of pain, such as age and gender differences in the response to and the reporting of pain (12; 14).

When studying pain in healthy humans, the methods used include testing of pain threshold (PT), pain tolerance threshold (PTT) and rating to supra-threshold (ST) stimulation. The International Association of the Study of Pain's (IASP) definition of pain threshold is that "*the least experience of pain which a subject can recognize*; and

pain tolerance threshold is *the greatest level of pain which a subject is prepared to tolerate"* (15). The descriptions to these pain thresholds are clarified as follows. During experimental pain studies, when a stimulus is delivered to the skin of a human, one first feels some sensation, and the intensity at this level is called sensory threshold. With increasing intensity, one starts to feel slight pain, and the intensity at this level is called 'pain threshold'. As intensity continue to increase, one will feel pain getting stronger and to a degree, one does not want to tolerate the pain any more, this level of stimulation is called 'pain tolerance threshold'. The pain ratings in response to the stimulations between the PT and PTT levels are called the 'ratings to supra-threshold stimulations' (13). In the thesis, the term 'pain perception study' refers to studies testing any of these pain thresholds.

Observation of changes in pain thresholds provides direct evidence of acupuncture analgesia in humans (16). Such studies have been conducted since the 1970s to better our understanding of acupuncture analgesia. The literature review in chapter 3 summarises the observations of these studies. By comparing human pain thresholds before and after interventions, i.e. manual acupuncture (MA) or electro-acupuncture (EA), many studies have shown that EA and MA both have greater analgesic effect than sham-acupuncture (SA). Furthermore, some of these studies also provided evidence of the spatial distribution of the analgesic effect (17; 18), the involvement of naloxone (19), and the analgesic mechanisms involved in EA (20; 21; 22).

However, there are three limitations of previous pain perception studies. Firstly, most of the previous studies tested pain thresholds to single stimulus which may not mimic clinical pain; these pain perception studies are different from clinical pain conditions in neurophysiologic aspects (23). One of the significant differences between pain threshold

studies and clinical pain is central sensitisation (12). Central sensitisation means the central nervous system is amplifying the activity-dependent afferent signals (24). Studying the central sensitisation is important because it underlies clinical pain, such as pain on light touch, pain on pressure or pain on movement (12). Secondly, in most cases, only the effect immediately after acupuncture was investigated. However, in clinical practice the acupuncture analgesic effect is thought to last for 24 to 72 hours (25; 26). Thirdly, the spatial distribution of the acupuncture analgesic effect is rarely studied to explain the general effect of acupuncture. In a recent study, the comparison between neural Segmental Inhibition Theory and traditional Meridian Theory was studied by measuring the change in pressure pain threshold at various sites of the body (17). MA on LI4, one classic analgesic acupoint, led to significant increases in the pain threshold at all measured sites; the result partially supports both Segmental Inhibition Theory and Meridian Theory. Therefore, further study is required to determine how the actions of acupuncture differentially affect different parts of the body i.e. the spatial distributing action of acupuncture.

1.2 Temporal summation of pain

Temporal summation (TS) of pain describes a central phenomenon in neurophysiology. A non-painful stimulus when repeated at a certain frequency can induce a painful sensation. It is understood that repeated afferent signals cause gradually enhanced activities of the wide dynamic range (WDR) neurons leading to painful sensation; and its underlying mechanism is associated with the activation of N-methyl-D-aspartic (NMDA) receptors (27; 28; 29; 30; 31). TS of pain can be induced with thermal (28), mechanical (32) or electrical stimulation (33). The commonly used 2-hertz electrical TS pain model was developed by Arendt-Nielsen and his colleagues in 1994 (33). Its reproducibility and reliability have also been tested (31; 34; 35; 36; 37). This model has been used in a number of studies to quantitatively assess the effectiveness and the central inhibitory effects of analgesics in healthy pain-free humans (see Chapter 2 for detail of these studies). For example, ketamine (38), codeine (34; 39), and venlafaxine (36; 40).

Employing this electrical TS pain test model to study acupuncture analgesia can help to address the limitations mentioned above (section 1.1). Using this valid pain model to study acupuncture analgesia allows researchers to quantitatively evaluate the analgesic effects of different acupuncture techniques and identify their central inhibitory properties associated with central sensitisation. Thereby, this approach may contribute to the overall understanding of the mechanisms of acupuncture analgesia.

There are different models that can be used to mimic clinical pains in healthy humans other than TS model, such as hyperalgesia model. Hyperalgesia is *an increased response to a stimulus which is normally painful* (15). The stimulation used for the hyperalgesia test is usually induced by prolonged, noxious heat, mechanical or electrical stimulation. The hyperalgesia model is different from the TS model as the stimulations are noxious,

i.e. at supra-threshold level. TS and hyperalgesia pain models can be used to evaluate the different levels of sensitivity of the central nervous system. TS model has a few advantages than a hyperalgesia model. First, the stimulations of TS are usually not painful and can be easily accepted by the participants. Second, the frequency of the repeated stimulation can be readily controlled so as to indicate the levels of central sensitisation (33). Third, the methods of TS pain model are well validated (see paragraph 1 in this section).

1.3 Selection of stimulations for the study

There are a few types of stimulations that can be used to test TS. They are electrical, mechanical and thermal stimulations. Electrical stimulation has obvious advantages of easy control and recording. However, electrical stimulation method has three major shortcomings. Firstly, it is unnatural and non-physiological. Secondly, it does not selectively activate certain afferent fibres. Thirdly, the information during the transduction processes is lost because electrical stimulations directly activate the sensory nerve endings (41). The mechanical and thermal stimulations are categorized as natural stimulations, i.e., physiologic stimulations (41; 42). Modern instruments can provide precise control for the delivery of mechanical and thermal stimuli. These stimulation methods have potential for TS tests. However, we can only choose the electrical stimulation method for this experiment due to two reasons. First, the methods using electrical stimulation to induce TS had been systematically evaluated by other researchers. It has been used in many studies and has been shown to be reliable and sensitive to analgesics (34; 36; 38; 39; 40). Second, we do not have any instrument that can deliver heat stimuli reliably. Ideally both electrical and heat stimulations should be used.

1.4 Systematic reviews and randomised controlled trials

Large amounts of uncategorized information are published in healthcare areas, including updated information and information that is out of date, all of which can influence the medical decisions of patients, practitioners and researchers. Systematic reviews using explicit methods can refine the reliable evidence and hence benefit healthcare management and medical decisions; especially when each systematic review can focus on one specific aspect of healthcare (43; 44). The conduct of a systematic review is one of the approaches of contemporary evidence-base medicine (EBM), and the other approach of EBM is the randomised controlled trials (RCT) using valid methods of randomisation and double-blinding (45; 46). The RCT using valid method of randomisation and double-blinding can enhance the quality of acupuncture studies (47; 48; 49). The proper randomisation methods and double-blinding procedures for acupuncture studies have been clarified in the Cochrane Reviewer's Handbook for Systematic Reviews of Interventions (44). The use of computer generated sequence numbers and sealed envelops are considered the proper procedures for the method of randomisation. Blinding the subjects and evaluator to the group assignment and blinding the acupuncturist to the performance of the subjects are considered proper double-blind methods.

1.5 Aims of the present project

The present study utilises two commonly accepted EBM approaches, systematic review and RCT, to assess the current data from acupuncture analgesia studies in healthy humans and to investigate the effects of acupuncture on TS of pain. An experimental RCT using double-blinding method aims to evaluate the effectiveness of acupuncture analgesia in healthy pain-free human subjects is conducted. The major comparisons are the effects of MA, EA (2/100 hertz) and non-invasive SA on electrical stimulation induced SPT, TST and pain ratings to ST levels of TS stimulation. These TS pain tests also take into consideration the central-peripheral expression on different limbs (i.e. the same dermatome of lumbar-5 segment on treatment leg and non-treatment leg and a second dermatome of Cervical-7 segment on the forearm), and the temporal factors (i.e. immediately after intervention and 24-hours after intervention).

The aims are:

1) to systematically review acupuncture studies in health humans to understand whether real acupuncture is better than SA and EA is better than MA in terms of improving pain thresholds as indicated by various stimulations, such as mechanical, thermal or electrical;

2) to compare the analgesic effect among EA, MA and SA by assessing the pain thresholds to single electrical stimulus (i.e. SPT) and repeated electrical stimulations (i.e. TST);

3) to evaluate the temporal effects of acupuncture analgesia by conducting pain assessments at before, 30 minutes after and 24 hours after interventions; and

4) to assess the spatial distribution of acupuncture analgesia by conducting pain assessments on the treatment leg, non-treatment leg and forearm.

1.6 About the thesis

In the thesis, the content is arranged as follows:

- Chapter 1 introduces the background and aims of the present study;
- Chapter 2 discusses the TS of pain and presents a literature review examining the existing studies using the electrical TS pain model;
- Chapter 3 introduces the acupuncture interventions used in present study and examines the literature explaining the mechanisms of these techniques of acupuncture;
- Chapter 4 focuses on systematically reviewing RCTs published in the English literature of acupuncture analgesia in healthy humans;
- Chapter 5 describes the methods of the RCT in the present study;
- Chapter 6 provides the results of the present study; and
- Chapter 7 encompasses the discussion and conclusion of the present study.

Chapter 2: Temporal summation of pain - experimental models and neural mechanisms

2.1 Definition of temporal summation of pain

In neurobiological and neurophysiological pain research, 'temporal summation' (TS) is a phenomenon which entails an successive increase of pain perception and / or pain response upon repeated nociceptive stimuli being delivered to the skin surface (31; 33; 50), or to muscle tissues (51; 52; 53). The TS pain threshold (i.e. TST) refers to the intensity of repetitive stimulation which is minimumly sufficient to cause a sensation identified as pain. This intensity is lower than the pain threshold (PT) which is tested with the same type of stimulation but delivered as a single episode, i.e. the single-stimulus pain threshold (SPT). Repeating a non-painful stimulus a few times can cause a painful sensation due to the process of central amplification (29; 31; 33).

TS of pain can be reliably elicited in healthy human with repeated mechanical, heat or electrical stimuli (31). TS of pain is frequency-dependent. A few human and animals studies indicate that TS of pain can only occur when electrical stimuli are delivered repeatedly at or above 0.5-hertz, thermal stimuli are delivered repeatedly at or above 0.3-hertz (28; 31), and mechanical stimuli are delivered repeatedly at or above 0.3-hertz (32).

Arendt-Nielsen and colleagues studied the parameters required for an electrical TS pain model in 1994. In a quantitative study, the pain ratings and the electromyogram (EMG) of withdrawal reflexes of healthy humans were recorded when trains of electrical stimuli (ES) were delivered at various frequencies (0.1-, 1-, 2-, 3-, 4- and 5- hertz) and at five or

ten numbers of stimuli to the skin surface on the retro-malleolar pathway of the sural nerve. Each train of ES consisted of five individual 1-ms pulses delivered at 200-hertz. This study found that the peak EMG reading was at the fourth and fifth train and ES with a frequency of 2-hertz and 3-hertz reliably elicited TS of pain (33).

2.2 Mechanisms of temporal summation

2.2.1 Neural responses to repeated stimulations

The repeated TS stimulations specifically activate C-fibres' responses in peripheral nerves and then trigger the excitability of the wide dynamic range (WDR) neurons located in the dorsal horn of the spine (28; 54; 55; 56). WDR neurons respond to information from a variety of afferents, including those of low and high intensity. These are different from the smaller neurons called nociceptive specific cells, which selectively respond to high intensity afferent signals (55; 57). As the stimuli to the afferent fibres is repeated, the excitability of the WDR neurons gradually increases; this phenomenon of the central neurons is called windup (55), and is a form of neuronal plasticity (58; 59). Windup has been observed and evaluated in a large numbers of animal and human studies over 40 years. Consistent results have been demonstrated which indicate that windup is the pathway of the central amplification effect in response to the repeated TS-mode stimulations (31; 59; 60). This central amplification effect is called central sensitisation (24). The courses between TS stimulations and central nerves responses are summarised in the Figure 2.1 as followed.

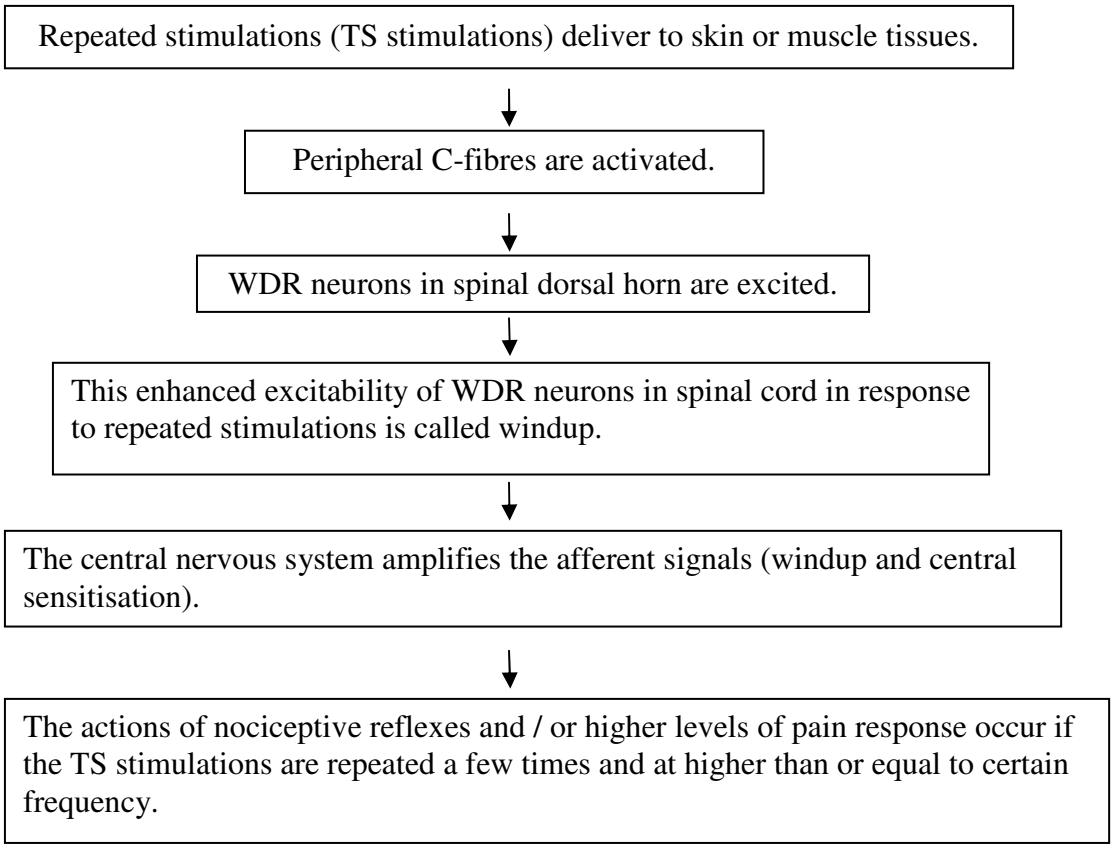


Figure 2.1 Neural mechanism of temporal summation of pain

2.2.2 Neural mechanism: temporal summation of pain and NMDA receptors

The activations of N-methyl-D-aspartic (NMDA) receptors in TS of pain has been reported in many studies (31; 38; 61). Upon a tissue injury, C and A-delta afferents are activated, causing prolonged discharge of the central neurons, such as nociceptive specific neurons. These discharges in turn activate the NMDA type of amino acid receptors, open up the ion channels (mainly the calcium flux) of the NMDA receptors on the WDR neurons (62), and the hyperexcitability of these neurons in the dorsal horn is therefore enhanced (63; 64). A similar mechanism is observed in TS of pain. Repeated stimulations activate the WDR neurons via opening up the ion channels (mainly the calcium flux) of the NMDA receptors (62). The NMDA receptor mechanism of TS pain was validated using ketamine studies. Ketamine, an NMDA receptor antagonist medication, which specifically blocks the ion channel on the NMDA receptors and thereby inhibit the hyperexcitability of the dorsal horn neurons (38; 64). The central mechanism of NMDA antagonist medications (i.e. ketamine, nitrous oxide and dextromethorphan) have been tested in studies using TS stimuli. An animal study demonstrated that the NMDA antagonist medications can decrease the dorsal horn neurons' firing in response to a train of stimuli in rats (56). In two human studies, both ketamine and dextromethorphan successfully suppressed the evocation of TS pain (TST) but had no effect on single-stimulus induced PT (SPT) (38; 61); a further study used ketamine tested fibromyalgia patients and also confirmed this observation (52). These results indicate that the central mechanism of TS pain is due to the activation of NMDA receptors (31; 38; 52; 61).

Inferred from the neural mechanisms of TS pain, if an intervention inhibits TST but has no effect on SPT, the intervention is likely to have central inhibitory effect, and potentially block the activation of NMDA receptors. If an intervention is effective on both SPT and TST, it may suggest that the analgesic effect is via not only inhibiting the activation of NMDA receptors but also other receptors, and this analgesic effect possibly involves both central and peripheral mechanisms. Studies examining the effects of various analgesics on TS of pain are summarised in Section 2.4.2.

2.3 Temporal summation and clinical pain

It is believed that TS closely relates to the central sensitisation underlying clinical pain symptoms for two reasons. First, both central sensitisation and temporal summation of pain show central amplification effects in response to nociceptive impulses (31). Second, the TS of pain has been shown to be enhanced in various clinical pain conditions (31; 52; 65). Two studies showed fibromyalgia patients had significantly higher pain responses to repeated heat stimuli than the pain-free humans control (65; 66). Another study found that fibromyalgia patients had significantly higher pain responses to repetitive mechanical stimuli when compared with normal subjects (67). A recent study showed 62% of 42 Caucasian adolescents with complex regional pain syndromes had enhanced TS of pain to repeated mechanical stimuli when compared to the normal pain-free controls (68). Central sensitisation is understood to be the main neural mechanism underlying the symptoms evaluated in the above studies. Enhanced TS of pain in these pain symptoms suggests a central contribution to the TS. In a clinical trial using the 2-hertz electrical TS pain model, ketamine reduced the pain and increased the TST in fibromyalgia patients (52). This study provides further evidence that the reproducible and reliable 2-hertz electrical TS model can be used as a good indicator to evaluate the effectiveness of analgesic interventions.

There is one type of clinical pain, hyperpathia, which is similar to TS. Hyperpathia is commonly seen in neuropathic pain. Patients with such pain experience sensory deficit however when a stimulus is delivered repeatedly, an exacerbation of pain is presented, due to deafferentation-related central sensitisation (69). TS is not hyperpathia, although both present pain upon repeated stimulation. The former can happen in normal humans and neuropathic pain patients, whereas hyperpathia only occurs in neuropathic pain

patients with sensory deficit. The underlying mechanisms of the two are also likely to be different (68; 70).

2.4 Analysing clinical trials using electrical temporal summation pain model

A literature search was conducted to examine the use of electrical TS pain model and the impacts of analgesics on it. The term 'temporal summation' was searched in four major electronic databases, Pubmed, Embase, CINAHL and Cochrane, in order to locate the clinical trials using the electrical TS pain model. In total 12 papers were found and selected (34; 35; 36; 37; 38; 39; 40; 52; 71; 72; 73; 74). Information of their methods and results is extracted into Table 2.1 and Table 2.2.

2.4.1 Characteristics of temporal summation electrical stimulation model

The variety of methods used in these studies is listed in Table 2.1. All of the authors claimed their TS models were reproducible, and could be used to identify the central effect of medications underlying specific NMDA receptor inhibition mechanisms.

2.4.1.1 Sites of assessment and analgesic effects of medications

Nearly all studies had the assessment site on the skin along the sural nerve path. Ten studies had at least one of their assessment sites on the sural nerve along its retromalleolar path, one study had one assessment site at the tibia anterior muscle along the sural nerve path (52), and the remaining one study conducted the TS pain assessment on the painful area which was reported by the individual chronic pain patient (40).

Nearly all studies had one assessed site, except for two studies which tested TS on multiple sites. One study assessed TST at one of each patient's neuropathic pain affected skin surfaces, according to the individual's report. This study reported a significant effect of venlafaxine, an antidepressant (serotonin-norepinephrine reuptake inhibitor), on suppressing the TST when compared with placebo (40). The other study had two assessment sites at the right Sacrum-1 (S1) on the foot (distal to the lateral malleolus along the sural nerve), and at Lumbar-4 (L4), in the middle of patella. This study compared three medications delivered via epidural injection and reported the increased TST were significantly higher at L4 than at the S1 areas. The author hypothesised that this result might be due to the larger nerve root size in S1 than in L4, affecting the strength of injection (35). This study offered evidence of variation in TS pain responses in different segmental areas. So far, few studies have addressed the segmental effects of an intervention on TS of pain.

2.4.1.2 Stimulation methods

All of the studies delivered a train of five stimuli at either 2-hertz or 3-hertz, except for one study, which delivered a train of four stimuli at 3-hertz (34). Three commonly used methods for TS of pain assessment were TST test, the electromyogram (EMG) recording of withdrawal reflexes and the pain response to supra-threshold (ST) level of TS stimulations reported by the subjects. Nine studies assessed the TST. Their definition of TST was whenever the subject experienced increased intensity and felt pain at the fourth or fifth train (34; 35; 36; 39; 40; 52; 72; 73; 74). All these nine studies tested the TST alone, except for one which also tested the pain tolerance threshold (PTT) of TS of pain (39). Three studies recorded both EMG and the subjects' pain responses to ST or PTT level of TS stimulations (37; 38; 71).

Table 2.1 Methods of 2-hertz electrical temporal summation pain model

(No.) Study	Assessed site	TS stimuli	TS pain assessment
(1) Curatolo M., 1997b	Sural nerve, behind the lateral malleolus	Single stimulus was repeated 5 times at 2Hz	TST; experienced increased intensity and the 4th or 5th stimuli as painful
(2) Curatolo M., 1998	Segmental areas: (1) Right S1 (foot, just distal to the lateral malleolus). (2) Right L4 (middle of the patella).	2Hz (as above)	As above
(3) Curatolo M., 1997a	Sural nerve, just distal to the right lateral malleolus. (EMG reflex recording: over the rectus femoris and the biceps femoris)	2Hz (as above)	TST; experienced increased intensity and the 4th or 5th stimuli as painful and the EMG amplitudes increase in the 4th or 5th reflexes.
(4) Peterson-felix S., 1995	Sural nerve was stimulated behind the right lateral malleolus. (EMG reflex recording: over the rectus femoris and the biceps femoris).	2Hz (as above)	As above
(5) Arendt-Nielsen L., 1995	Over sural nerve along its retromalleolar path (EMG reflex recording: over the rectus femoris and the biceps femoris).	2Hz (as above)	*Reflex threshold: defined from the EMG amplitudes increase to the 5th stimulus.
(6) Graven-Nielsen T., 2000	Tibia anterior muscle along the sural nerve path	2Hz (as above)	TST, experienced increased intensity and the 4th or 5th stimuli as painful
(7) Arendt-Nielsen L., 2000	Sural nerve along its retromalleolar path	Single stimulus was repeated 4 times at 3Hz.	As above
(8) Enggaard T.P., 2001a	Sural nerve along its retromalleolar path	Single stimulus was repeated 5 times at 3Hz.	TS pain tolerance threshold, the increase in perception of intensity at the 4th or 5th stimulation that the subject can tolerate.
(9) Enggaard T.P., 2001b	Sural nerve along its retromalleolar path	3Hz (as above)	TST, experienced the 4th or 5th stimuli as painful
(10) Yucel A., 2005	Within the neuropathic pain affected skin	Single stimulus was repeated 5 times at 2Hz	As above
(11) Enggaard T.P., 2006a	Sural nerve along its retromalleolar path	Single stimulus was repeated 5 times in 3Hz	As above
(12) Enggaard T.P. 2006b	Sural nerve along its retromalleolar path	As above	TST, experienced the 4th or 5th stimuli as painful

2.4.2 Effects of analgesics on temporal summation and single stimulus induced pain tests

In Table 2.2, seven out of 12 studies assessed both electrical SPT induced by a single stimulus and electrical TST induced by repeated stimuli, and recorded TST values that were lower than the SPT values (34; 36; 37; 40; 52; 71; 72). This indicates that a train of five electrical stimuli delivered at 2-hertz or 3-hertz (i.e. stimulations to test TST), using an intensity at sub-threshold can cause a painful sensation (33).

The TS of pain was more difficult to block than pinprick and cold sensitivity tests or single stimulus induced pain after epidural injection of bupivacaine, lidocain, epinephrine, and Clonidine (35; 71; 72), and inhalation of isoflurane (37). For example, in a study which tested the effect of isoflurane from 0.25% to 1.5%, the pain responses to electrical SPT pain test, pinprick and cold sensitivity tests were attenuated by administration of 0.75% isoflurane, but only 1.25% and 1.5% of isoflurane could suppress the pain response to TST pain test (37). Hence, these authors argued the pinprick and brief stimulation tests were not sufficient to test anaesthesia medications used in surgical environments (35; 37; 71; 72).

In eight analgesic medication studies that were not for anaesthesia, six medications were tested using TS of pain in human subjects (studies 5 to 12 in Table 2.2), and the reported actions of the medications can be divided into four types. Type one includes the effects of, ketamine, imipramine and venlafaxine, which reduced the TST and strong (ST and PTT) electrical or mechanical single stimulus thresholds but had less effect on SPT or pinprick and cold sensitivity tests (36; 38; 39). Their specific effects on TST and strong pain other than SPT suggest that they inhibit NMDA receptors and central nervous system responses. Type two refers to the effects of levetiracetam and tramadol, which

increased the SPT significantly but had no effect on the TST (73; 74). Tramadol is an opioid analgesic, when levetiracetam is a non-opioid medication. Type three was the effect of codeine, which was significantly more effective than the placebo in all the pain assessments of electrical and mechanical single stimulus induced SPT and PTT tests, as well as the TST tests and cold sensitivity tests (39). Codeine exerts the analgesic effect via endo-morphine (endogenous morphine) mechanism, thus it suggests that analgesic medications involving endo-morphine mechanism may be effective on both SPT and TST tests. Type four was the effect of UP 26-91, which had no better effect on either the TST or SPT (34). The author argues that the analgesic mechanism of UP 26-91 is related to an inhibitory action on 5-HT absorption which is similar to the mechanism of tramadol. It is notable that the analgesic medications have dose-dependent characteristics. For example, a study observed no analgesic effect of 100 mg codeine on electrical stimulation induced SPT and TST (34), whereas another study observed a strong analgesic effect of codeine 125mg on all of the SPT and TST pain tests (39). Hence, the type four analgesic effect of UP 26-91 may need to be further evaluated using a higher dosage to reach a firm conclusion.

2.4.3 Testing temporal summation in healthy humans and clinical pain patients

There are no human pain models that can perfectly mimic all features of clinical pains (13). As a result, the effect of any analgesics on pain-free humans can not be readily translated into its clinical efficacy. However, TS as a pain model seems to indicate the sensitivity of the central nervous system in both health humans and patients. In Table 2.2, in the studies number 5 and 6, the analgesic effect of ketamine was tested on TS pain model using healthy human subjects and clinical pain patients respectively (38; 52). The studies showed that ketamine significantly suppressed TST in healthy humans (study

number 5) and clinical pain patients (study number 6). Another example is the studies number 9 and 10 in Table 2.2. The authors tested the analgesic effect of velafaxine on TS pain model using healthy human subjects and clinical pain patients respectively (36; 40). They showed that velafaxine significantly suppressed TST in healthy humans (study number 9) and clinical pain patients (study number 10). These examples suggest that TS pain model can be used in health humans to assess the potential central inhibition functions of analgesics on clinical pain.

Table 2.2 Results of the studies using 2-hertz electrical temporal summation pain model

(No.) Study	Interventions	Mechanism of the medication	Subjects	Baseline SPT vs. TST	Single stimulus sensitivity test versus TS threshold
Using medications with anaesthesia purposes					
(1) Curatolo et al.,1997b	Epidural injection: Plain 0.5% bupivacaine 18mg was injected at L2-3 over 40-45s	Spinal anaesthesia can prevent sensitization and hence prevent hyperalgesic states.	10 ASA patients (no detail)	Baseline TST was sig. lower than SPT.	Within 10 minutes after the injection, electrical SPT, pinprick and cold induced pain were attenuated, TST was reduced but remained.
(2) Curatolo et al., 1998	L2-3, epidural injection: (1) lidocain hydrochloride, (2) lidocain + CO_2, (3) lidocain hydrochloride + sodium bicarbonate.	To add sodium into the anaesthetic solution can enhance the depth of epidural blockade; TS is hard to block.	24 patients were tested before surgery	SPT was not tested.	20 minutes after the injection, a large number of subjects reported no pain response to pinprick and cold tests. All TST tested in LI4 were significantly higher than at S1.
(3) Curatolo et al., 1997a	Epidural injection: (1) 20ml epinephrine (100 mg) (2) 20ml clonidine (8 mg) (3)20ml saline	Both clonidine and epinephrine anaesthesia relates to cerebrospinal fluid absorption in the spinal cord.	16 healthy humans	Baseline TST was lower than SPT	Epinephrine significantly reduced sensitivity to pinprick at L1-L4-S1 but had no effect on any other tests. Clonidine significantly reduced pinprick and cold at L1-L4-S1, and increased SPT and TST assessed at sural nerve.
(4) Peterson-felix et al., 1995	Breathe in isoflurane from 0.25%-1.5% in the mask and increased in step of 0.25%.	Isoflurane suppresses central hyperexcitibility via the increase of NMDA receptors' magnesium block.	6 healthy humans	Baseline TST was lower than SPT	Electrical SPT, pinprick and cold sensitivities were attenuated from 0.75% isoflurane, but only 1.25% or higher dosage of isoflurane can suppress the TST.
Analgesic medications using without sedation purpose					
(5) Arendt-Nielsen et al., 1995	(1) Ketamine (0.5mg/kg), (2) placebo. I.V. over 3 minutes	Ketamine blocks the NMDA receptor controlled ion channel, when this channel is opened by nociceptive stimulations.	12 healthy humans	SPT was not tested.	Supra-threshold response to electrical single stimulus, supra-threshold mechanical SPT and electrical TST were significantly suppressed by ketamine (**type 1 effect**).
(6) Graven-Nielsen et al., 2000	(1) Ketamine hydrochloride (0.3 mg/kg), (2) saline I.V. within 30min.	Ketamine inhibits central sensitisation and the central characteristics of neuropathic patients have been found.	29 female fibromyalgia patients	Baseline TST was lower than SPT	Ketamine significantly reduced electrical TST but not SPT. Ketamine significantly suppressed single mechanical stimulus induced pain tolerance threshold but not pain threshold (**type 1 effect**).

(7) Arendt-Nielsen et al., 2000	(1) UP 26-91 300mg, (2) codeine 100mg, (3) placebo. Orally.	Codeine produces endo-morphine. UP 26-91 relates to inhibition of 5-HT absorption which may similar to tramadol.	15 healthy males	Baseline TST was lower than SPT	Neither UP 26-91 (**type four effect**) nor codeine significantly reduced SPT or TST.
(8) Enggaard et al., 2001a	(1) Codeine 125mg (2) imipramine 100mg (3) placebo. Orally.	1) Imipramine relates to NMDA antagonistic mechanisms. 2) Codeine relates to u-opioid action via endo-morphine, and NMDA antagonist action is observed.	18 healthy humans	Baseline TS tolerance threshold was lower than single pain tolerance.	Imipramine reduced TST, and electrical and mechanical induced pain tolerance thresholds, but had no effect on single electrical or mechanical SPT or any cold PT (**type 1 effect**). Codeine reduced all of the pain thresholds in all tests (**type 3 effect**).
(9) Enggaard et al., 2001b	(1) Venlafaxine 37.5mg, (2) placebo	Venlafaxine is similar to imiprame. It involves the reuptake of serotonin inhibition mechanism and noradrenaline mechanism.	16 healthy humans	Baseline TST threshold was lower than SPT	Venlafaxine significantly reduced TST and single electrical pain tolerance threshold compared with placebo, but had no effect on any SPT, pressure pain tolerance thresholds or cold pain sensitivity tests (**type 1 effect**).
(10) Yucel et al., 2005	(1) Venlafaxine XR 75mg/day, (2) Venlafaxine XR 150mg/day, (3) placebo.	Venlafaxine may have a similar mechanism to imiprame.	55 neuropathic pain patients	Baseline TST was lower than SPT.	Both dosages of venlafaxine significantly reduced pinprick sensitivity, electrical SPT, electrical and heat induced TST compared with placebo. No difference between two medication dosages in all tests.
(11) Enggaard et al., 2006a	(1) Levetiracetam (1500mg) (2) placebo	Levetiracetam, an antidepressant medication, lack of opioid effect.	16 healthy humans	Baseline TST was lower than SPT.	Levetiracetam significantly increased single pain tolerance threshold and largely increased SPT compared with placebo but had no effect on TST (**type 2 effect**).
(12) Enggaard et al., 2006b	(1) Tramadol serum 100mg injection, (2) placebo intravenously.	Tramadol analgesia involves the mechanisms of CYP2D6 metabolite.	20 healthy humans	Baseline TST was lower than SPT.	Tramadol significantly increased electrical single pain tolerance threshold compared with placebo, but had no effect on TST or cold sensitivity (**type 2 effect**).

