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; and, any editorial work, paid or unpaid, carried out by a third party is acknowledged. And ethics procedures and guidelines have been followed.

Signature:

Name: Linghan Bai

Date: 28 August 2008
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28 August 2008
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Summary

Background

One of the key features of clinical pain is central sensitization, which is an enhanced activity in the central nervous system (CNS). It underlies a common pain-related neurological phenomenon, called hyperalgesia.

Previous studies mainly focused on testing the analgesic effects of acupuncture in healthy humans using transient painful stimulation, the lack of central sensitization in the pain test rendered these studies with limited clinical implication. To solve this problem, hyperalgesia model needs to be introduced into acupuncture studies.

Aims

The current project aimed to bridge this gap by employing a validated human hyperalgesia model to assess the analgesic effect of acupuncture.

This project consists of three stages: at the first stage, literature regarding acupuncture and hyperalgesia was critically reviewed to identify the optimal acupuncture parameters. At the second stage, an experiment was designed to assess the reproducibility of the topical capsaicin model, a commonly used hyperalgesia model in humans for this study. At the final stage, a validated hyperalgesia model, called heat/capsaicin model was used to evaluate the anti-hyperalgesia effect of electroacupuncture.
Methodology

For the literature review, “acupuncture”, ”electroacupuncture”, “hyperalgesia” and ”allodynia” and their combinations were used to search the major databases including PubMed, Proquest and CINHAL. Inclusion and exclusion criteria were applied to select relevant papers.

The pilot experiment (Stage 2) was a two-session, non-randomised study. Twelve (12) healthy human subjects were recruited and completed two sessions of test. In each session, capsaicin solution (0.1 ml, 1mg/ml) was applied topically onto the non-dominant forearm (1×1 cm²) for 45 minutes. Area of secondary hyperalgesia, mechanical pain threshold and the visual analogue scale (VAS) were used to determine the time-course of the hyperalgesia in these subjects. The two sessions were undertaken with a minimum of one month interval. The data from the two sessions were analysed to determine the within group reproducibility of this model.

Experiment 2 (The Final Stage) was a one-session, randomised, sham electroacupuncture-controlled study in healthy human subjects. Twenty (20) subjects were recruited and completed the experiment without any dropouts. The heat/capsaicin model was induced by heating (45˚C) an area of skin (3×3 cm²) in the middle of the forearm for 5 minutes followed with application of a thick layer of capsaicin cream (0.075%) in the sensitised site for 30 minutes. The sensitised area was then rekindled with heat stimulation (40˚C for 5 minutes) for four times. Real or sham electroacupuncture (REA or SEA) intervention was given for 25 minutes on eight acupuncture points located on the four extremities. Pain rating to long
thermal stimulation (40°C for 1 minute), area of secondary hyperalgesia and heat pain threshold were measured once before and twice after EA after the rekindling.

**Results**

The current project was reviewed and approved by the Human Research Ethic Committee of RMIT University (Reference No. 13/07)

In the literature review, 32 papers were selected. All of them were animal studies in rats. There was no human study regarding the anti-hyperalgesia effect of acupuncture. All studies used EA as the intervention. Hyperalgesia models used in these studies included inflammatory pain, neuropathic pain, and irritable bowel syndrome (IBS) and cancer models. Overall, EA significantly reduced both mechanical and heat hyperalgesia compared with sham procedures. The optimal EA parameter was identified for two specific types of hyperalgesia model; however, no universal optimal EA parameter was identified.

It was found in the pilot experiment, the overall reproducibility of the topical capsaicin model was not appropriate to assess the anti-hyperalgesia of acupuncture. The area of secondary hyperalgesia in the two sessions correlated well only at the first post-capsaicin measurement ($Pearson r = 0.643, p = 0.024$), but not at the second measure ($Pearson r = 0.456, p = 0.136$). Mechanical pain threshold, on the other hand, correlated well only in the second post-capsaicin measurement ($Pearson r = 0.745, p = 0.005$ in the primary hyperalgesia area; $Pearson r = 0.644, p = 0.024$ in the secondary hyperalgesia area). Only VAS ratings to three out of five von Frey filaments showed moderate correlation during both post-capsaicin
measurements.

As a result, a previously validated heat/capsaicin model was used in Experiment 2. It was found that both mechanical and thermal hyperalgesia decreased significantly in the REA and SEA groups with a similar magnitude. Area of secondary hyperalgesia (mechanical hyperalgesia) decreased to 30% of the baseline in REA group and 24% of the baseline in SEA group. Two-way ANOVA showed statistically significant reduction in the area in both groups ($F (2, 36) = 10.209, p < 0.001$) but without difference between the groups ($F (2, 36) = 2.146, p = 0.988$). Similar result was found in the heat pain threshold and pain rating to long thermal stimulation.

**Conclusion**

There was strong evidence in the animal studies supporting REA being associated with significantly better anti-hyperalgesia effect when compared with SEA. The current experiment 2 was the first one to assess the anti-hyperalgesia effect of acupuncture using a human hyperalgesia model (heat/capsaicin model). However, REA was not better than SEA in suppressing hyperalgesia. Further studies need to address a few short-comings (including the lack of the use of suprathreshold stimulation high frequency EA parameter) of the current experiment and to verify this finding.

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Chapter 1 introduction

1.1 What is pain?

According to the International Association for the Study of Pain (IASP), pain is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage”. Pain has its biological function for alarming the body of potential tissue damage. However, it can become so intense that it impairs other function of the body, and severely reduces the quality of life (Ferrell 1995; Tuzun 2007).

1.2 Acupuncture in treating pain

Acupuncture, an ancient treatment, has been using in China for more than two thousands years. Nowadays this therapy is accepted and practiced around the world. A review by the WHO in 2002 concluded that ‘acupuncture has been proved effective’ for 28 medical conditions, of which 15 are painful conditions (Zhang 2002).

1.3 Neural mechanisms of electroacupuncture analgesia

The current understanding of the analgesic effect of acupuncture is mainly based on neural mechanisms of endogenous pain control and endogenous opioid peptides (Han and Terenius 1982).

Three popular neurophysiologic theories have been used to explain the analgesic effect of acupuncture: gate control theory or segmental inhibition (Bekkering and Bussel 1998), diffuse noxious inhibitory control (Le Bars, Dickenson et al. 1979) and limbic system attenuation (Campbell 1999).
Gate control theory hypothesise that high frequency EA activates large myelinated afferent fibres (A-β) and prevent pain transmission by inhibiting signes from small afferent (C) fibres (Melzack 1984). Segmental inhibition theory, which is developed from gate control theory, suggests that EA not only reduces pain at the spinal segment level, but also suppress subsequent actions such as withdrawal reflex and local muscle contraction, following a painful stimulation (Bekkering and Bussel 1998). These theories explain the immediate analgesic effect of acupuncture in and around the area of treatment.

Diffuse noxious inhibitory control theory, which suggests painful stimuli applied to one part of the body inhibit pain responses in another part of the body (Le Bars, Dickenson et al. 1979), can explain why EA can produce widespread analgesic effect (Zaslawski, Rogers et al. 1997).

The third theory “limbic system attenuation” suggests that the deactivation effect of acupuncture on the limbic system could contribute to the feelings of calm and relaxation (Campbell 1999), consequently reduces the impact of pain.

Apart from the above mentioned theories, studies suggest that EA stimulation can induce the release of opioid peptides in the central nervous system (Cheng and Pomeranz 1979). This theory explains why acupuncture has effects lasting beyond the time of stimulation and up to a few days as often seen in the clinic.

All of these theories could play important roles in the overall analgesic effects of EA.
1.4 Laboratory research about acupuncture and pain

Though there is traditional Chinese medicine theory explaining and guiding the practice of acupuncture, its fundamental mechanism for treating pain remains unclear. Previous human studies mainly focused on acupuncture’s effect on pain threshold in healthy subjects (Lin, Chandra et al. 1981; Himuro, Tubaki et al. 1987; Cao, Wang et al. 1990; Farber, Tachibana et al. 1997; Wang and Hui-Chan 2003; Downs, Kirk et al. 2005). However, the test of transient pain sensation has little clinical relevance, as most pain conditions tend to last for a long time, from days to years. One of the key features that differentiate clinical pain from normal pain is central sensitization, a heightened state of activity in the central nervous system (CNS). For this reason, it is important to conduct acupuncture study on models that mimic clinical pain and reproduce central sensitization state.

1.5 Animal hyperalgesia models and acupuncture study

A feasible way to reproduce clinical pain condition is using animal models, in which, a neurological phenomenon called hyperalgesia is produced, with central sensitization being its key underlying mechanism. It is defined as an increased response to a stimulus which is normally painful. It can appear in a simple skin cut or as referred pain from heart disease.

In the past several decades, animal hyperalgesia models of persistent inflammatory pain and neuropathic pain have been developed and successfully used in the pharmaceutical researches to evaluate the efficacy of analgesics (Helyes, Szabo et al. 2004; Hsueh, Lu et al. 2004; Menendez, Lastra et al. 2004; Xu, Huang et al. 2007; Dong, Jia et al. 2008). Inspired by their
success in pharmaceutical studies, researchers began to use these animal models to evaluate
the anti-hyperalgesia effect of acupuncture, and to investigate its possible neurological
mechanism in treating pain (LaMotte 1992; Oliveira 2000; Dai 2001; Cui 2005).

1.6 Aims of the project

Despite the increasing evidence in the animal studies suggesting the anti-hyperalgesia effect
of acupuncture, there is little human study being conducted. The present study aimed to assess
the anti-hyperalgesia effect of acupuncture in human subjects.

The specific objectives were

1) To review current literature to identify an ideal set of acupuncture parameters for the anti-
   hyperalgesia effect;

2) To identify a stable and reproducible human model for testing anti-hyperalgesia effect of
   acupuncture;

3) To conduct a human study testing anti-hyperalgesia effect of acupuncture using the
   identified hyperalgesia model and parameters; and

4) To provide directions for future research in this area.

1.7 Outline of the thesis

Chapter 2 has two main parts: the first part reviews the available literatures regarding
hyperalgesia and its neurological mechanism, and some commonly used human hyperalgesia
models and their application in the clinical trial for analgesic evaluation. The second part
reviews the all studies assessing anti-hyperalgesia effect of acupuncture using various models,
providing a set of optimal parameters of acupuncture for the human study.
Chapter 3 describes the methodology of the two experiments, including overall design, subjects recruitment and screening, producing of hyperalgesia models, outcome measurements, intervention, blinding and statistical methods.

Chapter 4 presents the result from experiment 1 in which the reproducibility of the commonly used topical capsaicin model was assessed.

Chapter 5 presents the finding of experiment 2 in which the anti-hyperalgesia of REA and SEA was compared using heat/capsaicin model.

Chapter 6 summarizes the whole projects, discusses its strength and limitation, interprets the findings and provides recommendation for future study.
Chapter 2 Literature review

2.1 Hyperalgesia

2.1.1 Definition of hyperalgesia

Hyperalgesia means beyond (hyper) pain (algesia). Willis 1992 described the history of the term hyperalgesia and indicated this term first appeared in the late 19\textsuperscript{th} century (Willis 1992) and was defined as “a state of increased intensity of pain sensation induced by either noxious or ordinarily non-noxious stimulation of peripheral tissue” (Hardy, Wolff et al. 1950). According to the International Association for the Study of Pain (IASP), the definition of hyperalgesia has been revised several times since 1979. In 1994 hyperalgesia was defined as "an increased response to a stimulus which is normally painful". Furthermore, it has also been described as a leftward shift of the stimulus-response function (Figure 2.1), resulting in a lowering of pain threshold and /or an increase in pain intensity to suprathreshold stimuli (Campbell, Raja et al. 1988).

![Diagram of stimulus-response function](image)

Figure 2.1 Diagram of stimulus-response function
2.1.2 Hyperalgesia in clinical pain

Willis 1992 described the history of hyperalgesia and indicated this term first appeared in the late 19th century, the phenomenon of hyperalgesia has been recognised clinically for several centuries (Willis 1992). There are several references dating back to the 17th and 18th centuries that refer to cutaneous tenderness with visceral disease and nerve injuries (Bonica 1992).

From a functional point of view, hyperalgesia serves as a protective mechanism, alerting the individual to avoid contact with noxious stimulus to the injured site (Treede, Meyer et al. 1992). Hyperalgesia, by itself, is not a disease, but a neurological phenomenon or feature associated with a range of clinical pain syndromes and visceral diseases. However, in cases such as chronic pain, hyperalgesia can be a major clinical problem.

In clinical settings, hyperalgesia is observed in inflammatory and neuropathic conditions of both superficial and deep somatic structures, such as injury to skin and mucous membranes, diseases of peripheral and central nervous system, referred pain caused by visceral diseases, certain types of systemic disorders and certain psychological and psychiatric disorders (Bonica 1992).
2.1.3 Types of hyperalgesia

Experimentally, hyperalgesia can be classified as primary vs. secondary hyperalgesia and thermal vs. mechanical hyperalgesia.

2.1.3.1 Primary and secondary hyperalgesia

In 1935, Lewis was the first to discover the two distinctive areas following an experimentally-induced cutaneous injury, i.e. electrical stimulation, the site of injury and the undamaged skin around the injury site (Lewis 1935). He hypothesized that there might be a peripheral mechanism underlying this phenomenon. Later, in 1950, another pioneer Hardy, further examined the feature of hyperalgesia and used primary and secondary hyperalgesia to name evoked pain at the injury site and at the surrounding undamaged skin area, respectively (Figure 2.2). However, Hardy proposed different neural mechanisms for the two types of hyperalgesia. Firstly, he suggested that primary hyperalgesia was due to sensitisation of the peripheral nervous system (PNS), whereas secondary hyperalgesia was resulted mainly from sensitisation of the central nervous system (CNS) (Hardy, Wolff et al. 1950). Hardy’s view was developed and confirmed by later studies (Treede, Meyer et al. 1992). The terms primary and secondary hyperalgesia, continue to be used to describe such phenomena.
2.1.3.2 Heat and mechanical hyperalgesia

As researchers working in the field of hyperalgesia began to use different modalities of stimulation to explore the characteristics and mechanisms of primary and secondary hyperalgesia, heat and mechanical hyperalgesia were named accordingly, since these two types of stimulation were most commonly utilized by researchers. It was first described by Raja in 1984 (Raja, Campbell et al. 1984) and later by LaMotte (LaMotte, Shain et al. 1991) that primary hyperalgesia was characterized by a heightened responses to heat and mechanical stimuli, whereas secondary hyperalgesia only showed increased pain to mechanical stimuli. However, later studies demonstrated that heat hyperalgesia was also present in the area of secondary hyperalgesia in the topical capsaicin model (Yucel, Andersen et al. 2002).

Furthermore, subcategories of mechanical hyperalgesia have also been discovered. In 1992,
Koltzenburg identified static and dynamic components of mechanical hyperalgesia, based on the method of delivering mechanical stimulation (Koltzenburg, Lundberg et al. 1992). Static hyperalgesia is induced with a mechanical device such as a pressure algometer, von Frey filaments or a safety pin at a single point, whereas dynamic hyperalgesia is induced by a moving stimulus, such as brush stroking across the skin surface (Kilo, Schmelz et al. 1994).

According to the stimulation modality tested, punctate, stroking, pressure and impact hyperalgesia were classified (Kilo, Schmelz et al. 1994). Punctate hyperalgesia is assessed by poking the skin surface with a plastic filament or safety pin (Raja, Campbell et al. 1984). Stroking hyperalgesia is assessed by moving a brush across the skin (LaMotte, Shain et al. 1991). Pressure hyperalgesia, similar to punctate hyperalgesia, is measured with a pressure algometer. Impact hyperalgesia is tested with a plastic cylinder or bullet shooting against the skin (Kilo, Schmelz et al. 1994).

These subtypes of mechanical hyperalgesia manifest differently, according to the type of experiment models utilised. For example, stroking hyperalgesia is prominent in the topical capsaicin model, but almost absent in cold injury model. Furthermore, impact hyperalgesia is found in the cold injury model but absent in the capsaicin model (Kilo, Schmelz et al. 1994).

### 2.1.4 Different types of cutaneous hyperalgesia and their mechanisms

The knowledge of the neural mechanisms of hyperalgesia have been developed mainly based on studies using heat and capsaicin models in animal and human, as these two models have
been extensively studied.

2.1.4.1 Primary hyperalgesia

In his pioneering studies on hyperalgesia, Lewis attributed primary hyperalgesia to the sensitisation of the nociceptors by local release of the inflammatory factors from the damaged tissue (Lewis 1935). This view is supported by later studies conducted by Hardy and colleagues (Hardy, Wolff et al. 1950).

Primary hyperalgesia, according to the stimulus modality, is characterized by sensitisation to both thermal and mechanical stimuli (Lewis 1935; Hardy, Wolff et al. 1950), therefore, both heat and mechanical hyperalgesia co-exist in the area of primary hyperalgesia. Thus its mechanisms should be discussed in terms of both of them.

2.1.4.1.1 Peripheral neural mechanisms of heat hyperalgesia

Studies have shown that sensitisation of A-fiber and C-fiber mechano-heat nociceptors (AMHs and CMHs) account for heat hyperalgesia (Reeh, Kocher et al. 1986) following stimuli such as heat and mechanical injury, inflammation, and various chemical stimulations. However, the action of nociceptor is different between hairy skin and glabrous skin. Both AMHs and CMHs develop sensitisation to heat stimuli in hairy skin (LaMotte, Thalhammer et al. 1982), whilst AMHs are sensitised after injury in glabrous skin only (Meyer and Campbell 1981). Furthermore, preferential A-fiber blockade showed that the time course of heat hyperalgesia is not affected by the blockade in hairy skin whilst it is suppressed in a model of glabrous skin after the blockade (Meyer and Campbell 1981; LaMotte, Thalhammer et al.
This suggests that CMHs may play a dominant role in hairy skin, however may not contribute to heat hyperalgesia in glabrous skin.

**2.1.4.1.2 Peripheral neural mechanisms of primary mechanical hyperalgesia**

Mechanical hyperalgesia, such as pressure, punctate and stroking hyperalgesia also develop after heat injury or capsaicin application. However, evidence of nociceptor sensitisation is contradictory (Treede, Meyer et al. 1992). Sensitisation of CMHs to mechanical stimuli has been found in both human (Torebjork and Hallin 1978) and cats (Bessou and Perl 1969) however, not in monkeys and rats (Treede, Meyer et al. 1992), whereas sensitisation of AMHs to mechanical stimulation has only been found in rats (Reeh, Bayer et al. 1987). In addition, neither AMHs nor CMHs have displayed sensitisation to von Frey filaments stimulation in the microneurography test in humans (Baumann, Simone et al. 1991; Handwerker, Forster et al. 1991; LaMotte, Shain et al. 1991) or in monkeys (Baumann, Simone et al. 1991) following heat injury or capsaicin injection.

To an extent, further studies resolve the contradiction of nociceptor sensitisation underlying mechanical hyperalgesia within the area of primary hyperalgesia. The novel CMiHi nociceptors (mechano-heat insensitive C nociceptors) and CH nociceptors (heat sensitive C nociceptors) became sensitized to pressure (Schmidt, Schmelz et al. 1995) and punctate stimuli after injection of mustard oil and capsaicin (Schmelz, Schmid et al. 2000). An increase of mechanoreceptive field of CMH nociceptors after capsaicin or mustard oil application has also been reported (Schmelz, Schmidt et al. 1994). These findings may account for the
sensitivity change to mechanical stimuli within the area of primary hyperalgesia.

Importantly, evidence supporting nociceptor sensitisation to stroking stimuli has not been found within the region of primary hyperalgesia. Stroking hyperalgesia within the injury site may be due to central sensitisation.

### 2.1.4.2 Secondary hyperalgesia

Secondary hyperalgesia occurs in the uninjured region surrounding the injury site. Previously, it has been widely accepted that only mechanical hyperalgesia was present in this area (Treede, Meyer et al. 1992). However, recent studies have found that heat hyperalgesia also exists (Yucel, Andersen et al. 2002). Two early pioneers in the area, Lewis and Hardy, proposed different mechanisms underlying this phenomenon. Lewis suggested that a peripheral mechanism, involving the activation of primary afferent nociceptors by the release of substance from the activated nerve terminals, was responsible (Lewis 1935). Lewis proposed an unidentified peripheral nervous system called “nocifensor nerves” to support his view. However this hypothesis is rarely supported by later performed studies. Hardy, on the other hand, proposed that the spread of sensitisation within the central nerves system was the underlying mechanism (Hardy, Wolff et al. 1950). Hardy’s view, which was later referred as central sensitisation, has been supported by numerous studies in the last three decades and has become widely accepted as the mechanism for secondary hyperalgesia (Koltzenburg, Lundberg et al. 1992; Treede, Meyer et al. 1992; Kilo, Schmelz et al. 1994).
2.1.4.2.1 Peripheral mechanism of secondary hyperalgesia

It was found in Lewis’s early research that a proximal nerve block interrupting peripheral input to the central nervous system did not prevent the development of hyperalgesia and flare. Lewis then concluded that the spread of secondary hyperalgesia was due to a peripheral mechanism. He theorised a “nocifensor nerves” system (an unidentified peripheral nervous network) may account for these changes (Lewis 1935). A later study which identified electrical or chemical coupling between unmyelinated fibres in the periphery may support the existence of the nocifensor system (Meyer, Raja et al. 1985). However, no further evidence has been provided to support these findings.

In order to explain the characteristic of secondary hyperalgesia with peripheral mechanisms, two pieces of key evidence need to be identified. Firstly, the size of receptive fields of nociceptor is required to be at least half the size of the secondary hyperalgesia. Secondly, the nociceptors are required to become sensitised in the area of secondary hyperalgesia (Treede, Meyer et al. 1992). However, neither of these two findings was supported by further studies. It has been found that the mean size of a receptive field in both humans and moneys is 18 mm² (Campbell, Raja et al. 1989). This is significantly smaller than half of the size of secondary hyperalgesia which is up to 550 mm² (LaMotte, Shain et al. 1991). Furthermore, no sensitivity to mechanical stimuli (stroking or punctate) was found in CMiHi, CMHs, and AMHs nociceptors, or any other types of afferent fiber (LaMotte 1992; Schmelz, Schmidt et al. 1996). Therefore, secondary hyperalgesia cannot be readily explained by the peripheral mechanism alone.
2.1.4.2.2 Central mechanism of secondary hyperalgesia

Hardy and coworkers conducted a serial of psychophysical experiments on hyperalgesia using electrical nerve stimulation and local anesthetics. They found that proximal nerve block near the torso slowed the spreading of secondary hyperalgesia instead of preventing its development, whereas distal nerve block, near the extremity, prevented hyperalgesia spreading to the blocked area. These findings led Hardy and coworkers to conclude that the spread of the secondary hyperalgesia must be likely due to the changes in the central nervous system (Hardy, Wolff et al. 1950).

Evidence supporting central sensitization came from a series of electrophysiological experiments using animal hyperalgesia models. These findings included the heat injury model in monkeys showing the dorsal horn neurons to develop increased sensitivity to mechanical stimuli applied outside the injury site (Kenshalo, Leonard et al. 1982), decreasing mechanical thresholds reported in the receptive field of rat lamina I neurons (McMahon and Wall 1984) and sensitisation observed in the somatosensory cortex (Kayser and Guilbaud 1987) and spinothalamic tract (STT) neurons (Simone, Baumann et al. 1989; Simon, Sorkin et al. 1991) in animals under hyperalgesia state. Furthermore, following intradermal injections in monkeys, WDR and NS neuronal activity response was heightened due to both stroking and punctate stimuli (Simon, Sorkin et al. 1991) with changes also reported in the receptive field of central neurons, and an enlarged receptive field of STT neurons after burn injury (McMahon and Wall 1984).

Mechanical injury within the receptive field of dorsal horn neurons of rats was found to cause
the expansion of the receptive field of these neurons (Laird and Cervero 1989). Application of mustard oil outside of the receptive field of dorsal horn neurons of rats also led to its expansion (Woolf and King 1990).

More recently, studies in healthy human subjects using PET (Iadarola, Berman et al. 1998) and fMRI (Baron, Baron et al. 1999) demonstrated that under hyperalgesia state produced by capsaicin application, same amount of mechanical stimulations elicited an enhanced level of activity in the prefrontal cortex and somatosensory cortex. In another study, fMRI indicated different patterns of brain activation between thermal and mechanical hyperalgesia, which related to different psychophysical properties for these two types of hyperalgesia (Maihofner and Handwerker 2005). Another animal study using fMRI found that mechanical stimulation induced a decrease in blood oxygen levels dependent of signal intensity in periaqueductal grey (PAG), in capsaicin treated rats. This also demonstrates the roles of PAG in central sensitisation (Moylan Governo, Morris et al. 2006).

### 2.1.5 Experimental cutaneous hyperalgesia in humans

In a clinical setting, hyperalgesia can occur in both superficial and deep somatic tissue (Bonica 1992). Manifestations of hyperalgesia vary substantially between individuals, making it extremely difficult to study quantitatively. Inducing hyperalgesia in healthy human subjects under controlled experimental conditions provides the opportunity to study its underlying mechanisms and therapeutic effects of various analgesics.

The following methods were employed by early researchers to induce hyperalgesia in healthy
participants (Hardy, Wolff et al. 1950):

1. Ultraviolet light from a lamp applied over an area of skin.
2. High intensity thermal radiation applied onto an area of blackened skin.
3. Electrical stimulation on a small area of skin anaesthetized by procaine.
4. Crushing a small area of skin by forceps.
5. Injecting saline into the intraspinous ligament.

Skin, due to its easy accessibility, is the main site for producing hyperalgesia. However, some of these methods mentioned above cause significant tissue damage and hence, not widely used. For this reason, it is necessary to find a hyperalgesia model that resembles clinical pain conditions, yet with minimum tissue injury. Several models have been developed over the decades. These include chemical models, thermal models and a combination of these.

2.1.5.1 Chemical models

2.1.5.1.1 Capsaicin model

Topical application or intradermal injection of capsaicin, the pungent substance from chili peppers, can produce burning pain and distinct areas of both primary and secondary hyperalgesia without producing evident tissue injury (Simon, Baumann et al. 1989; LaMotte 1992). Due to this feature, capsaicin is one of the most commonly used models in the study of hyperalgesia.

It is understood that capsaicin has a unique selectivity for unmyelinated C-fibres and thinly myelinated Aδ primary sensory neurones (Dray and Dickenson 1993). Topical application or
local injection of capsaicin into healthy human skin produces a concentration-dependent, burning sensation and flare response. For example, an intradermal injection of capsaicin (100 µg capsaicin in 10µl vehicle) can produce punctate hyperalgesia for up to 24 hours, and stroking hyperalgesia for up to 6 hours (LaMotte, Shain et al. 1991). Within the area of application, mechanical and thermal hyperalgesia (primary hyperalgesia) are present. Around the area of application there is a region of secondary hyperalgesia, in which enhanced sensitivity to mechanical stimulation such as pinprick, is present (mechanical hyperalgesia) (Campbell, Bevan et al. 1993). These features of capsaicin-induced hyperalgesia serve as a criteria for measuring the analgesic efficacy of various interventions (Bonica 1992), such as Ketamine, alfentanil (Park, Max et al. 1995; Sethna, Liu et al. 1998), gabapentin (Iannetti, Zambreanu et al. 2005), and complementary therapies including chiropractic adjustment (Mohammadian, Gonsalves et al. 2004).

Properties of both intradermal and topical application models have been investigated through numerous studies. Zheng et al. (2000) studied the time-course of capsaicin-induced hyperalgesia in different age groups and found that punctate hyperalgesia lasts for a longer duration in older people than in the young (Zheng, Gibson et al. 2000). A study regarding intradermal capsaicin model concluded that the reproducibility of this model over a period of time (at least one week interval) was acceptable for pharmacological profiling of novel antinociceptive agents (Hughes, Macleod et al. 2002). However, the reproducibility of topical capsaicin model has not been studied yet.
2.1.5.1.2 Mustard oil

Like capsaicin, topical application of mustard oil can produce burning pain as well as primary and secondary hyperalgesia (Reeh, Kocher et al. 1986; Koltzenburg, Lundberg et al. 1992), its sensory changes is comparable to capsaicin model but develop faster (Koltzenburg, Lundberg et al. 1992). It takes effect by stimulate CMHs and make them sensitized to heat but not to mechanical stimulation (Reeh, Kocher et al. 1986; Schmelz, Schmidt et al. 1996).
2.1.5.2 Thermal models

2.1.5.2.1 Cold injury model

Lewis (Lewis 1935) was the first to introduce cold injury to produce hyperalgesia. They used two different stimulations, namely, copper bar at -15°C and rod of compressed carbon dioxide (CO₂) snow. The onset time of hyperalgesia for these two methods is approximately 20 minutes and 20 hours, respectively.

In 1994, Kilo et al. described an injury model in which the skin is briefly frozen to -28°C to cause moderate pain. Itching or burning sensation lasts for about 2 hours after the inflicted injury. Heat pain threshold is lowered within the injury site; however no hyperalgesia presented in the first hour after injury. Sensory tests conducted 22 hours after the procedure of freezing found that punctate hyperalgesia, but not stroking hyperalgesia, developed around the injury site (area of secondary hyperalgesia), whilst pressure and impact hyperalgesia only presented in the area of primary hyperalgesia (Kilo, Schmelz et al. 1994).

As revealed by differential nerve blocks, punctate hyperalgesia in this model is mediated via C fibers or thinly myelinated A-delta fibers, and impact hyperalgesia is most likely to be mediated by sensitized C fibers. Pressure hyperalgesia may be due to the involvement of sensitised nociceptor units (Kilo, Schmelz et al. 1994).

2.1.5.2.2 Heat injury model

Two different methods have been used to produce heat injury that include radiant heat (Hardy, Wolff et al. 1950; Raja, Campbell et al. 1984) and contact heat (LaMotte, Shain et al. 1991; Dirks, Petersen et al. 2003).
The temperature of heat stimulation varies between studies. In one study, heat stimulus of 49-50 °C was applied for five to seven minutes. This caused blister and heat hyperalgesia within the injury site, which lasted for over 24 hours. Stroking and punctate hyperalgesia in the area of secondary hyperalgesia developed after the injury and lasted for up to 6 and 24 hours, respectively (Dahl, Brennum et al. 1993; Moiniche, Dahl et al. 1993). In another study, the skin was heated to 47 °C for seven minutes with a computer controlled thermode. Similar sensory changes were induced at 49 °C, however minimal blistering occurred in young volunteers (Petersen, Brennum et al. 1997). During this study, it was found that heating the skin to 45 °C for three minutes caused mild to no pain with no spontaneous pain persisting after removal of the heat source from the skin. Stoking and punctate hyperalgesia in this model lasted for only a few minutes after the termination of the heat stimulation (Brennum, Dahl et al. 1994; Dirks, Petersen et al. 2003).

It is generally understood that heat injury causes greater degree of hyperalgesia in hairy skin than in glabrous skin (LaMotte, Thalhammer et al. 1982). In hairy skin, suprathreshold heat stimuli activate CMH nociceptors (LaMotte, Thalhammer et al. 1982). AMHs nociceptors can only be activated by heat stimuli above 51 °C. It is believed that the heat alldynia by heat injury attributes to the lowering of activation threshold of CMH nociceptors, while heat hyperalgesia may be due to lowering of activation thresholds and increasing of suprathreshold response in both CMHs and AMHs nociceptors (LaMotte, Thalhammer et al. 1982; Brennum, Dahl et al. 1994). On the other hand, in the glabrous skin, heat hyperalgesia is caused by sensitisation of AMHs nociceptors (Handwerker and Reeh 1991).
2.1.6 Heat/capsaicin model

2.1.6.1 Introduction

The heat/capsaicin model is a newly developed model by Petersen et al. (Petersen and Rowbotham 1999). This model involves the use of two different nociceptive stimuli, that include topical capsaicin and heat, which produces a longer lasting and stable hyperalgesia model when compared to using either method individually (Dirks, Petersen et al. 2003).

When using the heat/capsaicin model, capsaicin cream of low concentration (0.075%) is applied topically to the medial aspect of the forearm for 30 minutes, immediately after heating the site to 45°C for five minutes using a thermode. The sensitised skin is rekindled at 40°C at 40-45 minute intervals, four to fives times during the procedure, to maintain the area of secondary hyperalgesia (Petersen and Rowbotham 1999; Dirks, Fabricius et al. 2000; Petersen, Maloney et al. 2003; Frymoyer, Rowbotham et al. 2007). The area of punctate hyperalgesia is better maintained throughout rekindling than that of stroking hyperalgesia (Petersen and Rowbotham 1999; Dirks, Petersen et al. 2003).

Rekindling is the key to marinating the area of secondary hyperalgesia. An area of secondary hyperalgesia will shrink markedly without the application of rekindling (Petersen and Rowbotham 1999; Dirks, Petersen et al. 2003). Previous studies have demonstrated that applied cooling will result in the secondary area to shrink, whereas reheating the sensitised skin will cause it to expand (LaMotte, Shain et al. 1991; Koltzenburg, Lundberg et al. 1992). Rekindling in combination with stimulation from capsaicin of a low concentration can
produce sufficient C-nociceptor input to partially counteract the natural reduction of the secondary hyperalgesia, thus achieving maintenance (Petersen and Rowbotham 1999).