2.5 Summary of the electrical temporal summation pain model

In summary, previous studies confirmed that the electrical TS of pain stimulation and the assessment methods (i.e. electrical TS pain model) developed in 1994 by Arendt-Nielsen and his colleagues (33) was a reliable and reproducible model. This TS pain model can be used as a good indicator for testing the central effect of analgesic interventions. So far, no acupuncture study has employed this electrical TS pain model to evaluate acupuncture analgesia in either healthy humans or patients with pain.

Chapter 3: Acupuncture techniques and their neural mechanisms

3.1 Definitions and descriptions of acupuncture

The definition of acupuncture described in a publication of the World Health Organisation (WHO) in 1995 was that acupuncture "*Involves the act of needle insertion, although there are many other non-invasive techniques for acupuncture point stimulation. Points may be selected according to: traditional medical system, symptoms, point selection based on the scientific relationships of point function, and point prescription*" (75). Whereas, the definition of acupuncture given by National Institutes of Health (NIH) in 1997 was "*Stimulation, primarily by the use of solid needles, of traditionally and clinically defined points on and beneath the skin, in an organised fashion for therapeutic and / or preventive purpose*" (76). Both these two official definitions address the importance of stimulating the acupoint (s) and recognise that acupuncture is a traditional medical technique.

The most popular acupuncture techniques are the invasive techniques of manual acupuncture (MA) and electro-acupuncture (EA). The other non-invasive acupuncture techniques which stimulate the acupoints in collaboration with the rationale of MA and EA are: laser acupuncture (using light stimulation), acupressure (using mechanical stimulation), transcutaneous electrical acupoint stimulation (TEAS) (using electrical stimulation), etc. This thesis mainly addresses the invasive acupuncture techniques of MA and EA.

Needling manually is the most traditional technique of acupuncture practice. The techniques of MA are detailed in textbooks on acupuncture and involve the correct depth of needle insertion; the techniques of manipulation and the selection of acupoints for various syndromes (77).

EA was first introduced to Western countries as an anaesthesia technique by the Peking Acupuncture Anaesthesia Co-ordinating group of China in 1973 (78). The techniques of EA were developed on the basis of MA, hence they have the same procedures of acupoint selection and needle insertion. MA requires that needles are manipulated manually; whereas during EA, needles are stimulated with an electrical stimulator. The intensity and frequency of the electrical pulses can be adjusted at the preference of the acupuncturists; the intensity is usually increased to a strong but tolerable or strong but comfortable level. The frequencies of EA vary between 1- to 1,000- hertz depending on the function of the stimulator and the needs of the acupuncturist. The most popular EA frequencies used for clinical trials vary between 2- to 100- hertz in a continuous mode or a dense-disperse mode (D-D). EA of certain frequency, such as alternating 2/100 hertz EA in D-D mode, is believed to exert the best effect of EA because this combination maximize the release of various types of endogenous opioid peptides (79). Details are discussed in the followed sections.

When a needle is inserted into certain depth in an acupoint, the subject can feel some sensations which are different from painful sensation, such as numbness, distension, heaviness and soreness (80; 81; 82; 83). These sensations can be elicited and amplified if the needle is manipulated, and are recognised as *de qi*, or “the arrival of Qi” in Chinese (77). *De qi* is considered to be essential in acupuncture practice and the indicator of the treatment being effective.

3.2 The involvement of endogenous opioid peptides in EA

3.2.1 Endogenous opioid peptides and 2-hertz and 100-hertz EA

Opioids have morphine-like actions in the human body. Endogenous opioid peptides (EOPs) bind to their corresponding receptors (84). Met-enkephalin and Leu-enkephalin bind to delta receptors, beta-endorphin to mu and delta receptors (85; 86), dynorphins to kappa receptors (87), and endo-morphins (endogenous morphins) to mu receptors (88).

A few lines of evidence have indicated that different areas of the central nervous system respond to 2-hertz and 100-hertz EA stimulations. Firstly, a Functional Magnetic Resonance Imaging (fMRI) study showed different brain regions were activated when acupoints were stimulated with 2-hertz or 100-hertz TEAS; 2-hertz TEAS activated primary and supplementary motor areas and hippocampus areas in the brain and 100-hertz TEAS activated the brodmann area, pons, nucleus accumbens and amygdala regions in the brain (89). Secondly, the types of EOPs released in cerebrospinal fluid depend on the frequency of EA. In a human study, 2-hertz and 100-hertz of TEAS were applied to two randomly allocated groups before and after interventions; 2-hertz TEAS significantly increased immunoreactive Met-enkephalin-Arg-Phe (MEAP) but not immunoreactive dynorphin-A, whereas the 100-hertz TEAS significantly increased immunoreactive dynorphin-A but not immunoreactive MEAP (90). Thirdly, a study confirmed that 2-hertz EA facilitated the release of enkephalin, beta-endorphin and endo-morphin, but 100-hertz EA specifically increased the release of dynorphin (91). Fourthly, a radioimmunoassay gene study confirmed the differences between 2-hertz and 100-hertz of EA stimulations. The study used cRNA probes to assess the activities of mRNA encoding prepro-enkephalin (PPE), prepro-dynorphin (PPD) and

proopiomelanocortin in rats' brains; EA of both frequencies increased PPE mRNA in rostromedial reticular formation cells, 2-hertz EA increased PPE mRNA expression in supraoptic nucleus, suprachiasmatic nucleus, arcuate nucleus, paraventricular hypothalamic nucleus, ventromedial nucleus and the nucleus of the lateral lemniscus, whereas 100-hertz EA significantly increased PPD mRNA levels in the supraoptic nucleus, paraventricular hypothalamic nucleus, ventromedial nucleus and parabrachial nucleus (92). In summary, the differences between 2-hertz and 100-hertz EA lie in the types of EOPs released. 2-hertz EA enhances the release of the opioid peptides of enkephalin, beta-endorphin and endomorphins, whereas 100-hertz EA increases the release of dynorphins.

3.2.2 The effect of 2/100 hertz EA

A previous study compared the effects of alternating 2/15 hertz mode EA and continuous mode EA of 2-hertz and 100-hertz on pain tolerance in rats, and found the alternating mode EA exerted the best analgesic effect (93). A further study used alternating 2/100 hertz mode EA and confirmed this observation (91). Therefore, 2/100 hertz D-D mode EA might maximize the release of all three types of EOPs and provide a strong analgesic effect (79).

In addition, the effects of mu, kappa and delta receptor agonists are antagonised by naloxone dose-dependently. Naloxone, in a small dose has a strong blockage effect on mu receptor agonists but little effect on delta and kappa receptor agonists (84). It is hypothesized that naloxone would be more effective to antagonise the analgesic effect produced with continuous mode of low frequency (2-hertz) EA than that of high frequency (100-hertz) EA. This hypothesis has been confirmed by human studies. Naloxone at 1.2 milligram failed to reverse high frequency EA analgesia (94); and the same dosage of naloxone reversed the analgesic effect of low frequency electrical stimulations successfully (95). Animal studies also supported the notion that naloxone dose-dependently blocks EA analgesia induced by different frequencies (96; 97; 98; 99).

The corresponding relationships between 2-hertz, 100-hertz and 2/100 hertz EA and the effective receptors are summarised in Table 3.1 as followed.

Table 3.1 Summary of the correlation between EA frequencies, corresponding opioid peptides and receptors

EA frequency	Opioid peptides	Opioid receptors	Naloxone antagonism
2-hertz	Enkephalin, beta-endorphin and endomorphins	Delta and mu receptors	The analgesic effect acts via the delta, kappa and mu receptors and can be antagonised by naloxone dose-dependent; The analgesic effect acting via mu receptors is more sensitively to blockage by naloxone than the effect acting via other receptors.
100-hertz	Dynorphins	Kappa receptor	
2/100 hertz	Enkephalin, beta-endorphin, endomorphins and dynorphins	Delta, mu and kappa receptors	

3.3 Neural mechanisms of acupuncture

3.3.1 Mechanism of needle insertion

Both EA and MA insert needles into the acupoints. Needle insertion activates the polymodal nociceptors in the skin and muscles (100), which respond to mechanical stimulations indicative of potential tissue damage (101). This notion is the only explanation found in the literature which addresses the mechanism of needle insertion.

3.3.2 Gate Control Theory

Gate Control Theory contends that the gates located at the spinal level controls the transmission of pain signals from the peripheral to the central nervous system. The gate can be opened or closed in response to different types of somatic signal (102). The activation of small myelinated A-delta afferents and unmyelinated C-fibres opens the gate; whereas the activation of large myelinated A-beta afferents closes the gate so as to suppress pain (57; 103). Based on this theory, Melzack hypothesised the mechanisms of TEAS and EA (104). Unfortunately, we have not found a human experiment that can prove specifically that acupuncture analgesia is via A-beta fibre activation. In addition, the gate is closed during the time when A-beta afferents are concurrently activated while pain signals are transmitted, and the analgesic effect from this action does not last beyond the termination of A-beta activation. This theory alone can not explain acupuncture analgesia, which often lasts up to a few days (25).

3.3.3 Segmental Inhibition Theory and Meridian Theory

Segmental Inhibition Theory argues that acupuncture inhibits the spinal neurons at the segment of the needling site, and this affects the transmission of pain signals from the dermatome, myotome, sclerotome or viscerotome that are innervated by the same spinal nerves (57; 79; 105; 106). This theory potentially explains the action of acupuncture using anatomical knowledge instead of classical Meridian Theory. Meridian Theory argues that the spatial distribution of the acupuncture effect follows the classical defined meridian paths. Results from a recent RCT in healthy humans indicates that neither Segmental Inhibition Theory nor Meridian Theory could fully explain the distributions of the acupuncture analgesic effect (17). The study found that stimulating LI4 increased mechanical pain thresholds at 10 body sites located on various meridians and on the same or different segments to the site of simulation. The results indicate that the effect of acupuncture is general and diffusely distributed in the body.

3.3.4 Diffuse Noxious Inhibitory Control Theory

Diffuse Noxious Inhibitory Control (DNIC) Theory argues that any noxious stimulation can inhibit wide dynamic range neurons in the central nervous system and induce analgesia in a distant body area (107). This theory is supported by studies which showed a widespread analgesia after electrical or ice stimulations (108; 109). Many acupuncture studies also supported the involvement of DNICs in acupuncture analgesia (17; 110). However, since neural activities only last for a few seconds to a few minutes, DNIC theory is not able to explain the long-lasting effect of acupuncture (110).

3.3.5 Acupuncture effect on central limbic system

The limbic system, including hypothalamus and amygdala, controls humans' emotional and motivational activities, and has been recognised as the centre controlling the feeling of pleasure (111). A human fMRI study used TEAS on ST36 and found 100-hertz but not 2-hertz electrical stimulation enhanced activities of nucleus accumbens in hypothalamus (89). Another fMRI study evaluated the effect of 4-hertz EA at GB34 and observed that EA significantly de-activated activities at the limbic system areas (112). A recent fMRI study found that MA and 2-hertz EA suppressed the activities of amygdala areas but enhanced the activities of hypothalamus areas in chronic pain patients and confirmed that acupuncture inhibit the limbic system in both healthy and chronic pain patients (113).

3.3.6 Neurohumoral mechanisms of acupuncture

Various EOPs are released after acupuncture (sections 3.2.1 and 3.2.2), the analgesic effect of acupuncture can be antagonisted by naloxone (section 3.2.2), and the effects of EA can last for 72 hours (25); this evidence suggests a neurohumoral mechanism which involves the enhanced production of the precursor of EOPs (79). This neurohumoral mechanism explains that acupuncture can release EOPs, and the EOPs exert their effects as the agonists of opioid receptors throughout the whole nervous system (central and peripheral nervous systems) (79; 114).

Since EOPs exert their analgesic effects by agonising opioid receptors (84), if the number of opioid receptors is insufficient to uptake the available EOPs, the analgesic effect would be lower than when there is a sufficient number of opioid receptors. So far, a map indicating the amount of opioid receptors in different regions of the human body under normal conditions (i.e. quiet and without stimulation) has not been discovered.

With regard to the neurohumoral mechanisms of acupuncture a number of questions arise. Firstly, are there any spatial characteristics in the activation of opioid receptors after acupuncture? Secondly, does acupuncture stimulation also activate other non-opioid receptor like the action of TS stimulation? As discussed in a previous section (see section 2.2.2), TS stimulation can activate NMDA receptor in the body and thereby increases the number of NMDA receptors in the stimulated area (62). These questions need to be answered by further human experiments.

Chapter 4: A systematic review of acupuncture analgesia assessed in healthy humans

4.1 Introduction

Although acupuncture analgesia has been studied in healthy humans extensively, to date, there has only been one 'systematic review' in this area (16). The review argues the analgesic effect of electro-acupuncture (EA) is better than manual acupuncture (MA). However, this review has some limitations. Firstly, the authors did not distinguish invasive control from non-invasive control but accepted both of them as SA control. The use of an invasive or non-invasive control for SA has been debated, and some studies argue that the invasive control can produce analgesia (1; 115; 116). Secondly, the review included both randomised and non-randomised studies, and the authors did not assess the reporting quality of the included studies. Consequently, the conclusion was made without considering the validity and reliability of the studies. Thirdly, the data analysis was not sufficiently detailed. Percentages of PT change were summarised without considering the various forms of acupuncture stimulation used in these studies. Different acupuncture stimulations might cause different analgesic effect. This issue has been discussed in Chapter 3. Hence, it is necessary to conduct a systematic review on reliable and valid data to assess acupuncture analgesia in healthy pain-free human subjects which takes the various acupuncture techniques into consideration.

4.2 Aims

The present review aimed:

1) To examine the effect of MA or EA on experimentally induced pain, when compared with non-invasive sham acupuncture and invasive sham acupuncture.

2) To compare the effect of MA on experimentally induced pain when compared with EA.

3) To summarise the acupuncture techniques and pain models (i.e. pain stimulation and assessment methods) used in these studies.

4.3 Methods of the systematic review

The methods adopted in this review, including a literature search and selection, methodological quality assessments, extensive data extraction and effectiveness estimation analysis. They followed the recommendations from the Cochrane Reviewer's Handbook for Systematic Reviews of Interventions (44).

4.3.1 Search methods for identification of studies

Literature was electronically searched in four major databases (Pubmed, Cochrane Library, CINAHL and EMBASE) from their respective inceptions to 01 January 2006. The key words were 'acupuncture', 'analgesia', 'randomised controlled trial' and 'healthy humans'. The strategies and results of the literature search are presented in Table 4.1.

Table 4.1 Literature search strategies

Strategy / Database	Steps	Result
Pubmed	#1 search acupuncture #2 search human OR healthy subject OR healthy human OR humans OR healthy humans OR healthy subjects #3 search #1 AND #2 #4 search analgesia OR analgesic #5 search #4 AND #3 #6 search #4 AND #3 Field: All Fields, Limits: Animals #7 search #4 AND #3 Field: All Fields, Limits: Humans #8 search #6 NOT #7 #9 search #5 NOT #8 #10 search #5 NOT #8 Field: All Fields, Limits: Randomized Controlled Trial	222
Cochrane library	#1 search acupuncture #2 search control OR control! #3 search analgesi! #4 search healthy subject! AND human* #5 search #1 AND #2 #6 search #3 AND #5 #7 search #4 AND #6	66
ScienceDirect (Embase)	#1 search acupuncture #2 search control OR control! #3 search analgesi! #4 search healthy subject! AND human* #5 search #1 AND #2 #6 search #3 AND #5 #7 search #4 AND #6	141
CINAHL	#1 search (acupuncture) and (analgesi*) and (control*) #2 search (human*) or (healthy subject*) or (healthy human*) #3 search #1 AND #2	189
Total result	618 papers were collected, with 13 duplicated papers.	605

4.3.2 Inclusion and exclusion criteria for considering studies for this review

The protocol for literature selection is provided in Figure 4.1. Information on excluded studies is listed in Appendix 11.

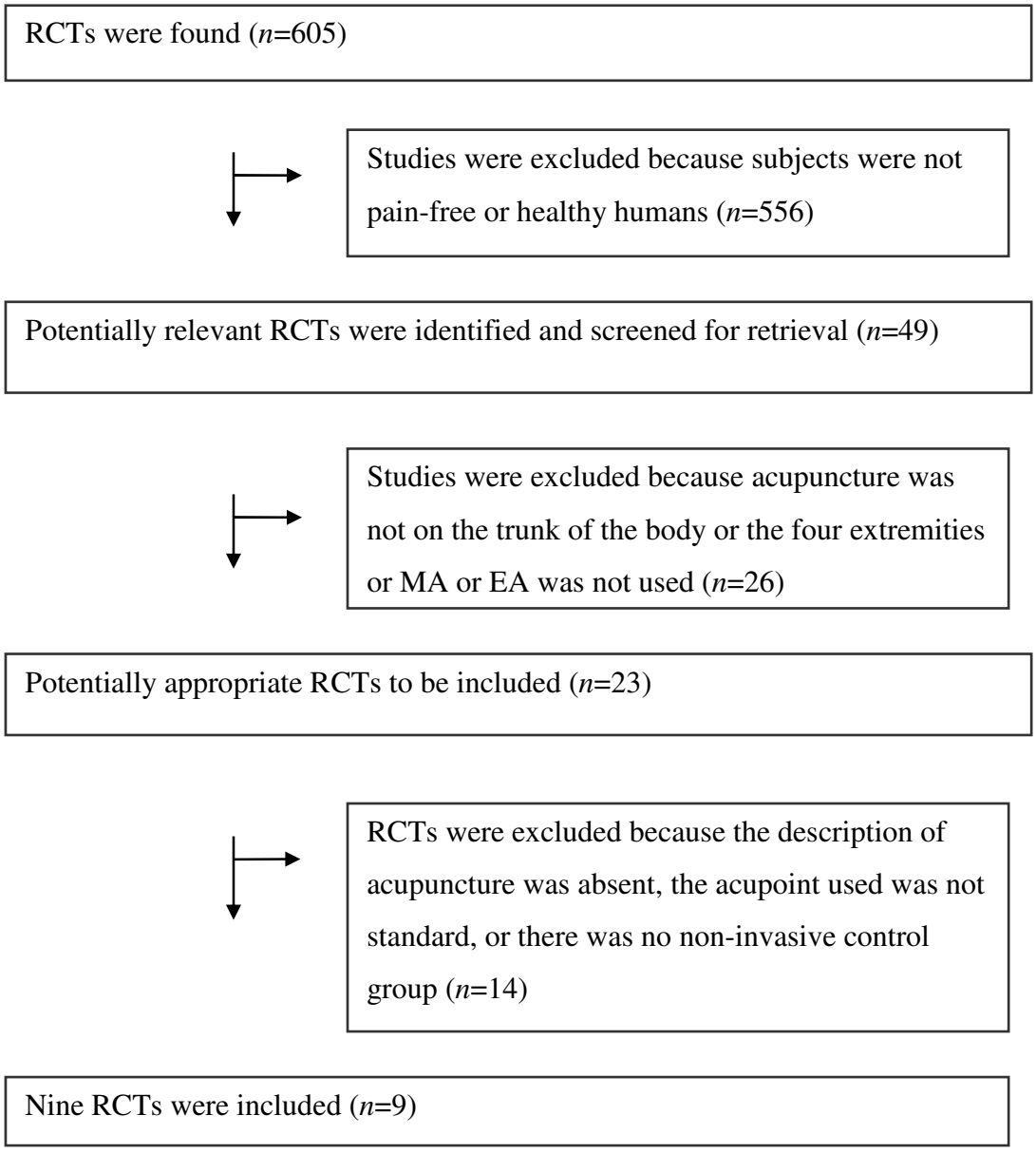
Studies were included if they met all of the following criteria:

1. Randomised controlled trials including quasi-randomised controlled trials;
2. including a placebo or SA control;
3. the study interventions were MA or EA or both (excluding auricular or scalp acupuncture);
4. healthy pain-free humans were used as study subjects; and
5. pain perceptions (including PT, PTT or pain ratings to ST stimulations) were reported.

Studies were excluded if they met one of the following criteria:

1. Description of the acupuncture technique was absent;
2. acupuncture intervention did not involve needle insertion (i.e. laser acupuncture);
3. acupuncture points used were not standard points described in acupuncture textbooks; and
4. the study was not published in the English language. None of the authors could read literature other than those in English and Chinese, and the Chinese literature was not included due to time limitations.

Figure 4.1 Protocols of literature selection



4.3.3 Assessment of methodological quality

The methodological quality was assessed with the Jadad Scale (117) and the Internal Validity Scale (IVS) (118). These scales have been validated and used previously in other acupuncture systematic reviews by other researchers (6; 119; 120).

4.3.3.1 Jadad scale

The items of the Jadad Scale include:

- Random allocation (1 point if allocation was described as random + 1 point if an adequate method to generate the random sequence was described)
- Double-blind (1 point if there was a statement that patients and evaluators were blinded + 1 point when the procedure was described and adequate)
- Reporting of dropouts/exclusions (1 point if dropouts or withdrawals, as well as the reasons, were listed independently for each treatment group)

The maximum score is five; studies scoring three or more points were considered high quality. The use of valid randomisation methods, for example computer generated sequence of numbers with a central random method or with sealed envelops, were considered adequate randomisation methods. Studies were considered to have inadequately reported the method of randomisation if they mentioned the use of a randomisation method but failed to provide a description of how the random numbers were generated. Studies with blinded participants and evaluator and / or data collector were considered as adequate as double-blind studies. The points achieved for each of the above three items are listed in order for each study in Table 4.2 'Methodological quality of included studies'. For a trial receiving full points on all aspects, the score is displayed as 2-2-1.

4.3.3.2 Internal validity scale

The six items of the scale are described as:

- Method of allocation to groups;
- concealment of allocation;
- baseline comparability;
- blinding of patients;
- blinding of evaluator; and
- likelihood of selection bias after allocation to groups by dropouts, etc.

Each item is scored as 0 (criterion not met or insufficient information provided), 0.5 (criterion partially met), or 1 (criterion met). The points achieved for each of the six items are listed for each trial in Table 4.2 'Methodological quality of included studies'. For a trial receiving full points on all items, the scores are displayed as 1-1-1-1-1-1.

4.3.4 Acupuncture adequacy assessment

A scale was developed for this review. The assessed items were selected from the checklist of 'Standards for Reporting Interventions in Controlled Trials of Acupuncture' (STRICTA) which has been validated for acupuncture systematic reviews (121). It takes into consideration the important features of acupuncture techniques as described in textbooks (77; 122).

All five items on this scale are associated with the nature and accuracy of the acupuncture stimulation and are described as:

(a) Use of standardised acupuncture point (s) in accordance with an acupuncture textbook.
(b) Proper depth of needle insertion.
(c) *De qi* sensations were reported.
d) Duration of acupuncture treatment was no less than 15 minutes.
(e) The needle was manipulated at least three times during MA or EA stimulation was use. - (77; 121; 122)

Each item is scored as 0 (criterion not met), 0.5 (insufficient information provided) or 1 (criterion met). The points achieved for each of the five items are listed in order for each trial in Table 4.3 'Acupuncture adequacy assessment'. For a trial receiving full points on all items, the scores are displayed as 1-1-1-1-1. A study that rated '4' or more points was considered as using adequate acupuncture.

Using standard points is a part of acupuncture practice. The special locations of acupoints have been clearly documented and used for a long period. Acupuncture textbooks were used to identify the proper depth of stimulation for each acupoint (77; 122), and the information is

listed in Table 4.3. If the depth of any acupoint used in the studies is shallower than the recommended depth of this point, it would be considered shallow needle insertion. An acupuncture treatment should not be shorter than 15 minutes, and three times of manipulation during 15 minutes (at five minute intervals) should be a minimum requirement for MA treatment. EA stimulation should not be shorter than 15 minutes.

4.3.5 Data extraction

All accessible data including information on participants, sample size, intervention techniques, control techniques, noxious painful stimulations, outcome measurements and reported results were extracted by the author and checked by a second researcher.

4.3.6 Data analysis

4.3.6.1 The factors preventing overall meta-analysis

After data extraction, the design of the included studies was found to be too diverse to perform meta-analysis. Factors preventing meta-analysis are listed as follows:

- Different interventions and controls: EA or MA with various techniques and frequencies of stimulation were applied and compared with non-invasive control, invasive control with or without manual manipulation, or invasive control with different electrical frequencies of electrical stimulations.
- Different temporal effects of acupuncture were assessed, such as: instant effects (during acupuncture) and immediate effects (within 60 minutes after acupuncture).
- Different types of noxious stimulation were used in each study to induce pain, such as: electrical stimulation induced dental pain, transcutaneous electrical stimulation induced pain, transcutaneous thermal stimulation induced pain, and transcutaneous mechanical pressure pain.
- Different pain perceptions were assessed: PT, ST and PTT.

As a result, the standardised mean difference (SMD) of each study was calculated and presented. The explanation to 'standardised mean difference (SMD)' is provided in the following section.

4.3.6.2 Comparisons of acupuncture analgesic effect

The data analysis package, Review Manager (also named 'RevMan', version 4.2 for Windows, The Cochrane Library), was used to compare the effects between the interventions in a same study via calculation of estimated SMD if mean and standard deviation values or numbers of responders were presented. Sub-category comparisons were also conducted to determine the temporal characteristics of acupuncture analgesia, including instant effects (during the intervention) and immediate effects (within 60 minutes after intervention) or examine the effect of acupuncture on different pain perceptions (i.e. PT, ST and PTT). Studies that did not report SD values were not included in the calculation of SMD estimates. Instructions for the use of RevMan and the explanations of its calculations are provided in Cochrane Reviewer's Handbook for Systematic Reviews of Interventions. The term 'standardised mean difference' (SMD) is used in this chapter and the appendices because the Cochrane Reviewer's Handbook for Systematic Reviews of Interventions recommends using the term "standardised mean difference" instead of the term 'effect size' to avoid confusion (44). The SMD is calculated using Hedges' g. Hedges' g examines the sample sizes of the respective standard deviations and also adjusts the overall effect size based on the sample sizes (123).

4.4 Results

4.4.1 Description of selected studies

The literature search provided 605 papers (Table 4.1 in above section 4.3.1), and in total nine papers (17; 18; 19; 20; 21; 22; 83; 124; 125) were included after applying the inclusion and exclusion criteria. All of the included studies had a non-invasive control group, and five of them also had an invasive control group (17; 18; 20; 21; 22). In addition, the study by Mayer et al. contained two trials (19); the first trial assessed the change of PT during MA treatment and the authors selected the subjects who had greater than 20% PT increase in response to acupuncture for the second trial. The second trial, which was during MA treatment, assessed the change of PT after administering either naloxone or saline injections to evaluate the involvement of endogenous opioid peptides during acupuncture. The present review only included the data of the first trial for analysis.

4.4.2 Methodological quality

Table 4.2 shows that the scores of the IVS and Jadad scale were consistent; none of the studies with a low Jadad score (Jadad score < 3) had an IVS score higher than 3.5. The overall median value of the Jadad score was 3 and the IVS score was 4.5, suggesting an overall high methodological quality for these studies. However, only two high quality studies had both an adequate randomisation method and a double-blind design; they were published in 1974 and 2003 respectively (study number 1 and 7 in Table 4.2) (17; 18). The other four high quality studies were deficient in either randomisation method or double-blind design (study number 2, 3, 4 and 8 in Table 4.2) (19; 20; 22; 124). None of the three low methodological quality studies used an adequate randomisation method or double-blind design (study number 5, 6 and 9 in Table 4.2) (21; 83; 125).

Table 4.2 Methodological quality assessments

Study	Jadad*	IVS*
1) Anderson, et al., 1974	2-2-1 =5	1-1-1-1-1-1 =6
2) Berlin et al., 1975	1-2-1 =4	0.5-0-1-1-1-1 =4.5
3) Chapmen et al., 1976	1-2-1 =4	0.5-0-1-1-1-1 =4.5
4) Mayer et al., 1977	0-2-1 =3	0-0-1-1-1-1 = 4
5) Stewart et al., 1977	1-0-1 =2	0.5-0-0-1-0-1 =2.5
6) Johnson et al., 1996	1-0-1 =2	0.5-0-0-1-0-1 =2.5
7) Zaslawski et al., 2003	2-2-1 =5	1-1-1-1-1-1 =6
8) Downs et al., 2005	2-0-1 =3	1-0.5-1-1-0-1 = 4.5
9) Kong et al., 2005	1-0-1 =2	0.5-0-1-1-0-1 = 3.5
Median value:	3	4.5
Mean ± SD value:	3.33±1.23	4.22±1.28

*Jadad items: Randomisation - Blinding - Dropouts.

*IVS items: Randomisation - Concealment of allocation - Baseline comparability - Blinding of patients - Blinding of evaluator - Dropouts.

4.4.3 Acupuncture techniques

The information on acupuncture techniques is summarised in Table 4.3, and described in the following paragraphs.

4.4.3.1 The types of acupuncture intervention

Four studies employed only MA (study number 1 - 4 in Table 4.3) (17; 19; 124; 125). Another four studies used continuous mode EA with different frequencies; two studies used 2- and 2.5- hertz low frequencies of EA respectively (study number 7 and 8 in Table 4.3) (20; 21), one study used 120-hertz high frequency EA (study number 6 in Table 4.3) (22), and one study did not report the EA frequency (study number 5 in Table 4.3) (18). The remaining one study compared the effects of 2/15 hertz EA and MA (study number 9 in Table 4.3) (83).

4.4.3.2 Acupuncture adequacy assessment

The median value of acupuncture treatment duration was 24.56 (SD 7.2) minutes. All studies used recognised classical acupoints. Four studies used a proper depth of needle insertion (study number 3, 6, 7 and 8 in Table 4.3) (17; 20; 21; 22), and two studies used shallow insertion (study number 2 and 9 in Table 4.3) (83; 125). The depth of needle insertion in another three studies was indeterminable because of the absence of information (study number 1, 4 and 5 in Table 4.3) (18; 19; 124). The overall median score of acupuncture adequacy was four. Two studies used MA and had acupuncture adequacy scores lower than four points. In these two studies, one study had absent information on two assessed items and the *de qi* sensations were not achieved in four of the subjects (study number 4 in Table 4.3) (124), another study used a shallow depth for needle insertion and had few subjects were achieved *de qi* sensations (study number 2 in Table 4.3) (125).

4.4.3.3 Adverse events in response to acupuncture

Only one study reported two out of 31 participants experiencing dizziness after acupuncture (study number 9 in Table 4.3) (83). None of the remaining studies reported any adverse event or side effects from the acupuncture treatments.

Table 4.3 Acupuncture adequacy assessments

	Duration	Acupoint	Recommended depth of acupoints (122)	Depth of needle inserted	Current authors' comment	Manipulation	*De qi* sensation	*Rating scores
MA study								
1) Mayer et al., 1977	30min	Bilateral, LI4.	LI4 (1.27-2.54cm)	Information was absent	None	Yes, 6 times	Information was absent	1-0.5-0.5-1-1 = 4
2) Johnson et al., 1996	20min	Unilateral, (1) GB20, (2) PC6.	GB20 (2.03-2.54cm) PC6 (1.27-2.54cm)	0.5cm	Shallow	Yes, 20 times	3 out of 12 subjects obtained *de qi*.	1-0-0-1-1 = 3
3) Zaslawski et al., 2003	21min	Unilateral LI4.	LI4 (1.27-2.54cm)	1.5-2 cm	Proper depth	Yes, 7 times	Yes	1-1-1-1-1 = 5
4) Downs et al., 2005	25min	Unilateral, (1) TB5, (2) LI11.	TB5 (1.27-3.81cm) LI11 (2.54-3.81cm)	Information was absent	None	Info. absent	14 out of 18 subjects obtained *de qi*.	1-0.5-0.5-1-0.5 = 3.5
EA study								
5) Anderson, et al., 1974	15min	Unilateral, (1) LI11- LI5, (2) SI5-SI8.	LI11 (2.54-3.81cm) LI5 (0.76-1.27cm) SI5 (0.76-1.27cm) SI8 (1.27-1.54cm)	Information was absent	None	Yes (EA, frequency not provided)	Information was absent	1-0.5-0.5-1-1 = 4
6) Berlin et al., 1975	20min	Unilateral, (1) LI4, (2) TB5.	LI4 (1.27-2.54cm) TB5 (1.27-3.81cm)	LI4 (1.25cm) TB5 (4cm)	Proper depth	Yes (120 Hz EA)	Yes	1-1-1-1-1 = 5
7) Chapmen et al., 1976	20min	Bilateral, LI4.	LI4 (1.27-2.54cm)	2 cm	Proper depth	Yes (2 Hz EA)	Information was absent	1-1-0.5-1-1 = 4.5
8) Stewart et al., 1977	35min	Bilateral, (1) LI4, (2) ST36.	LI4 (1.27-2.54cm) ST36 (2.54-5.08cm)	LI4 (2.0-2.5cm) ST36 (3.0-3.5 cm)	Proper depth	Yes (2.5 Hz EA)	Information was absent	1-1-0.5-1-1 = 4.5
EA & MA Study								
9) Kong et al., 2005	35min	Unilateral, (1) LI4, (2) ST36, (3) SP6.	LI4 (1.27-2.54cm) ST36 (2.54-5.08cm) SP6 (2.54-5.08cm)	1cm (original author was confirmed)	Shallow	Yes, 5 times	Yes	MA: 1-0-1-1-1 = 4
						Yes (2/15Hz, every 30sec)	Yes	EA: 1-0-1-1-1 = 4
	Median duration: 24.56±7.2 minutes.							Median score: 4 (range 3 – 5)

*Assessed items for rating scores: (1) Used standardised acupuncture point (s). (2) Proper depth of needle insertion. (3) *De qi* sensations were achieved in each subject. (4) Duration of acupuncture treatment was no less than 15 minutes. (5) The needle was manipulated at least three times during MA or use EA stimulation.

4.4.4 Procedures to enhance the precision of pain assessments

Table 4.4 lists information relating to the experimental procedures used to enhance the precision of pain assessments in these studies. The procedures were pre-testing training, temperature control in the testing environment, and the interval between two treatment sections if a subject is assigned to more than one intervention groups (this factor relates to the wash-out period for the analgesic effect after the first acupuncture treatment).

4.4.4.1 Pre-testing training

Four studies employed a training session before the start of the pain perception tests (study number 3,7,8 and 9 in Table 4.4) (17; 20; 83; 124). This was not reported in the other five studies (study number 1, 2, 4, 5 and 6 in Table 4.4) (18; 19; 21; 22; 125).

4.4.4.2 Control of the room temperature

Only one study reported that the room temperature was controlled during the pain tests. The study reported the room temperature during the experiment, which was 21 degrees Celsius (study number 6 in Table 4.4) (125).

4.4.4.3 Intervals between interventions

Each of the participants in five of the studies was assigned to receive one type of intervention (study number 1, 2, 3, 4 and 6 in Table 4.4) (18; 19; 20; 22; 125). In the other four studies, each participant experienced more than one intervention, the interval between the interventions was at least two days in two studies (study number 5 and 7 in Table 4.4) (17; 21), and at least one week in the other two studies (study number 8 and 9 in Table 4.4) (83; 124).

Table 4.4 Procedures for precise pain tests

	Pre-test training	Participant assignment for interventions		Room temperature
		Numbers of interventions received by a single subject	Length of intervals between acupuncture sections	
1) Anderson, et al., 1974	/	1	/	/
2) Berlin et al., 1975	/	1	/	/
3) Chapman et al., 1976	Yes	1	/	/
4) Mayer et al., 1977	/	1	/	/
5) Stewart et al., 1977	/	2	At least 2 days	/
6) Johnson et al., 1996	/	1	/	21 degrees Celsius
7) Zaslawski et al., 2003	Yes	3	At least 2 days	/
8) Downs et al., 2005	Yes	3	At least 1 week	/
9) Kong et al., 2005	Yes	3	At least 1 week	/

/ : No information available.

4.4.5 Pain models

Table 4.5 provides information on the types of stimulation, the methods of pain assessment and the values reported in the studies. Seven types of stimulation were used to evoke pain and each study employed only one type of these stimulations. In the studies using the same pain stimulation method, the pain assessment in each study varied for except two studies (study number 06 and 07 in Table 4.5) (21; 22). However, these two studies had employed different frequencies of EA stimulation (study number 06 and 07 in Table 4.6). The variations in the pain models and acupuncture techniques are summarised in Table 4.6.

4.4.5.1 Electrical stimulation induced pain and assessment

Two types of single electrical stimulus models were used in three studies. Single electrical stimulus induced dental pain was used in two studies, but their pain assessments varied. One study assessed pain ratings to three levels of ST intensity immediately after EA (study number 1 in Table 4.5) (20), and the other study assessed PT during MA (study number 2 in Table 4.5) (19). Another pain stimulation method was the transcutaneous single electrical stimulus induced pain. One study employed this stimulation method and assessed PT and PTT immediately after MA (study number 3 in Table 4.5) (125).

4.4.5.2 Thermal stimulation induced pain and assessment

The thermal pain stimulation methods were transcutaneous ice-water cold pain stimulation, transcutaneous cold-heat prolonged stimulation and transcutaneous prolonged heat stimulation. One study employed ice-water pain stimulation. Subjects were asked to immerse their forearms in zero degree Celsius ice-water and give pain ratings every 10 seconds within one minute (study number 4 in Table 4.5) (18). The transcutaneous prolonged cold-heat pain stimulation was used in another study (study number 5 in Table 4.5) (124). The name ‘cold-heat prolonged stimulation’ was used because the intensity (temperature) of the stimuli

was continuously changed; the study firstly assessed the cold stimulation induced PT and then increased the temperature until the heat stimulation induced PT was reached. The transcutaneous prolonged heat stimulation method was employed in three studies. Their assessment methods and interventions are described as follows. One study used consistent heat stimulation to assess the time it took to reach PTT before and immediately after 120-hertz EA (study number 6 in Table 4.5) (22); another study assessed the time it took to reach PT and PTT immediately after 2.5-hertz EA (study number 7 in Table 4.5) (21); the remaining one study assessed the pain ratings in response to low, medium and high levels of ST stimulations (12 seconds of each stimulation) immediately after 2/15 hertz EA and MA (study number 8 in Table 4.5) (83).

4.4.5.3 Mechanical stimulation induced pain and assessment

One study used continuously increasing pressure (1 kg/s) as the mechanical stimulation to assess PT immediately after MA (study number 9 in Table 4.5) (17).

Table 4.5 Pain models and outcome assessments of the included studies

	Pain stimulation	Types of measurement	Assessed Unit	Presented values
(1) Chapmen et al. 1976	Single electrical stimulus induced dental pain	Pain ratings to pre-defined low, medium and high levels of ST stimulus - Immediate effect. (Baseline PT defined as low level, medium level = low+2μA, high level = low+4μA)	VAS rating (0-7)	Pain rating to the stimulus (Mean and SD values)
(2) Mayer et al. 1977	Single electrical stimulus induced dental pain	PT (The intensity of electrical stimulus). - Instant effect.	Electrical potential (Volts)	(1) Percentage changes of intensity (mean and confidence interval values); (2) the number of responders whose PT was increased more than 20%.
(3) Johnson et al. 1996	Transcutaneous single electrical stimulus	PT and PTT (electrical current) – Instant and immediate effects	Electrical current (μA)	Electrical current intensity (mean and SD values);
(4) Anderson, et al. 1974	Cold pressor (immersing the forearm in 0℃ ice-water)	Pain rating every 10 seconds within 1 minute (6 ratings). – Immediate effect	VAS (0-10)	Rating (Mean values presented in a chart. No SD values or any value that can be converted into SD value.)
(5) Downs et al. 2005	Transcutaneous cold-heat prolonged stimuli	Cold PT and heat PT– Immediate effect	Temperatu re (℃)	Temperature (Mean and SD values)
(6) Berlin et al. 1975	Transcutaneous prolonged heat stimuli	PTT (Duration from the onset of the continuous heat stimulation to when pain was reported) – immediate effect	Time (sec)	Changes of PTT (mean and SD values were provided in chart)
(7) Stewart et al. 1977	Transcutaneous prolonged heat stimuli	PT and PTT (Duration from the onset of the continuous heat stimulation, PT was recorded and the stimulation was not stopped until PTT was reached) – Immediate effect	Time (sec)	Changes of PT and PTT (Mean and SEM values)
(8) Kong et al. 2005	Transcutaneous prolonged heat stimulation (12 seconds duration each)	Pain ratings to pre-defined low, medium, and high levels of ST heat stimulus of 12 seconds duration. – Immediate effect. (Baseline low, medium and high levels were defined as magnitude 7-10, 11-14, 15-18 of VAS ratings respectively)	VAS (0-20)	(1) Pain rating (Mean and SEM values); (2) The numbers of individual responder whose pain was significantly reduced after acupuncture.
(9) Zaslawski et al. 2003	Transcutaneous mechanical stimulation	PT (pressure was increased at a speed of 1 kg/s until PT was reported). – immediate effect	Pressure (Kg/ cm^2)	Pressure (Mean and confidence interval values)

Note: PT = pain threshold, ST = supra-threshold, PTT = pain tolerance threshold.

Immediate effect = the effect assessed at within 30 minutes after acupuncture. Instant effect = the effect assessed at during acupuncture.

Table 4.6 Description of interventions and pain models

	Acupuncture	Control	Pain model (stimulation, assessed pain perception)	Assessed temporal effect
Single electrical stimulus				
(1) Chapmen et al. 1976	2Hz EA	(1) Non-invasive control. (2) Invasive control e+.	Electrical single stimulus induced dental pain, assessed pain responses to ST stimulations.	Immediately after interventions
(2) Mayer et al. 1977	MA	Non-invasive control.	Electrical single stimulus induced dental pain, assessed the intensity achieving PT	Instantaneously during interventions
(3) Johnson et al. 1996	MA	Non-invasive control.	Transcutaneous electrical single stimulus, assessed the intensity achieving PT and PTT	Instantaneously during and immediately after interventions
Prolonged thermal or mechanical stimuli				
(4) Anderson, et al. 1974	EA (unknown frequency)	(1) Non-invasive control. (2) Invasive control e+.	Ice-water cold stimuli, assessed pain response ratings.	Immediately after interventions
(5) Downs et al. 2005	MA	Non-invasive control.	Transcutaneous cold-heat stimuli, assessed the intensities achieving cold and heat PT	Immediately after interventions
(6) Berlin et al. 1975	120Hz EA	(1) Non-invasive control. (2) Invasive control e+.	Transcutaneous heat stimuli, assessed the duration achieving PTT	Immediately after interventions
(7) Stewart et al. 1977	2.5Hz EA	(1) Non-invasive control. (2) Invasive control e+.	Transcutaneous heat stimuli, assessed the duration achieving PT and PTT	Immediately after interventions
(8) Kong et al. 2005	(1) 2/15 Hz EA; (2) MA.	Non-invasive control.	Transcutaneous heat stimuli, assessed pain responses to ST stimulations	Immediately after interventions
(9) Zaslawski et al. 2003	(1) MA m+; (2) MA m-.	(1) Non-invasive control; (2) Invasive control m+; (3) Invasive control m-.	Transcutaneous mechanical stimulations, assessed the intensity to achieve PT	Immediately after interventions

Note: e+ = with EA-like electrical stimulation; m+ = manipulation was present; m- = manipulation was absent. Immediately after intervention = the effect assessed at within 30mins after the intervention. Instantaneously during intervention = the effect assessed at during the intervention.

4.4.6 Between interventions comparisons of effectiveness

Instructions for how to read the RevMan output figure is provided in Appendix 12; the RevMan output figures for the results of comparisons and original data are provided in Appendices 13 to 20. The following paragraphs describe these results according to the interventions. The results of effectiveness comparisons between interventions in each study are summarised in the Tables following each section.

4.4.6.1 MA versus non-invasive control

Five studies compared MA versus non-invasive control (Table 4.7) (17; 19; 83; 124; 125).

Instant effect: In these five studies using MA as intervention, two studies evaluated the analgesic effect during MA. One study reported the effect during MA was significantly higher than that of non-invasive SA by assessing PT to a single electrical stimulus induced dental pain model (study 01 in Table 4.7) (19). Another study used six subjects in each group and reported the analgesic effect of MA was not better than non-invasive SA by assessing PT and PTT using transcutaneous single electrical stimulus induced pain model (study 02 in Table 4.7) (125).

Immediate effect: In the five studies using MA as intervention, four studies evaluated the analgesic effect immediately after intervention (study 02, 03, 04 and 05 in Table 4.7); Three of the four studies reported the MA effect was not significantly higher than non-invasive control using the transcutaneous single electrical stimulus induced pain model (six subjects in each group), the transcutaneous prolonged cold-heat stimulation induced pain model (18 subjects in each group) and the transcutaneous prolonged heat

stimulation pain model (11 subjects in each group), respectively (study 02, 03, 04 in Table 4.7) (83; 124; 125). Whereas the remaining one study reported that MA significantly increased PT more than the non-invasive control when assessed using the transcutaneous mechanical stimulation pain model (13 subjects in each group) (study 05 in Table 4.7) (17).

In summary, MA significantly induced analgesia to electrical stimulus induced dental pain during intervention (19), and to transcutaneous mechanical pressure pain immediately after the interventions (17). However, MA did not produce analgesia better than non-invasive SA to transcutaneous electrical pain at, during, or immediately after intervention (125), and the analgesic effect of MA was not statistically higher than that of non-invasive SA to transcutaneous thermal heat-cold pain immediately after interventions (124).

Table 4.7 MA versus non-invasive control

Sub-category.	Result (*P* value*)
Study 01: Mayer et al. 1977 (single electrical stimulus induced dental pain assessed by percentage change of intensity) - (Figure in Appendix 13)	
Comparison: Instant effect of MA vs. non-invasive control	
01. The percentage change of PT (MA, *n* = 35; non-invasive control *n*=40)	Favours MA, *p*= 0.0003
Study 02: Johnson et al. 1996 (transcutaneous single electrical stimulus induced pain assessed by the intensity of electrical current) - (Figures in Appendix 15)	
Comparison: Instant effect of MA vs. non-invasive control	
01. PT (*n*=6)	Favours control. *P*= 0.23
02. PTT (*n*=6)	Favours control. *P*= 0.38
Comparison: Immediate effect of MA vs. non-invasive control	
01. PT (*n*=6)	Favours control. *P*= 0.25
02. PTT (*n*=6)	Favours control. *P*= 0.35
Study 03: Downs et al. 2005 (transcutaneous prolonged cold-heat stimuli induced pain assessed by the intensity of temperature) - (Figure in Appendix 16)	
Comparison: Immediate effect of MA vs. non-invasive control	
01. Cold stimulation induced PT (*n*=18)	Favours MA, *p*= 0.35
02. Heat stimulation induced PT (*n*=18)	Favours MA, *p*= 0.13
Study 04: Kong et al. 2005 (transcutaneous prolonged heat stimuli induced pain assessed by pain response rating to supra-threshold stimulations) - (Figures in Appendix 19)	
Comparison: Immediate effect of MA vs. non-invasive control	
01. Pain rating to low level ST stimulation (*n*=11)	Favours MA, *p*= 0.44
02. Pain rating to medium level ST stimulation (*n*=11)	Favours MA, *p*= 0.51
03. Pain rating to high level ST stimulation (*n*=11)	Favours MA, *p*= 0.76
Study 05: Zaslawski et al. 2003 (transcutaneous prolonged mechanical stimulus induced pain assessed by pressure pain threshold) - (Figures in Appendix 20)	
Comparison: Immediate effect of MA vs. non-invasive control	
PT assessed in 10 points. (*n*=13)	All 10 results significantly favour MA.

Note: PT = pain threshold; ST = supra-threshold; PTT = pain tolerance threshold.

* The statistical significance of the above comparisons is at $p < 0.05$.

4.4.6.2 Low frequency EA versus non-invasive control

Two studies compared continuous mode low frequency (2-hertz and 2.5-hertz) EA versus non-invasive control (Table 4.8) (20; 21). Both studies evaluated the effect immediately after interventions. The study, with 15 subjects in each group, reported the analgesia of 2-hertz EA was significantly stronger than non-invasive control when assessed with the electrical stimulus induced dental pain model (study 01 in Table 4.8) (20). The other study, with 12 subjects in each group, reported the 2.5-hertz EA analgesic effect was not significantly better than non-invasive control using the transcutaneous heat stimulation induced pain model, however, there was a trend favouring EA ($p = 0.07$ and $p = 0.08$) (study 02 in Table 4.8) (21).

In summary, low frequency EA showed pronounced analgesia to electrical dental pain model but not to transcutaneous heat pain model.

Table 4.8 Low frequency EA versus non-invasive control

Sub-category.	**Result (*P* value*)**
Study 01: Chapmen et al. 1976 (single electrical stimulus induced dental pain assessed by pain response rating) - (Figures in Appendix 14)	
Comparison: Immediate effect of 2Hz EA vs. non-invasive control	
01. Pain rating to low level ST stimulation (*n*=15)	Favours EA, *p*= 0.001
02. Pain rating to medium level ST stimulation (*n*=15)	Favours EA, *p*= 0.0001
03. Pain rating to high level ST stimulation (*n*=15)	Favours EA, *p*= 0.0008
Study 02. Stewart et al. 1977 (transcutaneous prolonged heat stimuli induced pain assessed by the stimulation duration required to achieve pain threshold and pain tolerance threshold) - (Figures in Appendix 18)	
Comparison: Immediate effect of 2.5Hz EA vs. non-invasive control	
01. The duration to reach PT (*n*=12)	Favours 2.5Hz EA, *P*= 0.08
02. The duration to reach PTT (*n*=12)	Favours 2.5Hz EA, *P*= 0.07

Note: PT = pain threshold; ST = supra-threshold; PTT = pain tolerance threshold.

* The statistical significance of the above comparisons is at $p < 0.05$.

4.4.6.3 High frequency EA versus non-invasive control

Only one study compared continuous mode 120-hertz EA versus non-invasive control (Table 4.9) (22). The effect immediately after EA was significantly higher than that of non-invasive control assessed by transcutaneous heat stimulation induced pain (n = 10 in each group).

Table 4.9 High frequency EA versus non-invasive control

Sub-category.	**Result (*P* value *)**
Study: Berlin et al. 1975 (transcutaneous prolonged heat stimuli induced pain assessed by the stimulation duration required to achieve pain tolerance threshold) - (Figures in Appendix 17)	
Comparison: Immediate effect of 120 Hz EA vs. non-invasive control	
01. The duration to reach PTT (*n*=10)	Favours 120Hz EA, *P*< 0.00001

Note: PTT = pain tolerance threshold.

* The statistical significance of the above comparisons is at $p < 0.05$.

4.4.6.4 EA (2/15 hertz) versus non-invasive control

Only one study compared alternating mode 2/15 hertz EA versus non-invasive control (Table 4.10) (83). The effect immediately after EA was not significantly higher than that of non-invasive control, when assessed with the transcutaneous heat stimulation induced pain model (n=11 in each group).

4.4.6.5 EA (2/15 hertz) versus MA

Only one study compared alternating mode 2/15 hertz EA versus MA (Table 4.10) (83). The effect immediately after EA was not significantly higher than that of MA when assessed with the transcutaneous heat stimulation induced pain model (n=11 in each group).

Table 4.10 EA (2/15 hertz) versus non-invasive control and MA

Sub-category.	**Result (*P* value*)**
Study: Kong et al. 2005 (transcutaneous prolonged heat stimuli induced pain assessed by pain response rating to supra-threshold stimulations) - (Figures in Appendix 19)	
Comparison: Immediate effect of 2/15Hz EA vs. non-invasive control	
01. Pain rating to low level ST stimulation (*n*=11)	Favours 2/15Hz EA, *p*=0.47
02. Pain rating to medium level ST stimulation (*n*=11)	Favours 2/15Hz EA, *p*=0.22
03. Pain rating to high level ST stimulation (*n*=11)	Favours 2/15Hz EA, *p*=0.33
Comparison: Immediate effect of 2/15Hz EA vs. MA	
01. Pain rating to low level ST stimulation (*n*=11)	Favours MA, *p*=0.92
02. Pain rating to medium level ST stimulation (*n*=11)	Favours 2/15Hz EA, *p*=0.43
03. Pain rating to high level ST stimulation (*n*=11)	Favours 2/15Hz EA, *p*=0.43

Note: ST = supra-threshold.

* The statistical significance of the above comparisons is at $p < 0.05$.

4.4.6.6 A comparison of the studies including results of acupuncture versus invasive control and acupuncture versus non-invasive control

Four studies employed both invasive and non-invasive controls (Table 4.11) (17; 20; 21; 22). All of the invasive controls used in these studies were inserted needles into non-acupoints.

One study compared the effects of 2-hertz EA, invasive control with 2-hertz electrical stimulation (named '2-hertz e+ invasive control'; 'e+' represents 'with electrical stimulations') and non-invasive control using the electrical dental pain model. EA significantly reduced the pain ratings to low, medium and high ST levels of electrical stimulation compared with non-invasive control, but the effect of EA on high level ST stimulation was not statistically higher than that of 2-hertz e+ invasive control (study 01 in Table 4.11) (20). Another study compared the effects of 2.5-hertz EA, 2.5-hertz e+ invasive control and non-invasive control using the transcutaneous heat pain model. The PT immediately after EA was not significantly higher than that of either non-invasive control or 2.5-hertz e+ invasive control (study 02 in Table 4.11) (21). The above two studies showed that e+ invasive controls induced some level of analgesia and at times their effect was indistinguishable from that of EA on real acupoints.

Another study observed no difference in results between 120-hertz EA versus 120-hertz e+ invasive control and 120-hertz EA versus non-invasive control; this study assessed the PTT using the transcutaneous heat pain model (study 03 in Table 4.11) (22).

One study compared the effects of MA, invasive control with manipulations (named 'invasive control m+'), invasive control without manipulations (named 'invasive control m-') and non-invasive control (study 04 in Table 4.11) (17). This study found the results of MA versus non-invasive control were similar to those of MA versus invasive control m-, which was that MA analgesia was significantly higher than that of either of the controls assessed at all 10 sites. A comparison of MA with invasive control m+ showed that the MA effect was significantly greater at nine out of 10 sites.

In summary, 2-hertz e+ invasive control and invasive control m+ had strong analgesic effects. When they were used as SA control, the difference between real acupuncture and the control was reduced.

Table 4.11 A comparison of the studies including results of acupuncture versus invasive control and acupuncture versus non-invasive control

Sub-category.	Result (*P* value*)
Study 01: Chapmen et al. 1976 (single electrical stimulus induced dental pain assessed by pain response rating) - (Figures in Appendix 14)	
Comparison 01. Immediate effect of 2Hz EA vs. non-invasive control	
01. Pain rating to low level ST stimulation (*n*=15)	Favours EA, *p*= 0.001
02. Pain rating to medium level ST stimulation (*n*=15)	Favours EA, *p*= 0.0001
03. Pain rating to high level ST stimulation (*n*=15)	Favours EA, *p*= 0.0008
Comparison 02. Immediate effect of 2Hz EA vs. 2 Hz e+ invasive control	
01. Pain rating to low level ST stimulation (*n*=15)	Favours EA, *p*= 0.03
02. Pain rating to medium level ST stimulation (*n*=15)	Favours EA, *p*= 0.008
03. Pain rating to high level ST stimulation (*n*=15)	Favours EA, *p*= 0.1
Study 02: Stewart et al. 1977 (transcutaneous prolonged heat stimuli induced pain assessed by stimulation duration required to achieve pain threshold and pain tolerance threshold) - (Figures in Appendix 18)	
Comparison 01. Immediate effect of 2.5Hz EA vs. non-invasive control	
01. The duration to reach PT (*n*=12)	Favours 2.5Hz EA, *P*= 0.08
02. The duration to reach PTT (*n*=12)	Favours 2.5Hz EA, *P*= 0.07
Comparison 02. Immediate effect of 2.5Hz EA vs. 2.5Hz e+ invasive control	
01. The duration to reach PT (*n*=12)	Favours 2.5Hz EA, *p*= 0.27
02. The duration to reach PTT (*n*=12)	Favours 2.5Hz EA, *p*= 0.5
Study 03: Berlin et al. 1975 (transcutaneous prolonged heat stimuli induced pain assessed by stimulation duration required to achieve pain tolerance threshold) - (Figures in Appendix 17)	
Comparison 01. Immediate effect of 120 Hz EA vs. non-invasive control	
01. The duration to reach PTT (*n*=10)	Favours 120Hz EA, *P*< 0.00001
Comparison 02. Immediate effect of 120 Hz EA vs. 120 Hz e+ invasive control	
01. The duration to reach PTT (*n*=10)	Favours 120Hz EA, *P*< 0.00001
Study 04: Zaslawski et al. 2003 (transcutaneous prolonged mechanical stimulus induced pain assessed by pressure pain threshold) - (Figures in Appendix 20)	
Comparison 01. Immediate effect of MA vs. non-invasive control	
Intensity of PT assessed in 10 points. (*n*=13)	All 10 results significantly favour MA.
Comparison 02. Immediate effect of MA vs. invasive control m+	
Intensity of PT assessed in 10 points. (MA, *n*=13; Invasive control m+, *n*=9)	All 10 results favour MA; with 9 significant and 1 insignificant results.
Comparison 03. Immediate effect of MA vs. invasive control m-	
Intensity of PT assessed in 10 points. (*n*=13)	All 10 results significantly favour MA.

Note: e+ = with EA-like electrical stimulation; m+ = manipulation was present; m- = manipulation was absent. PT = pain threshold; ST = supra-threshold; PTT = pain tolerance threshold.
* The statistical significance of the above comparisons is at $p < 0.05$.

4.4.6.7 A direct comparison between invasive control and non-invasive control

A direct comparison of the standardised mean differences of the invasive and non-invasive controls in each of the four studies was conducted (Table 4.12) (17; 20; 21; 22).

In one study, 2-hertz e+ invasive control significantly reduced the pain ratings to medium and high level ST stimulations compared with non-invasive control in the electrical stimulus dental pain model (study 01 in Table 4.12) (20). In another study, 2.5-hertz e+ invasive control did not significantly increase PT or PTT more than non-invasive control did using the transcutaneous heat pain model (study 02 in Table 4.12) (21). In a third study, 120-hertz e+ invasive control significantly increased PTT compared with non-invasive control in the transcutaneous heat pain model (study 03 in Table 4.12) (22). The last study assessed transcutaneous mechanical stimulation induced pain at ten sites and reported the invasive control m+ produced better analgesic effect than non-invasive control at all ten sites. The analgesic effect reached statistical significance at seven of ten sites in the invasive-control m+ group; whereas the invasive control m- produced significantly greater effect than non-invasive control at two of these sites (study 04 in Table 4.12) (17).

In summary, three out of four studies showed that invasive controls produced significant change in pain perception which was better than that the change produced by non-invasive control (17; 20; 22).

Table 4.12 A direct comparison between invasive control and non-invasive control

Sub-category.	Result (*P* value*)
Study 01: Chapmen et al. 1976 (single electrical stimulus induced dental pain assessed by pain response rating) - (Figures in Appendix 14)	
Comparison: Immediate effect of 2 Hz e+ invasive control vs. non-invasive control	
01. Pain rating to low level ST stimulation (*n*=15)	Favours 2Hz e+ invasive control, *p*= 0.07
02. Pain rating to medium level ST stimulation (*n*=15)	Favours 2Hz e+ invasive control, *p*= 0.04
03. Pain rating to high level ST stimulation (*n*=15)	Favours Invasive control e+, *p*= 0.04
Study 02. Stewart et al. 1977 (transcutaneous prolonged heat stimuli induced pain assessed by stimulation duration required to achieve pain threshold and pain tolerance threshold) - (Figures in Appendix 18)	
Comparison: Immediate effect of 2.5Hz e+ invasive control vs. non-invasive control	
01. The duration to reach PT (*n*=12)	Favours 2.5Hz e+ invasive control, *p*= 0.34
02. The duration to reach PTT (*n*=12)	Favours 2.5Hz e+ invasive control, *p*= 0.29
Study 03. Berlin et al. 1975 (transcutaneous prolonged heat stimuli induced pain assessed by stimulation duration required to achieve pain tolerance threshold) - (Figures in Appendix 17)	
Comparison: Immediate effect of 120 Hz e+ invasive control vs. non-invasive control	
01. The duration to reach PTT (*n*=10)	Favours 120Hz e+ invasive control, *P*< 0.00001
Study 04. Zaslawski et al. 2003 (transcutaneous prolonged mechanical stimulus induced pain assessed by pressure pain threshold) - (Figures in Appendix 20)	
Comparison: Immediate effect of invasive control m+ vs. non-invasive control	
Intensity of PT assessed in 10 points. (Invasive control m+, *n*=9; non-invasive control, *n*=13)	All 10 results favour invasive control m+; with 7 significant and 3 insignificant results.
Comparison: Immediate effect of invasive control m- vs. non-invasive control	
Intensity of PT assessed in 10 points (*n*=13)	2 results significantly favour invasive control m-. 8 results insignificantly favour non-invasive control (no effect).

Note: e+ = with EA-like electrical stimulation; m+ = manipulation was present; m- = manipulation was absent. PT = pain threshold; ST = supra-threshold; PTT = pain tolerance threshold.

* The statistical significance of the above comparisons is at $p < 0.05$.

4.4.7 Description of the study with no SD value for effectiveness estimation

In Table 4.8, information from a study with no SD value for standardised mean difference estimation is extracted (18). This study employed zero degree Celsius cold water pain stimuli, and the pain ratings were recorded every 10 seconds within one minute (6 ratings). This study only provided the mean values in a chart without the SD values. It was reported the EA (the information of EA frequency was absent) significantly reduced the pain ratings compared with either e+ invasive control or non-invasive control on the treatment side of the forearm but no significant result was detected on the non-treatment side of the forearm.

Table 4.13 The study with absent value for standardised mean difference estimation

Study	Intervention	Acupuncture techniques	Measurement	Results
Anderson, et al. 1974	(1)EA (no frequency was provided). (2) Non-acupoint invasive control with EA-like electrical stimulation. (3) Group with no treatment.	Unilateral, right forearm (1) LI11-LI5, (2) SI5-SI8. The treatment duration was 15 minutes.	Ice-water pain: immerse the forearm into zero Celsius degree ice-water, subjects rated the pain response in a VAS (0-10) every 10 seconds in a 60 seconds period. Pain assessments were conducted before and immediately after intervention. Firstly, test the treatment side of forearm (right). Secondly, test the non-treatment side forearm (left). Finally, test the treatment side forearm (right) once again.	Mean values were calculated and presented in a chart. No SD value was published. EA significantly reduced the pain response to ice-water pain compared with either the invasive control with electrical stimulation or non-invasive control on the treatment limb but not on the other non-treatment limb.

4.5 Discussion

4.5.1 Summary of results

In summary, the median values of the methodological quality score and the acupuncture adequacy assessment score were high. In these studies, due to the diverse treatment methods, pain models used and control intervention employed, a meta-analysis was not conducted. The analgesic effect during MA and immediately after low frequency EA were significantly greater than non-invasive control, in an electrical stimulus induced dental pain model. The analgesic effect immediately after MA was significantly better than non-invasive control in a transcutaneous mechanical stimulation induced pain model. The effect immediately after continuous mode high frequency EA was significantly higher than non-invasive control in a transcutaneous heat stimulation pain model. There was no difference in the analgesic effects of 2/15 hertz EA, MA and non-invasive control in a study using transcutaneous heat pain model. Three out of four studies reported that the invasive controls induced significantly stronger analgesia than non-invasive controls. Furthermore, the findings have to be interpreted with caution because in most cases there is only one study in each sub-group comparison.

4.5.2 Strengths and limitations

The literature search found two previous reviews which addressed acupuncture analgesia studies on healthy humans (16; 23). One of these reviews did not publish the methods and data analysis in the article (23). The other review compared the effectiveness of EA and MA respectively with SA, but there were some deficiencies in the methods which were discussed in a previous section (section 4.1) (16). Neither of the two reviews drew conclusions concerning the invasive and non-invasive controls, and neither made their conclusions distinguishing between RCTs and non-RCTs. Hence, the current review may be the first review of pain perception studies evaluating acupuncture analgesia which makes comparisons between EA, MA, non-invasive control and invasive control. Furthermore, the included studies of this review were RCTs with relatively high methodological quality and were published in English speaking countries. This afforded the review further reliability since a previous review concerning certain countries indicated bias in result reporting (126). In addition, it has been advocated that the acupuncture qualifications of experimenters and reviewers need to be considered (127). The present reviewers have sufficient qualifications and clinical background in acupuncture practice.

This review is different from the Cochrane Systematic Review in some aspects and these differences may be criticised. A Cochrane Systematic Review requires at least three reviewers in different locations to review literature covering all major languages, and must include the literature of RCT and non-RCT, as well as published and unpublished literature. In the present review, due to the limited human resources and time constraints, only the published RCT papers in English were reviewed, but the methods, results and interpretations of this review were examined and discussed within the RMIT Chinese Medicine research group. These review procedures ensured the interpretations made in

this study properly reflect the relevant published English language experimental RCT literature.