There have been various studies performed to determine the reproducibility of this model. To determine the within-day reproducibility, coefficients of variance were calculated by using area of secondary hyperalgesia of first and third rekindling. The between-day reproducibility was determined by calculating the coefficient of variance with data from two separate study days. Results demonstrate that heat/capsaicin model has better within-day reproducibility (CV, 23%) on punctate hyperalgesia than either heat model (CV, 36%) or capsaicin model (CV, 36%) alone, but relatively poor within day reproducibility on stroking hyperalgesia than either of the stimulation alone. No significant difference was found in the size of area of punctate hyperalgesia between the two study days. However, it was determined that within-day reproducibility is higher than the between-day reproducibility (Dirks, Petersen et al. 2003).

Gender differences of the heat/capsaicin model have also been explored. Studies have shown that the area of punctate hyperalgesia is the same in both genders, while area of stroking hyperalgesia is larger in females than in males. No gender difference was found to exist in pain rating and heat pain thresholds. Overall, there are minimal gender differences in the heat/capsaicin model (Jensen and Petersen 2006).
2.1.6.2 Measurements

Both mechanical and heat hyperalgesia can be assessed in this heat/capsaicin model. Area of secondary hyperalgesia was measured with both von Frey filaments and foam paintbrush along four linear paths arranged vertically and horizontally around the stimulation site (Petersen, Maloney et al. 2003). The heat pain detection threshold was measured using a computer-controlled thermode, whereby subjects indicate the threshold by pressing a mouse button (Mathiesen, Imbimbo et al. 2006). Subjects were also asked to rate pain sensation during the rekindling procedure. The rating was then continuously recorded with an electronic VAS (Dirks, Fabricius et al. 2000).

2.1.6.3 Application in the clinical trials for analgesics

Since 1999, the heat/capsaicin model has been used in 10 pharmaceutics studies (Table 2.1). The measurements include the area of punctate or stroking hyperalgesia, allodynia, heat pain thresholds, VAS rating to the rekindling (40 °C, 1 minute) and pain rating to long thermal stimulation (45 °C, 1 minute) (Petersen and Rowbotham 1999; Dirks, Petersen et al. 2003). Anti-hyperalgesia effects of various medications have been evaluated using these measurements.

Table 2.1 (below) summarises the pharmaceutical studies that have used this model. Several types of analgesic medications were used including opioid, NMDA receptor antagonist, local anesthetics, anticonvulsants, COX-2 inhibitor, and others agents. Three studies showed that opioids, such as Remifentanil, Hydromorphone and morphine reduced both mechanical and heat hyperalgesia (Petersen, Jones et al. 2001; Duedahl, Dirks et al. 2005; Frymoyer,
Rowbotham et al. 2007). These results indicate their effect on both peripheral and central sensitisation. NMDA receptor antagonists, such as dextromethorphan and CHF3381, only reduced punctuate hyperalgesia but not heat hyperalgesia (Duedahl, Dirks et al. 2005; Mathiesen, Imbimbo et al. 2006), indicating their specific anti-central sensitisation effect.

There was only one study that tested systemic lignocaine, a local anaesthetic agent. Results found that it reduced stroking hyperalgesia but not punctuate or heat hyperalgesia (Dirks, Fabricius et al. 2000). The effect of anti-hyperalgesia of anticonvulsants was not consistent amongst the studies. For example, oral gabapentin reduced both mechanical (stroking and punctuate hyperalgesia) and heat hyperalgesia (Dirks, Petersen et al. 2002), whereas oral lamotrigine had no effect over these two types of hyperalgesia (Petersen, Maloney et al. 2003). Other medications such as valdecoxib (COX-2 inhibitor) (Burns, Hill et al. 2006), adenosine (Dirks, Petersen et al. 2001) and magnesium (Mikkelsen, Dirks et al. 2001), although having NMDA receptor antagonist-like effect (Poleszak, Wlaz et al. 2007), showed no anti-hyperalgesia function.

In brief, this model provides a non-invasive, non-injury yet reliable and reproducible way to evaluate the efficacy of analgesic interventions. It is also possible to use this model to test the specific anti-central or peripheral sensitization effects of medications and interventions.
### Table 2.1 Summary of the studies using heat/capsaicin model for testing anti-hyperalgesia effects of analgesics

<table>
<thead>
<tr>
<th>Paper No.</th>
<th>Design</th>
<th>Types of medications</th>
<th>Intervention</th>
<th>Medication time point</th>
<th>Subject No. (total)</th>
<th>Measurement</th>
<th>Result (compared to control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Dirks, Fabricius et al. 2000).</td>
<td>double blinded, randomised order of infusion, crossover, placebo controlled.</td>
<td>local anaesthetic</td>
<td>Systemic lidocaine (bolus 2 mg/kg over 10 minutes, followed by infusion 3 mg/kg/h for 75 minutes)</td>
<td>immediately after the heat/capsaicin model</td>
<td>24</td>
<td>Area of stroking hyperalgesia</td>
<td>Reduced</td>
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<td>Area of punctate hyperalgesia</td>
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<td>Heat pain detection thresholds</td>
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<td>Pain rating to long thermal stimulation (45°C)</td>
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<td>Pain rating to rekindling (40°C),</td>
<td>No effect</td>
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<tr>
<td>2 (Dirks, Petersen et al. 2001)</td>
<td>double blinded, randomised, crossover, placebo controlled</td>
<td>Antiarrhythmic agents, has anti-central sensitisation effect</td>
<td>Systemic adenosine (intravenous 60 µg/kg/min for 85 minutes)</td>
<td>immediately after the heat/capsaicin model</td>
<td>25</td>
<td>Area of stroking hyperalgesia</td>
<td>No effect</td>
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<td>Pain rating to rekindling (40°C),</td>
<td>No effect</td>
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<tr>
<td>3. (Mikkelsen, Dirks et al. 2001)</td>
<td>Double blinded, randomised, crossover, placebo controlled</td>
<td>Has NMDA antagonist like effect</td>
<td>Intravenous magnesium (bolus 2 mg/kg over 10 minutes, followed by infusion)</td>
<td>immediately after the heat/capsaicin model</td>
<td>25</td>
<td>Area of stroking hyperalgesia</td>
<td>No effect</td>
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<td>Pain rating to long</td>
<td>Increased</td>
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<tr>
<td>Study (Year)</td>
<td>Design</td>
<td>Intervention</td>
<td>Method</td>
<td>Outcome</td>
<td>Results</td>
<td></td>
<td></td>
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<tr>
<td>-------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4. (Petersen, Jones et al. 2001)</td>
<td>Double blinded, randomised, crossover, placebo controlled</td>
<td>Opioid medication</td>
<td>Intravenous Remifentanil (0.05 mg/kg/ min for 5 minutes, followed by 0.1 mg/ kg/ min for 35 minutes)</td>
<td>Immediately after the heat/capsaicin model</td>
<td>Pain rating to long thermal stimulation (45°C), No effect</td>
<td>Area of stroking hyperalgesia, Reduced</td>
<td>Area of punctate hyperalgesia, Reduced</td>
</tr>
<tr>
<td>5. (Dirks, Petersen et al. 2002)</td>
<td>Double blinded, randomised, crossover, placebo controlled</td>
<td>Anticonvulsant</td>
<td>Oral gabapentin (1,200 mg)</td>
<td>Immediately after the heat/capsaicin model</td>
<td>25</td>
<td>Area of stroking hyperalgesia, Reduced</td>
<td>Area of punctate hyperalgesia, Reduced</td>
</tr>
<tr>
<td>6. (Petersen, Maloney et al. 2003)</td>
<td>Double blinded, randomised, crossover, placebo controlled</td>
<td>Anticonvulsants</td>
<td>Oral Lamotrigine (400 mg), 90 minute before and immediately after the heat/capsaicin model</td>
<td>Area of stroking hyperalgesia, No effect</td>
<td>Area of punctate hyperalgesia, No effect</td>
<td>Pain rating to long thermal stimulation (45°C), No effect</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Opioid</td>
<td>Oral hydromorphone (8 mg), immediately after the heat/capsaic</td>
<td></td>
<td>Area of stroking hyperalgesia, Reduced</td>
<td>Area of punctate Reduced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study (Author et al.)</td>
<td>Intervention</td>
<td>NMDA receptor Antagonist</td>
<td>COX-2 Inhibitor</td>
<td>Oral Agent</td>
<td>NMDA receptor</td>
<td>COX-2 Inhibitor</td>
<td>Area of Punctate Hyperalgesia</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------</td>
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<td>-------------</td>
<td>----------------</td>
<td>----------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>7. (Duedahl, Dirks et al. 2005)</td>
<td>Opioid</td>
<td>Remifentanil (0.05 mg/kg/min for 10 minutes, followed by 0.1 mg/kg/min for 25 minutes)</td>
<td>NMDA receptor antagonist</td>
<td>Intravenous dextromethorphan, 0.5 mg/kg,</td>
<td>Intravenous dextromethorphan, 0.5 mg/kg,</td>
<td>Area of Punctate Hyperalgesia</td>
<td>Pain Rating to Long Thermal Stimulation (45°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Immediately after the heat/capsaicin model</td>
<td>24</td>
<td>Reduced</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Heat Pain Detection Thresholds</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>8. (Burns, Hill et al. 2006)</td>
<td>COX-2 Inhibitor</td>
<td>Oral valdecoxib (40 mg)</td>
<td>COX-2 Inhibitor</td>
<td>Oral valdecoxib (40 mg)</td>
<td>Oral valdecoxib (40 mg)</td>
<td>Area of Punctate Hyperalgesia</td>
<td>Pain Rating to Long Thermal Stimulation (45°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 hours before the heat/capsaicin model</td>
<td>20</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hot/Cold Pain Threshold</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>9. (Mathiesen, Randomised)</td>
<td>NMDA receptor</td>
<td>Oral CHF3381</td>
<td>NMDA receptor</td>
<td>Oral CHF3381</td>
<td>Oral CHF3381</td>
<td>Area of Punctate Hyperalgesia</td>
<td>Pain Rating to Long Thermal Stimulation (45°C)</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Intervention</td>
<td>Measures</td>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------</td>
<td>-----------------------------</td>
<td>-------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>---------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imbimbo et al. 2006</td>
<td>double-blind, crossover, placebo controlled</td>
<td>antagonist (500 mg), oral gabapentin (1,200 mg), after first rekindle</td>
<td>Heat pain detection threshold, pain rating to long thermal stimulation (45°C)</td>
<td>No effect, Reduced</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. (Frymoyer, Rowbotham et al. 2007)</td>
<td>randomize double-blind, placebo-controlled, crossover</td>
<td>NMDA receptor antagonist and opioid medication</td>
<td>Oral dextromethorphan (30 mg) and morphine (30 mg) combination immediately after the heat/capsaicin model</td>
<td>Area of stroking hyperalgesia, area of punctate hyperalgesia, pain rating of long thermal stimulation (45°C)</td>
<td>Reduced, Reduced, Reduced</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.2 Acupuncture and hyperalgesia

2.2.1 Introduction

Previous acupuncture studies have mainly focused on pain threshold, which has limited clinical significance. Central sensitisation is an important characteristic of clinical pain however, cannot be tested using the non-injury pain model. To solve this problem, hyperalgesia animal models were introduced into acupuncture studies. Models such as Complete Freud’s Adjuvant (CFA), Chronic Constrictive Injury (CCI) and Irritable Bowel Syndrome (IBS) mimic painful, clinical conditions for a period of time, often lasting several days to months. These models can provide researchers with the opportunity to test medications and other interventions in a more clinically orientated setting, therefore producing more clinically significant results.

There are two major types of animal models used in acupuncture studies: inflammatory and neuropathic models, each with different neurological mechanisms and clinical significance. Inflammation models, including those induced by CFA, carrageenan and formalin, mimic inflammatory pain conditions, such as arthritic pain.

Neuropathic models, including CCI, Spinal Nerve Ligation (SNL) and Inferior Caudal Trunk Resection (ICTR) models, aim to reproduce clinical neuropathic pain conditions, such as sciatica and trigeminal neuralgia. Several other models have also been used, including a cancer pain model that reproduces pain symptoms caused by cancer, and an incision model to replicate post-operative pain, and IBS model that mimics the irritable bowel syndrome.
Most of these models produce thermal and mechanical hyperalgesia, which are cardinal features of human hyperalgesia models and clinical pain conditions. Therefore, by measuring hyperalgesia, it is possible to assess the analgesic efficacy of novel interventions.

2.2.2 Aim

The aims of the review were to:

1) Identify the animal models used,

2) Assess the effect of acupuncture on hyperalgesia in animal pain models,

3) Identify the optimal acupuncture parameters for anti-hyperalgesia effect

2.2.3 Searching strategy

“Acupuncture AND hyperalgesia” or “acupuncture AND allodynia” or “electroacupuncture AND hyperalgesia” or “electroacupuncture AND allodynia” in text word were terms used to search the major databases: PubMed, Proquest and CINHAL. There were no limits placed on publication date, subjects or publication types. Studies published up to October 2007 were included.

Studies that tested the effect of acupuncture on hyperalgesia, such as paw withdrawal latency to thermal or mechanical stimuli, were included. Included studies must have had at least one control group. Studies on animals or humans were included. Acupuncture in the studies was required to be invasive, with no drugs injected into the acupoint. Case studies, review and studies that did not include a valid acupuncture group were excluded.
As shown in Figure 2.3, the search generated 55 papers, of which 32 were relevant. All of these studies were animal (rat) studies and used electroacupuncture. Twenty-three papers were excluded according to selection criteria: nine of these did not assess hyperalgesia, five did not use acupuncture, five used bee venom and laser acupuncture, three were case studies and one was a review. Included and excluded studies are listed in Tables 2.2 and 2.3, respectively.

Figure 2.3  A flowchart illustrating the process of study selection
Table 2.2  Titles of the included studies

<table>
<thead>
<tr>
<th>Paper No.</th>
<th>Title and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Electroacupuncture combined with indomethacin enhances antihyperalgesia in inflammatory rats (Zhang, Lao et al. 2004)</td>
</tr>
<tr>
<td>2</td>
<td>Excitatory amino acid receptor antagonists and electroacupuncture synergistically inhibit carrageenan-induced behavioral hyperalgesia and spinal Fos expression in rats (Zhang, Ji et al. 2002)</td>
</tr>
<tr>
<td>3</td>
<td>Electro-acupuncture attenuates behavioral hyperalgesia and selectively reduces spinal Fos protein expression in rats with persistent inflammation (Lao, Zhang et al. 2001)</td>
</tr>
<tr>
<td>4</td>
<td>Involvement of opioid receptors in electroacupuncture-produced anti-hyperalgesia in rats with peripheral inflammation (Zhang, Lao et al. 2004)</td>
</tr>
<tr>
<td>5</td>
<td>Electro-acupuncture relieves chronic visceral hyperalgesia in rats (Cui, Li et al. 2005)</td>
</tr>
<tr>
<td>6</td>
<td>Ketamine potentiates the effect of electroacupuncture on mechanical allodynia in a rat model of neuropathic pain (Huang, Li et al. 2004)</td>
</tr>
<tr>
<td>7</td>
<td>A parametric study of electroacupuncture on persistent hyperalgesia and Fos protein expression in rats (Lao, Zhang et al. 2004)</td>
</tr>
<tr>
<td>8</td>
<td>Attenuation of mechanical but not thermal hyperalgesia by electroacupuncture with the involvement of opioids in rat model of chronic inflammatory pain (Huang, Hu et al. 2004)</td>
</tr>
<tr>
<td>9</td>
<td>A minimal stress model for the assessment of electroacupuncture analgesia in rats under halothane (Wen, Yeh et al. 2007)</td>
</tr>
<tr>
<td>10</td>
<td>Anti-hyperalgesic effect of electroacupuncture in a model of post-incisional pain in rats (Oliveira and Prado 2000)</td>
</tr>
<tr>
<td>11</td>
<td>Corticosterone mediates electroacupuncture-produced anti-edema in a rat model of inflammation (Li, Zhang et al. 2007)</td>
</tr>
<tr>
<td>12</td>
<td>Corticotropin-releasing factor and interleukin-1β are involved in the electroacupuncture-induced analgesic effect on inflammatory pain elicited by carrageenan (Sekido, Ishimaru et al. 2004)</td>
</tr>
<tr>
<td>13</td>
<td>Differences of electroacupuncture-induced analgesic effect in normal and inflammatory conditions in rats (Sekido, Ishimaru et al. 2003)</td>
</tr>
<tr>
<td>14</td>
<td>Disruption of glial function enhances electroacupuncture analgesia in arthritic rats (Sun, Chen et al. 2006)</td>
</tr>
<tr>
<td>15</td>
<td>Down-regulation of GFRα-1 expression by antisense oligodeoxynucleotide attenuates electroacupuncture analgesia on heat hyperalgesia in a rat model of neuropathic pain (Dong, Ma et al. 2006)</td>
</tr>
<tr>
<td>16</td>
<td>Effects of electroacupuncture on the mechanical allodynia in the rat model of neuropathic pain (Hwang, Min et al. 2002)</td>
</tr>
<tr>
<td>17</td>
<td>Effects of pertussis toxin on electroacupuncture-produced anti-hyperalgesia in inflamed rats (Liu, Zhang et al. 2005)</td>
</tr>
<tr>
<td>18</td>
<td>Electroacupuncture (EA) modulates the expression of NMDA receptors in primary sensory neurons in relation to hyperalgesia in rats (Wang, Zhang et al. 2006)</td>
</tr>
<tr>
<td>19</td>
<td>Electro-acupuncture attenuates stress-induced defecation in rats with chronic visceral hypersensitivity via serotonergic pathway (Tian, Bian et al. 2006)</td>
</tr>
<tr>
<td>20</td>
<td>Electroacupuncture combined with MK-801 prolongs anti-hyperalgesia in rats with peripheral inflammation (Zhang, Wang et al. 2005)</td>
</tr>
</tbody>
</table>
| 21        | Electroacupuncture inhibits inflammatory edema and hyperalgesia through regulation of cyclooxygenase synthesis in both peripheral and central nociceptive
<table>
<thead>
<tr>
<th>No.</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Electroacupuncture suppresses spinal expression of neurokinin-1 receptors induced by persistent inflammation in rats (Zhang, Liu et al. 2005)</td>
</tr>
<tr>
<td>25</td>
<td>Involvement of nociceptin/orphanin FQ and its receptor in electroacupuncture-produced anti-hyperalgesia in rats with peripheral inflammation (Fu, Wang et al. 2006)</td>
</tr>
<tr>
<td>26</td>
<td>Is functional state of spinal microglia involved in the anti-allodynic and anti-hyperalgesic effects of electroacupuncture in rat model of monoarthritis? (Shan, Qi-Liang et al. 2007)</td>
</tr>
<tr>
<td>27</td>
<td>Kynurenic acid enhances electroacupuncture analgesia in normal and carrageenan-injected rats (Zhang, Ji et al. 2003)</td>
</tr>
<tr>
<td>28</td>
<td>Mu opioid receptor-containing neurons mediate electroacupuncture-produced anti-hyperalgesia in rats with hind paw inflammation (Zhang, Wang et al. 2005)</td>
</tr>
<tr>
<td>29</td>
<td>Stage-dependent analgesia of electro-acupuncture in a mouse model of cutaneous cancer pain (Mao-Ying, Cui et al. 2006)</td>
</tr>
<tr>
<td>30</td>
<td>Involvement of peripheral opioid mechanisms in electroacupuncture analgesia (Zhang, Yu et al. 2005)</td>
</tr>
<tr>
<td>31</td>
<td>The anti-inflammatory effects of 2 Hz electroacupuncture with different intensities on acute carrageenan-induced inflammation in the rat paw (Lee, Choi et al. 2005)</td>
</tr>
<tr>
<td>32</td>
<td>Effects of electroacupuncture on the pain threshold and NMDA R1 mRNA in DRG on neuropathic pain rats (Chen, Yang et al. 2003)</td>
</tr>
<tr>
<td>34</td>
<td>Effect of electroacupuncture on GDNF positive cell immunoreactivity in local dermal tissue of the inflammatory pain focus in the rat of adjuvant arthritis (Liu, Li et al. 2006=)</td>
</tr>
<tr>
<td>35</td>
<td>Effect of varying frequency and duration of electroacupuncture stimulation on carrageenan-induced hyperalgesia (Taguchi and Taguchi 2007)</td>
</tr>
</tbody>
</table>

Note: Paper No. 22, 24, 33 were excluded
Table 2.3  Excluded studies and reasons for exclusion

<table>
<thead>
<tr>
<th>Excluded paper</th>
<th>Reason for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Dai and Xu 1993)</td>
<td>Hyperalgesia not tested</td>
</tr>
<tr>
<td>(Hamba 1988)</td>
<td>Hyperalgesia not tested</td>
</tr>
<tr>
<td>(Kong, Fufa et al. 2005)</td>
<td>Hyperalgesia not tested</td>
</tr>
<tr>
<td>(Dickhaus, Pauser et al. 1978)</td>
<td>Hyperalgesia not tested</td>
</tr>
<tr>
<td>(Clark, Yang et al. 1986)</td>
<td>Hyperalgesia not tested</td>
</tr>
<tr>
<td>(Raevskaia 1992)</td>
<td>Hyperalgesia not tested</td>
</tr>
<tr>
<td>(Omana, Olvera et al. 1994)</td>
<td>Hyperalgesia not tested</td>
</tr>
<tr>
<td>(Wang, Zhu et al. 1998)</td>
<td>Hyperalgesia not tested</td>
</tr>
<tr>
<td>(Cha, Choi et al. 2006)</td>
<td>Hyperalgesia not tested</td>
</tr>
<tr>
<td>(Gracely, Dubner et al. 1983)</td>
<td>Acupuncture not used</td>
</tr>
<tr>
<td>(Liu, Li et al. 2006)</td>
<td>Acupuncture not used</td>
</tr>
<tr>
<td>(Fu, Zhu et al. 2007)</td>
<td>Acupuncture not used</td>
</tr>
<tr>
<td>(Ma, Xie et al. 2003)</td>
<td>Acupuncture not used</td>
</tr>
<tr>
<td>(Dong, Wang et al. 2006)</td>
<td>Acupuncture not used</td>
</tr>
<tr>
<td>(Kwon, Lee et al. 2001)</td>
<td>Bee venom</td>
</tr>
<tr>
<td>(Kwon, Lee et al. 2002)</td>
<td>Bee venom</td>
</tr>
<tr>
<td>(Roh, Kwon et al. 2004)</td>
<td>Bee venom</td>
</tr>
<tr>
<td>(Giuliani, Fernandez et al. 2004)</td>
<td>Laser acupuncture</td>
</tr>
<tr>
<td>(Zhu, Li et al. 1990)</td>
<td>Laser acupuncture</td>
</tr>
<tr>
<td>(Zanon, Garetto et al. 2004)</td>
<td>Case study</td>
</tr>
<tr>
<td>(Biedermann, Lapeer et al. 1986)</td>
<td>Case study</td>
</tr>
<tr>
<td>(Luo 1996)</td>
<td>Case study</td>
</tr>
<tr>
<td>(Vinik 2005)</td>
<td>Review</td>
</tr>
</tbody>
</table>
The design of the study is summarised in Table 2.4. Listed are the sham control, randomisation and blinding details of the included studies.

Table 2.4 Summary of study design

<table>
<thead>
<tr>
<th>Design</th>
<th>Paper No.</th>
<th>Number of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham acupuncture was used</td>
<td>1, 2, 3, 4, 5, 7, 8, 10, 11, 14, 16, 17, 18, 20, 23, 25, 26, 27, 28, 29, 30, 34, 35</td>
<td>23</td>
</tr>
<tr>
<td>Randomisation was used</td>
<td>3, 4, 6, 7, 8, 15, 17, 18, 20, 23, 25, 28, 29, 30, 34</td>
<td>15</td>
</tr>
<tr>
<td>Blinding (assessor)</td>
<td>1, 3, 4, 5, 7, 14, 15, 17, 19, 20, 23, 26, 27, 28</td>
<td>14</td>
</tr>
<tr>
<td>Sham, randomisation and blinding</td>
<td>3, 7, 17, 20, 23, 28</td>
<td>6</td>
</tr>
</tbody>
</table>

2.2.4 Animal models

Animal pain models used in acupuncture studies are listed in Table 2.5, which include inflammatory, neuropathic and cancer pain models and visceral hyperalgesia (Dai, Kondo et al. 2001; Hwang, Min et al. 2002; Huang, Hu et al. 2004; Sekido, Ishimaru et al. 2004; Lee, Choi et al. 2005). The most commonly used models were for inflammation, such as CFA and carrageenan models. Other models including neuropathic, IBS and cancer models are not often used.
Table 2.5  Types of animal models used in acupuncture studies

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>No. of study</th>
<th>Outcome measures</th>
<th>Usage No.</th>
<th>Injury site</th>
<th>Usage No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>CFA</td>
<td>16</td>
<td>PWL to radiant heat</td>
<td>14</td>
<td>Plantar surface of one hind paw</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Response Latency to hot plate test</td>
<td>1</td>
<td>Left tibio-tarsal joint</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mechanical withdraw threshold to von Frey filaments</td>
<td>3</td>
<td>Unilateral ankle articular cavity</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Foot-bend score</td>
<td>2</td>
<td>Left malleolus articular cavity</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Carrageenan</td>
<td>7</td>
<td>PWL to radiant heat</td>
<td>2</td>
<td>Plantar surface of one hind paw</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Response latency to hot plate test</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PPT using analgesy-meter</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>formalin</td>
<td>1</td>
<td>Weighted pain score (behavioral hyperalgesia)</td>
<td>1</td>
<td>Plantar surface of left hind paw</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>incision</td>
<td>1</td>
<td>Mechanical withdraw threshold to von Frey filaments</td>
<td>1</td>
<td>1cm longitudinal, through skin and fascia of the plantar region of heel</td>
<td>1</td>
</tr>
<tr>
<td>Neuropathic</td>
<td>CCI</td>
<td>2</td>
<td>PWL to radiant heat</td>
<td>2</td>
<td>Left sciatic nerve</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SNL</td>
<td>1</td>
<td>Mechanical withdraw threshold to von Frey filaments</td>
<td>1</td>
<td>L5-L6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>ICTR</td>
<td>1</td>
<td>Mechanical alldynia to von Frey filaments</td>
<td>1</td>
<td>Between S3 and S4 of right superior caudal trunk</td>
<td>1</td>
</tr>
<tr>
<td>Visceral Hyperalgesia</td>
<td>IBS</td>
<td>2</td>
<td>AWR to CRD</td>
<td>2</td>
<td>Colorectal</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EMG to CRD</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PTP to CRD</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fecal pellet output to WAS</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer pain</td>
<td>Cutaneous cancer pain</td>
<td>1</td>
<td>Response latency to hot plate test</td>
<td>1</td>
<td>Plantar region of the unilateral hind paw</td>
<td>1</td>
</tr>
</tbody>
</table>
2.2.4.1 Inflammation models

Three different chemicals have been used to produce the inflammation model. These include CFA, carrageenan and formalin models. All three are used to model human arthritis in studies to evaluate efficacy of various anti-inflammation medications.

Unlike an acute pain model, which uses heat or electrical stimulation to create transient noxious stimuli in normal animals, these inflammation models utilise chemical agents to produce a persistent, local inflammation state, lasting for days to weeks, thus mimicking sub-acute and chronic pain conditions. These models not only produce local tissue inflammation but also central sensitisation, which is an important feature of chronic pain.

2.2.4.1.1 CFA

CFA model is the most frequently used model in acupuncture studies and appeared in 16 out of 32 included papers. The Complete Freund’s adjuvant (CFA), which is named after an American immunologist, Jules T. Freund (1890-1960), was originally used as immunopotentiator for boosting immune system. It is made by emulsifying an inactivated pathogenic agent of tuberculosis (mycobacterium tuberculosis) in mineral oil.

When injected subcutaneously or intraperitoneally, CFA induces painful reactions in animals (Zhang, R. Lao et al. 2002) (Zhang, Ji et al. 2002; Zhang, Lao et al. 2004). However, due to its severe toxicity, it is forbidden to be used in human subjects. To produce the hyperalgesia state in a rat, a small amounts of CFA solution (0.05 or 0.08ml, suspended in 1:1 oil/saline emulsion, containing 40 or 50 µg Mycobacterium tuberculosis) is
subcutaneously injected into the plantar surface of one hind paw, or injected into the joint
cavity (Sun, Chen et al. 2006), using a 25-gauge hypodermal needle. In some studies, before
the injection, the animals were briefly anaesthetised with chlorohydrate (Huang, Hu et al.
2004) or isoflurane (Fu, Wang et al. 2006; Sun, Chen et al. 2006).

Inflammation induced by the local injection of CFA manifests as redness, edema and
hyperalgesia of the local tissue. These conditions become evident within 2 hours after the
injection and maximize during 6 to 24 hours. Manifestations then generally disappear after
2 weeks.

Outcome measures of the CFA model are the Paw Withdrawal Latency (PWL), Response
Latency (hot plate test) and mechanical withdrawal threshold using von Frey filaments and
Foot-bend score.

2.2.4.1.2 Carrageenan

Another commonly used inflammation model is carrageenan model. Carrageenan, a
polysaccharide extracted from seaweed, has been used as food additive for centuries. When
injected subcutaneously, it can cause local swelling and pain, thus being used to produce
animal models of inflammation.

Carrageenan-produced hyperalgesia is of a similar magnitude to CFA. However, its
duration is much shorter. The inflammatory hyperalgesia appears within 1 hour and lasts for
about 24 hours (Ren and Dubner 1999).
The carrageenan model is produced by subcutaneously injecting 0.1 ml of carrageenan solution into the plantar surface of the hind paw, using a 26-gauge needle. In some studies, rats are anaesthetised during administration of the injection (Sekido, Ishimaru et al. 2003; Sekido, Ishimaru et al. 2004; Taguchi and Taguchi 2007). Measurements used in this include PWL, mechanical withdrawal threshold using Analgesy-meter and Response Latency to hot plate tests.

2.2.4.1.3 Formalin

The formalin model was firstly used in cats by Frankstein and later modified by O’Keefe (Dubuisson and Dennis 1977). This model is produced by intraplantar injection of 50 µl diluted formalin (5%) via a 26-gauge needle. The injection produces a distinct biphasic behavioural changes and heightened activity of dorsal horn nociceptive neurons (Hogan 2002).

Compared with CFA and carrageenan models, formalin model is short lived for one hour only. However, it has two distinct behavior phases with different underlying neurological mechanisms. The first phase is observed during the initial 5 minutes after injection whereby, the rats lift their injected paw to prevent it from touching the surface of the cage. During the following phase, which last for about 40 minutes, rats began to use the paw to bear weight, however constantly shaking and licking. Only behavioral hyperalgesia is feasible to be measured in this model using weighted pain score. Mechanical/heat hyperalgesia or allodynia are not usually tested.
2.2.4.2 Neuropathic pain model

There are three types of neuropathic pain models used in the selected studies: Chronic Constrictive Injury (CCI) model, Spinal Nerve Ligation (SNL) model and Inferior Caudal Trunk Resection (ICTR) model.

2.2.4.2.1 Chronic Constrictive Injury (CCI) model

Two studies used the model of Chronic Constrictive Injury (CCI) to evaluate the effect of EA. This method is described by Bennett and Xie (Bennett and Xie 1988). Essentially, the sciatic nerve is exposed at the level of middle of the thigh via blunt dissection through biceps femoris. The sciatic nerve is loosely tied with four 4-0 chromic gut sutures. Hyperalgesia to noxious radiant heat stimulation on the injured limb appears on the second day after the operation and last for approximately two months. Allodynia, and possibly, spontaneous pain also present. However, due to the limitation of this model it is difficult to properly measure the spontaneous pain based on the behavioral observation (Bennett and Xie 1988). It is understood that hyperalgesia and allodynia peak around 10 to 14 days after operation (Hogan 2002). However, the tightness of the ligature, the operator factor and the degree of the injury of the fascicles in the same nerve are the major variants influencing the consistency of this model (Hogan 2002). Measurement used on this model includes PWL.

2.2.4.2.2 Spinal Nerve Ligation (SNL) model

One study used this model following the procedure described by Kim and Chung (Kim and Chung 1992). Rats are anesthetised with 10% chlorohydrate and the dorsal vertebral columns of Lumbar 5 (L5) to L6 spinal nerves are exposed. Tight ligation of these nerves is
made distally to the dorsal root ganglion with 4-0 silk sutures. Animals are then allowed to recover for 5 days. Hyperalgesia and allodynia develop in the affected hind foot shortly after the ligation and last for about 4 months. Behavioral signs of spontaneous pain are also present (Wang and Wang 2003).

Compared to the CCI model, this model achieves better consistency by choosing a relatively fixed location and applying complete ligation. However, the model does require more extensive surgical procedures (Wang and Wang 2003). The measurement used includes mechanical withdrawal threshold.