Another limitation of this review was the diversity in study designs, procedures and outcome measures precluded the possibility of meta-analysis. So that an overall standardised mean difference could not be obtained (see section 4.3.6). In the present review, the comparisons in the analyses were valid because there was no overlap of different pain assessments within the studies. This limitation would be resolved if more studies in this area are conducted in the future. Moreover, a similar situation occurred in a previous Cochrane Systematic Review. This review had a limited number of included studies and meta-analysis was not possible, but this did not affect the interpretation when the results of the included studies were analysed (2).

4.5.3 Acupuncture analgesia and pain models

This review showed that each of the acupuncture techniques of MA, continuous mode low frequency EA and continuous mode high frequency EA were reported to produce significantly greater analgesia than non-invasive control according to assessments using different pain models; and the effect of MA was similar to that of low frequency of EA when assessed with the same pain model. The strong analgesic effect of acupuncture has been advocated in a number of reviews of clinical trials of patients (1; 10; 128; 129; 130). However, a recent review written by Staud and Price examining both clinical trials and experimental studies concluded that acupuncture analgesia showed strong evidence in experimental pain studies but there was less convincing evidence for chronic pain conditions in clinical trials. Unfortunately, this article did not publish the details of the data-analysis (23). The present review showed each form of acupuncture technique was effective in some pain models but not in all of these pain models. Therefore, it is possible that different acupuncture techniques might suit different types of clinical pain conditions. It is still too early to draw conclusions on the overall effects of acupuncture analgesia for clinical pain.

In the comparison between 2/15 hertz EA, MA and non-invasive control in a study using a thermal pain model immediately after intervention, it is surprising that the analgesic effect of alternating mode 2/15 hertz EA was not better than non-invasive control. Three factors, the heat pain model, the acupuncture technique used and the experimental method should be considered. Regarding the heat pain model, other included studies in this review show MA has less effect on heat pain, and the 2/15 hertz EA may be not effective on heat pain as well. This hypothesis needs to be confirmed with further experiments. Regarding the second factor, the acupuncture technique used in this study had a deficiency, which was the shallow needle insertion. This aspect of acupuncture

technique has been widely accepted as an important factor and a standard for acupuncture practice (121). Besides this, the study has a methodological deficiency in the concealment of allocation and the blinding of the evaluator.

In the present review no studies using a TS pain model, which delivers standardised repetitive stimulations at high frequencies, were located.

4.5.4 The use of controls in acupuncture studies

The present review shows that the analgesia effect induced by invasive controls with acupuncture-like stimulation was stronger than that of non-invasive controls. The consideration here is certain analgesic effects are activated during the needle insertion. In relation to this consideration, one of the included studies has addressed this issue of type of control. Zaslawski and his colleagues compared the effects of MA, invasive control m+, invasive control m- and non-invasive control; this study showed the importance of needle manipulations in producing MA analgesia, and also identified that the use of invasive control without manipulations can produce a certain degree of analgesia which is better than non-invasive control but was less likely to induce the reporting of MA analgesia (17). Future publications should distinguish invasive control and non-invasive control in instead of calling both of them ‘sham-acupuncture’.

4.5.5 The necessity of long term acupuncture effect evaluation

Pain perception studies investigating the analgesic effect 24-hours after an acupuncture treatment can help to answer the questions “How long can the effect of acupuncture last?” and “What is the change in pain perceptions over night after acupuncture?”. Studying these issues can also help identify the wash-out period (the time it takes for the treatment to no longer have any effect on the participant) of an acupuncture treatment in order to avoid any carry-over influence if the study uses the same participant for more than one intervention. For example, the interval between different interventions was at least two days in two of the included studies (17; 21). This incites the critical question of whether this wash out period is enough. In this review, no experimental study was found which evaluated the analgesic effect hours after an acupuncture treatment. A review regarding clinical trials on patients found that only one clinical trial had evaluated the effects after a single acupuncture treatment at longer than 24 hours after the treatment (26). Hence the long lasting analgesic effect after a single acupuncture treatment needs to be further explored in human studies.

4.5.6 Safety and side effects of acupuncture

Only a minimum number of adverse events with mild symptoms were reported in association with acupuncture. The result of this review is in accordance with previous reviews which found that acupuncture is a relatively safe treatment when using clean needles and trained practitioners (128; 129; 131; 132). In addition, one of the studies excluded from this review reported that the application of morphine was associated with significant adverse events and ketamine (5 mg/kg) was associated with greatest severity of adverse events whereas there were no adverse eventts in association with acupuncture in the pain-free healthy participants (133).

4.5.7 Recommendations for future research

4.5.7.1 The selection of acupuncture techniques for research

A variety of acupuncture techniques have been used in clinical trials, such as shallow or deep needle insertions, various durations and techniques of manipulation used in MA, various modes of electrical stimulation used in EA and there has been a wide selection of acupoints used (8; 116; 134). When the results of these diverse trials are interpreted together, a complexity of factors is introduced. This could lessen the likelihood of a valid evaluation of the acupuncture effect with the result that the effectiveness of acupuncture would remain controversial. Therefore, improved strategies that consider these various factors are required when conducting acupuncture experiments. Consequently, it is recommended that studies should investigate each of the various acupuncture techniques in a systematic series of experiments using validated assessment methods with high quality design. This approach may enable conclusions to be drawn regarding the effectiveness of acupuncture.

4.5.7.2 Evaluate acupuncture analgesia using a temporal summation pain model

The TS of pain model has relatively clear mechanisms and well defined methods of stimulation and assessment which were introduced in Chapter 2. Therefore, an experimental RCT which quantitatively compares acupuncture with non-invasive control could be used to assess acupuncture analgesia on TST in healthy pain-free subjects. Such studies might provide data that could be applied to explain the effects of acupunture in clinical pain conditions. In addition, future studies could conduct pain assessments at different segmental areas and at time periods longer than 24 hours after interventions using this pain model.

4.6 Conclusion

In acupuncture, in the area of RCT studies in healthy pain-free humans in the English language, the published acupuncture analgesia pain perception studies with non-invasive control have been systematically evaluated. Within these studies, the invasive control produced greater analgesia than non-invasive control. When comparing acupuncture with non-invasive control, significant acupuncture effects were reported in the studies which used both valid pain assessment methods and high quality of acupuncture techniques. The observation, which suggests the analgesic effect of EA is better than MA, can not been firmly concluded. The long term effects of acupuncture analgesia and the comparison between EA and MA needs to be further evaluated. Acupuncture is a relatively safe treatment. The model of temporal summation of pain has not been employed in acupuncture RCT quantitative studies using non-invasive control.

Chapter 5: Methods

The current project is a randomised, double-blinded, sham-acupuncture controlled experimental study. This project was reviewed and approved by the Human Research Ethics Committee of RMIT University (Reference No. 24/05) (Appendix 01), of which the principles were in accordance with the 'National Statement on Ethical Conduct in Research Involving Humans 1999' issued by National Health and Medical Research Council. All the tests were conducted at the Clinical Research Lab of RMIT Chinese Medicine Research Group, Bundoora West Campus. The laboratory room was a quiet, temperature-controlled room. The room temperature was adjusted to 22 - 25 degrees Celsius.

In the thesis, 'Investigator A' refers to the evaluator, 'Investigator B' refers to the acupuncturist and 'Investigator C' refers to the person in responsible for randomisation and data analysis.

5.1 Volunteer recruitment

The volunteers were recruited via advertisements placed on the RMIT University website and posted at the University's Bundoora and City campuses (Appendix 02). RMIT staff and students or members of the public who answered the advertisements were recruited as long as they met the selection criteria. They were volunteers with no payment or any other benefit for their participation.

5.1.1 Inclusion and exclusion criteria

The selection of volunteers was based on the information provided by the volunteers (Appendix 03), and according to the inclusion and exclusion criteria detailed below.

Volunteers were included if they

(a) were aged between 18 and 40 years old and healthy at the time of recruitment;

(b) agreed to fully participate in the study;

(c) provided a written consent form for participation; and

(d) never had acupuncture prior to this study.

Volunteers were excluded from the study if they

(a) did not comprehend English;

(b) were currently pregnant at the time of recruitment;

(c) had severe heart disease, wore a pacemaker, or had a high risk of cardiovascular diseases as assessed using the Cardiovascular Risk Questionnaire (Appendix 04);

(d) had a brain tumour or epilepsy

(e) had the tendency to bleed, such as being haemophilia;

(f) had drug addiction;

(g) had taken any analgesics in the previous two weeks;

(h) suffered from chronic pain or recurrent pain; or

(i) had skin problems at the proposed acupuncture sites. Investigator A checked if there was any ulcer or dermatitis at the proposed stimulation sites.

Volunteers were given written information (i.e. Plain Language Statement, Appendix 05) and a verbal explanation concerning the study. Full explanation to any questions raised was given by Investigator A. Signed Informed Consent (Appendix 06 and 07) was then

obtained from each volunteer. Every volunteer was also notified that he/she was free to withdraw from the study at any time.

5.2 Method of randomisation and double-blinding

Randomisation and double-blinding were employed. Each volunteer was randomly assigned to one of three groups, i.e. manual acupuncture (MA), electro-acupuncture (EA) and sham acupuncture (SA), by drawing a sealed envelop which contained a random number that indicated the group allocation. Investigator C created these random numbers by using Microsoft Excel software (Microsoft Office 2002, Windows version) (Appendix 08). Investigator C was not involved in any of the testing procedures. He passed the random numbers only to the acupuncturist, i.e., Investigator B. The acupuncturist (Investigator B) delivered the interventions of MA, EA and SA, and was blinded to the pain assessments. The evaluator, i.e., Investigator A, who conducted pain assessments, was blinded to treatment allocation. During the treatment period, the volunteers lay on a treatment bed in a supine position and their vision to the sites of acupuncture was blocked by an object placed at their waist level. At the conclusion of the whole experiment, Investigator C collected the data from Investigator A and used the random numbers to identify the group assignment and conduct the data analysis.

5.3 Interventions

There are two active intervention groups, EA and MA, and a sham control intervention group. Acupuncture needles were two 0.25 x 40mm sterile single-use needles with guide tube (Hwato, Suzhou Medical Appliance Company, China). Table 5.1 shows the comparisons between the techniques of these three interventions.

5.3.1 Selection of acupoints

Acupoints Zusanli (ST 36) and Fenglong (ST 40) were selected for the intervention as they are often used for pain reduction. Methods of locating these acupoints are described as follows:

ST36: "*The point is located in the fossa one finger breadth lateral to the anterior margin of the tibia, and 3 inches inferior to ST35. The location of ST35 is, with the knee flexed at 90^{o}, at the inferior margin of the patella in the fossa lateral to the tendon of the patella*." - Page 63 and 68, (122).

ST40: "*At the midpoint between the inferior margin of the patella and the skin crease of the ankle joint, 1.5 inches lateral to the anterior margin of the tibia, and between the tibia and fibula.*" - Page 73, (122).

5.3.2 Manual acupuncture

Needles were inserted into acupoints to a depth of 20-25 mm. The manipulation technique involved needle rotation between the fingers at a medium rate of stimulation with 180 to 360 degrees in a bidirectional manner, first clockwise then anticlockwise. This action was repeated nine times, and lasted approximately 10 seconds. *De qi* sensations, described as soreness, numbness, or distension at the needling site, were produced. This manipulation was repeated every five minutes over a period of 25 minutes; so in total six episodes of manipulation were performed in 25 minutes. Similar MA techniques were used in a recent acupuncture study (17).

5.3.3 Electro-acupuncture

After needles were inserted into the correct depth, needles were manipulated to achieve the *de qi* sensations. A modified acupuncture electrical stimulator (Myer 501, Australia) was then connected to the two needles via two electrodes. The mode of EA used was dense-disperse (D-D) mode with alternating frequency between 2- and 100- hertz every six seconds. The stimulus intensity was adjusted to a strong but tolerable level with visible muscle contraction. When the intensity of electrical stimulation had been adjusted, no further change of the intensity was made during the rest of the EA period. The duration of EA treatment was 25 minutes. The same machine and the mode of EA were used in a previous clinical trial (79).

5.3.4 Sham acupuncture

In the SA group, a non-invasive method was used (81). An empty plastic guide tube was tapped at ST36 and ST40 on the dominant leg to produce some discernible sensation, and then two bent needles, each with a piece of adhesive bandage (see Figure 5.1) were then taped to the dermal surface of the two acupoints respectively for 25 minutes. Manipulations were made by pressing the bent needles to produce a pressing sensation on the skin surface of the acupoints every five minutes. A non-functioning electrical acupuncture stimulator was connected to the end of the two needles via wires, and was placed on a table within the volunteers' eyesight, showing a continuously flashing light. *De qi* sensations were not intended and were avoided.

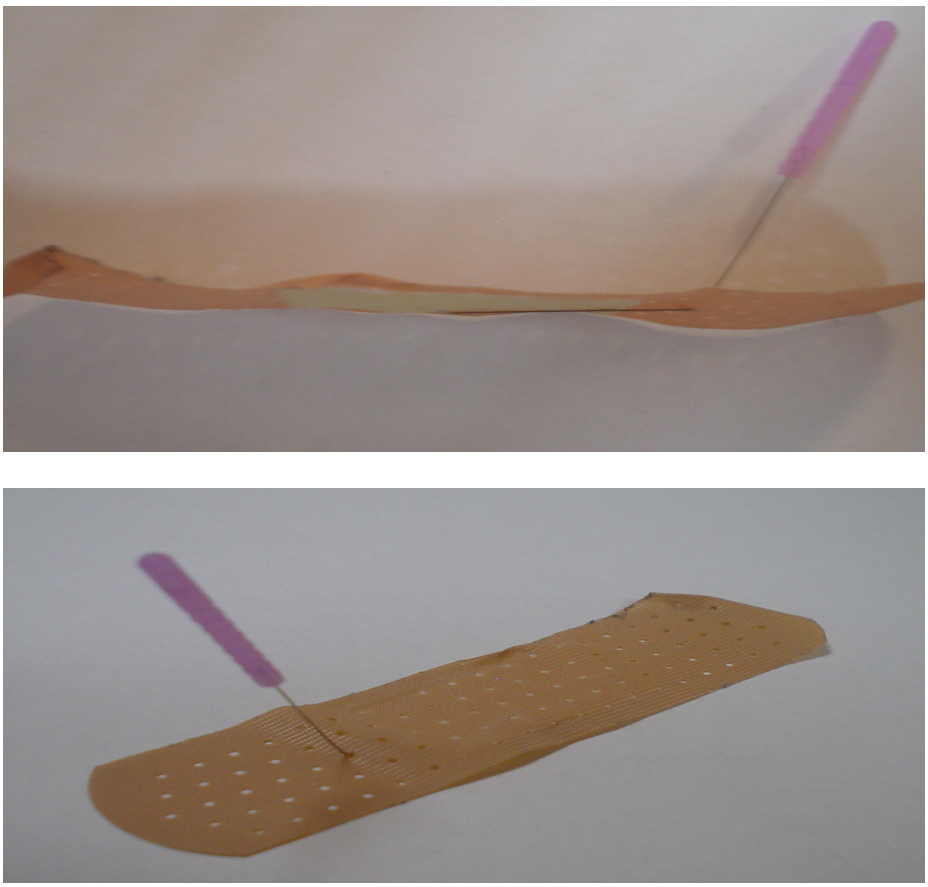


Figure 5.1 Sham-acupuncture design

The top panel shows the reverse side of the sham needle. The needle is bent and the tip is hidden under the bandage. The lower panel shows the top side of the sham needle.

Table 5.1 Summary of interventions

	Manual acupuncture	**Electro-acupuncture**	**Sham acupuncture**
Posture of the subjects	Supine	Supine	Supine
Locations	ST 36 and ST 40 on the dominant leg	ST 36 and ST40 on the dominant leg	1-2 cm next to ST36 and ST40 on the dominant leg
Insertion	20-25 mm	20-25 mm	Non-invasive mock insertion
De qi	Yes	Yes	No
Duration	25 min	25 min	25 min
Schedule of needle manipulations	Every 5 minutes.	Once (at the beginning).	Every 5 minutes.
Conduction of manipulations	Needles were twirled at a moderate speed with 180 to 360 degrees of rotation in a bidirectional manner, first clockwise then anticlockwise. This action was repeated nine times in each episode of manipulation, and lasted about 10 seconds.	After needle insertion and *de qi* sensations, a modified EA stimulator was connected to the two needles via two electrodes. Frequency: Dense-Disperse mode with alternating frequencies at 2- and 100- Hz,every 6 seconds. Intensity: a strong but tolerable intensity with visible muscle contraction. The intensity of electrical stimulation was kept consistent during the treatment period.	Manipulations were made by pressing the bended needles to produce a pricking sensation. A mock electrical acupuncture stimulator was connected to the end of the two needles via wires, and was placed on a table within volunteers' eyesight, showing a continuously flashing light.

5.4 Primary outcome measures – pain assessment

Primary outcome measures were: 1. pain threshold to single electrical stimulation (i.e. SPT); 2. pain threshold to repeated electrical stimulation (i.e. TST); and 3. pain ratings to supra-threshold stimulation at 1.2 X TST and 1.4 X TST intensity, using the methods developed by Arendt-Nielsen et al., 1994 (33). SPT and TST were assessed at three body sites during baseline, immediately after the intervention and 24-hours after the intervention.

5.4.1 Sites of assessment

The following three sites were selected for assessment (Figures 5.2 – 5.4).

Treatment leg site: At the skin surface of the tibia anterior muscle along the sural nerve path of the dominant leg and parallel to the mid point between the acupoints ST36 and ST40 (dermatome Lumbar-5 segment).

Non-treatment leg site: At the skin surface of the tibia anterior muscle along the sural nerve path of the non-dominant leg and parallel to the mid point between the acupoints ST36 and ST40 (dermatome Lumbar-5 segment).

Forearm (non-dominant side): At the dorsal skin surface of the non-dominant forearm along the median nerve path and 3-4 cm above the wrist crease (dermatome Cervical-7 segment).

The following pictures illustrate the locations of assessment sites and the locations of acupuncture points; no picture was taken of the volunteers during the experimental period due to privacy considerations. The pictures are edited uding Adobe Photoshop 8.0 software (Adobe software company, USA).

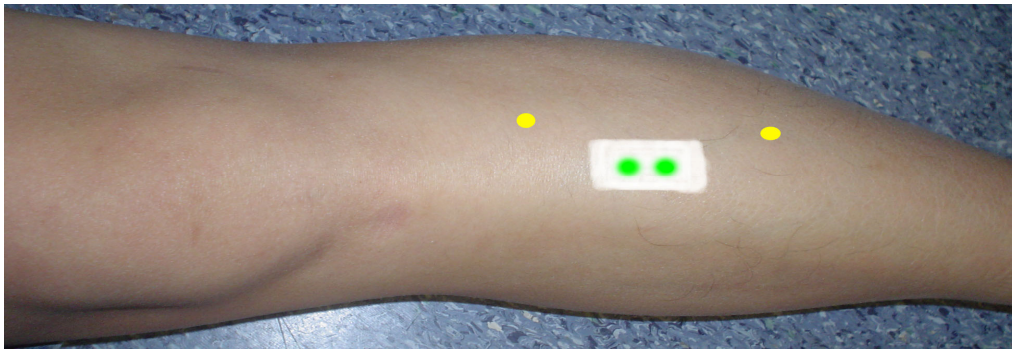


Figure 5.2 Sites of assessment and acupuncture on the treatment leg (dominant leg)

Two green spots represent the assessment sites, i.e. the location of the two adhesive electrodes. Two yellow spots represent the acupuncture sites. The digitally whitened area represents the skin area that has been prepared for pain assessment.

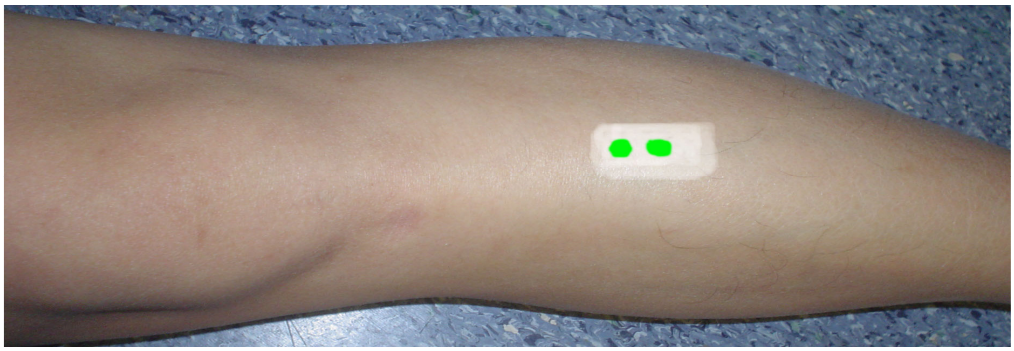


Figure 5.3 Sites of assessment on the non-treatment leg (non-dominant side)

Two green spots represent the assessment sites, i.e. the location of the two adhesive electrodes. The digitally whitened area represents the skin area that has been prepared for pain assessment.

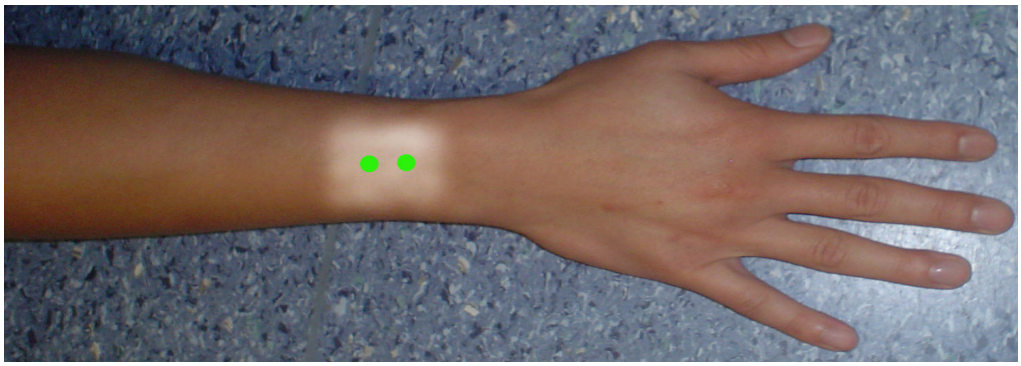


Figure 5.4 Sites of assessment on the forearm (non-dominant side)

Two green spots represent the assessment sites, i.e. the location of the two adhesive electrodes. The digitally whitened area represents the skin area that has been prepared for pain assessment.

5.4.2 Electrical stimulation and instruments

Electrical stimulations were delivered with an electrical stimulator (Grass S88, USA) that was connected to an isolation unit (Grass SIU5, USA) and a constant current unit (Grass CCU1, USA). The isolation unit was connected between the stimulator and the constant current unit to avoid electricity surge. The constant current unit controlled the magnitude of the electrical current and was connected to the assessment sites via electrodes. Constant current pulses were delivered to the proposed skin surface via two adhesive ECG electrodes (1x1 cm diameter, Dantec Medical, USA), which were filled with electrode gel. A standard pulse train, consisting of five individual 1-ms pulses delivered at 200-Hz was used as a single stimulus throughout the experiment. This single stimulus was repeated five times at 2-hertz to form a train of stimuli, and these repeated stimuli were used for TST tests. The frequency of the electrical impulses was calibrated with an oscilloscope (HM1007, HAMEG, Germany) before the start of the pain assessments. The instruments and their connections are displayed in Figure 5.5 as follows.

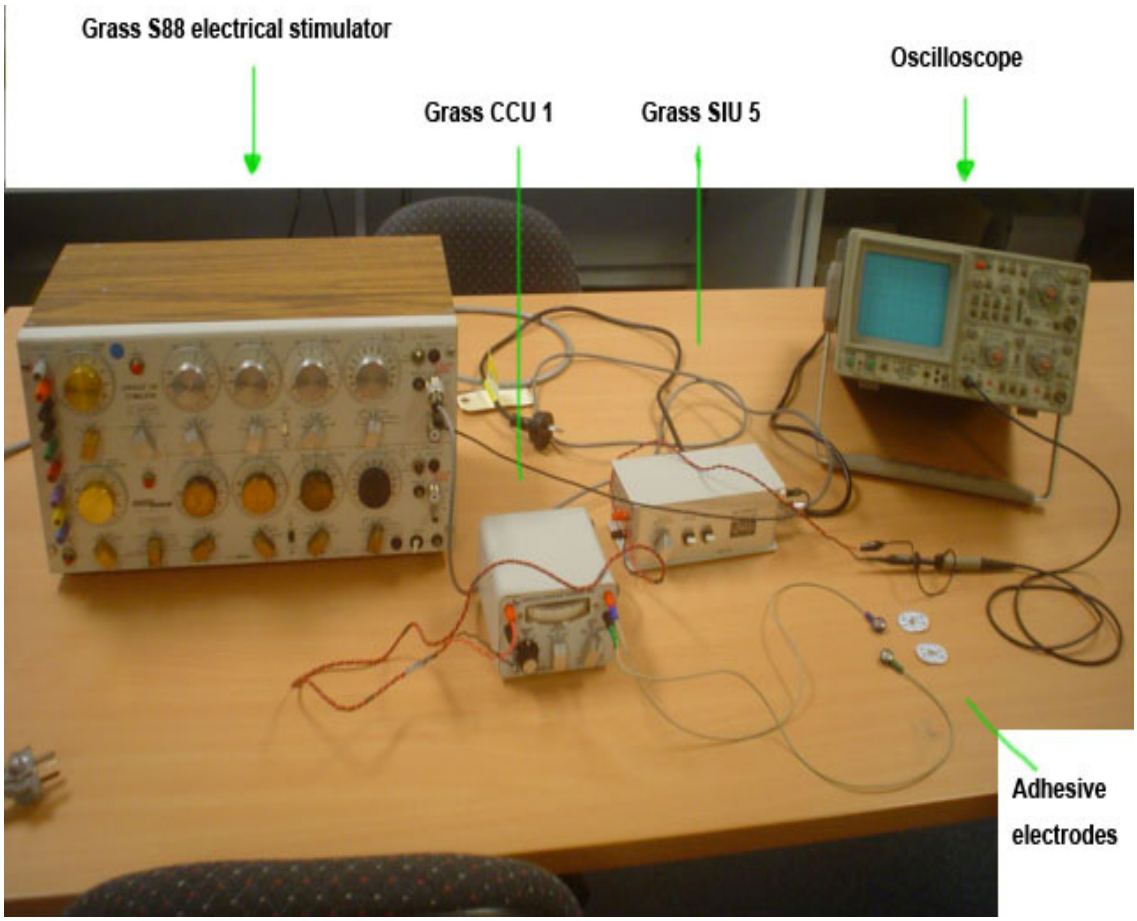


Figure 5.5 The instruments used to produce electrical stimulations

Grass CCU1 is the model of the constant current unit. Grass SIU5 is the model of the isolation unit.

5.4.3 Procedures of pain assessment

Three steps were involved in assessing the intensities of electrical current induced SPT and TST and the pain response ratings to ST levels of TS stimuli (at 1.2 x TST and 1.4 x TST intensities). They were performed at each assessed site before (baseline), immediately after and 24-hours after the intervention session. Each step is explained as follows.

Step 1: Testing SPT. The intensity of the current was increased from zero milliampere (mA) in steps of 2 mA. If no pain was recorded at 20 mA, the current intensity was increased in steps of 5 mA until a pain threshold could be recorded, or a maximum 50 mA was reached, whichever was reached first. The lowest current value of a single stimulus to elicit a sensation of pain (via verbal report) was recorded. Pain was defined as a definite sharp or pin prick sensation, like an injection. Inter-stimulus interval was 30 seconds. The SPT test was measured twice. The mean value was accepted for data analysis.

Step 2: Testing TST. TST was tested after SPT. The intensity of the current was increased from zero mA in steps of 2 mA, if no TST could recorded at 20 mA, the current intensity was increased in steps of 5 mA until a TST could be recorded, or a maximum 50 mA was reached. The lowest current value to elicit a painful sensation in response to the 4th or 5th stimulus of a train of five stimuli (via verbal report) was recorded. Inter-stimulus interval was 30 seconds. TST was tested twice, and the mean value was accepted for data analysis.

Step 3: Pain ratings in response to supra-threshold stimulation. According to the protocol, after TST was obtained, Investigator A delivered ST stimulation at 1.2 and 1.4 times the baseline TST. After each train of stimuli, the volunteers were asked to rate the intensity of their responses to the first and the fifth stimulus within a train of five stimuli on a Visual Analogue Scale (VAS). Stimulation at each intensity (1.2 x TST, 1.4 x TST) was measured twice. The mean values of pain ratings to the fifth stimulus (highest intensity perceived) were used for data analysis. The VAS is a standard instrument for measuring the intensity of pain. One end of the scale is '0', indicating 'no pain' at all; while '100' is at the other end of the scale, indicating "worst pain imaginable" (Appendix 09).

5.5 Secondary outcome measures

The secondary outcome measures include:

1) Spielberg State and Anxiety Inventory (SSAI), administered before and after the intervention by Investigator A (the evaluator); and
2) a post-treatment questionnaire, administered once after the intervention by Investigator A.

The SSAI was used to measure volunteers' level of anxiety before and after the intervention (135). A total of 20 questions with 10 negative and 10 positive questions listed in random order were answered by each volunteer. Each question had four possible answers: 1. not at all; 2. some what; 3. moderately so; 4. very much so. An answer key form provided the true score to each answer of each question and this answer key was kept away from the volunteers. The total score was calculated by adding up the true scores. Due to copyright reason, a copy of this questionnaire and the answer key form are not presented in this thesis.

Immediately after the intervention session, Investigator A asked the volunteer to complete the post-treatment questionnaire. This questionnaire had three questions. In the first question, the volunteer was asked to describe the sensation he / she perceived during the intervention using a standardised verbal pain categorical scale: (1) no pain at all; (2) mild pain; (3) moderate pain; (4) severe pain. The other two questions were developed by Lao and colleagues in 1999 (80), to evaluate whether the volunteers were blinded successfully (Appendix 10).

5.6 Description of experimental procedures

One day prior to the experiment, the volunteer was asked not to miss breakfast or lunch and not to drink coffee or take any stimulants on the day of the experiment. On the experiment day, after arrival and after obtaining the written consent form, Investigator A prepared the skin area for testing and conducted the training session. Investigator A gently shaved the body hair at the proposed stimulation sites if necessary, cleaned the skin with a moisturised tissue and alcohol swab (Briemar, Australia) to remove any oil on the skin and enhance electrical conduction.

The procedure of the experiment is illustrated in Table 5.4. During the 30 minutes training session, the volunteer was asked to feel and be familiarised with the sensation and mild pain induced by electrical stimulation of various intensities. The volunteer was also trained to use VAS.

The volunteer then had a five-minute rest. In the following 45 minutes, the baseline pain assessments were conducted, this was followed by the completion of the baseline SSAI by the volunteer. Investigator A then left the room and Investigator B entered the room. The volunteer was asked to pick one of the sealed envelops which contained a random number. Investigator B then conducted the allocated intervention in the following 25 minutes with the volunteer in a supine position. The volunteer and Investigator B were free to communicate as if in a clinical situation. Once Investigator B had finished the intervention and left the room, Investigator A came back. Immediately after the intervention, Investigator A asked the volunteer to complete the SSAI questionnaire once again, followed by the post-treatment questionnaire. These procedures were finished within 10 minutes followed by a 20-minute rest in the chair. At 30 minutes after the intervention, pain assessments were conducted within 45 minutes using the same

methods. The next day, at 24 hours after the intervention, the volunteer came back to the clinical trial room. Investigator A prepared the skin area once again and conducted another 45 minutes of pain assessments using the same methods.

Table 5.2 Experimental procedures

Step No.	Timeline (minutes)	Title of Activity	Activity description
1.	Prior to test	Preparation	Obtain written consent. Skin preparation.
2.	0 - 30	Training session	The volunteer was trained to familiarise themselves with the sensation induced by electrical stimulation and learn how to use VAS.
3.	30 - 35	A rest period	The volunteer was seated and rested for 5 minutes.
4.	35 - 80	The baseline pain assessments	Firstly, the pain threshold to a single electrical stimulus (SPT) was tested, and then the pain threshold to repetitive stimuli (TST) was tested at each of the three assessed sites in a sitting position.
5.	80 - 90	Baseline SSAI questionnaire	Spielberg state anxiety inventory (SSAI) was completed by the volunteer.
6.	90 - 120	Intervention procedures	Volunteer was randomly allocated to one of the three groups, and then one of the interventions (MA, EA and SA) was delivered to the volunteers in a supine position.
7.	120 - 130	Post-treatment SSAI and the post-treatment questionnaires	SSAI was completed by the volunteer once again. Then they completed the post-treatment questionnaires.
8.	130 - 150	A rest period	The volunteer was seated and had a rest for 20 minutes.
9.	150 - 195	Pain assessments 30 minutes after intervention	Pain assessments were conducted 30 minutes after the end of the intervention using the same methods and schedules as the baseline measurement.
10.	24 hours after the end of intervention	Pain assessments 24-hours after intervention	Pain assessments were repeated 24 hours after the end of the intervention using the same methods and schedules as the baseline measurement.