**2.2.4.2.3 ICTR (inferior caudal trunk resection) model**

This model is described by Na and colleagues et al (Na, Han et al. 1994). Under sodium pentobarbital anaesthesia, the right superior caudal trunk is transected between the Sacrum 3 (S3) and S4 spinal nerves, which innervate the tail. This model produces cold or thermal hyperalgesia and mechanical allodynia in the tail within a day after operation. These changes can last for several weeks.

In this model, thermal and mechanical stimulation can be easily applied to the tail, and blind behaviour assessment is possible due to a lack of deformity of the tail by the surgery. Measurement used in this model includes tail response frequency.
2.2.4.3 Other models

2.2.4.3.1 Irritable Bowel Syndrome (IBS) model

The Irritable Bowel Syndrome (IBS) model was first developed by Al-chaer et al. in 2000 (Al-Chaer, Kawasaki et al. 2000). This model mimics IBS symptoms in humans by applying repetitive colorectal distention (CRD) in male neonatal Sprague-Dawley rats. When these rats become adults, they develop chronic visceral hypersensitivity, which is characterised by hyperalgesia and allodynia to CRD in abdominal and colorectal areas.

In this model (Cui, Li et al. 2005; Tian, Bian et al. 2006), neonatal rats (8 days old) receive mechanical colon distention on a daily basis until they are 21 days old. An inflatable silica balloon (length 20.0 mm; diameter 3.0/2.0 mm) is inserted into the descending colon via the rectum. The balloon is distended with 2 ml air, exerting 60 mm Hg pressure on the colon, as measured by a sphygmomanometer. After one minute duration, the balloon is deflated and withdrawn from the colon. The stimulation is repeated twice, at 30 minutes intervals within one hour. After the colon distention treatment, the rat is fed until adulthood, which is generally six weeks old.

Measurements used to test this model include an Abdominal Withdrawal Reflex (AWR) score, Pain Threshold Pressure (PTP), Electromyogram (EMG) and Water Avoidance Stress (WAS) test.
2.2.4.3.2 Incision model

Brennan et al. (1996), developed the paw incision model in 1996 (Brennan, Vandermeulen et al. 1996) In this model, under halothane anaesthesia, a 1-cm cut is made through the skin and fascia of the plantar region using a surgical blade. The incision starts 0.5 cm from the proximal edge of the heel. The plantaris is elevated with its origin and insertion left intact. The skin is stitched with two 5-0 nylon sutures after hemostasis. The animal is then placed in the cage for recovery.

This model produces reliable and quantifiable mechanical hyperalgesia around the wound for several days. The measurement used is mechanical withdrawal threshold to von Frey filaments.

2.2.4.3.3 Cancer pain model

This model is recently developed by Sasamura et al. (Sasamura, Nakamura et al. 2002). The B16-BL6 melanoma cells are inoculated into plantar surface of the hind paw, and moderate hyperalgesia appears on day 7 to 10 and becomes marked at day 14 after inoculation. Measurements used include PWL and hot plate test.

2.2.5 Measurements

2.2.5.1 Measurement of spontaneous pain

Spontaneous pain is one of the cardinal features of chronic pain, and is likely to be present in some pain models such as CCI model (Bennett and Xie 1988). For example, in the CCI model, researchers assumed the behavior of sudden lifting the affected hind paw and
protecting it can be a sign of spontaneous pain. However, it can also be a result from some undetected simulation like a slight shift of weight (Bennett and Xie 1988). In principle, researchers have to rely on indirect observation of the animal behaviors such as autotomy (gnaw claw tips) and sudden lifting and holding the injured hind paw in guarding position, to infer the existence of spontaneous pain, which can be imprecise and sometimes inappropriate. Therefore, the reliability of current measurements of spontaneous pain, which heavily rely on observation of the animal behavior, is limited.

It is interesting to note that none of the selected acupuncture studies in this review reported or measured spontaneous pain.

2.2.5.2 Measurements of hyperalgesia

Table 2.6 summarises the measurements used in different animal models and their equivalence in human.

In human studies, hyperalgesia is always classified as primary hyperalgesia (occurring at the site of injury and characterised by hyperalgesia by both thermal and mechanical stimuli) and secondary hyperalgesia (around the injury site, characterised by hyperalgesia to mechanical stimuli). While in animal studies, hyperalgesia state is usually defined according to the behavioral response to different types of stimuli (thermal or mechanical). Researchers use PWL as the test for thermal hyperalgesia, and algesiometry as the test for mechanical hyperalgesia. Therefore, the thermal hyperalgesia in inflammation animal model is possibly equivalent to primary hyperalgesia in human model, and mechanical
hyperalgesia is equivalent to secondary hyperalgesia. For the neuropathic model, the test site is remote from the injury site, thus both thermal and mechanical hyperalgesia can be understood as being equivalent to secondary hyperalgesia in human.
Table 2.6  Tests used in different animal model

<table>
<thead>
<tr>
<th>Test</th>
<th>Types of measurements</th>
<th>Human equivalence</th>
<th>Model</th>
<th>Model site</th>
<th>Test site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paw withdrawal latency</td>
<td>Thermal hyperalgesia</td>
<td>Primary hyperalgesia</td>
<td>CFA</td>
<td>Plantar surface of hind paw</td>
<td>Plantar surface of hind paw</td>
</tr>
<tr>
<td></td>
<td>Thermal hyperalgesia</td>
<td>Primary hyperalgesia</td>
<td></td>
<td>Articular cavity between the tibiofibular and tarsus bone</td>
<td>Plantar surface of hind paw</td>
</tr>
<tr>
<td></td>
<td>Thermal hyperalgesia</td>
<td>Primary hyperalgesia</td>
<td>CAR</td>
<td>Plantar surface of hind paw</td>
<td>Plantar surface of hind paw</td>
</tr>
<tr>
<td></td>
<td>Thermal hyperalgesia</td>
<td>Secondary hyperalgesia</td>
<td>CCI</td>
<td>Left sciatic nerve</td>
<td>Plantar surface of hind paw</td>
</tr>
<tr>
<td>Hot plate test (Response Latency)</td>
<td>Thermal hyperalgesia</td>
<td>Primary hyperalgesia</td>
<td>CFA</td>
<td>Plantar surface of hind paw</td>
<td>Plantar surface of hind paw</td>
</tr>
<tr>
<td></td>
<td>Thermal hyperalgesia</td>
<td>Primary hyperalgesia</td>
<td>CAR</td>
<td>Plantar surface of hind paw</td>
<td>Plantar surface of hind paw</td>
</tr>
<tr>
<td>Mechanical withdraw threshold</td>
<td>Mechanical hyperalgesia</td>
<td>Primary and secondary hyperalgesia</td>
<td>CFA</td>
<td>Plantar surface of hind paw</td>
<td>Plantar surface of hind paw</td>
</tr>
<tr>
<td></td>
<td>Mechanical hyperalgesia</td>
<td>Secondary hyperalgesia</td>
<td>SNL</td>
<td>L5-L6</td>
<td>Plantar surface of hind paw</td>
</tr>
<tr>
<td></td>
<td>Mechanical hyperalgesia</td>
<td>Secondary hyperalgesia</td>
<td>Incision</td>
<td>1cm longitudinal, through skin and fascia of the plantar region of heel</td>
<td>Mid-plantar right hind paw</td>
</tr>
<tr>
<td>Foot-bend score</td>
<td>Behavior hyperalgesia</td>
<td>Primary hyperalgesia</td>
<td>CFA</td>
<td>Left malleolus’ articular cavity</td>
<td>Ankle joint</td>
</tr>
<tr>
<td>Analgesy-meter</td>
<td>Mechanical hyperalgesia</td>
<td>Secondary hyperalgesia</td>
<td>CAR</td>
<td>Plantar surface of hind paw</td>
<td>Dorsal surface of the hind paw</td>
</tr>
<tr>
<td>Weighted pain score</td>
<td>Mechanical hyperalgesia</td>
<td>Primary and secondary hyperalgesia</td>
<td>Formalin</td>
<td>Plantar surface of hind paw</td>
<td>N/A</td>
</tr>
<tr>
<td>Tail response frequency</td>
<td>Mechanical allodynia</td>
<td>Secondary hyperalgesia</td>
<td>ICTR</td>
<td>Between S3 and S4 of right superior caudal trunk</td>
<td>The sensitive spot on the tail</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------------</td>
<td>------------------------</td>
<td>------</td>
<td>--------------------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>AWR test</td>
<td>Visceral hyperalgesia</td>
<td>N/A</td>
<td>IBS</td>
<td>IBS Colorectal area</td>
<td>Colorectal area</td>
</tr>
<tr>
<td>EMG test</td>
<td>Visceral hyperalgesia</td>
<td>N/A</td>
<td>IBS</td>
<td>IBS Colorectal area</td>
<td>Colorectal area</td>
</tr>
<tr>
<td>Water-avoidance stress</td>
<td>Visceral hyperalgesia</td>
<td>N/A</td>
<td>IBS</td>
<td>IBS Colorectal area</td>
<td>N/A</td>
</tr>
</tbody>
</table>
2.2.5.2.1 Paw withdrawal latency (PWL)

This test is first described by Hargreaves et al. (Hargreaves, Dubner et al. 1988). It determines the hyperalgesia state of the affected paw to noxious thermal stimulus. The Paw Thermal Stimulator System (UCSD, San Diego) (Lao, Zhang et al. 2004; Zhang, Lao et al. 2004; Li, Zhang et al. 2007) or equivalent (Fu, Wang et al. 2006) is used. Rats are placed on a glass surface enclosed in a plastic chamber and are allowed to acclimatise for 30 minutes before the test. To deliver the thermal stimulation, a projector lamp bulb (50w) controlled by a timer is focused on the plantar surface of each hind paw underneath the glass surface. The latency is automatically determined by the timer to the nearest 0.1 s when the paw is withdrawn. The intensity of the lamp is adjusted to derive a baseline of 10 s (Lao, Zhang et al. 2004; Li, Zhang et al. 2007) or 20 s (Wang, Zhang et al. 2006) in naive animal. To prevent tissue damage, a 20 s cut-off is used.

Mean latency is achieved by averaging results from four tests with 5-minutes intervals (Zhang, Liu et al. 2005).

2.2.5.2.2 Response Latency

The hot plate test is used to determine the thermal hyperalgesia. Rats are placed on a hot plate of 52°C, the latency time is recorded when the rats lift its paws or jump. A 60-s cut-off is used to prevent tissue damage. The thermal hyperalgesia latency is determined by averaging results from three tests (Huang, Hu et al. 2004).

2.2.5.2.3 Mechanical withdrawal threshold to von Frey filaments

This test uses a serial of von Frey filaments ranging from 1 to 26 g to determine the paw withdrawal threshold. The individual rat is placed onto a wire mesh floor enclosed in a Plexiglas chamber, which enables the researchers to apply the von Frey filaments to the
plantar surface of one paw through the hole of the wire floor. After a period of acclimatisation, the von Frey filaments are applied in ascending order. If the stimulation of a filament does not elicit a valid response by completely lifting the stimulated paw from the floor within five repeated stimulations, the next filament with a higher bending force is applied. The bending force of the filament that elicits the lifting response is recorded as the withdrawal force. The paw withdrawal threshold is either defined as the average results of three measurements (Huang, Hu et al. 2004), or as the force of a particular filament that produce valid response more than twice out of five applications (Sun, Chen et al. 2006; Shan, Qi-Liang et al. 2007).

2.2.5.2.4 Foot-bend score

This measure is used in the model when CFA is injected into the tibio-tarsal joint (Wang, Zhang et al. 2006) or malleolus joint (Liu, Li et al. 2006). The hyperalgesia is defined as the score obtained in flexion test or extension test with 15 minutes interval between them. The flexion or extension score (1 or 0) is given according to the response, such as squeaking or withdrawing leg, of the animals to flexion or extension manipulation of the hind paw. Each manipulation is applied 5 times at 5-s interval (Wang, Zhang et al. 2006).

2.2.5.2.5 Analgesy-meter

Analgesy-meter (Ugo Basile) is used to evaluate the nociceptive threshold. Rats are restrained in a jacket whilst pressure is incrementally applied to the dorsal surface of the hind paw. Paw Pressure Threshold (PPT) is defined as the pressure that elicits paw withdrawal response. The mean PPT is the average of the result of two consecutive measurements with a 2-minutes interval. A cut-off of 250g is used to prevent tissue damage.

2.2.5.2.6 Weighted pain score

This assessment is used in the formalin test to evaluate pain based on the behavioral response.
Three prominent pain-induced behaviors that include favouring, elevation and licking or biting of the paw, are given weights of 1, 2 and 3, respectively, for a period of one hour (weighted every 5 minutes, 12 times). The pain score is calculated by multiplying these weights with the number of each behaviors presented (Abbott, Franklin et al. 1995).

### 2.2.5.2.7 Tail response frequency (Mechanical allodynia)

The Tail response frequency is evaluated using a von Frey filament with the bending force of 2.0g. During the test, the rat is restrained in a plastic holder with its' tail laid on a plate. The area of allodynia is determined by stroking different areas on the tail with the von Frey filament. Once the sensitive area is spotted, the von Frey filament is applied to the most sensitive spot for ten times at 10-20s intervals. A valid response is defined as an abrupt tail movement of more than 0.5 cm to the gentle poking of the filament. The degree of response, which attribute to the mechanical allodynia, is expressed as the percentage of valid response out of ten stimulations (Hwang, Min et al. 2002).

### 2.2.5.2.8 Abdominal withdrawal reflex (AWR) score

After light anaesthesia with halothane, a deflated balloon is inserted into the descending colon. The rat is then placed into a clear plastic chamber (20×8×8cm) on a platform, and allowed to wake up and adapted for 20-30 minutes. Colorectal Distention (CRD) is achieved by inflating the balloon to a designated pressure (20, 40, 60, 80 mmHg) for 10s. The pressure is applied in an ascending order at 4-minutes intervals. Abdominal Withdrawal Reflex (AWR) is evaluated based on the observation of the response to a certain grade. The score is assigned according to the scale described by Al-Chaer et al.

### 2.2.5.2.9 EMG

Under pentobarbital anaesthesia (40/mg/kg, intraperitoneal), EMG of rectus abdominis is
recorded by electrodes placed on them bilaterally. The signal is amplified and fed into computer for analyse. The CRD is applied in the same manner as the AWR test. During the complete test, the body temperature is kept at approximately 37°C by a servo-controlled heat blanket.

2.2.5.2.10 Pain threshold pressure (PTP)

To determine the pain threshold pressure (PTP), pressure is increased by 5 mmHg for duration of 30s. The pressure that elicits pain behavior, such as obvious contraction of abdominal wall, is defined as PTP. The maximum pressure of 80 mmHg is applied to avoid tissue damage.

2.2.5.2.11 Water-avoidance stress (WAS)

This test is used in the IBS model. In this test, rat is placed on a platform (8×6×6cm) fixed in the middle of a water tank. The tank is filled with water 7cm deep and within 1 cm on top of the platform. The rat stays on the block for 1 hour between 10:00AM and 13:00PM. The fecal pellet output is countered every 15 minutes for 1 hour.

2.2.6 Mechanism of animal models

2.2.6.1 Inflammation models

2.2.6.1.1 CFA model

Systemic application of CFA leads to inflammation of multiple joints eyes, nose, ears and penis. It also causes lymph node enlargement and liver dysfunction. These changes are thought to be T-cell mediated delayed-type hypersensitivity reactions (Pearson and Wood 1959).
Injected locally, CFA leads to local inflammation and produces an algogenic substance, such as prostaglandins, bradykinin and substance P. These chemicals sensitise the peripheral sensory fibers, causing spontaneous Aδ and C-fiber activity and an increased responsiveness of ipsilateral nociceptive lamina I projection neurons (Stein, Millan et al. 1988).

2.2.6.1.2 Carrageenan model

Local injection of carrageenan causes inflammation and edema. Evidence suggests that carrageenan injection alters the C-fiber responsiveness to thermal as well as mechanical stimuli (Handwerker, Anton et al. 1987). Furthermore, the recording from the nociceptive neurons in the ventrobasal thalamus, following carrageenan injection, showed decrease in the thermal threshold of these neurons and increased responsiveness to thermal stimulation (Benoist, Kayser et al. 1984).

2.2.6.1.3 Formalin model

The two-phase pain-induced behaviors seen in a formalin model have different neurological mechanism underlying each phase. The first phase, which lasts for approximately 5 minutes, results from direct activation of primary afferent fibers of both low-threshold mechanoreceptive and nociceptive at the injection site. The secondary phase, which lasts for about 40 minutes, is more complex and less understood. It is generally accepted that this phase attributes to the increased activity in the spinal dorsal horn neurons, being initiated by the peripheral afferent input during the first phase (Ren and Dubner 1999; Hogan 2002).