5.7 Statistical analysis

The data of SPT, TST and anxiety assessment (SSAI) were analysed by analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS, Windows Version 13.0) to detect between treatment group differences. Significance for each of the ANOVA's was assessed at $\alpha = 0.05 / 3 = 0.0167$ (Bonferroni Correction). Post-hoc tests via multiple-comparisons using a Bonferroni correction were used to detect significant differences among the three interventions when a significant ANOVA was obtained. Equivalence of the groups on demographic variables, i.e. age, gender, dominant hand; and on the answers provided in the post-treatment questionnaires were assessed by ANOVA and *chi*-square tests. Statistical power analysis and sample size calculations were done via the MINITAB statistical package (MINITAB, Windows Version 15.0).

Chapter 6: Results

6.1 General information about the volunteers

The experiment was conducted from May to September in 2006. A total of 27 volunteers were recruited according to the inclusion/exclusion criteria. All volunteers signed the written consent forms and completed all experimental procedures. No one withdrew from the study, and no side effects were reported. The demographic data about the volunteers are provided in Table 6.1. All volunteers were University students, including 15 males and 12 females. The average age was 24.81 years (SD 5.4), with a range from 18 to 41 years. Three volunteers had a left-dominant side and 24 volunteers had a right-dominant side; the dominant side of leg and forearm were consistent in every person. The three groups were comparable for demographic characteristics.

Every step of the experiment was conducted according to the trial protocol except for the rating to supra-threshold stimulation. An error was made when conducting this step of the experiment and this is described in the Methods. This error did not affect other steps of the study. There was no missing data.

In this thesis, the terms “treatment leg”, “non-treatment leg” and “forearm” represent the dominant-leg, non-dominant leg and the non-dominant forearm respectively. In addition, the term single pain threshold (SPT) represents the pain threshold to single electrical stimulus, and the term temporal summation threshold (TST) represents the pain threshold to a train of five repeated stimuli delivered at 2-hertz.

Table 6.1 Demographic variables at baseline in each group

	SA (*n* = 9)	**MA** (*n* = 9)	**EA** (*n* = 9)	**Statistical tests**	***p***	**Total** (*n*=27)
Age (years) (Mean ± SD)	24.67 ±5.03	25.22 ±4.32	24.56 ±7.11	*F* value (2, 24) = 0.036	0.964 *	24.81 ± 5.4
Gender (Male : Female)	3 : 6	5 : 4	7 : 2	χ^2 (*df*=*2*) = 3.6 **a**	0.165 ▲	15:12
Dominant hand (Right : Left)	8 : 1	9 : 0	7 : 2	χ^2 (*df*=*2*) = 2.25 **b**	0.325 ▲	24:3

* The significance level for the ANOVA was set at $\alpha = 0.05/3 = 0.0167$ (Bonferroni Correction).
df: degrees of freedom.
χ^2: *chi*-square value.
a 3 cells (50.0%) have expected count less than 5. The minimum expected count is 4.00.
b 3 cells (50.0%) have expected count less than 5. The minimum expected count is 1.00.
▲ The significance level for the above *chi*-square calculations was at $p < 0.05$.

6.2 A comparison of the baseline variables among the three groups

There was no statistically significant difference among the three groups in any of the baseline pain tests or the anxiety test (Table 6.2). The baseline mean values of TST were always lower than those of SPT assessed at each site (Figure 6.1).

Table 6.2 A comparison of the baseline values of all pain assessments among the three intervention groups – One-way ANOVA

		Mean ± SD	*n*	*df*	*F*	*p**
SPT baseline - Treatment leg	Between Groups	SA: 1.98 ± 0.71	9	2, 26	0.475	0.627
		MA: 1.78 ± 1.05	9			
		EA: 2.19 ± 0.88	9			
TST baseline - Treatment leg	Between Groups	SA: 1.16 ± 0.35	9	2, 26	0.796	0.463
		MA: 0.92 ± 0.75	9			
		EA: 1.26 ± 0.55	9			
SPT baseline - Non-treatment leg	Between Groups	SA: 1.97 ± 0.59	9	2, 26	0.002	0.998
		MA: 1.96 ± 0.91	9			
		EA: 1.98 ± 0.83	9			
TST baseline - Non-treatment leg	Between Groups	SA: 1.18 ± 0.32	9	2, 26	0.153	0.859
		MA: 1.10 ± 0.66	9			
		EA: 1.04 ± 0.50	9			
SPT baseline - Forearm	Between Groups	SA: 2.31 ± 0.80	9	2, 26	0.842	0.443
		MA: 1.81 ± 0.96	9			
		EA: 2.21 ± 0.82	9			
TST baseline - Forearm	Between Groups	SA: 1.41 ± 0.62	9	2, 26	1.151	0.333
		MA: 0.98 ± 0.66	9			
		EA: 1.13 ± 0.55	9			
SSAI Baseline	Between Groups	SA: 33.33 ± 9.50	9	2, 26	0.561	0.578
		MA: 32.22 ± 11.10	9			
		EA: 29.00 ± 5.50	9			

* The significance level for each of the ANOVAs was set at $\alpha = 0.05/3 = 0.0167$ (Bonferroni Correction).
SPT – single pain threshold
TST - temporal summation threshold
SSAI - Spielberg state anxiety inventory
df: degrees of freedom

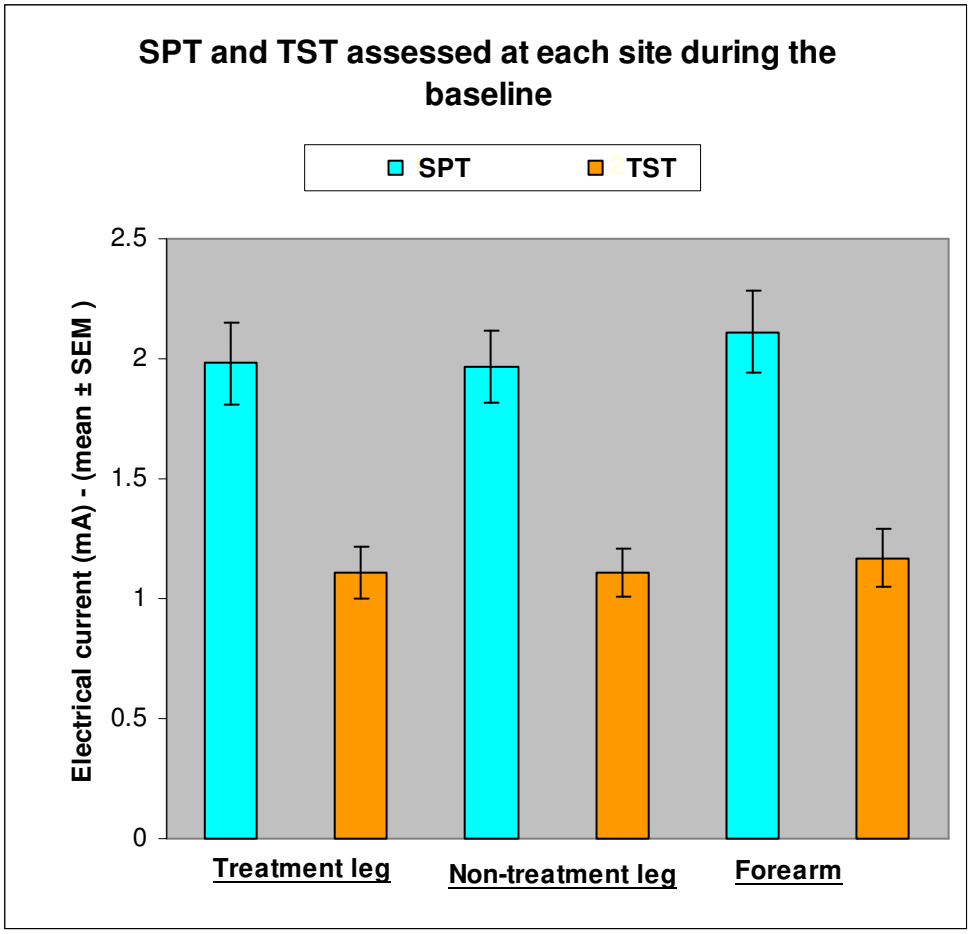


Figure 6.1 SPT and TST assessed at each site during the baseline

Figure 6.1 shows the single pain threshold and temporal summation threshold assessed at three sites during the baseline (n = 27).

SPT – single pain threshold

TST - temporal summation threshold

6.3 Single pain thresholds

6.3.1 The effect of acupuncture on single pain thresholds tested immediately after the interventions

Table 6.3 shows the one-way ANOVA results of the effect immediately after intervention on SPT among the three intervention groups. Although SPT in the MA and EA groups were higher than those in the SA group at the treatment leg, no statistically significant differences were detected.

Table 6.3 A comparison of single pain thresholds assessed immediately after interventions among the three groups – One-way ANOVA.

		Mean ± SD	*n*	*df*	*F*	*p**
SPT immediately after the interventions - Treatment leg	Between Groups	SA: 1.92 ± 0.81	9	2, 26	4.585	0.021
		MA: 2.41 ± 1.55	9			
		EA: 4.27 ± 2.44	9			
SPT immediately after the interventions - Non-treatment leg	Between Groups	SA: 1.83 ± 0.64	9	2, 26	2.791	0.081
		MA: 2.27 ± 0.87	9			
		EA: 2.98 ± 1.44	9			
SPT immediately after the interventions - Forearm	Between Groups	SA: 1.99 ± 0.67	9	2, 26	2.777	0.082
		MA: 2.08 ± 1.11	9			
		EA: 3.22 ± 1.71	9			

* The significance level for each of the ANOVAs was set at $\alpha = 0.05/3 = 0.0167$ (Bonferroni Correction).
SPT – single pain threshold
TST - temporal summation threshold
df: degrees of freedom

6.3.2 The effect of acupuncture on single pain thresholds tested 24-hours after the interventions

Table 6.4 shows the one-way ANOVA results of SPT assessed 24-hours after the interventions. There was statistically significant group difference in SPT, assessed on the treatment leg (p = 0.01). Bonferroni corrected post-hoc comparisons indicated that EA increased the SPT significantly greater than SA on the treatment leg 24-hours after intervention (Table 6.5) (EA versus SA, p = 0.012), and there was no statistically significant difference between MA and SA or EA and MA. Although EA also increased the SPT on the non-treatment leg and forearm, they failed to reach a statistical significance level.

Table 6.4 A comparison of single pain threshold assessed 24-hours after the interventions among the three groups – One-way ANOVA

		Mean ± SD	*n*	*df*	*F*	*p**
SPT 24-hours after the interventions - Treatment leg	Between Groups	SA: 1.98 ± 0.76	9	2, 26	5.601	0.010 #
		MA: 2.82 ± 1.70	9			
		EA: 5.82 ± 4.03	9			
SPT 24-hours after the interventions - Non-treatment leg	Between Groups	SA: 1.88 ± 0.61	9	2, 26	4.623	0.020
		MA: 2.37 ± 1.08	9			
		EA: 3.42 ± 1.45	9			
SPT 24-hours after the interventions - Forearm	Between Groups	SA: 2.01 ± 0.68	9	2, 26	2.644	0.092
		MA: 2.29 ± 1.13	9			
		EA: 3.26 ± 1.62	9			

* The significance level for each of the ANOVAs was set at $\alpha = 0.05/3 = 0.0167$ (Bonferroni Correction).
# indicates statistically significant.
SPT - single pain threshold
TST - temporal summation threshold
df: degrees of freedom

Table 6.5 Between-group comparisons of single pain threshold assessed on the treatment leg 24-hours after the interventions between groups - Bonferroni corrected post-hoc tests

Bonferroni corrected post-hoc tests (*n* = 9 in each group)						
Dependent Variable	**Comparison**	**Mean Difference**	**Std. Error**	***p****	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
SPT 24-hours after the interventions - Treatment leg	SA vs. MA	-0.84	1.21	1.000	-3.95	2.26
	MA vs. EA	-3.00	1.21	0.061	-6.11	0.11
	SA vs. EA	-3.84	1.21	0.012 #	-0.74	-6.95

* The significance level for each of the ANOVAs was set at $\alpha = 0.05/3 = 0.0167$ (Bonferroni Correction).
# indicates statistically significant.

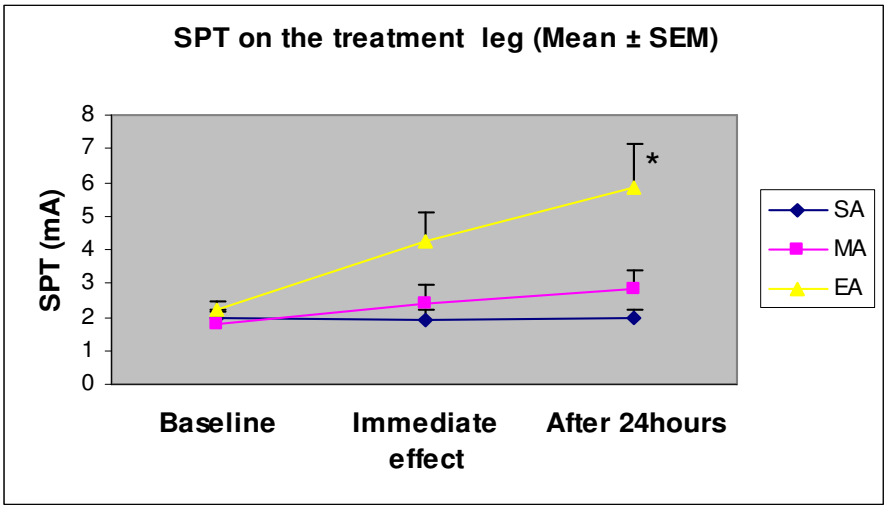
6.3.3 The time effect of acupuncture on the single pain thresholds within 24-hours

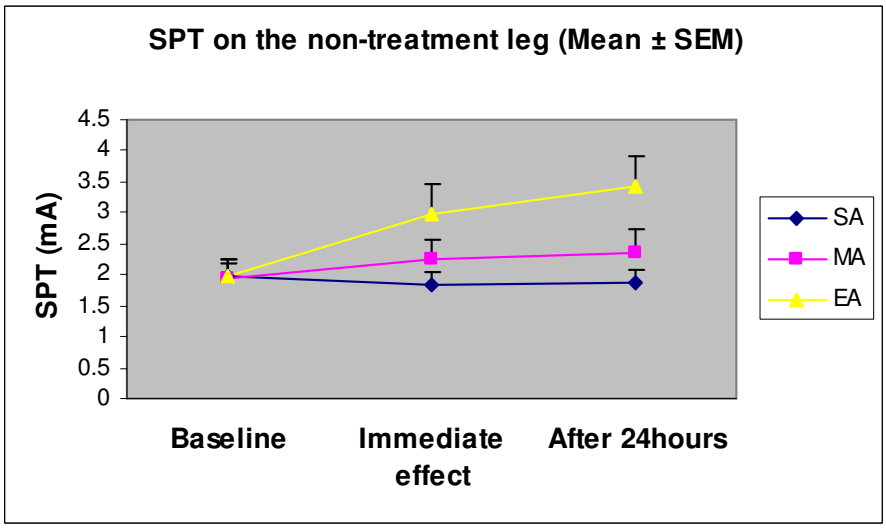
Figure 6.2 shows the change of SPT values assessed at baseline, immediately after and 24-hours after the interventions at the three sites, i.e. the treatment leg, the non-treatment leg and the forearm.

On the treatment leg, the SPT in the SA group did not change over time, whereas those in the EA and MA groups increased, and the increase in the EA was much higher than that in MA. Only the result of EA versus SA at 24-hours after the interventions showed statistically significant differences (see sections 6.3.1 and 6.3.2).

On the non-treatment leg, after the interventions, the SPT in the SA group did not change over time, whereas those in the EA and MA groups were increased. However, no statistical significance between the three groups in the SPT assessed on the non-treatment leg was detected (see sections 6.3.1 and 6.3.2).

On the forearm, after the interventions, the SPT in SA and MA groups did not change much over time, when those in the EA group were increased immediately after the intervention and maintained that level at 24-hours after. No statistical significance between the three groups in the SPT assessed on the forearm was detected (see sections 6.3.1 and 6.3.2).





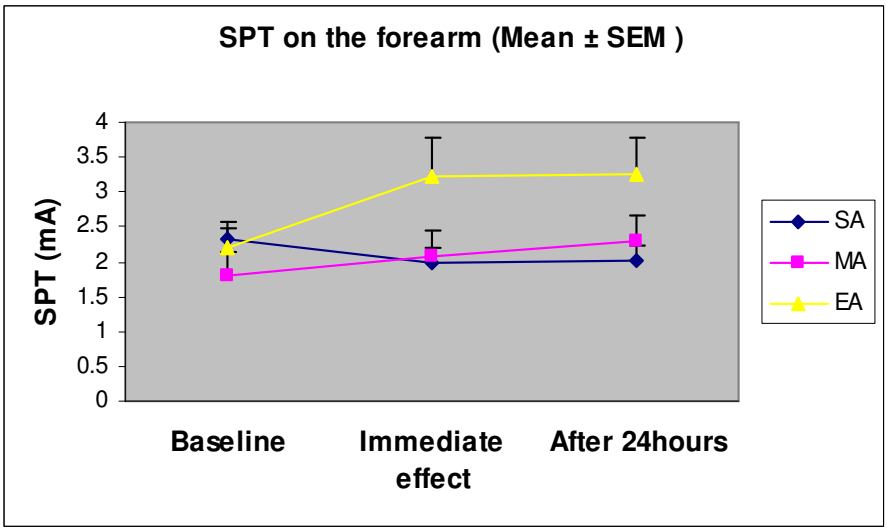


Figure 6.2 Single pain thresholds of the three intervention groups tested at the three sites at baseline, immediately after the interventions and 24-hours after (*n* = 9 in each group)

SA = sham-acupuncture, MA = manual acupuncture, EA = electro-acupuncture.

Immediate effect = immediately after interventions; After 24hours = 24 hours after interventions.

6.4 Temporal summation pain threshold

6.4.1 The effect of acupuncture on temporal summation thresholds tested immediately after the interventions

Table 6.6 shows the one-way ANOVA results of the effect immediately after intervention on TST among the three intervention groups. Although TST in the MA and EA groups were much higher than those in the SA group at the treatment leg, no statistically significant difference was detected.

Table 6.6 A comparison of temporal summation thresholds assessed immediately after interventions among the three groups – One-way ANOVA.

		Mean ± SD	*n*	*df*	*F*	*p**
TST immediately after the interventions - Treatment leg	Between Groups	SA: 1.03 ± 0.43	9	2, 26	4.309	0.025
		MA: 1.28 ± 0.92	9			
		EA: 2.32 ± 1.38	9			
TST immediately after the interventions - Non-treatment leg	Between Groups	SA: 1.07 ± 0.41	9	2, 26	0.826	0.450
		MA: 1.37 ± 0.78	9			
		EA: 1.43 ± 0.69	9			
TST immediately after the interventions - Forearm	Between Groups	SA: 1.27 ± 0.55	9	2, 26	0.475	0.627
		MA: 1.31 ± 0.81	9			
		EA: 1.57 ± 0.73	9			

* The significance level for each of the ANOVAs was set at $\alpha = 0.05/3 = 0.0167$ (Bonferroni Correction).
SPT - single pain threshold
TST - temporal summation threshold
df: degrees of freedom

6.4.2 The effect of acupuncture on temporal summation thresholds tested at 24-hours after the interventions

Table 6.7 shows the one-way ANOVA results on TST assessed 24-hours after interventions. There was a statistically significant group difference in TST, assessed on the treatment leg ($p = 0.013$). Bonferroni corrected post-hoc comparisons indicated that EA increased the TST significantly greater than SA on the treatment leg 24-hours after interventions (Table 6.8) (EA versus SA, $p = 0.011$), and there was no statistically significant difference between MA and SA or EA and MA. Although EA also increased the TST on the non-treatment leg and forearm, they failed to reach a statistical significance level.

Table 6.7 A comparison of temporal summation threshold assessed 24-hours after the interventions among the three groups – One-way ANOVA

		Mean ± SD	*n*	*df*	*F*	*p**
TST 24-hours after the interventions - Treatment leg	Between Groups	SA: 1.06 ± 0.40	9	2, 26	5.233	0.013 #
		MA: 1.66 ± 0.96	9			
		EA: 2.4 ± 1.12	9			
TST 24-hours after the interventions - Non-treatment leg	Between Groups	SA: 1.07 ± 0.43	9	2, 26	4.551	0.021
		MA: 1.40 ± 0.86	9			
		EA: 2.01 ± 0.66	9			
TST 24-hours after the interventions - Forearm	Between Groups	SA: 1.24 ± 0.59	9	2, 26	2.452	0.107
		MA: 1.32 ± 0.82	9			
		EA: 1.91 ± 0.66	9			

* The significance level for each of the ANOVAs was set at $\alpha = 0.05/3 = 0.0167$ (Bonferroni Correction).
# indicates statistically significant.
SPT – single pain threshold
TST - temporal summation threshold
df: degrees of freedom.

Table 6.8 Comparisons of temporal summation threshold assessed on the treatment leg 24-hours after interventions within groups - Bonferroni post hoc tests

Bonferroni corrected post-hoc tests (*n* = 9 in each group)						
Dependent Variable	**Comparisons**	**Mean Difference**	**Std. Error**	***p****	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
TST 24-hours after the interventions - Treatment leg	SA vs. MA	-0.60	0.42	0.488	-1.67	0.47
	MA vs. EA	-0.74	0.42	0.259	-1.82	0.33
	EA vs. SA	1.34	0.42	0.011 #	0.27	2.42

* The significance level for each of the ANOVAs was set at $\alpha = 0.05/3 = 0.0167$ (Bonferroni Correction).
# indicates statistically significant.

6.4.3 The time effect of acupuncture on the temporal summation thresholds within 24-hours

Figure 6.3 shows the change of TST values assessed at baseline, immediately after and 24-hours after the interventions at the three sites, i.e., the treatment leg, the non-treatment leg and the forearm.

On the treatment leg, the TST in the SA group did not change over time, whereas those in the EA and MA groups increased, and the increase in the EA was much higher than that in MA. Only the result of EA versus SA at 24-hours after the interventions showed statistically significant differences (see sections 6.4.1 and 6.4.2).

On the non-treatment leg, after the interventions, the TST in the SA group did not change over time, whereas those in the EA and MA groups were increased. However, no statistical significance between the three groups in the TST assessed on the non-treatment leg was detected (see sections 6.4.1 and 6.4.2).

On the forearm, after the interventions, the TST in the SA and MA groups did not change much over time, whereas those in the EA increased immediately after the intervention and maintained that level 24-hours after. No group differences in the TST assessed on the forearm were detected (see sections 6.4.1 and 6.4.2).

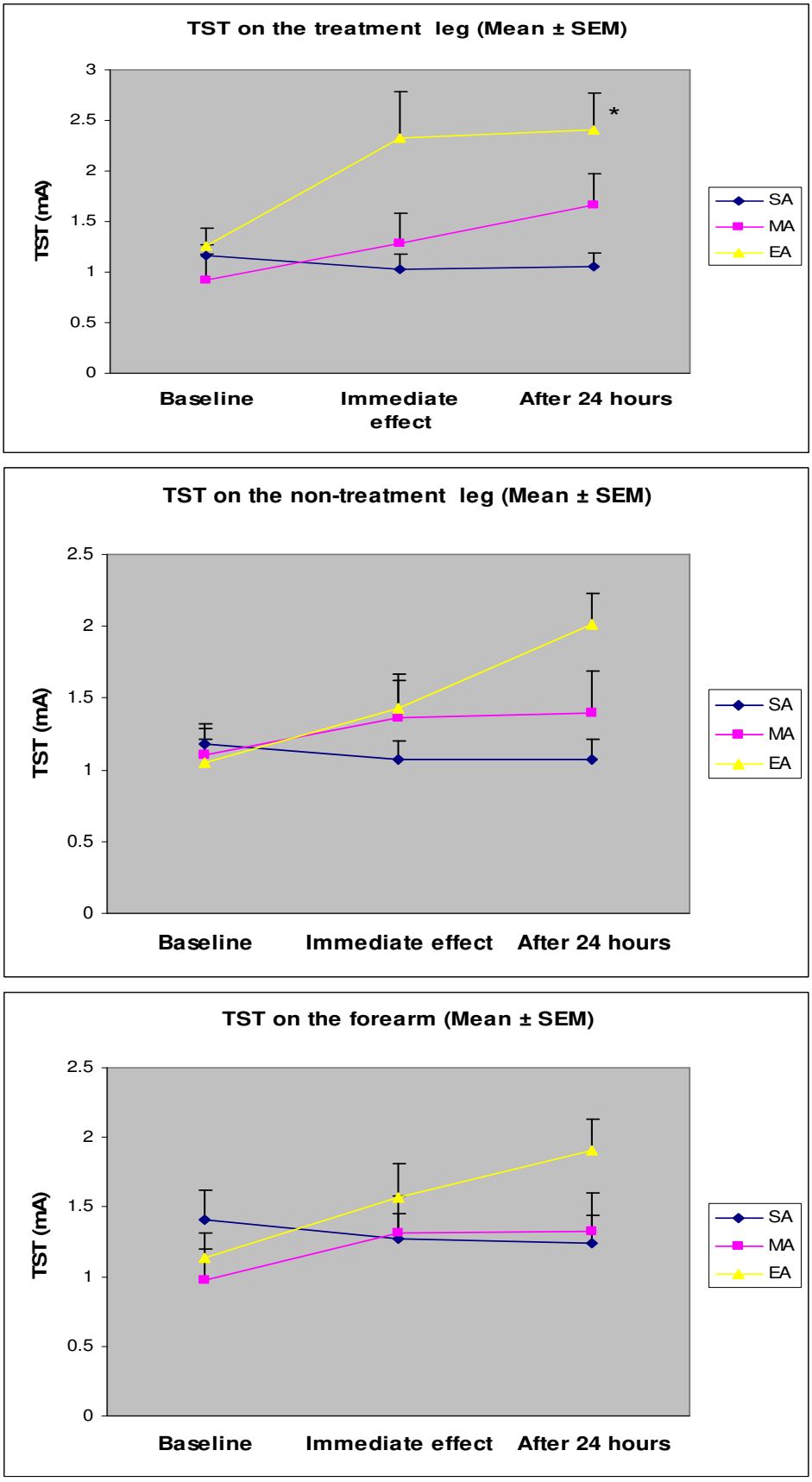


Figure 6.3 Temporal summation thresholds of the three intervention groups tested at the three sites at baseline, immediately after the interventions and 24-hours after (*n* = 9 in each group)

SA = sham-acupuncture, MA = manual acupuncture, EA = electro-acupuncture.
Immediate effect = immediately after interventions; After 24hours = 24 hours after interventions.

6.5 The number of responders in the three groups

In a previous acupuncture study in healthy humans, the volunteers who had a greater than 20% increase in pain thresholds from the baseline were classified as a responder (19). The same method was used in the current study, and the result is summarised in Table 6.9. No subject in the SA group was classified as responders. Overall, more subjects in the EA group than in the MA groups were responders.

Table 6.9 The number of responders in each group

			Numbers of responders in each intervention group		
			SA (*n*)	**MA (*n*)**	**EA (*n*)**
SPT	**Immediately after interventions**	**Treatment Leg**	0	6	7
		Forearm	0	2	3
		Non-treatment Leg	0	2	7
	24-hours after interventions	**Treatment Leg**	0	8	9
		Forearm	0	5	4
		Non-treatment Leg	0	5	7
TST	**Immediately after interventions**	**Treatment Leg**	0	7	9
		Forearm	0	4	8
		Non-treatment Leg	0	4	6
	24-hours after interventions	**Treatment Leg**	0	9	8
		Forearm	0	6	7
		Non-treatment Leg	0	5	8

6.6 Post-hoc power analyses and sample size calculations

In Tables 6.10, 6.11 and 6.12, the effective power of the statistical tests with the existing sample size and the required number for 80% power for the comparisons of EA versus MA, EA versus SA and MA versus SA in SPT and TST tests are presented.

6.6.1 EA versus MA

For the comparison between EA and MA (Table 6.10), none of the statistical power values of the pain assessments was above 49%. The least number of subjects in both groups required to achieve an effective power of 80% would be: 21 subjects for SPT test immediately after interventions, 18 subjects for SPT test 24-hours after interventions, 21 subjects for TST test immediately after interventions and 26 subjects for TST test 24-hours after interventions.

Table 6.10 Post-hoc power analyses and required sample sizes for EA versus MA comparisons

EA versus MA					
		EA	**MA**	**Effective power**	***n* required for 80% power in each group**
		n	***n***		
SPT immediately after	**Treatment leg**	9	9	44%	21
	Non-treatment leg	9	9	22%	45
	Forearm	9	9	35%	26
SPT 24-hours after	**Treatment leg**	9	9	49%	18
	Non-treatment leg	9	9	38%	25
	Forearm	9	9	28%	34
TST immediately after	**Treatment leg**	9	9	43%	21
	Non-treatment leg	9	9	5%	1943
	Forearm	9	9	10%	144
TST 24-hours after	**Treatment leg**	9	9	30%	32
	Non-treatment leg	9	9	35%	26
	Forearm	9	9	35%	27

* The sample size that would be required in each group for 80% power.

6.6.2 EA versus SA

For the comparison between EA and SA (Table 6.11), the effective statistical power of the pain assessments were from 15% to 92%. The TST pain assessments after 24-hours at the non-treatment leg and treatment leg had effective statistical powers above 80%; these were 92% and 89% respectively. The SPT pain assessment after 24-hours at the non-treatment leg had 79% effective power with the sample sizes used. The least number of subjects in both groups that could have achieved an effective power of 80% would be: 11 subjects for immediate effect SPT test, 10 subjects for after 24-hours SPT test, 11 subjects for immediate effect TST test and seven subjects for after 24-hours TST test.

Table 6.11 Post-hoc power analyses and required sample sizes for EA versus SA comparisons

EA versus SA					
		EA	**SA**	**Effective power**	*** *n* for 80% power required in each group**
		n	***n***		
SPT immediately after	**Treatment leg**	9	9	73%	11
	Non-treatment leg	9	9	54%	16
	Forearm	9	9	47%	19
SPT 24-hours after	**Treatment leg**	9	9	75%	10
	Non-treatment leg	9	9	79%	10
	Forearm	9	9	52%	17
TST immediately after	**Treatment leg**	9	9	71%	11
	Non-treatment leg	9	9	25%	39
	Forearm	9	9	15%	75
TST 24-hours after	**Treatment leg**	9	9	89%	8
	Non-treatment leg	9	9	92%	7
	Forearm	9	9	58%	15

* The sample size that would be required in each group for 80% power.

6.6.3 MA versus SA

For the comparison between MA and SA (Table 6.12), none of the statistical power values of the pain assessment statistical tests was above 37%. The least number of subjects in both groups that could have achieved an effective power value of 80% would be: 50 subjects for immediate effect SPT test, 40 subjects for after 24-hours SPT test, 69 subjects for immediate effect TST test and 25 subjects for after 24-hours TST test.

Table 6.12 Post-hoc power analyses and required sample sizes for MA versus SA comparisons

MA versus SA					
		MA	**SA**	**Effective power**	*** *n* for 80% power required in each group**
		n	***n***		
SPT immediately after	**Treatment leg**	9	9	12%	101
	Non-treatment leg	9	9	20%	50
	Forearm	9	9	5%	1675
SPT 24-hours after	**Treatment leg**	9	9	25%	40
	Non-treatment leg	9	9	20%	52
	Forearm	9	9	9%	178
TST immediately after	**Treatment leg**	9	9	10%	138
	Non-treatment leg	9	9	16%	69
	Forearm	9	9	5%	3851
TST 24-hours after	**Treatment leg**	9	9	37%	25
	Non-treatment leg	9	9	11%	132
	Forearm	9	9	6%	1328

* The sample size that would be required in each group for 80% power.

6.7 Percentage change in pain threshold after the interventions

6.7.1 Percentage change of single pain threshold

Table 6.13 provides the percentage change of SPT after each intervention from the baseline value of each group. SA showed no effect on SPT, whereas the effects in EA and MA showed increases. EA had apparent higher percentage increases than MA on each site. The increase rates 24-hours after acupuncture were always higher than the increase rates immediately after acupuncture.

Table 6.13 Percentage change of single pain threshold from baseline

		Percentage change of SPT immediately after interventions (%)	**Percentage change of SPT 24-hours after interventions (%)**
Treatment leg	**EA**	+ 95.0%	+ 165.8%
	MA	+ 35.4%	+ 58.4%
	SA	- 3.0%	0%
Non-treatment leg	**EA**	+ 50.5%	+ 72.7%
	MA	+ 15.8%	+ 20.9%
	SA	- 7.1%	- 4.6%
Forearm	**EA**	+ 45.7%	+ 47.5%
	MA	+ 14.9%	+ 26.5%
	SA	- 13.9%	- 13.0%

6.7.2 Percentage change of temporal summation threshold

Table 6.14 provides the percentage change of TST after each intervention from the baseline value of each group. SA showed no effect on TST, whereas the effects of EA and MA showed increases. The percentage increases of TST were similar to the percentage increases of SPT. EA had higher percentage increases than MA on each site. The increase rates 24-hours after acupuncture were always higher than the increase rates immediately after acupuncture.