2.2.6.2 Neuropathic pain model

Neuropathic pain models such as CCI, SNL and ICTR model share similar neurological mechanisms.
Injury to the peripheral nerve system causes functional changes in the somatosensory neurons in both peripheral and central nervous system (Porreca, Ossipov et al. 2002; Bennett 2005). Neuropathic pain involves two pathological changes in the nervous system: spontaneous discharge in nociceptors evoked by nerve injury; and central sensitisation initiated and maintained by ongoing discharge from C nociceptors (Bennett 2005).

Several mechanisms were discovered to explain the ongoing discharge in nociceptors and low-threshold mechanoreceptors. Firstly, several afferent axons begin to discharge spontaneously and ectopically. This is understood to be caused by oscillations in the neurons membrane potential (Devor and Seltzer 1999). Secondly, intact afferent neurons traveling in a nerve (C fibers) adjacent to the degenerating nerve, discharges spontaneously which may be due to the peripheral input that maintain central sensitisation (Bennett 2005). Thirdly, sensitised nociceptors may produce ongoing discharge however, no tangible evidence supports this hypothesis (Cline, Ochoa et al. 1989; Bennett 2005). Forthly, tumor necrosis factor-α (TNF-α) may also contribute to the ongoing ectopic discharge, as anti-TNF-α neutralising antibodies can reduce neuropathic pain in CCI and PSL models and exposing nerve fiber to TNF-α can elicit neuropathic pain (Sorkin and Doom 2000; Sommer, Lindenlaub et al. 2001).

Central nervous system also plays an important role in the neuropathic pain. It is now clear that central sensitisation is triggered by the release of glutamate from the C fiber terminals that acts on glutaminergic synapses of the NMDA type (Bennett 2005). Another important change in the spinal dorsal horn is disinhibition, which inhibits neurons that exert negative feedback control over the message sent to the brain. Evidence showed anatomical changes in this negative feedback system that are significantly decreased in the CCI model (Sugimoto, Bennett et al. 1990). Later, studies have been found to suggest that GABAergic inhibition is
2.2.6.3 Other models

2.2.6.3.1 Incision model

In the incision model, mechanical hyperalgesia develops after the operation. The initiative afferent input of mechanical hyperalgesia from the incision area (plantar region starting 0.5 cm from the proximal edge of the heel) is transmitted by both sural and tibial nerves. Resection of the tibial nerve blocks the mechanical withdrawal response in the medial proximal side of the rat foot, while the resection of both sural and tibial nerves blocked the mechanical withdrawal response in the lateral proximal side of the rat foot (Brennan, Vandermeulen et al. 1996).

In this model, probing the incision site and a site about 10 mm distal to the incision site one can detect decreased withdrawal thresholds, suggesting that mechanical hyperalgesia presents in the wounded and surrounding uninjured areas. Hyperalgesia, at the intact area around the incision, is considered as secondary hyperalgesia (Brennan, Vandermeulen et al. 1996).

2.2.6.3.2 IBS

The nociceptive neural circuits undertake significant development in the neonatal stage, thus become very susceptible to plasticity. Noxious stimulation in this stage produces permanent alteration in afferent pathways (Al-Chaer, Kawasaki et al. 2000). The IBS model is a result of the neonatal colon irritation which causes permanent plasticity change in the central nervous system in the adult. On the contrary, if colon irritation is applied in adults, it does not produce the permanent state of central sensitisation (Cui, Li et al. 2005).
2.2.7 The effect of EA on hyperalgesia in different animal model

All the included studies used electro-acupuncture as intervention. No manual acupuncture was used. This could be due to the reason that electro-acupuncture delivers consistent stimulation with accurate intensity and frequency.

2.2.7.1 Immediate effect of EA

2.2.7.1.1 Single application of EA

Compared with sham or no treatment, single EA treatment significantly reduced both mechanical (PPT, MWT) and thermal (PWL, hot plate test) hyperalgesia in inflammatory, neuropathic, IBS and cancer models.

For the single application, the most commonly used models are CFA and carrageenan models. Both high (100 Hz) and low (2 Hz) frequencies were used. Only distal acupoints (away from the injection site, in the same limb) were used, and stimulation was given bilaterally, ipsilaterally or contralaterally. Commencing time for EA treatment varied from minutes to days, and EA showed its effect usually within one hour and the effect lasted up to 20 hours.
Table 2. 7 A summary of the immediate effect of single EA application in different studies

<table>
<thead>
<tr>
<th>No.</th>
<th>Model</th>
<th>Frequency</th>
<th>Current</th>
<th>Pulse</th>
<th>Duration</th>
<th>Acupoint</th>
<th>Time point (EA)</th>
<th>Tests</th>
<th>Effect time</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Formalin</td>
<td>4 Hz, 10/20 v,</td>
<td>0.5 ms square pulse</td>
<td>30 min</td>
<td>ST36, 5 mm below ST36, contralateral to formalin (left)</td>
<td>end 10 min before formalin</td>
<td>Weighted pain score</td>
<td>Both 10, 20V: 20-60 min (second phase) compared with no treatment</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Car</td>
<td>A DS of 60 and 2 Hz</td>
<td>1-2-3 mA for 10 min each</td>
<td>n/a</td>
<td>30 min</td>
<td>ST36, UB60, Contralateral</td>
<td>3 h post carrageenan</td>
<td>PWL</td>
<td>10, 20, 30 min after EA begin, 10 min after EA end, compared with NS control</td>
</tr>
<tr>
<td>12</td>
<td>Car</td>
<td>3 Hz</td>
<td>1-2-3 mA for 20 min each</td>
<td>0.1 ms</td>
<td>60 min</td>
<td>ST36, 5 mm from ST 36, ipsilateral</td>
<td>3 h after carrageenan</td>
<td>PPT</td>
<td>At least 20 h after EA end, compared to no treatment</td>
</tr>
<tr>
<td>13</td>
<td>Car</td>
<td>3 Hz</td>
<td>1-2-3 mA for 20 min each</td>
<td>0.1 ms</td>
<td>60 min</td>
<td>ST36, 5 mm from ST 36, ipsilateral</td>
<td>3 h after carrageenan</td>
<td>PPT</td>
<td>At least 20 h after EA end, compared with non treatment</td>
</tr>
<tr>
<td>21</td>
<td>Car</td>
<td>2, 15, 120 Hz</td>
<td>1-2-3 mA for 10 min for each</td>
<td>30 min</td>
<td>ST36, SP6, bilateral</td>
<td>immediate after injection</td>
<td>Hot plate test</td>
<td>At least 30 min to 120 min after EA end, compared with no treatment</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Car</td>
<td>A DS of 60 and 2 Hz,</td>
<td>1-2-3 mA for 10 min each</td>
<td>30 min</td>
<td>ST36, UB60, contralateral</td>
<td>3 h post carrageenan injection</td>
<td>PWL</td>
<td>10, 20, 30, 40 min after EA start, compared with sham</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Car</td>
<td>2 Hz,</td>
<td>0.5, 1 or 3 mA</td>
<td>30 min</td>
<td>ST36, SP6, bilateral</td>
<td>immediately after carrageenan injection</td>
<td>Hot plate test</td>
<td>At least 0 min to 3 h after EA end, compared with non treatment</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Car</td>
<td>3, 15 or 100 Hz,</td>
<td>1-2-3 mA for 3 biphasic square</td>
<td>for 1, 15</td>
<td>ST36,</td>
<td>3 h after</td>
<td>PPT</td>
<td>1 min EA:</td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>Hz, 0.5-1-1.5 mA for 15, 100 Hz</th>
<th>0.1 ms or 60 min</th>
<th>5mm from ST36, ipsilateral</th>
<th>carrageenan</th>
<th>no effect for all FQ, compared with no treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>CFA</td>
<td>100 Hz</td>
<td>0.5-1-1.5 mA for 10 min each</td>
<td>0.2 ms 30 min</td>
<td>ST36, SP6, bilateral</td>
<td>48 h after CFA</td>
<td>MWT 15 min after EA start, compared with no treatment</td>
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<td></td>
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<td></td>
<td></td>
<td>Hot plate test No effect, compared with no treatment</td>
</tr>
<tr>
<td>14</td>
<td>CFA</td>
<td>A 100 and 2 Hz</td>
<td>0.2 ms 30 min</td>
<td>GB30, GB34, ipsilateral</td>
<td>48h after CFA injection</td>
<td>PWL 45 min after EA end, compared with sham</td>
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<td></td>
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<td></td>
<td>MWT 45 min after EA end, compared with sham</td>
</tr>
<tr>
<td>25</td>
<td>CFA</td>
<td>A DS 60 and 2 Hz, 0.6 ms pulse width</td>
<td>less than 1 mA bidirectional asymmetric pulse</td>
<td>30 min</td>
<td>GB30, GB34, unilateral</td>
<td>72 h after CFA</td>
<td>PWL 10, 20, 40, 60 min after EA start, compared with sham</td>
</tr>
<tr>
<td>30</td>
<td>CFA</td>
<td>30 Hz, 2 mA, 0.1 ms pulse width</td>
<td>30 min</td>
<td>GB30, 10 mm below GB30, ipsilateral</td>
<td>5 day after CFA injection</td>
<td>PWL 30 min to 3h after EA end, compared with non treatment and vehicle</td>
<td></td>
</tr>
</tbody>
</table>
2.2.7.1.2 Carrageenan

In this model, EA was applied immediately after the injection or three hours post injection. EA reduced both mechanical and thermal hyperalgesia and produced a lasting effect. For example, a single treatment of acupuncture applied immediately after carrageenan injection produced a lasting alleviation of the latency in the hot plate test for 30 minutes to 3 hours after the cessation of EA treatment (Lee, Choi et al. 2005; Lee, Jang et al. 2006). A 60-minute EA intervention (3 hours after carrageenan injection) at 3 Hz significantly increased the PPT for at least 20 hours. However, EA at 5 or 15Hz did not produce any significant effect. Thirty minutes EA intervention of alternating frequency of 2 to 60 Hz significantly increased PWL.
at 10, 20 and 30 minutes during the treatment and 10 minutes post treatment (Zhang, Ji et al. 2002; Sekido, Ishimaru et al. 2003; Zhang, Ji et al. 2003; Sekido, Ishimaru et al. 2004; Taguchi and Taguchi 2007).

2.2.7.1.3 CFA

In the CFA model, EA was usually applied at 2 to 5 days post injection. EA with different parameters produce different effects at the same time point after injection of CFA. For example, at 48 hours post CFA, EA with alternating frequency of 2 and 100 Hz at GB30 and GB 34 elevates mechanical hyperalgesia (MWT) 45 minutes after the end of treatment, while EA of 100 Hz at ST36 and SP6 elevates MWT at only 15 minutes during the treatment (Sasamura, Nakamura et al. 2002; Huang, Hu et al. 2004; Sun, Chen et al. 2006). This suggests that the anti-hyperalgesia effect of EA is parameter dependent.

Interestingly, in an additional study, EA successfully increases MWT (mechanical hyperalgesia) but fails to elevate the PWL (thermal hyperalgesia) by single or repetitive treatment (Huang, Hu et al. 2004). However, this result contradicts with other completed studies.

In conclusion, EA applied 3 days and 5 days after CFA injection produces significant alleviation of PWL for 30 minutes and 3 hours respectively (Zhang, Yu et al. 2005; Fu, Wang et al. 2006)

2.2.7.1.4 Incision

In this model, 15 minutes EA treatment increases MWT for at least 30 minutes after the cessation of the treatment (Oliveira and Prado 2000).

2.2.7.1.5 Formalin
One study used this model reported thirty minutes of EA application that finished 10 minutes before the formalin injection significantly reduced the formalin-induced hyperalgesia (weighted pain score) during the first 60 minutes. It is notable that EA at 20V produce similar effect as morphine (2 mg/kg, given 40 minutes before formalin injection) (Wen, Yeh et al. 2007).

### 2.2.7.1.6 ICTR model (inferior caudal trunk resection)

EA applied 21 hours after the operation increases the MWT in the rat tail at 15 minutes during the treatment and 15 minutes post treatment. This effect was similar to the effect of morphine (1.5 mg/kg). Furthermore, there was no synergetic effect of EA combined with morphine was observed in this study (Hwang, Min et al. 2002).

### 2.2.7.1.7 IBS and Cancer

Single EA treatment alleviates hyperalgesia in both models including IBS and Cancer, however, EA shows little effect when applied in the late stage of the cutaneous cancer model.

### 2.2.7.1.8 Two sessions of EA

In order to achieve maximum effect, EA was given twice, once immediately after the CFA injection and the other 2 hours after the injection. Several studies utilise this procedure. According to different parameters, EA significantly increases thermal hyperalgesia (PWL) at 10 minutes to several days after the treatment (Lao, Zhang et al. 2004; Zhang, Lao et al. 2004). Table 2.8 summarises the immediate effect of two sessions of EA application in different studies.

<table>
<thead>
<tr>
<th>Table 2.8</th>
<th>Effect of two sessions of EA treatments</th>
</tr>
</thead>
</table>