Table 6.14 Percentage change of temporal summation threshold from baseline

		Percentage change of TST immediately after interventions (%)	**Percentage change of TST 24-hours after interventions (%)**
Treatment leg	**EA**	+ 84.1%	+ 90.5%
	MA	+ 39.1%	+ 80.4%
	SA	- 11.2%	- 8.6%
Non-treatment leg	**EA**	+ 37.5%	+ 93.3%
	MA	+ 24.5%	+ 27.3%
	SA	- 9.3%	- 9.3%
Forearm	**EA**	+ 38.9%	+ 69.0%
	MA	+ 33.7%	+ 34.7%
	SA	- 9.9%	- 12.0%

6.8 Summary of pain assessments

In comparison to SA, MA and EA consistently increased the SPT and TST within 24-hours after the intervention. Only the difference between the EA group and the SA group was, however, statistically significant on the treatment leg at 24-hours after EA treatment. These results were validated by the fact that the power of the statistical tests reached values of 75% and 89% respectively for these comparisons (see sections 6.3.2, 6.4.2 and Table 6.11). A small increase in the sample size would achieve 80% statistical power for the comparisons between EA versus SA on SPT and TST measured on the non-treatment leg and the forearm immediately after the treatment or 24-hours after (See Table 6.11). Comparisons of SPT and TST for the MA versus the SA, and the EA versus the MA showed no statistically significant differences at all of the three sites.

In the post-intervention pain assessments, SA showed no effect on either SPT or TST. However, EA and MA increased both SPT and TST in each post-intervention pain assessement. The percentage increases of SPT and TST after EA were always higher than those increases after MA. The increase rates 24-hours after acupuncture were always higher than the increase rates immediately after acupuncture (See sections 6.7.1 and 6.7.2).

6.9 Anxiety evaluation

The baseline values of the Spielberg State Anxiety Inventory (SSAI) were comparable among the three groups ($p = 0.578$) (see Table 6.2 in section 6.2). After interventions, the one-way ANOVA comparison among the three groups showed no statistically significant difference ($p = 0.493$) (Table 6.15).

Table 6.15 Post-intervention SSAI scores – One-way ANOVA

ANOVA						
		Mean ± SD	***n***	***df***	***F***	***p****
Post-treatment Spielberg state anxiety inventory (SSAI) test	Between Groups	SA: 31.67 ± 6.38	9	2	0.729	0.493
		MA: 36.33 ± 13.59	9			
		EA: 31.56 ± 7.06	9			

* The significance level for the ANOVAs was set at $\alpha = 0.05/3 = 0.0167$ (Bonferroni Correction).
df: degrees of freedom.

6.10 Success of blinding

Credibility of the blinding process was assessed with a post-treatment questionnaire. All 27 volunteers completed this one-item questionnaire. No statistically significant difference was detected among the three groups. The results indicated that the blinding procedure was successful (Table 6.16).

Table 6.16 Volunteers' perception of treatment in each group

The three choices for the answer	**Volunteers' perception of treatment in each group (number)**			**Statistical test**	
	EA (*n*)	**MA (*n*)**	**SA (*n*)**	**χ^2 (*df*)**	***p* value ▲**
(1) Real Acupuncture	5	5	6	0.318* (2)	0.853
(2) Placebo/sham acupuncture	0	0	0		
(3) Don't know	4	4	3		

▲ Significance for the above chi-square calculation is at $p < 0.05$.

*3 cells (100%) had an expected count less than 5. The minimum expected count was 3.33.

df: degrees of freedom.
χ^2: *chi*-square value.

6.11 Ratings of acupuncture needling and the side effects of acupuncture

The answers in response to the intensity of acupuncture stimulation are provided in Table 6.17. There were more subjects in the SA group (56%) reporting that acupuncture was not painful than those in the EA (22%) and in the MA (11%). 33% of subjects in each group reported mild pain in response to acupuncture. About 50% of subjects in the EA or MA group considered acupuncture stimulation to be of moderate pain in comparison to only one subject in the SA group. None of the subjects reported experiencing severe pain. There was no statistically significant difference in the ratings of acupuncture stimulation among the three groups.

None of the volunteers reported side effects such as nausea or dizziness during or after the experimental period.

Table 6.17 The intensity rating of response to acupuncture stimulation

	***The intensity of acupuncture stimulation**					
	No pain; *n*(%)	**Slight/mild pain; *n*(%)**	**Moderate pain; *n*(%)**	**Severe pain; *n*(%)**	**χ^2 (*df*)**	***P*** ▲
EA (*n*=9, 100%)	2 (22%)	3 (33%)	4 (44%)	0	5.85* (4)	0.211
MA (*n*=9, 100%)	1 (11%)	3 (33%)	5 (56%)	0		
SA (*n*=9, 100%)	5 (56%)	3 (33%)	1 (11%)	0		

▲ Significance for the above chi-square calculation is at $p < 0.05$.

* 9 cells (100%) had expected count less than 5. The minimum expected count was 2.67.

df: degrees of freedom.
χ^2: *chi*-square value.

6.12 Ratings of supra-threshold painful stimulation

An error was made when delivering the supra-threshold painful stimulation. The original intention was to deliver 1.2x and 1.4x the baseline TST at three time points (baseline, immediate after the interventions and 24-hours after). During the tests, stimulation with the intensity of 1.2x and 1.4x the TST obtained at each time point was delivered.

This mistake increased the level of stimulation significantly as shown by the two examples illustrated in Tables 6.18 and 6.19.

Table 6.18 An example using the correct method to assess pain ratings to supra-threshold levels of temporal summation stimulations

	Baseline pain assessments	**Immediately after acupuncture**	**24hours after acupuncture**
If the TST values assessed in step 2 was:	2 mA	4 mA	6 mA
The intensity used for 1.2 x TST supra-threshold pain test in step 3 should be:	2 x 1.2 = 2.4 mA	2 x 1.2 = 2.4 mA	2 x 1.2 = 2.4 mA
The intensity used for 1.4 x TST supra-threshold pain test in step 3 should be:	2 x 1.4 = 2.8 mA	2 x 1.4 = 2.8 mA	2 x 1.4 =2.8 mA

Table 6.18 shows an example of the intensity would have been used for the pain ratings to ST levels of TST tests. The pain ratings in response to the consistent intensity can be calculated to assess whether the pain responses to the same intensity of painful stimuli are changed in response to the interventions.

Table 6.19 An example using the incorrect method mistakenly employed in the present study to assess the pain response ratings to supra-threshold levels of temporal summation stimulations

	Baseline pain assessments	**Immediately after acupuncture**	**24hours after acupuncture**
If the TST values assessed in step 2 was:	2 mA	4 mA	6 mA
The intensity used for 1.2 x TST supra-threshold pain test in step 3 was:	2 x 1.2 = 2.4 mA	4 x 1.2 = 4.8 mA	6 x 1.2 = 7.2 mA
The intensity used for 1.4 x TST supra-threshold pain test in step 3 was:	2 x 1.4 = 2.8 mA	4 x 1.4 = 5.6 mA	6 x 1.4 = 8.4 mA

Table 6.19 shows an example of the intensity used in present study for the pain ratings to ST levels of TST tests. The pain ratings in response to the inconsistent intensities used for these pain assessments can not be calculated to detect the true effects of the intervention.

Due to this error, only descriptive data are presented in Table 6.20 and no inferential statistical analyses were conducted. In these supra-threshold pain tests, each of the baseline mean values of the pain ratings to 1.2x the TST level stimulations was lower than the corresponding mean value of the pain ratings to 1.4x the TST level stimulations. Hence, stimulation with higher intensity induced a stronger pain rating.

At the baseline, the ratings were similar among the three groups. After the interventions, it was expected that the ratings to supra-threshold stimulation would reduce as the pain thresholds increased. Contradictory to the expectation, the ratings did not either increase or decrease greatly in the three groups. This was due to the mistake in the delivered intensity of stimulation. For instance, the subjects in the EA group assessed on the treatment leg, the mean values of TST assessed at baseline (Table 6.2 in section 6.2), immediately after interventions (Table 6.6 in section 6.4.1) and 24-hours after interventions (Table 6.7 in section 6.4.2) were 1.26 mA, 2.32 mA and 2.4 mA respectively; and the TST increased by 84% immediately after interventions and 90% 24-hours after. As a result, the intensities of the stimulations of 1.2x the TST also increased by 84% and 90%. However, the subjects in the EA group reported ratings to the stimulations of 1.2x the TST delivered to the treatment leg and assessed at three time points of 2.92, 2.54 and 3.19; which indicated that their pain ratings decreased by 13% immediately after EA and increased by 9% 24-hours after EA. The mismatch in the percentage changes in ratings and pain thresholds suggests the analgesic effect induced by acupuncture.

Table 6.20 Descriptive data for supra-threshold pain rating tests

		(n = 9 in each of the three groups)	**Baseline (Mean ± SD)**	**Immediately after interventions (Mean ± SD)**	**24-hours after interventions (Mean ± SD)**
Pain rating to the stimulation of 1.2xTST intensity	**assessed on the treatment leg**	SA	2.65 ± 0.42	2.43 ± 0.94	2.8 ± 0.94
		MA	2.85 ± 1.39	2.24 ± 1.28	2.47 ± 1.07
		EA	2.92 ± 1.86	2.54 ± 1.61	3.19 ± 1.04
	assessed on the non-treatment leg	SA	2.36 ± 0.74	2.60 ± 1.14	2.88 ± 0.89
		MA	3.03 ± 1.49	2.38 ± 1.01	2.6 ± 1.33
		EA	2.79 ± 1.11	2.37 ± 1.12	2.79 ± 1.53
	assessed on the forearm	SA	2.35 ± 1.02	2.23 ± 0.97	2.60 ± 1.14
		MA	2.19 ± 1.17	2.01 ± 0.97	2.13 ± 1.08
		EA	2.66 ± 1.49	2.74 ± 1.32	3.07 ± 1.11
Pain rating to the stimulation of 1.4xTST intensity	**assessed on the treatment leg**	SA	3.33 ±0.89	3.18 ± 1.12	3.23 ± 0.90
		MA	3.46 ± 1.75	2.72 ± 1.55	3.28 ± 1.49
		EA	3.55 ± 1.66	3.34 ± 1.60	3.68 ± 1.16
	assessed on the non-treatment leg	SA	3.25 ± 1.11	3.18 ± 0.90	3.41 ± 0.99
		MA	4.09 ± 1.89	3.55 ± 1.59	3.48 ± 1.70
		EA	3.51 ± 0.96	3.11 ± 1.16	3.36 ± 1.60
	assessed on the forearm	SA	2.91 ± 1.03	2.94 ± 0.89	3.38 ± 1.19
		MA	2.84 ± 1.26	2.73 ± 1.29	2.94 ± 1.42
		EA	3.31 ± 1.23	3.43 ± 1.21	3.87 ± 1.56

Chapter 7: Discussion and Conclusion

7.1 A summary of the results

In the study, the model of temporal summation of pain was successfully elicited. The statistical calculations showed that EA significantly increased SPT and TST 24-hours after the treatment on the treatment leg when compared with SA. There was a trend to show that EA also increased SPT and TST assessed on the treatment leg immediately after acupuncture, and on the non-treatment leg 24-hours after when compared with SA. There was no significant difference between EA versus MA or MA versus SA in pain thresholds measured at any body site. This may have been due to the small sample size.

This is the first study that examines the effect of acupuncture on TS of pain. The results indicate that the EA (2/100 hertz) can induce a strong analgesic effect on the central nervous system. This effect is expressed ipsilaterally on the same spinal segment as the acupuncture sites. This effect grows stronger 24-hours after the intervention.

7.2 Strengths

The baseline TST was lower than SPT at each assessed site across the three groups, suggesting that the model of TS of pain was successfully induced. The acupuncture naïve subjects were properly blinded to the treatment allocation, so was the evaluator (the author). An acupuncturist who did not know the treatment allocation delivered the treatment. This dummy double-blinding design ensures that performance bias on the part of the subjects and the researchers was well-controlled.

Other factors that might influence the results were also controlled. In a training session prior to the testing, the subjects were trained to be familiar with the electrical stimulation and the reporting of pain. The room temperature was controlled at 22 - 25 degrees Celsius as temperature can impact on human responses to pain stimulation (136). The non-invasive SA was particularly successful. The SA group showed contrary effects on SPT or TST to the acupuncture groups. None of the subjects in the SA group had increased pain thresholds. Moreover, subjects in the SA group were not aware they were experiencing a sham procedure.

Acupuncture is an invasive procedure, and can produce anxiety and stress. Previous studies have indicated that anxiety or stress can increase or decrease pain threshold (137; 138; 139). The level of anxiety in this study was measured with the well-accepted Spielberg state anxiety inventory (SSAI) (137; 140). There was no difference in the level of anxiety before and after acupuncture, indicating that the increased pain thresholds were not due to the stress associated with needling. This result is in accordance with those from other studies (1; 12; 16; 75; 79; 80; 83). The current experiment recruited young humans and hence no age group can be divided for analysis.

7.3 Limitations

There are two major limitations of the study, the small sample size and the error in delivering the supra-threshold stimulations.

As this is the first study evaluating the effect of acupuncture on the TS of pain, it was difficult to predict the proper sample sizes. Based on previous studies on SPT (the studies examined in Chapter 3), we proposed to recruit 45 subjects with 15 in each groups. We were only able to recruit 27 subjects with 9 subjects in each group by the end of experimental period. Various factors contributed to the difficulty in recruiting subjects. Firstly, the subjects were limited to being acupuncture naïve. Secondly, the subjects had to come twice within 24-hours. Thirdly, the experiment involves needle insertion, which would have excluded a group of people who are afraid of needles.

The small sample size limited the power of the statistical test analyses. For instance, to detect a difference in TST with 80% power on the non-treatment leg and forearm 24-hours after the treatment for the effect of EA versus SA, seven to fifteen subjects in each group would be needed. The interpretation of the results will take into account this limitation.

Pain ratings to supra-threshold stimulation at 1.2x and 1.4x the baseline TST were included in the design. Due to the error in the delivery of supra-threshold stimulations, the intensity of stimulation delivered immediately after the interventions and 24-hours after was much higher (over 80% higher) than planned. Consequently, pain ratings to supra-threshold stimulations remained the same or slightly increased instead of reduced as expected. The resultant data could not be statistically analysed to detect group differences. As explained in the 'Results' chapter (section 6.12), the increase of the TST

and the intensity of stimulation was over 80%, whereas the increase of the pain ratings was less than 10%. The results indicate that a strong analgesia was induced not only at the pain threshold level but also at the supra-pain threshold level.

This error does not, however affect the value of the pain thresholds and therefore does not limit the interpretation of the findings from TST and SPT tests.

7.4 Interpretation of the findings

7.4.1 The effects of acupuncture on SPT and TST

The EA and MA evaluated in present study increased both TST and SPT. Therefore, the acupuncture analgesic effect is unlike that of an NMDA antagonist, such as ketamine, imipramine or venlafaxine, which enhance TST but have little effect on SPT (36; 38; 39). This finding is in line with two recent animal studies which provide evidence that acupuncture analgesia does not inhibit NMDA receptors directly. One study reported ketamine (0.5 mg/kg), an NMDA receptor antagonist medication, was not antagonised by the analgesia effect of 100-hertz EA, but on the contrary it enhanced the rats' pain tolerance threshold (141). Another study reported that the effects of 3-hertz EA on ST36 of rats was not affected by the administration of ketamine or nitrous oxide, both of which are NMDA receptor antagonist medications (142). These animal studies showed acupuncture and NMDA antagonist medications (i.e. ketamine and nitrous oxide) had no competitive relationship to agonise NMDA receptors. So far, no study provides evidence that acupuncture specifically antagonises NMDA receptors immediately after a treatment.

In addition, the acupuncture analgesic effect is unlike the effects of levetiracetam and tramadol, which increase the SPT significantly but have no effect on the TST (73; 74). On the contrary, the 2/100 hertz EA effects on SPT and TST 24-hours after treatment are similar to the effects of codeine, which is significantly more effective than placebo on both SPT and TST (39). Codeine exerts its analgesic effect via an endo-morphine mechanism (39); thus this suggests the acupuncture analgesic effect involves an endo-morphine mechanism as well. This finding confirms the observation of a previous

animal study that 2/100 hertz EA enhances the release of endogenous opioid peptides (93).

In a previous section (section 2.4.2), the evidence for some medications showing dosage-dependent analgesic effects on SPT and TST was discussed. For example, the suppression of TST requires a high dose of isoflurane than for SPT (37). This seems also to be the case in the present study. At 24-hours after, EA increased SPT by 165.8% and TST by 90.5% on the treatment leg. However, this dose-dependent response was not the aim of the present study and was not examined. Future studies should investigate the effect of acupuncture on TST with various strengths of stimulation.

7.4.2 The spatial characteristics of acupuncture analgesia

The statistical calculations showed that EA significantly increased SPT and TST on the treatment leg 24-hours after the treatment when compared with SA, and this EA effect was not found on the non-treatment leg or the forearm. The sites of the pain assessments were in the same dermatome as the treatment site; thus, the present finding favours the Segmental Inhibition Theory. The current result is different from that of a study by Zaslawski and his colleagues, who assessed the analgesic effect of MA on right LI4 by testing the pressure pain thresholds on 10 sites within the treatment side or on the central line of the body. The study showed the analgesic effect of MA was significantly higher than non-invasive SA at all 10 sites, and the result does not support either traditional Meridian Theory or the Segmental Inhibitory Theory (17). However, another pain perception study reported a different observation. A RCT with 10 subjects in each group tested the effect of continuous mode EA (information of EA frequency was absent), which involved needling four acupoints on the right forearm. Immediately after the treatment, EA significantly reduced the pain ratings to pain induced by zero degree Celsius ice-water assessed on the treatment side of forearm but had no effect on the other side of forearm indicating a segmental effect of EA (18). It is important to note that the two studies and the current one employed different pain models and used different modes of acupuncture stimulation.

Electrical pain model was used in the current study. Previous studies showed that the effect immediately after MA did not increase electrical pain threshold on the skin (125); although it increased electrically induced dental pain (19). In the current study, MA increased SPT by 58.4% and TST by 80.4% at 24-hours after acupuncture on the treatment leg. However, the comparisons of MA versus SA on SPT or TST were not statistically significant. It is likely that the small sample size of the current study

compromises the potential MA-induced analgesia in the whole body. According to the power analysis, in order to demonstrate the effect of MA on SPT in the treatment leg, non-treatment leg and forearm, 101, 50 and 1675 subjects are required for the effect immediately after MA in each group, when 40, 52 and 178 subjects are required for the effect 24-hours after MA in each group. MA perhaps does not have a strong analgesic effect on electrically induced cutaneous pain.

7.4.3 The temporal characteristics of acupuncture analgesia

All the acupuncture studies in healthy humans tested its immediate effect. The non-significant results found for the immediate effect comparison in this study were, however, not able to be interpreted because the inadequate sample sizes limited the power of the statistical tests. To demonstrate the analgesic effect immediately after EA, 11 subjects for each group will be needed.

In the current study, the effects of EA and MA on TST grew stronger with time and their effects at 24 hours after the intervention were better than immediately after the intervention. Since no previous human RCT addressing the temporal effect of acupuncture could be found, it is not possible to place these results within the context of previous studies. Nevertheless, it seems that the enhanced central inhibitory effect of the EA and MA at 24-hours after may be due to a neurohumoral effect (i.e. activation of endogenous opioid peptides and opioid receptors) rather than a purely neural effect as the former acts slowly in hours whereas the latter acts within seconds and minutes of stimulation (57).

It has been hypothesed that the analgesic effect of a single acupuncture treatment might be more beneficial overnight (26). The significant EA effect after 24 hours found in the current study provides supporting evidence for the hypothesis. So far, this hypothesis has not been tested in a human study previously. In an animal study, preproenkephalin mRNA gene transcription in the brain was observed for 72 hours after a single session of EA; the preproenkephalin mRNA gradually increased in the brain with the peak occurring at 48 hours, and then a 50% decline after 72 hours (25). Such an effect has yet to be examined in humans.

7.5 Conclusion and implications for future studies

The D-D mode 2/100 hertz EA has strong inhibitory effects on both SPT and TST at 24 hours after a single session of treatment, and the effects are likely to be mediated via the central nervous system with peripheral contributions, and are more pronounced within same dermatome segment of the needling sites.

The mechanism of acupuncture actions need to be examined by using various medications, such as NMDA antagonists and opioid receptor antagonists. Future human studies should also assess the analgesia of single acupuncture session after 48 hours to understand the temporal characteristics of acupuncture.

On the basis of our study results, we calculated the sample size for the comparison between EA and MA to achieve 80% power in statistical tests (section 6.6.1). In future human studies, in order to compare the effects of EA and MA assessing with the electrical pain thresholds, at least 21 subjects and 18 subjects in each group would be required for the effects immediately after acupuncture and 24-hours after acupuncture, respectively. The result of this study is to be compared with future studies employing thermal and mechanical TS pain models. Future studies using this paradigm to test acupuncture analgesia in clinical pain patients are essential.

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Appendix

Appendix 01 Ethics Approval of the Study

RMIT UNIVERSITY

Phone: 9925 2251
Fax: 9925 2387
janet.gaffey@rmit.edu.au

PRO VICE-CHANCELLOR
RESEARCH & INNOVATION

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MEMORANDUM

To: Sam Feng

cc. Dr Zhen Zheng
Dr John Reece
A/Professor Barbara Polus

From: Janet Gaffey
Executive Officer
RMIT Human Research Ethics Committee

Date: 1 February 2006

Project No 24/05 The effect of acupuncture on temporal summation of pain: a randomised, single-blind, controlled-study

Thank you for making the amendments sought by the RMIT Human Research Ethics Committee to the above project. Your project is now approved.

This project is approved for 3 years from the date of this letter, that is, until 1 February 2008. This approval is conditional on the submission of annual reports. A final report should be provided at the conclusion of the project. If your work is completed within twelve months a final report, only, is required. Report forms are available from the Human Research Ethics Committee web site (http://www.rmit.edu.au/rd/hrec_apply).

If, as you proceed with your investigation you find reason to amend your research method, you should advise the RMIT Human Research Ethics Committee and seek approval for the proposed changes. If you decide to discontinue your research before its planned completion you must also advise the Committee of this and of the circumstances.

You should notify the Committee immediately of any serious or unexpected adverse effects on subjects, or unforeseen events, which may affect the ethical acceptability of your project.

You are reminded that:
any research data, which identifies people and is stored in electronic form, should not be held on a computer which is connected to the web or a network, but should be held on removable media such as a CD; and
the Principal Investigator is responsible for the retention and storage of the original data pertaining to the project for a minimum period of five years.

We wish you well with research.

Appendix 02 Advertisment for pariticipant recruitment

Division of Chinese Medicine Research Group
School of Health Science

Does Acupuncture Reduce Pain?

Do You Want To Contribute to an Acupuncture Study?

Welcome, Volunteers!

RMIT Chinese Medicine Research Group is conducting an acupuncture study at the RMIT Bundoora West Campus. **We urgently need 60 healthy volunteers to participate in this study.**

Aims of this study

The aims of the study are to investigate whether acupuncture reduces experiment-induced pain in health humans, how long the acupuncture analgesic effect lasts and how widely this effect is distributed in your body.

Criteria of participants

Any healthy human aged between 18-40 years old.
Never experienced acupuncture.

What will you be asked to do to help us?

We will test your rating to a few sets of painful and non-painful electrical stimuli delivered to your skin before and after acupuncture treatment. You will be asked to report the level of pain and strength of sensation. The stimulation will range from below your pain level to slightly above your pain level.

How long does the study take?

The study consists of a 3-hour test including 30 minutes acupuncture treatment on the first day and a 30-minute test on the next day.

Your participation in this study is voluntary. You are free to withdraw from the study at any stage of the study. If you are happy to take part in the study please contact:

Sam Feng
BH: 9925 7176; AH: 0432 214 011 (mobile)
Email: s3069785@student.rmit.edu.au

This project has been reviewed and approved by the Human Research Ethics Committee of RMIT University.

Appendix 03 Participants' self-reporting form

Participant Record

Project Title: The effect of acupuncture on experimental pain (temporal summation of pain): a randomised, double-blind, controlled-study

Date of Participation:

Name: **Age:** **ethnicity:**

Gender: **Occupation:**

Telephone:

Mobile:

Email:

Address:

Medical history:

Are you currently taking any medication? **Yes** **No**

If yes, please specify:

Have you had any acupuncture treatment before this experiment? **Yes** **No**

Are you pregnancy or malignancy? **Yes** **No**

Do you have any followed conditions? Mark a tick if yes.

- **Severe heart disease or wear a pacemaker**
- **Brain tumour or epilepsy**
- **Tendency to bleed**
- **Drug addiction**
- **Had any analgesics in the previous weeks**
- **Suffer from chronic pain or recurrent pain**
- **Skin problems at the proposed acupuncture sites**

Note:

Your personal information and relevant data will be stored in password protected computer. All other documents and records will be stored in the cabinet protected by key-lock. Only the investigators can access the information. No name will be referred to any reports or publications or discussions. Only group data will be reported. The information will be retained as required by RMIT for 15 years. At the end of the period, the documents will be destroyed according to the University document disposal procedure.

Signature of participant: **Date:**

Appendix 04 Cardiovascular Risk Questionnaire

CARDIOVASCULAR RISK FACTOR QUESTIONNAIRE

In order to be eligible to participate in the experiment you are required to complete the following questionnaire, designed to assess the risk of you having a cardiovascular event during the course of the trial.

ID: _____

Circle the most appropriate responses for the following questions:

1. Are you overweight? Yes No Don't Know
2. Do you smoke? Yes No Don't Know
3. Do you or your family have a history of premature cardiovascular problems (e.g. heart attack, stroke)? Yes No Don't Know
4. Do you have high blood cholesterol levels? Yes No Don't Know
5. Do you have high blood pressure? Yes No Don't Know
6. Do you have an arrhythmia? Yes No Don't Know
7. Do you have a heart murmur? Yes No Don't Know
8. Do you have impaired circulation in the hands or feet when cold?

   Yes No Don't Know
9. Are you on any medication Yes No
   If so, what is the medication? _____
10. Do you think you have any medical complaint or any other reason which you know of which you think may prevent you from participating in this trial? Yes No
    If yes, please elaborate. _____

I, _____, believe that the answers to these questions are true and correct.

Signed: Date: _____

Appendix 05 Plain Language Statement

Information about acupuncture and pain study

PROJECT TITLE: The effect of acupuncture on experimental pain (temporal summation of pain): a randomised, single-blind, controlled study in humans

INVESTIGATOR: Jian Qiang (Sam) Feng, Registered Acupuncturist, Masters Candidate

Dear Volunteer,

My name is Jian Qiang (Sam) Feng, a Masters student at the division of Chinese Medicine Research Group, RMIT University. My study is under the supervision of Dr. Zhen Zheng, Prof. Charlie Xue and A/Prof Chun Guang Li (RMIT, Chinese Medicine Research Group). In this study, I will use electrical stimulation as a means to assess the neural mechanism of acupuncture analgesic effect in healthy humans. This is to provide you with relevant information about my study.

1. Purpose of the study

The aims of the study are to evaluate whether manual or electro-acupuncture modifies your level of pain sensitivity, how long the acupuncture analgesic effect lasts and how widely this analgesic effect distributes in your body.

2. What will you be asked to do during the study?

The study includes a 3-hour session on the first day and a 30-minute session on the next day. During the first session, you will be asked to report your sensation to a few sets of painful and no-painful single or repeated electrical stimulation. You will then receive manual, electrical or sham acupuncture for 30 minutes. Finally the electrical stimulation tests will be repeated, and you will ask to report your sensation to those stimuli again. On the following day, the electrical stimulation tests will be repeated.

On the first day, you will also be asked to complete two questionnaires.

3. What kind of pain will you experience?

Electrical stimuli will be delivered to the skin of one of your forearms and both legs via surface electrodes (each is 0.5cm in diameter) with a standard electrical stimulator. When a single electrical stimulus is delivered to your skin, you will feel different sensations as the intensity of the stimulus increases. At first you will feel buzzing, tingling, or vibrating sensation. Then you may feel prickling, stinging, sharp, slightly burning or slightly discomfort sensation, and you may consider this sensation painful.

When five single non-painful electrical stimuli are delivered one after another within a short period of 2.5 seconds, your sensation to the stimulation may increase, and you may consider the sensation painful. It is this enhanced pain sensitivity that we are interested in, in this study. This enhanced pain sensitivity phenomenon plays an important role in our understanding of clinical pain.

Please note, **in this study, we only investigate your pain sensitivity and your ratings to some painful stimuli. We do not assess how much you can tolerate pain.**

4. Safety issues and potential discomfort of electrical pain tests

The electrical stimulator used will be connected to an isolation unit and a constant current unit to ensure your safety. The magnitude of current will be monitored and adjusted within a safe range.

A number of studies have used the current method with human participants in the last decade, and have proved this method to be safe and acceptable to humans.

5. The Real or Placebo Treatment

It is necessary to have an inactive treatment group who will undergo sham acupuncture treatment, so that the true effect of acupuncture can be demonstrated. Sham acupuncture is a form of placebo treatment with minimal effect on your body. It is used to show whether the real treatment has a true effect. Once you have met the inclusion and exclusion criteria, you will be allocated randomly into one of the three groups (two real acupuncture and one sham acupuncture groups). Please note that you will have a 1/3 chance of being placed in an inactive treatment group.

6. Safety issue and potential discomfort of acupuncture

Acupuncture procedure is widely used in everyday practice with an excellent safety profile. Only disposable needles will be used and they are much thinner than needles used for injections. Acupuncture has been reported to be associated, in a very few cases, with minor risks, such as fainting, infection, and hematoma. Needles may puncture small blood vessels during the procedures. Precautions will be taken to avoid inserting needles too deeply or into nerves or arteries. There is no evidence that acupuncture treatment may result in psychological damage.

In the current study, two sterilised and single-used needles will be used, and the acupuncture site will be on one of your legs. Some people may experience minor pricking sensations during the early phase of acupuncture. This sensation normally subsides after a few seconds. The sensation of soreness, numbness or distension at the needle site may be perceived at the acupuncture sites.

The electrical acupuncture stimulation machine to be used in this study has been approval by the Therapeutic Goods Administration of Australia.

The investigator who will deliver acupuncture treatment is a registered and experienced acupuncturist. And all researchers involved in the study have a level 2 First Aid certificate.

7. Discontinuation and termination of your participation

Your participation in this study is voluntary. You are free to withdraw from the study at any stage of the study.

8. Confidentiality of information you provide

All information provided by you and data collected through this study will be stored in a password protected computer program. Authorised auditors may inspect your records. You will have access to your records through the investigator. In any form of publication, all the personal information will be removed. Group results will be provided on request at the end of the study.

9. Benefit of your participation

Your participation will benefit human pain studies and enhance our understanding of acupuncture analgesic mechanisms underlying clinic pain. There is no direct benefit to you.

10. Your participation in other research projects

If you are participating in other research projects at the same time, please let us know before the commencement of acupuncture treatment.

This project has been reviewed and approved by the Human Research Ethics Committee of RMIT University.

Any question or complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476, Melbourne, 3001. Tel: 9925 1745.
Details of the complaints procedure are available also from the above address.

If you have any discomfort after the acupuncture treatment or electrical stimulation tests, please contact me (Sam) on 9925 7176 or E-mail s3069785@student.rmit.edu.au.

Appendix 06 Inform consent form-A

HREC Form 2a

RMIT HUMAN RESEARCH ETHICS COMMITTEE

Prescribed Consent Form For Persons Participating In Research Projects Involving Tests and/or Medical Procedures

PORTFOLIO OF	**Science, Engineering and Technology**
SCHOOL OF	**Health Sciences**
Name of participant:	
Project Title:	The effect of acupuncture on experimental pain (temporal summation of pain): a randomised, single-blind, controlled-study

Name(s) of investigators:

(1)	Jian Qiang (Sam) Feng	Phone:	9925 7167, 0432214011
(2)	Zhen Zheng	Phone:	9925 7176
(3)	Charlie Xue		9925 7745
(4)	Chun Guang Li		9925 7635

1. I have received a statement explaining the tests/procedures involved in this project.

2. I consent to participate in the above project, the particulars of which - including details of tests or procedures - have been explained to me.