75
<table>
<thead>
<tr>
<th>No.</th>
<th>Model</th>
<th>Frequency</th>
<th>Current</th>
<th>Pulse</th>
<th>Duration</th>
<th>Acupoint</th>
<th>Time point</th>
<th>Tests</th>
<th>Effect time point</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>CFA</td>
<td>10 or 100 Hz</td>
<td>3 mA</td>
<td>0.1 ms</td>
<td>20 min twice</td>
<td>GB30, bilateral</td>
<td>immediate after and 2h after injection</td>
<td>PWL</td>
<td>10 or 100Hz: 10 min after EA end (2.5h post-CFA), compared with vehicle and sham</td>
</tr>
<tr>
<td>3</td>
<td>CFA</td>
<td>10 Hz</td>
<td>3v</td>
<td>0.1 ms</td>
<td>20 min twice</td>
<td>GB30, bilateral</td>
<td>immediate after and 2h after injection</td>
<td>PWL</td>
<td>At 10 min and 120 h after EA end (2.5h post-CFA), compared with sham</td>
</tr>
<tr>
<td>4</td>
<td>CFA</td>
<td>10 or 100 Hz</td>
<td>3 mA</td>
<td>0.1 ms</td>
<td>20 min twice</td>
<td>GB30, bilateral</td>
<td>immediate after and 2h after injection</td>
<td>PWL</td>
<td>At 10 min after EA end (2.5h post-CFA), compared with sham</td>
</tr>
<tr>
<td>7</td>
<td>CFA</td>
<td>2Hz</td>
<td>2 mA</td>
<td>0.1 ms</td>
<td>20 min twice</td>
<td>GB30, bilateral</td>
<td>immediate after and 2h after injection</td>
<td>PWL</td>
<td>No effect at any time point, compared with sham</td>
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<td></td>
<td>No effect at any time point, compared with sham</td>
</tr>
<tr>
<td>10Hz</td>
<td>1 mA</td>
<td>2 ms</td>
<td>20 min twice</td>
<td>GB30, bilateral</td>
<td>immediate after and 2h after injection</td>
<td>PWL</td>
<td>At 2.5 and 5 h post CFA,</td>
<td></td>
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<tr>
<td>Current (mA)</td>
<td>Duration (ms)</td>
<td>Intensity (ms)</td>
<td>Time After Injection</td>
<td>PWL</td>
<td>Result</td>
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<tr>
<td>1</td>
<td>1</td>
<td>20 min</td>
<td>immediate</td>
<td>PWL</td>
<td>No effect at any time point, compared with sham</td>
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<tr>
<td>1</td>
<td>0.1</td>
<td>20 min</td>
<td>immediate</td>
<td>PWL</td>
<td>No effect at any time point, compared with sham</td>
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<tr>
<td>2</td>
<td>0.1</td>
<td>20 min</td>
<td>immediate</td>
<td>PWL</td>
<td>At 2.5h post CFA, compared with sham</td>
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<tr>
<td>3</td>
<td>0.1</td>
<td>20 min</td>
<td>immediate</td>
<td>PWL</td>
<td>At 2.5h, 1d, 5d, 7d post CFA, compared with sham</td>
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<tr>
<td>3</td>
<td>0.1</td>
<td>10 min</td>
<td>immediate</td>
<td>PWL</td>
<td>No effect at any time point, compared with sham</td>
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<tr>
<td>3</td>
<td>0.1</td>
<td>30 min</td>
<td>immediate</td>
<td>PWL</td>
<td>No effect at any time point, compared with sham</td>
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<tr>
<td>50Hz</td>
<td>1</td>
<td>20 min</td>
<td>immediate</td>
<td>PWL</td>
<td>No effect at any time point, compared with sham</td>
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<tr>
<td>Current</td>
<td>Frequency</td>
<td>Duration</td>
<td>Mode</td>
<td>Timing</td>
<td>Effect</td>
<td>Comparison</td>
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<tr>
<td>2 mA</td>
<td>0.1 ms</td>
<td>20 min</td>
<td>twice</td>
<td>immediate after and 2 h after injection</td>
<td>PWL</td>
<td>At 2.5 h post CFA, compared with sham</td>
<td></td>
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<tr>
<td>3 mA</td>
<td>0.1 ms</td>
<td>20 min</td>
<td>twice</td>
<td>immediate after and 2 h after injection</td>
<td>PWL</td>
<td>At 2.5 h post CFA, compared with sham</td>
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<tr>
<td>100 Hz</td>
<td>1 mA</td>
<td>2 ms</td>
<td>twice</td>
<td>immediate after and 2 h after injection</td>
<td>PWL</td>
<td>At 2.5 h post CFA, compared with sham</td>
<td></td>
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<tr>
<td>1 mA</td>
<td>1 ms</td>
<td>20 min</td>
<td>twice</td>
<td>immediate after and 2 h after injection</td>
<td>PWL</td>
<td>No effect at any time point, compared with sham</td>
<td></td>
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<tr>
<td>1 mA</td>
<td>0.1 ms</td>
<td>20 min</td>
<td>twice</td>
<td>immediate after and 2 h after injection</td>
<td>PWL</td>
<td>No effect at any time point, compared with sham</td>
<td></td>
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<tr>
<td>2 mA</td>
<td>0.1 ms</td>
<td>20 min</td>
<td>twice</td>
<td>immediate after and 2 h after injection</td>
<td>PWL</td>
<td>At 2.5 h post CFA, compared with sham</td>
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<tr>
<td>3 mA</td>
<td>0.1 ms</td>
<td>20 min</td>
<td>twice</td>
<td>immediate after and 2 h after injection</td>
<td>PWL</td>
<td>At 2.5 h, 5 h, 1 d post CFA, compared with sham</td>
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<tr>
<td>3 mA</td>
<td>0.1 ms</td>
<td>10 min</td>
<td>twice</td>
<td>immediate after and 2 h after injection</td>
<td>PWL</td>
<td>At 5 h, 1 d, 5 d post CFA, compared with sham</td>
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<tr>
<td>3 mA</td>
<td>0.1 ms</td>
<td>30 min twice</td>
<td>GB30, bilateral</td>
<td>immediate after and 2h after injection</td>
<td>PWL</td>
<td>No effect at any time point, compared with sham</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10 Hz</td>
<td>3 mA</td>
<td>0.1 ms</td>
<td>20 min twice</td>
<td>GB30, bilateral</td>
<td>immediate after and 2h after injection</td>
<td>PWL</td>
<td>At 2.5 and 24 h 120h, 168h post CFA, compared with sham</td>
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<tr>
<td>3 mA</td>
<td>0.1 ms</td>
<td>20 min twice</td>
<td>TE5, bilateral</td>
<td>immediate after and 2h after injection</td>
<td>PWL</td>
<td>No effect at any time point, compared with sham</td>
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<tr>
<td>3 mA</td>
<td>0.1 ms</td>
<td>20 min twice</td>
<td>Opposite aspect of GB30, bilateral</td>
<td>immediate after and 2h after injection</td>
<td>PWL</td>
<td>No effect at any time point, compared with sham</td>
<td></td>
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</tr>
<tr>
<td>11 CFA</td>
<td>10 Hz</td>
<td>3 mA</td>
<td>0.1 ms</td>
<td>20 min twice</td>
<td>GB30, Huantiao, bilateral</td>
<td>immediate after and 2h after injection</td>
<td>PWL</td>
<td>At 10 min after EA end (2.5h post-CFA), compared with sham</td>
<td></td>
</tr>
<tr>
<td>17 CFA</td>
<td>10 Hz</td>
<td>3 mA</td>
<td>0.1 ms</td>
<td>20 min twice</td>
<td>GB30, bilaterally</td>
<td>immediate after and 2h after injection</td>
<td>PWL</td>
<td>At 10 min after EA end (2.5h post-CFA), compared with sham and no treatment</td>
<td></td>
</tr>
<tr>
<td>20 CFA</td>
<td>10 or 100</td>
<td>3 mA</td>
<td>0.1</td>
<td>20 min</td>
<td>GB30,</td>
<td>immediate</td>
<td>PWL</td>
<td>At 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hz</td>
<td></td>
<td>ms</td>
<td>twice</td>
<td>bilateral</td>
<td>after and</td>
<td>2h after</td>
<td>injection</td>
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<tr>
<td>23</td>
<td>CFA</td>
<td>10 Hz</td>
<td>3 mA</td>
<td>0.1 ms</td>
<td>20 min twice</td>
<td>GB30, bilateral</td>
<td>immediate after and 2h after injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>CFA</td>
<td>10 Hz</td>
<td>3 mA, 0.1 ms</td>
<td>20 min twice</td>
<td>GB30, bilateral</td>
<td>immediate after and 2h after injection</td>
<td></td>
<td></td>
<td>PWL</td>
</tr>
</tbody>
</table>

Two sessions of 20 minutes EA at 10 Hz and 3 mA produces an anti-hyperalgesia effect that lasts for 7 days, while the same treatment with a lower intensity of 2 mA only produces a transient effect that lasts for 10 minutes. At an intensity of 1 mA, no significant effect is achieved. Similar results are observed at 100 Hz but not at 50 Hz, which produce transient effect even at a high intensity (3 mA) (Lao, Zhang et al. 2004). This suggests that there could be a optimal combination among frequency, intensity, and wave width (Lao, Zhang et al. 2004).

In the two-session protocol, EA was always delivered bilaterally. Results suggest there is evidence that bilateral EA is achieves a better anti-hyperalgesia effect rather than unilateral EA (Lao, Zhang et al. 2004). GB30, which was distal from the model site, was the only acupoint being used. Although TE5 was also used to test the point specification of EA
intervention, it did not produce any anti-hyperalgesia effect.

From these results it is difficult to establish which one is more effective, either the single treatment or repeated treatments, as the two treatments were not compared directly in a single study.
2.2.7.2 Cumulative effect of EA

In general, repetitive application of EA produces cumulative anti-hyperalgesia effect in inflammatory as well as neuropathic models. Both mechanical (MWT) and thermal (PWL) hyperalgesia was significantly reduced after EA intervention. Some studies followed up the effect after the end of EA treatments and found that EA showed increased lasting effect.

High frequency (100 Hz), low frequency (2 Hz) and alternating frequency (2 to 100 Hz) were used experimentally. Stimulation duration was 20 or 30 minutes on all occasions. The whole treatments consisted of 2 to 14 sessions given every day or every other day. Bilateral, ipsilateral or contralateral distal (away from the injection site, within the same limb) acupoints were used in the studies.

Table 2.9 is the summary of the effect of cumulative effect of repetitive EA application in different studies.
<table>
<thead>
<tr>
<th>No.</th>
<th>Model</th>
<th>Frequency</th>
<th>Current</th>
<th>Pulse</th>
<th>Duration</th>
<th>Acupoint</th>
<th>Time point</th>
<th>Tests</th>
<th>Effect time</th>
<th>Session times</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>CFA</td>
<td>100 Hz</td>
<td>0.5-1-1.5 mA for 10 min each</td>
<td>0.2 ms</td>
<td>30 min</td>
<td>ST36, SP6, bilateral</td>
<td>day 3 and 6 for 4 weeks</td>
<td>MW T</td>
<td>At 3 to 4 week during EA. No follow up, compared with no treatment</td>
<td>8 in 28 day</td>
</tr>
<tr>
<td>34</td>
<td>CFA</td>
<td>A DS 16 and 4 Hz</td>
<td>0.5-1-1.5v, each for 10 min</td>
<td>N/A</td>
<td>30 min</td>
<td>GB30, GB34, ipsilateral</td>
<td>every other day on from day 1 to day 13 after CFA</td>
<td>Foot bend score</td>
<td>Point: day 9-11 (5th to 6th EA) Contra point: day 8-12 (5th to 6th EA) Non point: day 8, 9, 11 (5th to 6th EA) , compared with sham</td>
<td>7 in 13 day</td>
</tr>
<tr>
<td>18</td>
<td>CFA</td>
<td>A DS 16 and 4 Hz</td>
<td>0.5-1-1.5v for 10 min each</td>
<td>30 min</td>
<td>GB30, GB34, ipsilateral</td>
<td>once a day form 1st day after CFA injection, 14 days</td>
<td>PWL</td>
<td>5d to 8d after EA, compared with sham and no treatment</td>
<td>14 in 14day</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>CFA</td>
<td>10 Hz</td>
<td>3 mA</td>
<td>0.1 ms</td>
<td>20 min</td>
<td>GB30, 4 mm off GB30, ipsilateral</td>
<td>immediate after, 2h after, and twice on day 2 after CFA injection</td>
<td>PWL</td>
<td>1-2d during EA. No follow up, compared with sham</td>
<td>2 in 2 day</td>
</tr>
<tr>
<td>26</td>
<td>CFA</td>
<td>Alternating 100 and 2 Hz</td>
<td>1-2-3 mA 10 min for each</td>
<td>0.2 ms square wave</td>
<td>30min</td>
<td>GB30, GB34, ipsilateral</td>
<td>pre CFA, 1 to 4 day post CFA, 5 treatments</td>
<td>MW T PWL</td>
<td>2-5 during EA (3rd to 5th EA). Last for 3-4 d after EA end, compared with sham</td>
<td>5 in 5 day</td>
</tr>
<tr>
<td>6</td>
<td>SNL</td>
<td>2 Hz</td>
<td>0.5-1-1.5 mA for 10 min each</td>
<td>0.6 ms</td>
<td>30 min</td>
<td>ST36, SP6, bilateral</td>
<td>5 d after SNL, on day 3 and 6 for 4 weeks</td>
<td>MW T</td>
<td>At 2 to 4 week during the EA treatment. No follow up, compared with vehicle</td>
<td>8 in 28 day</td>
</tr>
<tr>
<td>32</td>
<td>CCI</td>
<td>A DS</td>
<td>1-2-3v for 5,10,15 min</td>
<td>30 min</td>
<td>GB30, GB34, ipsilateral</td>
<td>Everyday from the first after operation for 14 days</td>
<td>PWL</td>
<td>Day 7, 10, 14 during EA. Peak at 14d. No follow up, compared with no treatment</td>
<td>14 in 14 day</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CCI</td>
<td>Alternating 60 and 2 Hz</td>
<td>less than 1 mA, 12 V</td>
<td>30 min</td>
<td>GB30, GB34 contralateral</td>
<td>once every day from 7th day after operation, 11 treatments</td>
<td>PWL</td>
<td>GB30, GB34</td>
<td>Contralateral once every day from 7th day after operation, 11 treatments</td>
<td>11 in 20 day</td>
</tr>
<tr>
<td>---</td>
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<td>------------</td>
<td>-----------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>15</td>
<td>Cutaneous cancer pain</td>
<td>Alternating DS 100 and 4 Hz</td>
<td>1 mA, 0.6 ms pulse width, bidirectional asymmetric pulse</td>
<td>30 min</td>
<td>ST36, BL60, ipsilateral</td>
<td>every other day, day 8-16 after inoculation, 5 treatments</td>
<td>PWL</td>
<td>4-8 day during EA (3rd to 5th EA). Peak at 4th EA and decrease thereafter, compared with sham and no treatment</td>
<td>5 in 8 day</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>IBS</td>
<td>Alternating DS 100 and 4 Hz</td>
<td>1 mA</td>
<td>30 min</td>
<td>ST36, ST37, bilateral</td>
<td>once every other day for 13 days, 7 treatments</td>
<td>AW, EMG</td>
<td>Appear 2-4d after EA start (1st to 2nd EA), peak at 8-12d, last 5 d after EA end (EA end at 13 d), compared with before EA</td>
<td>7 in 13d</td>
<td></td>
</tr>
</tbody>
</table>

### 2.2.7.2.1 Cumulative effect in CFA model

Several studies utilised the CFA model to evaluate EA anti-hyperalgesia effect. The protocol of EA treatments varies from study to study. The frequencies of the acupuncture treatment vary among studies, it ranged from twice a week for one month (Huang, Hu et al. 2004), daily or alternate day for about 14 days, to twice daily for 2 days. For most of the included studies, EA treatment started during the first day after the injection of CFA. One study initiated the first treatment 30 minute prior to the CFA injection (Shan, Qi-Liang et al. 2007). Different parameters of EA were used in these studies.

Due to the variety of the EA protocol, comparability among studies is limited. Therefore, it is difficult to verify which protocol produced the most significant results. However, the general finding is that repetitive EA treatment produces cumulative and lasting effect. A study showed that the cumulative effect could last for 3-4 days after the cessation of EA treatment (Shan, Qi-Liang et al. 2007). Another study continued to apply EA after the PWL and foot bend score return to the baseline. In these studies, no change in these two measurements occurred despite continuous EA application. This suggests that EA has ability to restore
function back to baseline (Wang, Zhang et al. 2006).

2.2.7.2.2 Cumulative effect in CCI model

In CCI model, EA is applied either on the first day (Chen, Yang et al. 2003) after the performed operation or one week later (Dong, Ma et al. 2006). Both studies achieved cumulative effect from their specific procedure. The EA effect peaked by the end of the study, however no follow up observation is carried out. Therefore, it is difficult to establish how long this cumulative effect lasted for after the cessation of the EA treatment.

2.2.7.2.3 Cumulative effect in SNL model

In the SNL model, EA (applied 5 days after the operation, twice a week for 4 weeks) increased MWT at 2 to 4 weeks after the commencement of treatment. However, due to a lack of follow up observation, the duration of the beneficial effect of EA is not known (Huang, Li et al. 2004).

2.2.7.2.4 Cumulative effect in IBS model

EA treatment was applied once every other day for 13 days. The anti-hyperalgesia effect appeared 2-4 days after EA started, peaked at 8-12 days, and lasted for 5 days after EA treatment ended (Cui, Li et al. 2005).

2.2.7.2.5 Cumulative effect in Cancer model

Five treatments were applied for 8 days after the inoculation. The anti-hyperalgesia effect (PWL and hot plate test) appeared at 3rd to 4th treatment and than peaked at 4th treatment and decreased thereafter despite the treatment continued (Mao-Ying, Cui et al. 2006).
2.2.8 Interactions with medications

More than half of the included papers studied the interaction between EA and drugs. The results either suggest a possible analgesic mechanism occurring from EA, or provided evidence for EA’s potential in drug-reduction.

A few lines of evidence support the involvement of spinal and peripheral opioid receptors in EA anti-hyperalgesic effect. Firstly, opioid receptor antagonists, CTOP (µ opioid receptor antagonist) and NTI (δ opioid receptor antagonist, naltrindole hydrochloride), inhibited the anti-hyperalgesic effect of EA in a dose-dependent manner (Zhang, Lao et al. 2004). Secondly, co-application of EA with sub-effective doses of morphine enhanced the anti-hyperalgesia of EA (Zhang, Lao et al. 2004). Thirdly, intraplantar injection of opioid antagonist naloxone methiodide blocked EA’s anti-hyperalgesia (Zhang, Yu et al. 2005).

Other studies also showed that co-application of EA with sub-effective does analgesia such as INDO (Zhang, Lao et al. 2004), AP5, DNQX (Zhang, Ji et al. 2002) and morphine (Zhang, Lao et al. 2004) additively or synergistically enhanced EA’s effects. These results provide support that EA has the potential to reduce the dosage of certain analgesia which may have serious side effects.