3. I authorise the investigator or his or her assistant to use with me the tests or procedures referred to in 1 above.

4. I acknowledge that:

   (a) The possible effects of the tests or procedures have been explained to me to my satisfaction.
   (b) I have been informed that I am free to withdraw from the project at any time and to withdraw any unprocessed data previously supplied (unless follow-up is needed for safety).
   (c) The project is for the purpose of research and/or teaching. It may not be of direct benefit to me.
   (d) The privacy of the personal information I provide will be safeguarded and only disclosed where I have consented to the disclosure or as required by law.
   (e) The security of the research data is assured during and after completion of the study. The data collected during the study may be published, and a report of the project outcomes will be provided to me. Any information which will identify me will not be used.

Participant's Consent

Name: _____ **Date:** _____
(Participant)

Name: _____ **Date:** _____
(Witness to signature)

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 1745.
Details of the complaints procedure are available from the above address.

Appendix 07 Inform consent form-B

HREC Form No 2b

RMIT HUMAN RESEARCH ETHICS COMMITTEE

Prescribed Consent Form For Persons Participating In Research Projects Involving Interviews, Questionnaires or Disclosure of Personal Information

PORTFOLIO OF		**Science, Engineering and Technology**		
SCHOOL OF		**Health Sciences**		
Name of participant:				
Project Title:		The effect of acupuncture on experimental pain (temporal summation of pain): a randomised, single-blind, controlled-study		
Name(s) of investigators:	(1)	Jian Qiang (Sam) Feng	Phone:	9925 7167, 0432214011
	(2)	Zhen Zheng	Phone:	9925 7176
	(3)	Charlie Xue		9925 7745
	(4)	Chun Guang Li		9925 7635

1. I have received a statement explaining the interview/questionnaire involved in this project.

2. I consent to participate in the above project, the particulars of which - including details of the interviews or questionnaires - have been explained to me.

3. I authorise the investigator or his or her assistant to interview me or administer questionnaires.

4. I acknowledge that:

   (a) Having read Plain Language Statement, I agree to the general purpose, methods and demands of the study.
   (b) I have been informed that I am free to withdraw from the project at any time and to withdraw any unprocessed data previously supplied.
   (c) The project is for the purpose of research and/or teaching. It may not be of direct benefit to me.
   (d) The privacy of the personal information I provide will be safeguarded and only disclosed where I have consented to the disclosure or as required by law.
   (e) The security of the research data is assured during and after completion of the study. The data collected during the study may be published, and a report of the project outcomes will be provided to me. Any information which will identify me will not be used.

Participant's Consent

Name: _____ **Date:** _____
(Participant)

Name: _____ **Date:** _____
(Witness to signature)

Participants should be given a photocopy of this consent form after it has been signed.

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 1745.
Details of the complaints procedure are available from the above address.

Appendix 08 Computer generated sequence for randomisation

1	B	1	0.6417
2	C	1	0.9690
3	A	1	0.7035
4	B	1	0.4860
5	C	1	0.4140
6	A	1	0.5695
7	B	1	0.9374
8	C	1	0.2249
9	A	1	0.8441
10	B	2	0.5147
11	A	2	0.0548
12	B	2	0.0317
13	C	2	0.5657
14	A	2	0.6897
15	C	2	0.6033
16	B	2	0.3091
17	A	2	0.9182
18	C	2	0.7516
19	A	3	0.3203
20	A	3	0.9066
21	C	3	0.0460
22	B	3	0.4187
23	B	3	0.5196
24	B	3	0.9072
25	A	3	0.8305
26	C	3	0.9486
27	C	3	0.2586
28	C	4	0.7603
29	B	4	0.5063
30	C	4	0.2936
31	A	4	0.4803
32	C	4	0.3412
33	B	4	0.2365
34	A	4	0.7667
35	B	4	0.1405
36	A	4	0.4936
37	B	5	0.8620
38	A	5	0.0259
39	C	5	0.7172
40	A	5	0.7489
41	B	5	0.2623
42	A	5	0.3646
43	C	5	0.3982
44	B	5	0.6585
45	C	5	0.9310

A = Sham-acupuncture

B = Manual acupuncture

C = Electro-acupuncture

RMIT University

Document: Sam Rand.xls, Sheet1
Print Date: 09/05/2006
Page 1 of 1

Appendix 09 Visual Analogue Scale (0-100)

Pain Intensity Rating (Visual Analogue Scale)

Name: **Date/time:**
Dominant hand: Right Left **Dominant leg: Right Left**
Electrical resistance btw electrodes: **Voltage:**
Measure Type: Baseline 0.5h 2h 24h **Measure area: Forearm R-leg L-Leg**

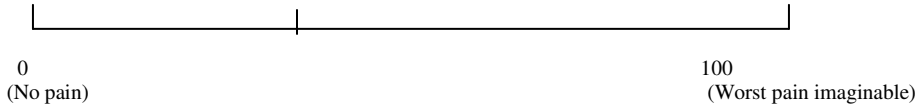
Acupuncture site: R-Leg L-Leg

Pain threshold: 1st 2nd **TS Threshold: 1st 2nd**

Level of stimulation: 1.2 x P T/H 1st 2nd 1.4 x p T/H 1st 2nd

The following is an example of how to use the scale to rate the intensity of your pain.

For example, if you draw a perpendicular line on the scale, the distance from 0 to the line is 35mm. The distance represents the intensity of your pain, which is 35 out of 100.



Test 1: Please indicate the intensity of your sensation in response to the **1st and 5th** electrical stimulus

1st:
0 (No Pain) 100 (Worst pain imaginable)
5th:

Test 2: Please indicate the intensity of your sensation in response to the **1st and 5th** electrical stimulus

1st:
0 (No Pain) 100 (Worst pain imaginable)
5th:

Test 3: Please indicate the intensity of your sensation in response to the **1st and 5th** electrical stimulus

1st:
0 (No Pain) 100 (Worst pain imaginable)
5th:

Test 4: Please indicate the intensity of your sensation in response to the **1st and 5th** electrical stimulus

1st:
0 (No Pain) 100 (Worst pain imaginable)
5th:

Appendix 10 Post-treatment questionnaire

Post-treatment questionnaire

Project title:

The effect of acupuncture on experimental pain (temporal summation of pain): a randomised, double-blind, controlled-study

Name: **Number:**

Please circle the answer.

Section A

How strong was your sensation of the acupuncture stimulation?

(1) No pain

(2) Slight / mild pain

(3) Moderate pain

(4) Severe pain

Section B

(modified from Lao et al., 1999)

Please indicate which treatment you believe you had received.

(1) Acupuncture

(2) Placebo/sham

(3) Don't know

If you answer either Acupuncture or Placebo/sham, what led to that belief?

(1) The manner, attitude, or words of the acupuncturist

(2) The manner, attitude, or words of the assistant

(3) The sensation of the acupuncture stimulation

(4) The results of the acupuncture treatment (eg, changes in pain threshold or rating)

(5) The experience of the acupuncture procedure (eg, what the acupuncturist did and how it felt)

Appendix 11 Reasons for exclusion of any experimental RCT

Study	Reason for exclusion
Li C. L. et al., 1975	No non-invasive control group.
Saletu B. et al.,1975	No non-invasive control group.
Stacher G. et al., 1975	No non-invasive control group.
Stern J. A. et al., 1977	No non-invasive control group.
Knox V. J. et al., 1979	No non-invasive control group.
Ashton H. et al. 1984	The needled acupoint was not a recognised acupoints (2cm above PC7).
Ernst M. et al.,1987	No non-invasive control group.
Lundeberg T. et al., 1988	No non-invasive control group.
Lundeberg T. et al., 1989	No non-invasive control group.
Brockhaus A. et al., 1990	No non-invasive control group.
Moret V. et al., 1991	No non-invasive control group.
Olausson B. et al., 2000	No non-invasive control group.
Xu W. D. et al., 2003	No non-invasive control group. No acupuncture technique description.
Leung A. et al., 2005	No non-invasive control group.

Note: The above studies are experimental RCTs using healthy pain-free human subjects to evaluate the analgesic effects of EA and MA (133; 143; 144; 145; 146; 147; 148; 149; 150; 151; 152; 153; 154; 155).

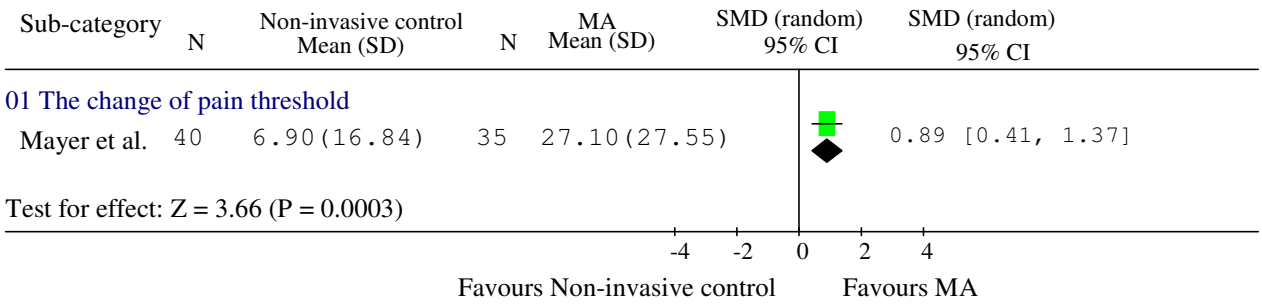
Appendix 12 Instructions for reading RveMan output figures showing the results of data analyses

The results of RevMan are saved as figure documents and exported to the appendices of the thesis (Appendices 13 - 20). When the RevMan output is read from the figures, the positive SMD value (+) favours the intervention presented on the right side of the output, whereas the negative (-) value favours the intervention presented on the left side of the output. In this review, the SMD value was used to indicate the standardised mean difference, and the interpretations of the results of data analyses depended on the *p* value of each comparison. The *p* value less than 0.05 indicates statistically significant differences between the two interventions. The SMD is calculated using Hedges' g. Hedges' g examines the sample sizes of the respective standard deviations and also adjusts the overall effect size based on the sample sizes (123) (also see section 4.3.6.2).

Appendix 13 Estimation of standardised mean differences, study 01

Pain model: Single electrical stimulus induced dental pain

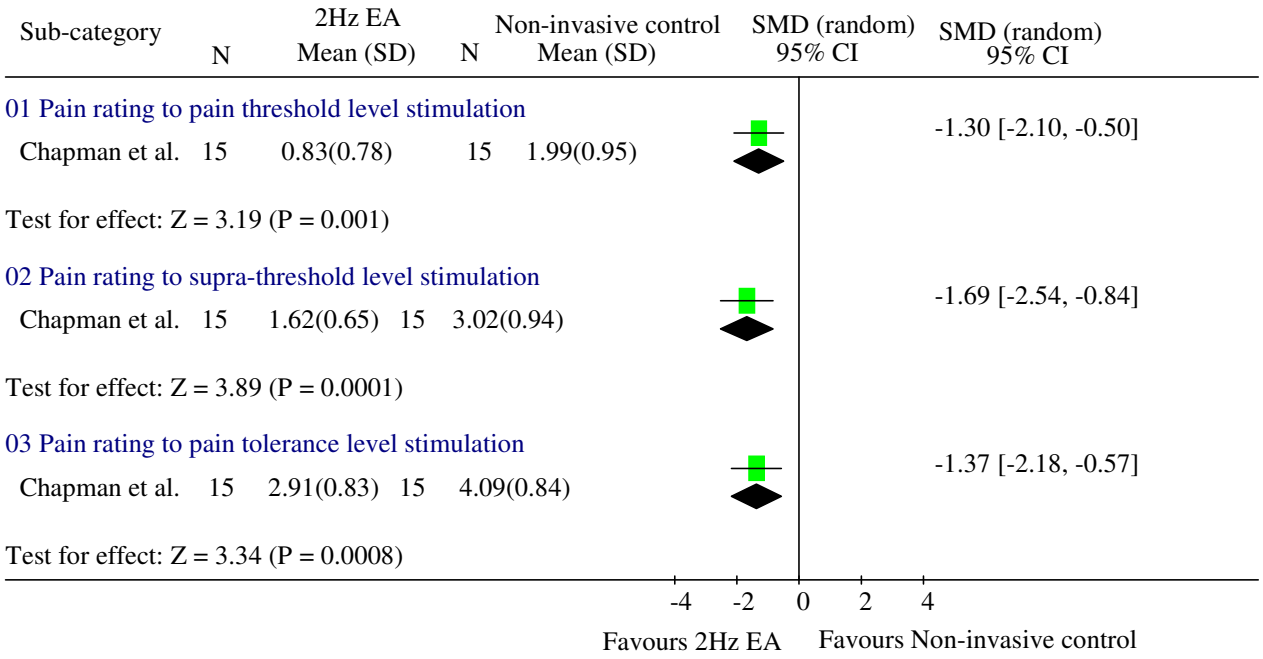
Comparison: MA vs. non-invasive control (the effect instantly during intervention)



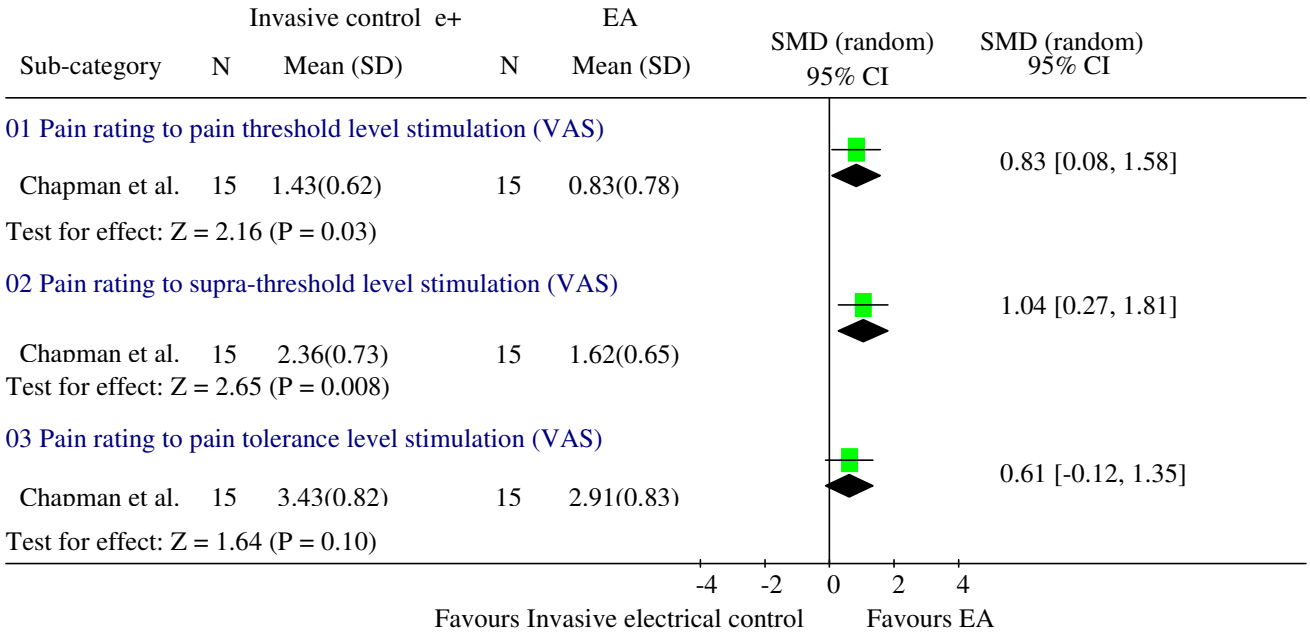
Appendix 14 Estimation of standardised mean differences, study 02

Pain model: Single electrical stimulus induced dental pain assessed by pain response rating (VAS)

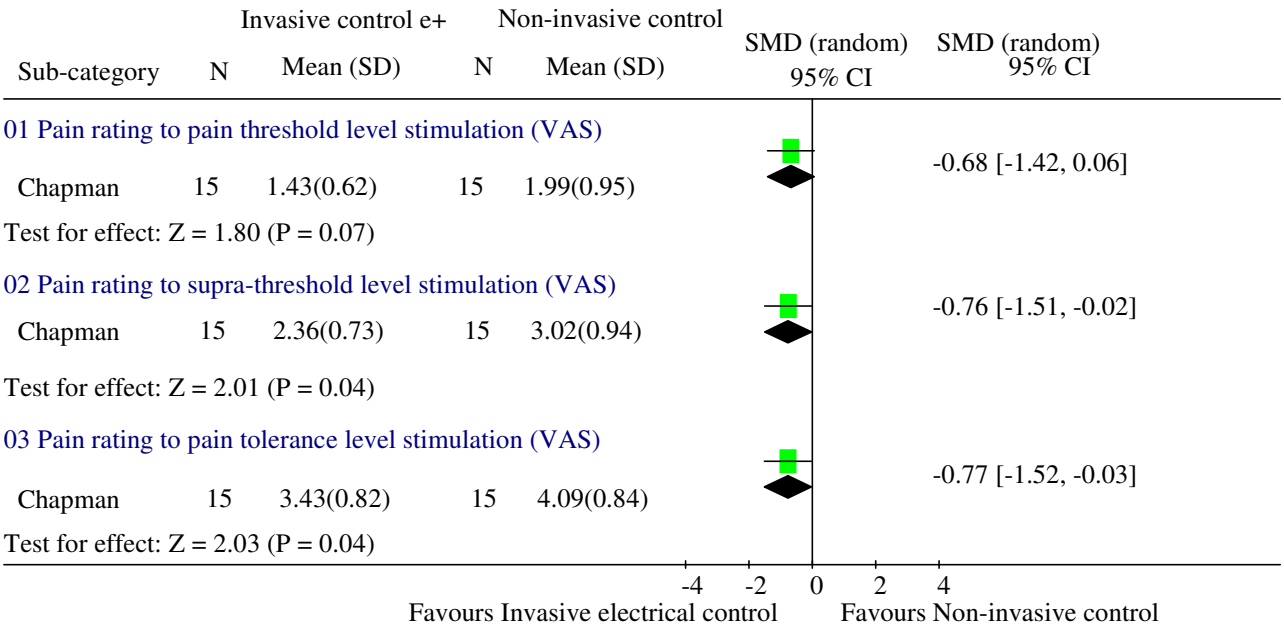
Comparison 01: 2Hz EA vs. non-invasive control (the effect immediately after inteverntion)



Comparison 02: 2Hz EA vs. invasive control with electrical stimulation (the effect immediately after inteverntion)



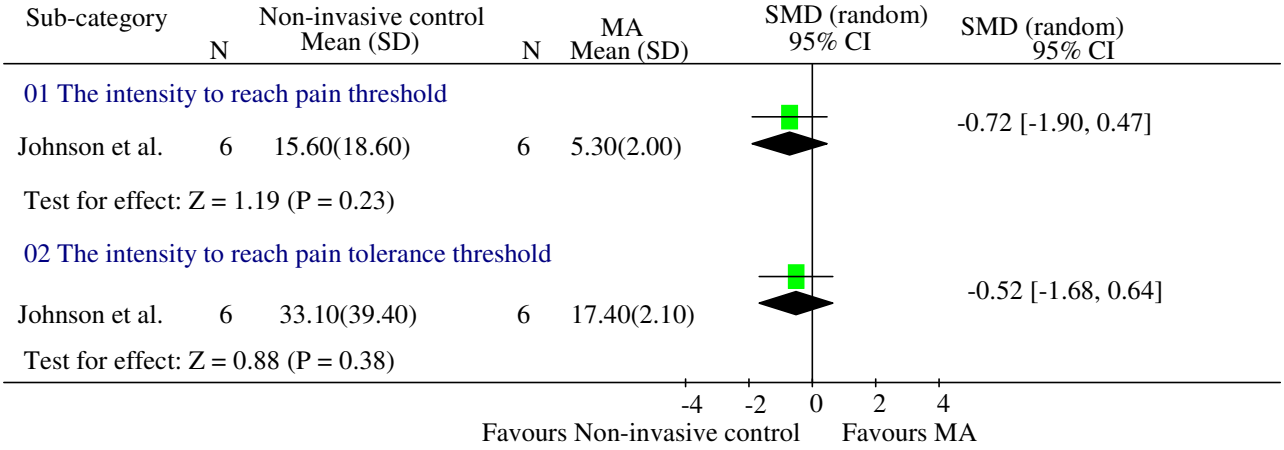
Comparison 03: Invasive control with electrical stimulation vs. non-invasive control (the effect immediately after inteverntion)



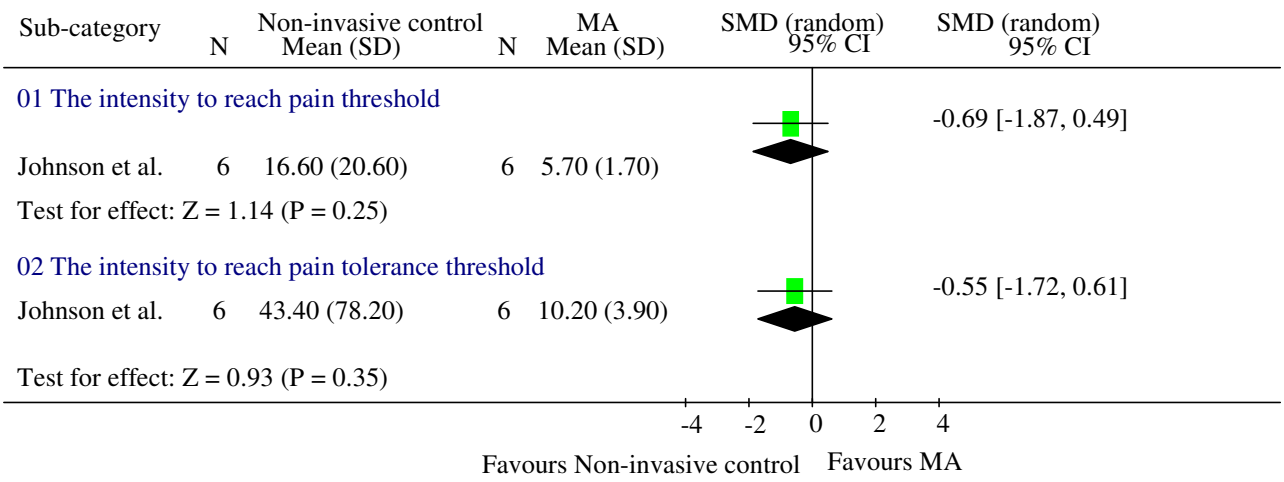
Appendix 15 Estimation of standardised mean differences, study 03

Pain model: Transcutaneous single electrical stimulus induced pain assessed by the change of intensity (μA) to achieve PT or PTT pain responses

Comparison 01: MA vs. non-invasive control (the effect instantly during intervention)



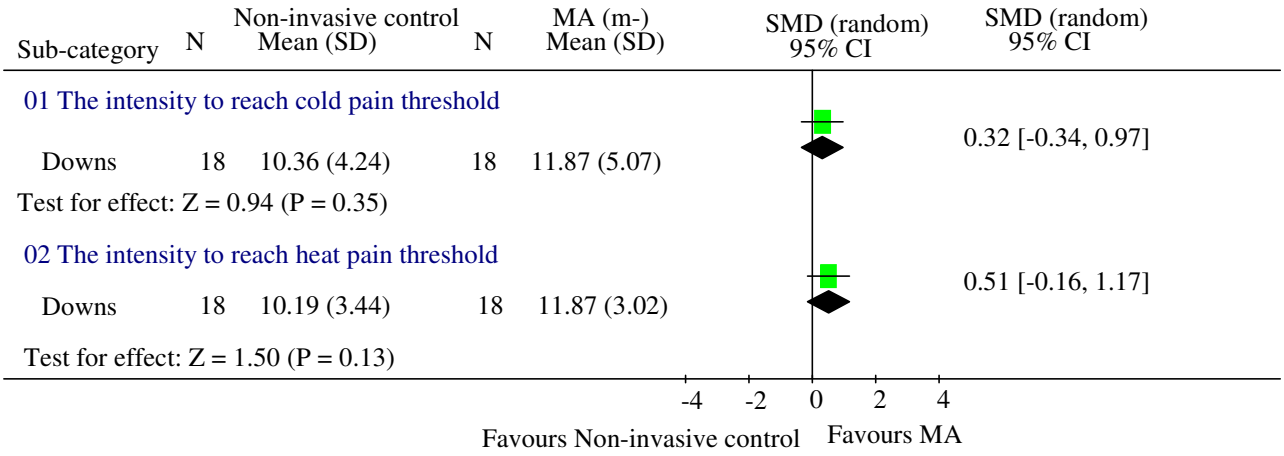
Comparison 02: MA vs. non-invasive control (the effect immediately after inteverntion)



Appendix 16 Estimation of standardised mean differences, study 04

Pain model: Transcutaneous cold-heat prolonged stimuli induced pain assessed by temperature (degree Celsius)

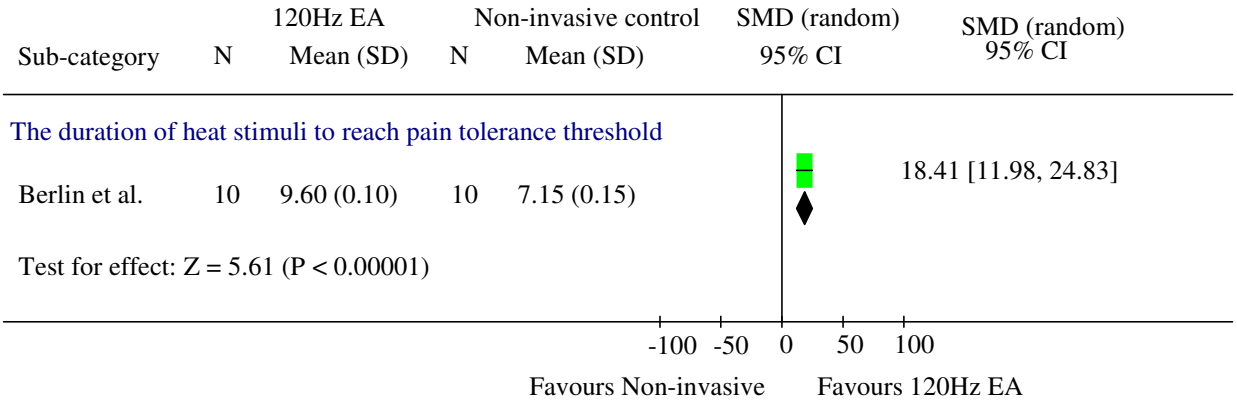
Comparison: MA vs. non-invasive control (the effect immediately after inteverntion)



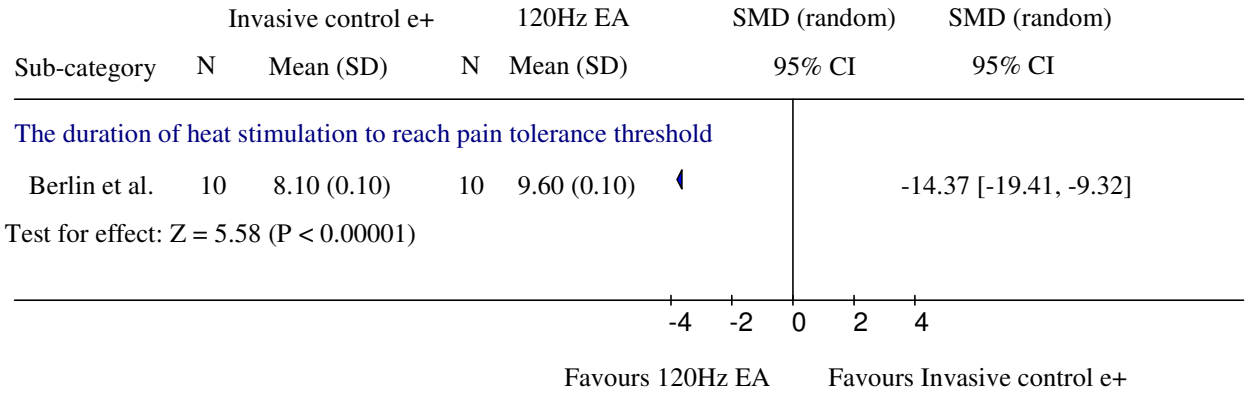
Appendix 17 Estimation of standardised mean differences, study 05

Pain model: Transcutaneous prolonged heat stimuli induced pain assessed by the stimulating duration to reach pain tolerance threshold

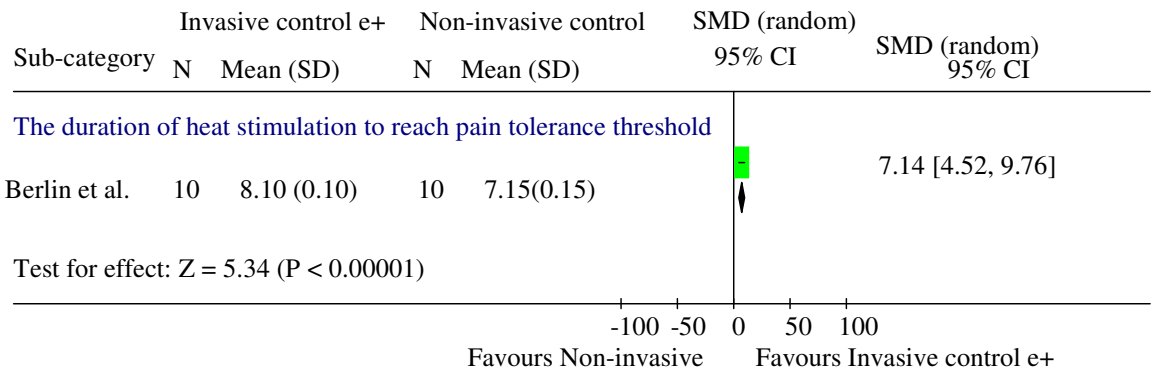
Comparison 01: 120Hz EA vs. non-invasive control (the effect immediately after inteverntion)



Comparison 02: 120Hz EA vs. invasive control with electrical stimulation (the effect immediately after inteverntion)



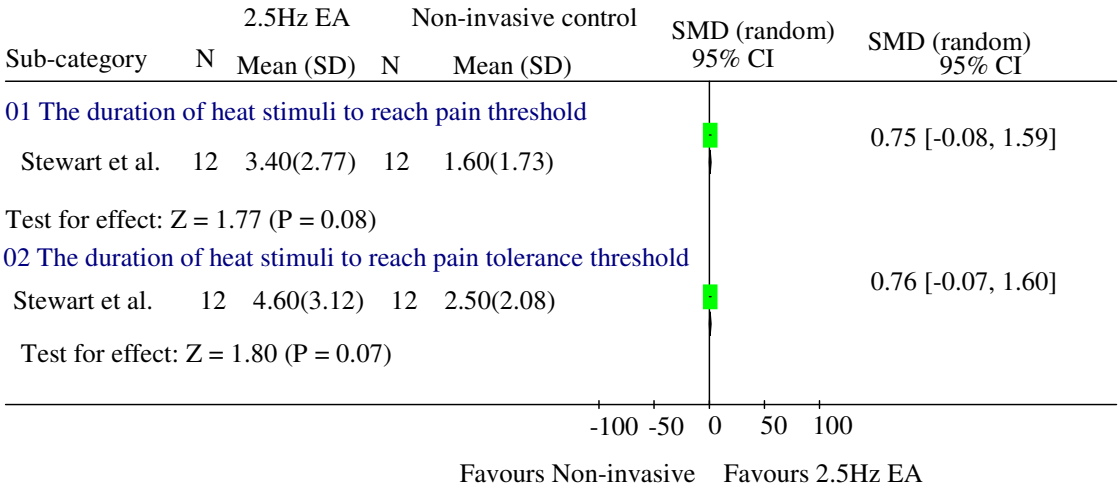
Comparison 03: Invasive control with electrical stimulation vs. non-invasive control (the effect immediately after inteverntion)



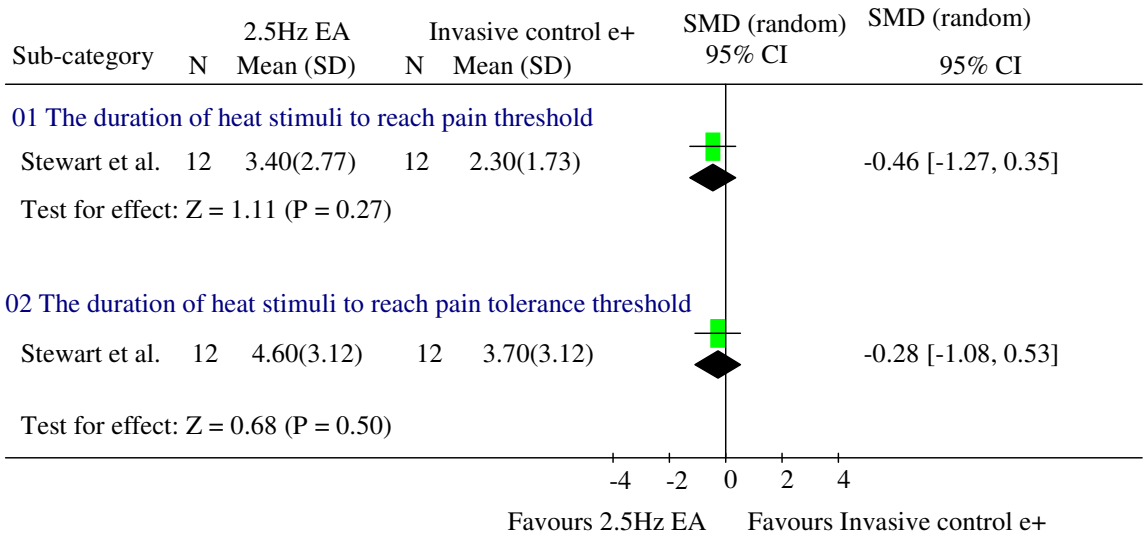
Appendix 18 Estimation of standardised mean differences, study 06

Pain model: Transcutaneous prolonged heat stimuli induced pain assessed by the stimulating duration to reach pain threshold and pain tolerance threshold

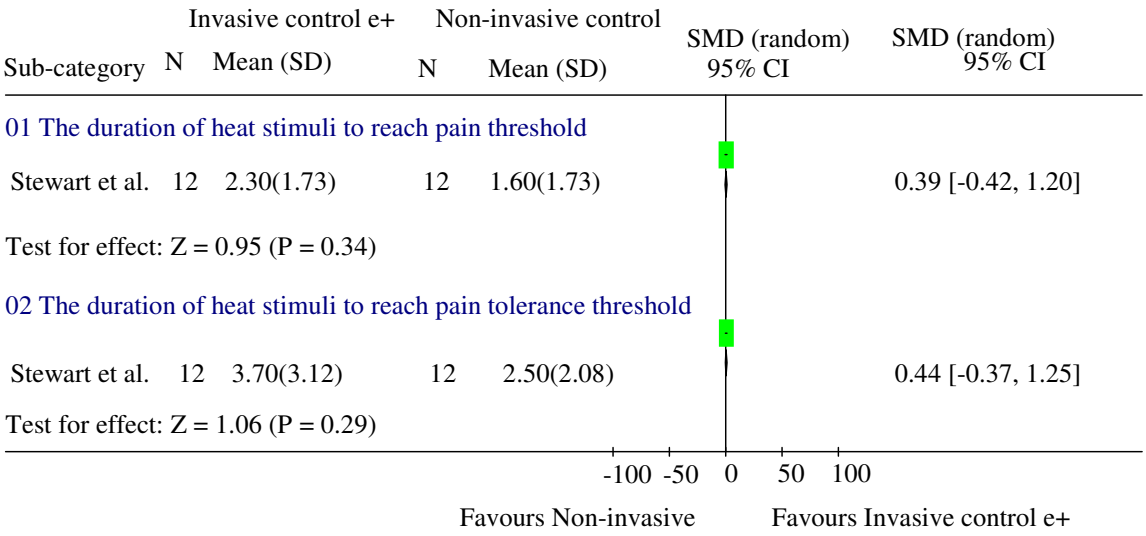
Comparison 01: 2.5Hz EA vs. non-invasive control (the effect immediately after inteverntion)



Comparison 02: 2.5Hz EA vs. Invasive control with electrical stimulation (the effect immediately after inteverntion)



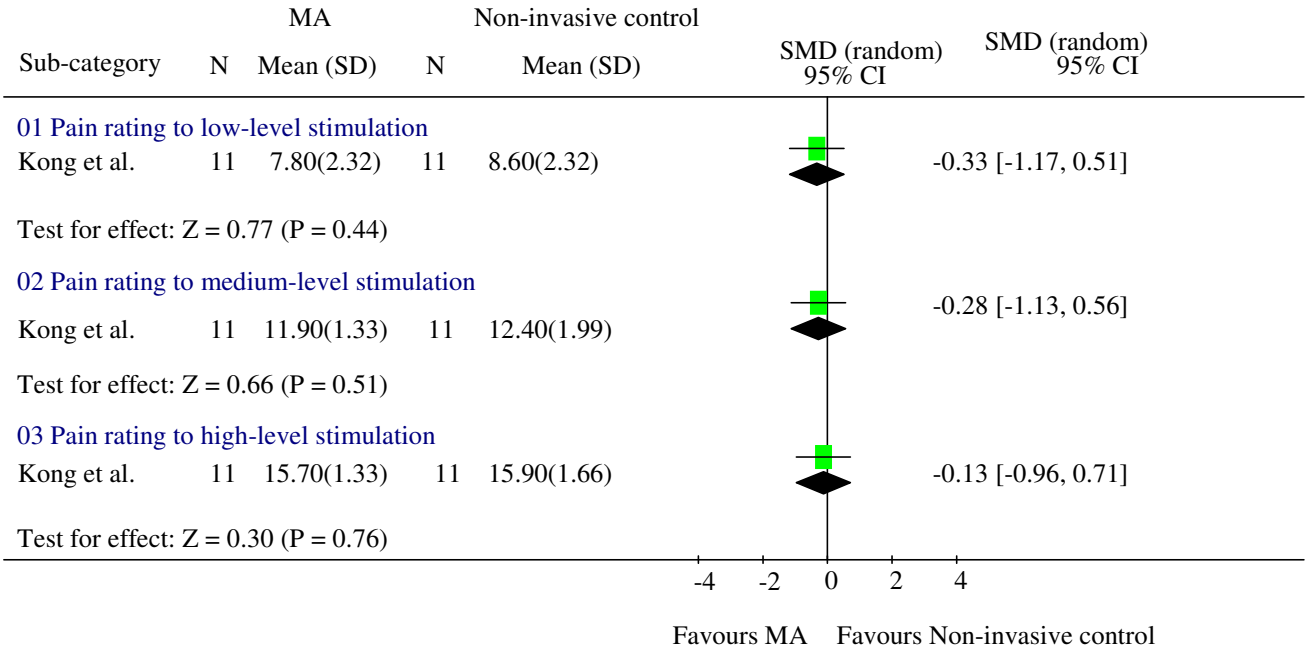
Comparison 03: Invasive control with electrical stimulation vs. non-invasive control (the effect immediately after inteverntion)



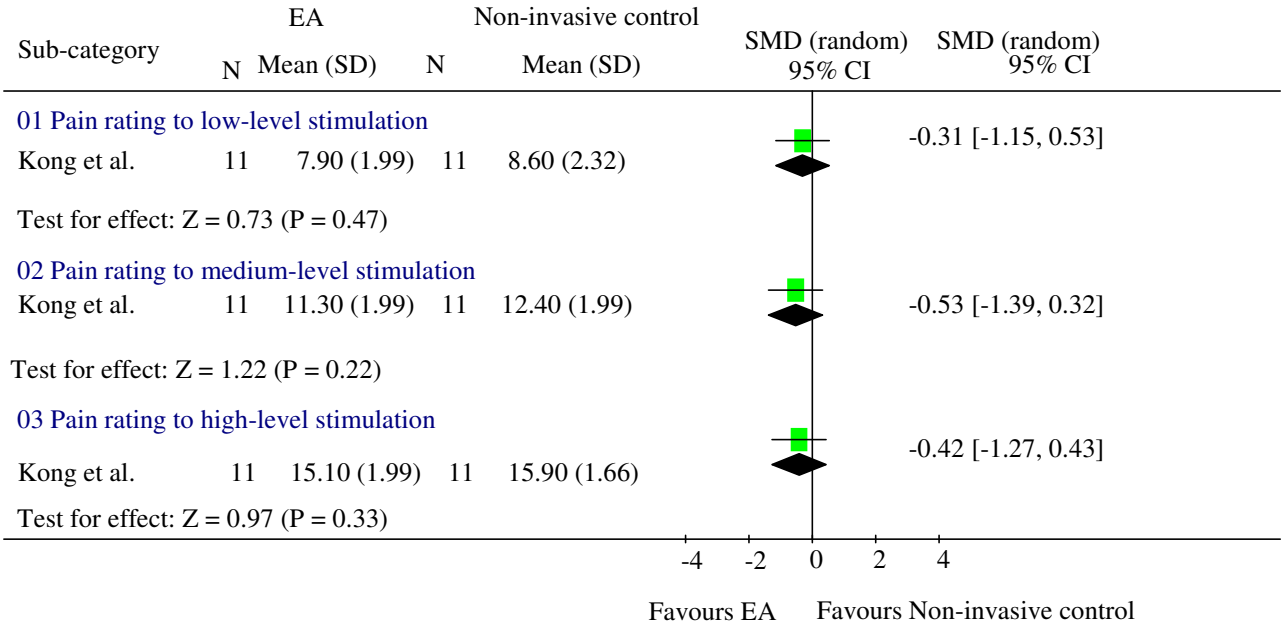
Appendix 19 Estimation of standardised mean differences, study 07

Pain model: Transcutaneous single heat stimulation (12 seconds duration each) induced pain assessed by pain response rating (VAS)

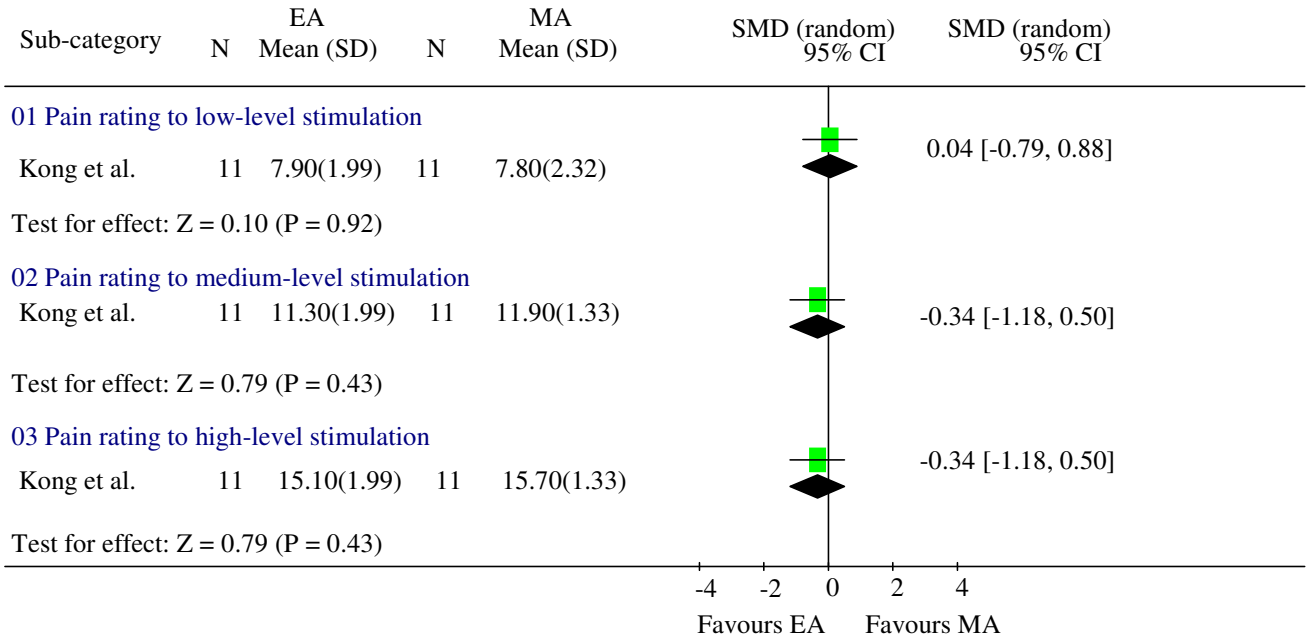
Comparison 01: MA vs. non-invasive control (the effect immediately after inteverntion)



Comparison 02: EA vs. non-invasive control (the effect immediately after inteverntion)



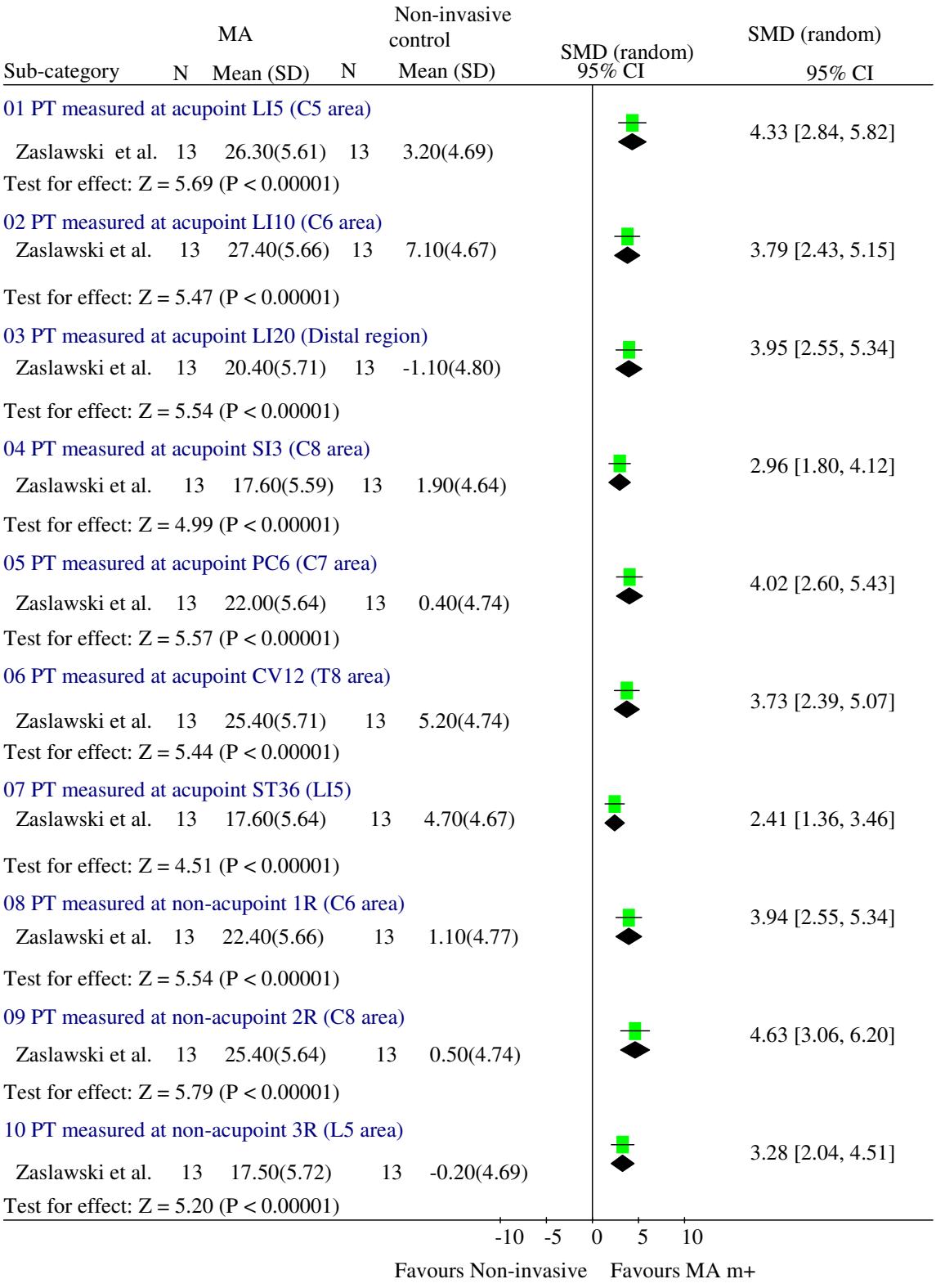
Comparison 03: EA vs. MA (the effect immediately after inteverntion)



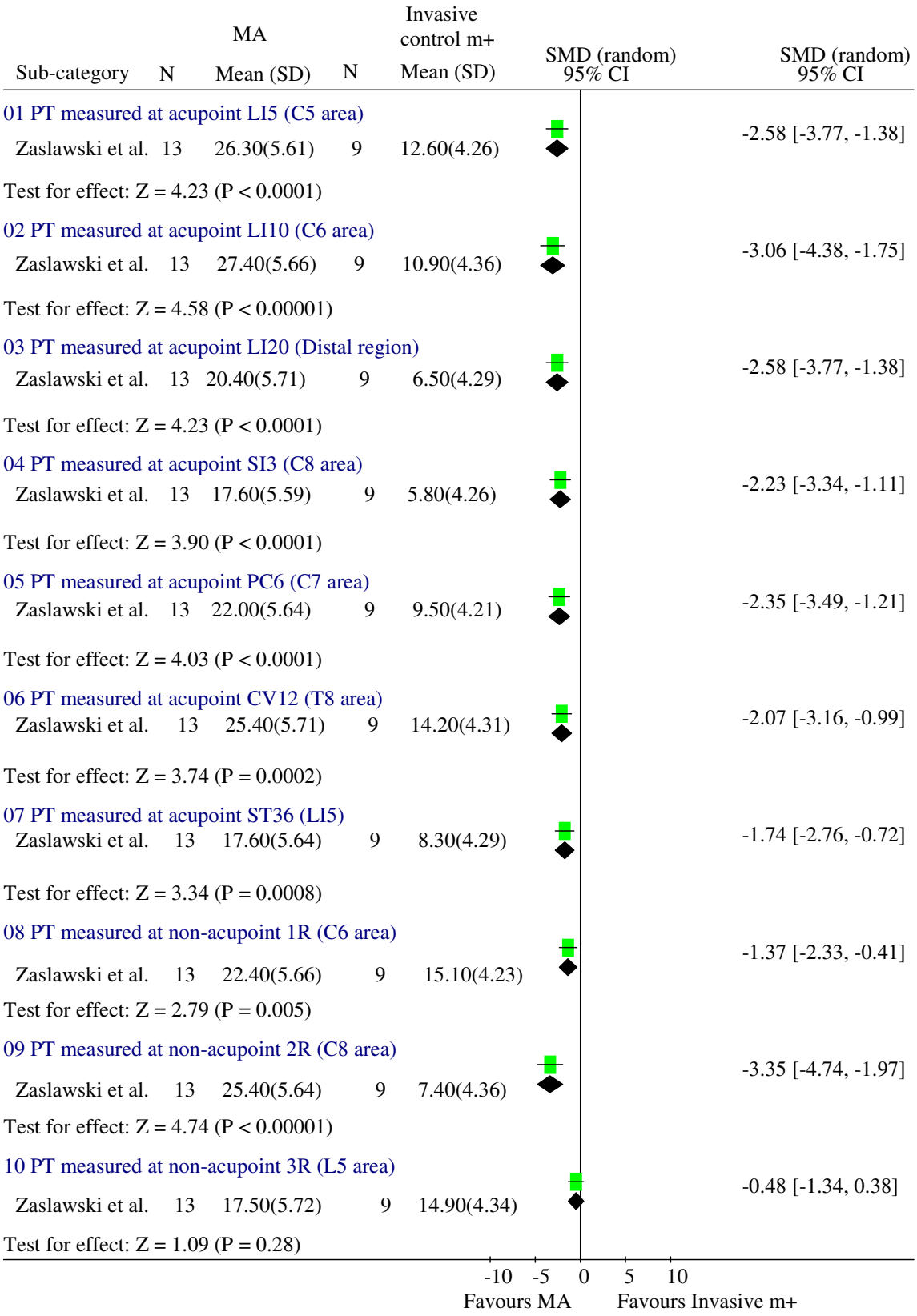
Appendix 20 Estimation of standardised mean differences, study 08

Pain model: Transcutaneous prolonged mechanical stimulus induced pain (pressure pain)

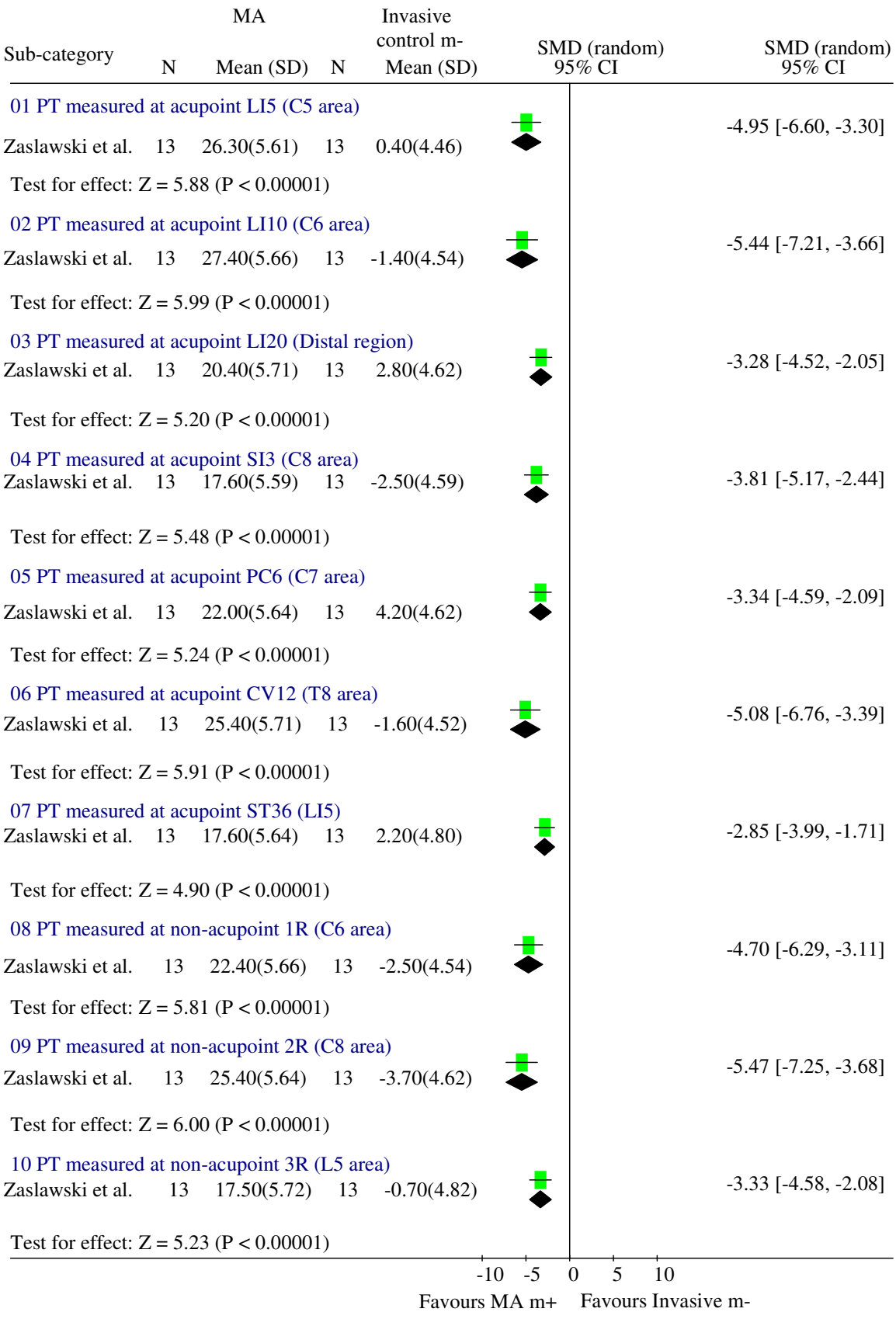
Comparison 01: MA vs. Non-invasive control (the effect immediately after inteverntion)



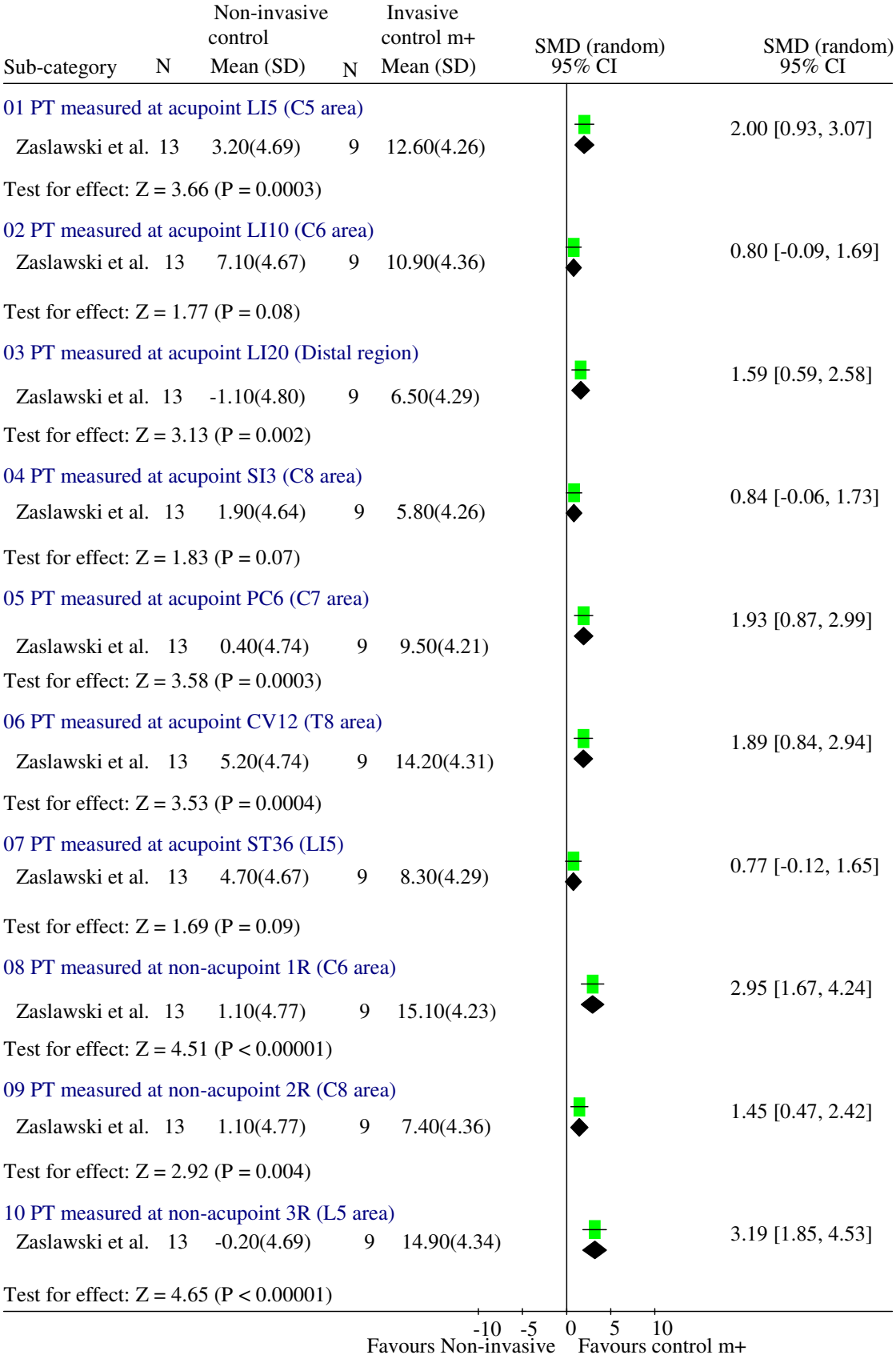
Comparison 02: MA vs. invasive control m+ (the effect immediately after inteverntion)



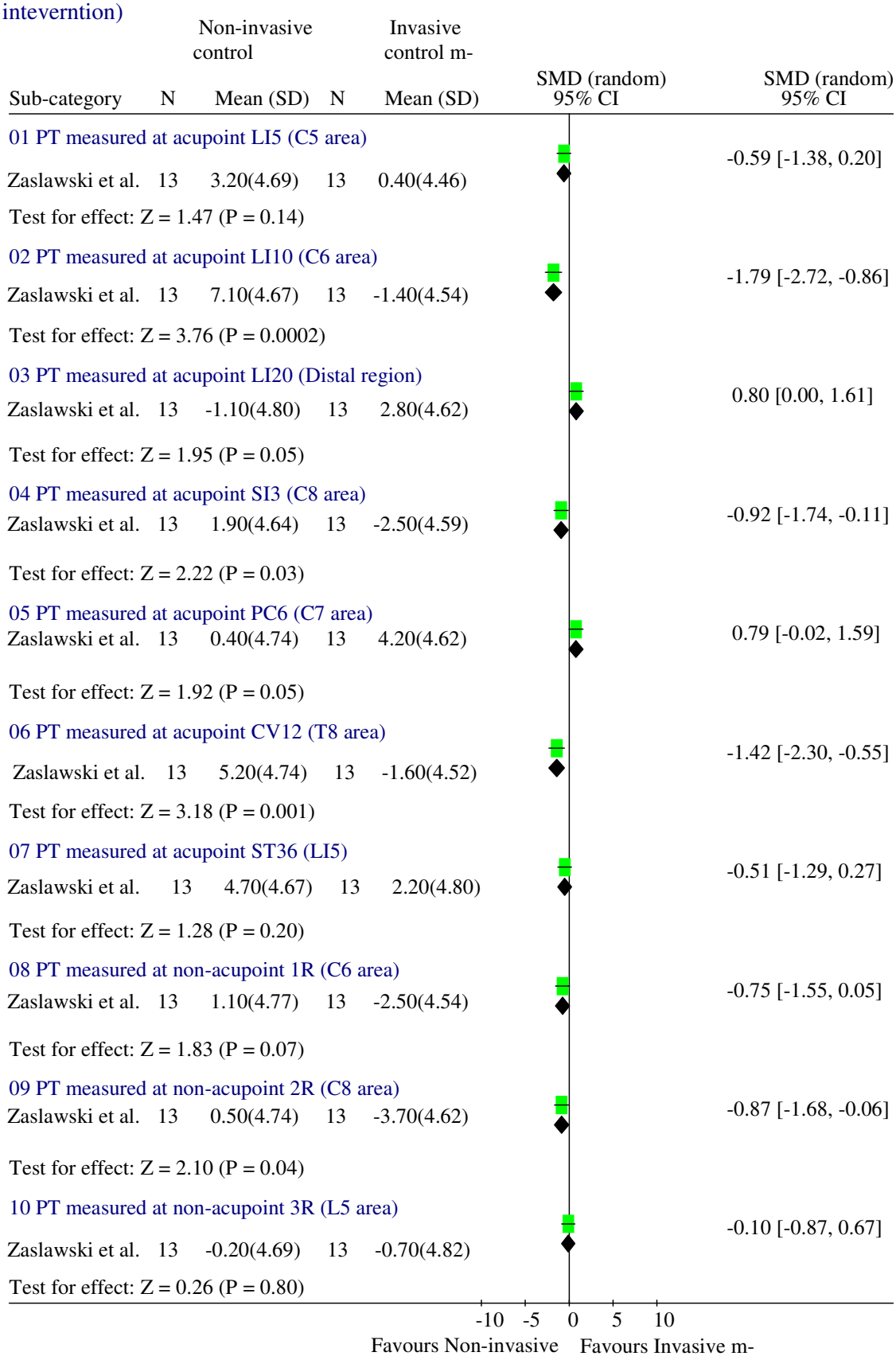
Comparison 03: MA vs. invasive control m- (the effect immediately after inteverntion)



Comparison 04: Invasive control m+ vs. Non-invasive control (the effect immediately after inteverntion)



Comparison 05: Invasive control m- vs. Non-invasive control (the effect immediately after inteverntion)



Appendix 21 Abbreviation list

Abbreviation List (in alphabetical order)

ANOVA = analysis of variance	mRNA = messenger ribonucleic acid
CNS = central nervous system	NIH = National Institutes of Health
cRNA = catalytic ribonucleic acid	NMDA = N-methyl-D-aspartic
D-D = dense-disperse	PLS = Plain Language Statement
DNIC = Diffuse Noxious Inhibitory Control	PPD = prepro-dynorphin
e+ = electro-acupuncture type of electrical stimulation is presented	PPE = prepro-enkephalin
ECG = electrocardiogram	PT = pain threshold
EA = electro-acupuncture	PTT = pain tolerance threshold
EBM = evidence base medicine	RCT = randomised controlled trials
EMG = electromyogram	RevMan = Review Manager
EOP = endogenous opioid peptide	SA = sham-acupuncture
ES = electrical stimuli	SD = standard deviation
fMRI = functional magnetic resonance imaging	SEM = stand error of mean
Hz = hertz	SMD = standardised mean difference
IASP = International Association for the Study of Pain	SPSS = Statistical Package for the Social Sciences
IVS = Internal Validity Scale	SPT = single-stimulus pain threshold
kg/s = kilogram per second	SSAI = Spielberg State and Anxiety Inventory
μA = microampere	ST = supra-threshold
m- = manipulation was absent	STRICTA = Standards for Reporting Interventions in Controlled Trials of Acupuncture
m+ = manipulation was present	TEAS = transcutaneous electrical acupoint stimulation
MA = manual acupuncture	TS = temporal summation
mA = milliampere	TST = temporal summation pain threshold
MEAP = Met-enkephalin-Arg-Phe	VAS = visual analogue scale
mg = milligram	WDR = wide dynamic range
mg/kg = milligram per kilogram	WHO = World Health Organisation