2.2.9 The effect of sham EA on hyperalgesia

Several types of sham acupuncture were used in the studies (Table 2.10). Most of the studies used the invasive sham acupuncture in which acupuncture needles were inserted into the same acupoint as in the real acupuncture but without electrical stimulation. Some researchers used invasive as well as non invasive sham acupuncture, such as taping the needles on the acupoints (Lao, Zhang et al. 2004; Tian, Bian et al. 2006). There were sham methods delivering electrical stimulation to non-acupoint several mm off the acupoint (Oliveira and
Prado 2000) or acupoint in the abdominal (Hwang, Min et al. 2002) or acupoint contralateral to the inflamed limb (Liu, Li et al. 2006). Sham acupuncture was not significantly differently from the no treatment group, and did not produce any significant anti-hyperalgesia effect when compared with real EA.
<table>
<thead>
<tr>
<th>No.</th>
<th>Mode</th>
<th>Mode</th>
<th>Sham method</th>
<th>Duration</th>
<th>Measure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>20 min for twice, immediate after and 2h post-CFA injection</td>
<td>PWL to radiant heat</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Carrageenan</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>30 min for once, 3h post carrageenan</td>
<td>PWL to radiant heat</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>CFA</td>
<td>Non-invasive</td>
<td>Non-invasive: taped on the G30, Invasive: insertion without stimulation</td>
<td>20 min for twice, immediate after and 2h post-CFA injection</td>
<td>PWL to radiant heat</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>20 min for twice, immediate after and 2h post-CFA injection</td>
<td>PWL to radiant heat</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>IBS</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>30 min, once every other day for 13 days, 7 treatments</td>
<td>AWR to CRD, EMG to CRD</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>SNL</td>
<td>N/A</td>
<td></td>
<td>5 d after SNL, 30 min, for once or on day 3 and 6 for 4 weeks</td>
<td>MWT to von Frey filaments</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>CFA</td>
<td>Non-invasive</td>
<td>Non-invasive: taped on the GB30, Invasive: insertion without stimulation</td>
<td>20 min for twice, immediate after and 2h post-CFA injection</td>
<td>PWL to radiant heat</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>30 min single: 48 h after CFA repetitive: day 3 and 6 for 4 weeks</td>
<td>PWL to hot plate test, MWT to von Frey filaments</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>Formalin</td>
<td>N/A</td>
<td></td>
<td>30 min for once, end 10 min before formalin</td>
<td>Weight pain score</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>Incision</td>
<td>Invasive</td>
<td>EA at 5mm lateral to sp6 in operated paw</td>
<td>15 min, 2h after incision</td>
<td>MWT to von Frey filaments</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>20 min for twice, immediate after and 2h post-CFA injection</td>
<td>PWL to radiant heat</td>
<td>Negative</td>
</tr>
<tr>
<td>12</td>
<td>Carrageenan</td>
<td>N/A</td>
<td>3h after carrageenan, 60 min for once</td>
<td>PPT using analgesymeter</td>
<td>Negativ e</td>
<td></td>
</tr>
<tr>
<td>---</td>
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<td></td>
</tr>
<tr>
<td>13</td>
<td>Carrageenan</td>
<td>N/A</td>
<td>3h after carrageenan, 60 min for once</td>
<td>PPT using analgesymeter</td>
<td>Negativ e</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>48h after CFA injection, 30 min for once</td>
<td>PWL to radiant heat, PWT to von Frey filaments</td>
<td>Negativ e</td>
</tr>
<tr>
<td>15</td>
<td>CCI</td>
<td>N/A</td>
<td>30 min, once every day from 7th day after operation, 11 treatments</td>
<td>PWL to radiant heat</td>
<td>Negativ e</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>ICTR</td>
<td>Invasive</td>
<td>Non-acupoint in abdominal area, with stimulation</td>
<td>30 min for once, 21 day after operation.</td>
<td>Mechanical allodynia to von Frey filaments</td>
<td>Negativ e</td>
</tr>
<tr>
<td>17</td>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>20 min for twice immediate after and 2h post-CFA injection</td>
<td>PWL to radiant heat</td>
<td>Negativ e</td>
</tr>
<tr>
<td>18</td>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>30 min, once a day from 1st day after CFA injection, 14 days</td>
<td>PWL to radiant heat, Foot-bend score</td>
<td>Negativ e</td>
</tr>
<tr>
<td>19</td>
<td>IBS</td>
<td>N/A</td>
<td>30 min for once, after IBS</td>
<td>PTP and AWR to colon stimulation, fecal pellet output to WAS</td>
<td>Negativ e</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>20 min for twice, immediate after and 2h post-CFA injection</td>
<td>PWL to radiant heat,</td>
<td>Negativ e</td>
</tr>
<tr>
<td>21</td>
<td>Carrageenan</td>
<td>N/A</td>
<td>30 min, immediate after injection</td>
<td>PWL to hot plate test</td>
<td>Negativ e</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>20 min/each, immediate after, 2h after, and twice on day 2 after CFA injection</td>
<td>PWL to radiant heat,</td>
<td>Negativ e</td>
</tr>
<tr>
<td>25</td>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>30 min, 72 h after CFA</td>
<td>PWL to radiant heat,</td>
<td>Negativ e</td>
</tr>
<tr>
<td>26</td>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without</td>
<td>30min/day, pre CFA, 1 to 4 day post CFA, 5 treatments</td>
<td>PWT to von Frey filaments,</td>
<td>Negativ e</td>
</tr>
<tr>
<td>Patient</td>
<td>Treatment</td>
<td>Intervention Type</td>
<td>Location</td>
<td>Timing</td>
<td>Outcome</td>
<td>Other Observations</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
<td>-------------------</td>
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</tr>
<tr>
<td>Carrageenan</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>30 min, 3 h post carrageenan injection</td>
<td>PWL to radiant heat</td>
<td>Negativen</td>
<td></td>
</tr>
<tr>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>20 min for twice, immediate after and 2h post-CFA injection</td>
<td>PWL to radiant heat</td>
<td>Negativen</td>
<td></td>
</tr>
<tr>
<td>Cutaneous cancer pain</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>30 min/day, every other day, day 8-16 after inoculation, 5 treatments</td>
<td>PWL to radiant heat, Response latency to hot plate test</td>
<td>Negativen</td>
<td></td>
</tr>
<tr>
<td>CFA</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>30 min once, 5 day after CFA injection</td>
<td>PWL to radiant heat</td>
<td>Negativen</td>
<td></td>
</tr>
<tr>
<td>Carrageenan</td>
<td>N/A</td>
<td>30 min for once, immediately after carrageenan injection</td>
<td>Response latency to hot plate test</td>
<td>Negativen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCI</td>
<td>N/A</td>
<td>30 min/day, everyday from the first after operation for 14 days</td>
<td>PWL to radiant heat</td>
<td>Negativen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFA</td>
<td>Invasive</td>
<td>Non-acupoint: 5 mm left to GB30, GB34 with stimulation Contralateral: right GB30, GB34 with stimulation</td>
<td>30 min/day, every other day on from day 1 to day 13 after CFA</td>
<td>Ankle joints dorsiflexion pain test</td>
<td>Negativen</td>
<td></td>
</tr>
<tr>
<td>Carrageenan</td>
<td>Invasive</td>
<td>Needle insertion at acupoints without stimulation</td>
<td>for 1, 15 or 60 min, 3h after carrageenan</td>
<td>PPT using analgesymeter</td>
<td>Negativen</td>
<td></td>
</tr>
</tbody>
</table>
2.2.10 Discussion

2.2.10.1 Ideal EA parameters

The majority of the studies used an inflammation model to assess the anti-hyperalgesia effects of EA. The use of the neuropathic model and other models, such as IBS model and cancer pain model, was limited to one or two studies for each type. Thus, the result of EA effect in the inflammation model is more conclusive than that of the neuropathic models. It is recommended that future studies should consider the use neuropathic models.

Both immediate effect from a single EA treatment and cumulative effect from a series of EA treatment were assessed in these studies. In general, single treatment of EA produces anti-hyperalgesia effect that last for hours to days; and repetitive EA treatments produce cumulative effect that last for days after the cessation of the treatment. There is also evidence suggest the restoring effect of EA (Wang, Zhang et al. 2006).

Whilst EA generally produces anti-hyperalgesia effects, its’ capacity depends on the parameter of the EA application which include duration of treatment, pulse frequency, pulse intensity, pulse wave length, and acupoint.

It was shown with fixed parameters that EA with high frequency (100 Hz) produces short lasing (within 1 day) but high potent anti-hyperalgesia effects, whilst low frequency (10 Hz) shows prolonged (1 to 7 days) but less potent effect on PWL (Lao, Zhang et al. 2004).

Generally, EA with low frequency (2 or 10 Hz) produces better anti-edema effects than high frequency 100 Hz effects (Zhang, Lao et al. 2004; Lee, Jang et al. 2006). However, the anti-edema effect of high frequency is contradictory. One study showed that 100 Hz did not produce any anti-edema effect (Zhang, Lao et al. 2004), whilst another demonstrated that 120
Hz produced significant anti-edema effects. This may be due to the variation in other parameters.

Data also suggested that the anti-hyperalgesia effect is intensity dependent. Higher intensity (3 mA) produced the most potent anti-hyperalgesic effect compared to lower intensity (1 or 2 mA) (Lao, Zhang et al. 2004).

Treatment duration is another important parameter. Results suggest that longer duration is not necessarily more effective. It was found in the CFA model that two 20-minute EA treatments produce the best result when compared to two 10-minute and two 30-minute EA treatments, at either high or low frequency (Lao, Zhang et al. 2004). Whilst in the carrageenan model, 60-minute EA treatment produced better result than the 15-minute treatment. The influence of treatment duration on the anti-hyperalgesia effect may depend on which models are used (Taguchi and Taguchi 2007).

There is evidence to suggest that increasing pulse width has the similar anti-hyperalgesic effect as increasing frequency. However, this effect has not been systematically studied (Lao, Zhang et al. 2004). Pulse width ranging from 0.1 to 2 ms was adopted by most studies (Sekido, Ishimaru et al. 2003; Huang, Hu et al. 2004; Lao, Zhang et al. 2004; Zhang, Lao et al. 2004; Taguchi and Taguchi 2007).

Taken together, no single optimal EA combination was found. The optimal EA profile varied according to different models. One study identified that 10 Hz EA, with a intensity of 3 mA, and pulse width of 0.1 ms for 20 minutes, was the ideal combination for the CFA model (Lao, Zhang et al. 2004). The other study demonstrated 3 Hz EA for 60 minutes was the optimal combination for carrageenan-induced hyperalgesia (Taguchi and Taguchi 2007).
2.2.10.2 Implication for human studies

Currently, there is no literature available on human studies assessing anti-hyperalgesic effect of acupuncture. It is worth mentioning, outcome measures of hyperalgesia differ between studies in animals and humans. Thus, the result from animal studies cannot be directly applied to humans. Nevertheless, reviewing animal studies on this topic has provided valuable information for the future direction of human studies.

Based on the evidence of animal studies, and the assumption of the human equivalent of the measurements, it can be inferred that EA may have the anti-hyperalgesia effect in human hyperalgesia model as well.

Although the models and outcome measures of hyperalgesia differ in animals to humans. Thus, a range of parameters including response to both thermal and mechanical should be tested. EA should be used as none of the selected study used manual acupuncture. EA effects can be tested immediately after the treatment. The intensity of stimulation should be high with either high or low frequency. Additionally, EA on the same side of the injury is important.
Chapter 3 Methodology

3.1 Overall design

In order to answer the research aims of this project, two steps need to be taken. First is to identify a reliable hyperalgesia model and second is to test the anti-hyperalgesia effect of acupuncture on the model.

In the literature review section, two promising hyperalgesia models have been identified, one is topical capsaicin model, and another is the heat/capsaicin model. The capsaicin model is validated, low-cost, and easy-to-use. It does not require an instrument to produce heat stimulation and also allows the researchers to assess spontaneous pain, heat and mechanical hyperalgesia. However, despite its popularity, its reproducibility over a period of one month was not investigated by any studies. On the other hand, the reproducibility of the heat/capsaicin model is acceptable (Dirks, Petersen et al. 2003), but the device to produce this model was not available in this department at the time of the initial design. Furthermore, it is a model only used to test mechanical hyperalgesia but not other parameters, such as spontaneous pain. To tackle the dilemma, we decided to test the reproducibility of topical capsaicin model to find out whether it was suitable for this project.

Thus, the whole project consists of two experiments. In experiment one, we designed a two-session protocol to compare the key parameters of the topical capsaicin model over one month interval. If the results from this experiment indicated that the reproducibility was acceptable, we would use this model to test the anti-hyperalgesia effect of acupuncture. Otherwise, we would use the heat/capsaicin model for the second experiment.
3.2 HREC approvals

Both experiments was reviewed and approved by the Human Research Ethic Committee of RMIT University (Reference No. 13/07) (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. Both experiments were conducted at the clinical research laboratory of RMIT Chinese Medicine Research Group, Bundoora West Campus. The laboratory was a quiet, well-lit and temperature controlled (20 – 22 °C) room.

3.3 Subjects

Subjects were recruited from the local community via advertisements (Appendix 02, 03) posted on the RMIT Bundoora west campus. Volunteers who responded to the advertisement were screened according to the following inclusion and exclusion criteria

Inclusion criteria:

1) Aged between 18 and 40 years old healthy volunteers;
2) Agree to make themselves available for the period of the study;
3) Provide a written consent for participation.

Exclusion criteria:

Volunteers who take one or more of the following medications were excluded:

1) Analgesics (Medication for relieving pain)
2) Anti-inflammatory agents (Medication for reducing inflammation)
3) Anti-anxiety agents (Medication for reducing anxiety)
4) Anti-depressants (Medication for reducing depression)
5) Anti-psychotic agents (Medication for psychosis)
 Volunteers who have one or more of the following conditions were excluded:

01) Stoke
02) Epilepsy
03) Diabetes
04) Severe Alcoholism
05) Peripheral Vascular Disease
06) Peripheral Neuropathy
07) Psychosis
08) Heart disease
09) Impaired circulation in hands or feet
10) Wearing a cardiac pacemaker
11) Pregnancy
12) Have metal implant
13) A history of chronic pain
14) Having any types of pain currently
15) Allergies to chilli pepper or adhesive paper

For Experiment two, an additional item, volunteers did not have acupuncture treatment in the past one year, was added.

Included subjects were given Plain Language Statement (Appendix 04, 05) and verbal explanation regarding any questions about the experiment. Subjects were notified that they were free to withdraw at any time. A signed consent form (Appendix 07) was obtained from each subject prior to the commencement of the experiments.
3.4 Hyperalgesia model

3.4.1 Topical capsaicin model

1 mg/ml capsaicin solution was prepared by dissolving capsaicin powder (sigma) in 50% ethanol 50% distilled water. 0.1 ml of the solution was absorbed onto a patch of filter paper (2×2 cm²). The patch was applied to the middle of the forearm, and then covered with a piece of transparent dressing (3×3 cm²) to prevent evaporation (Zheng, Gibson et al. 2000). After 50 minutes, the capsaicin patch was removed, and the site was cleaned with alcohol swabs.

Previous studies show that topical application of capsaicin can reliably induce pain at a concentration between 1 mg/ml and 10 mg/ml (Helme and McKernan 1985; Green and Flammer 1988), and produce mechanical hyperalgesia at a minimum concentration of 0.1 mg/ml (Morris, Cruwys et al. 1997; Morris, Cruwys et al. 1998).
3.4.2 Heat/capsaicin model

To produce the heat/capsaicin model, moderate thermal stimulation (45°C) was given for five minutes in the middle of the non-dominant forearm. Capsaicin cream (Zotrix HP cream, 0.075% capsaicin) was then applied topically to the heated area for 30 minutes to achieve further sensitisation. The sensitised area was rekindled at 40°C for 5 minutes periodically throughout the session to maintain the status of mechanical hyperalgesia (Petersen and Rowbotham 1999; Dirks, Petersen et al. 2003).

The thermal stimulation is delivered by the Medoc TSA 2001 (Medoc, Ramat Yishai, Israel) (Photo 3.1) with a computer controlled thermode with a surface area of 3×3 cm² (Photo 3.2).
3.5 Measurements

Table 3.1 lists the outcome measures used in Experiment 1 and Experiment 2. MPT and stimulus-response function were only used in Experiment 1, while HPT and pain rating to long thermal stimulation were only used in Experiment 2. The area of secondary hyperalgesia was measured in both experiments.

Table 3.1 Outcome measures used in experiment 1 and 2

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical pain threshold (MPT)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Stimulus-response function</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Area of secondary hyperalgesia</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Heat pain threshold (HPT)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Pain rating to long thermal stimulus</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
3.5.1 Mechanical pain threshold (MPT)

MPT and stimulus-response function to mechanical stimulation were tested with a series of von Frey filaments (Stoelting, USA) (Photo 3.3) at different stages of Experiment 1. Von Frey filaments are the standard tool widely used for measuring mechanical sensitivity in both healthy subjects and patients with pain.

![Photo 3.3 A set of von Frey filaments](image)

The MPT was measured according to the guideline below:

1) Hold the end of the filament bar with the dominant hand while the wrist was support by a soft cushion 2-5 cm above the test surface;

2) Apply the filament perpendicularly to the test surface;

3) On contact with the test surface, increase force gradually by pushing the bar of the applicator till the filament starts to bend. At this point, the bearing force is the designed force of that particular filament.

To determine the MPT, we tested the skin of volar aspect of the non-dominant forearm of the subject using a pseudo-double random staircase method. Subject was asked to close their eyes and report whether a stimulus was painful or not. The least force that evokes sharp pain on 50% of occasions was recorded as the MPT. Stimuli were applied at an interval of 30 seconds.
and each lasted for 3 seconds. Within one test session, stimuli were not to be applied on the same point.

After determining the area of secondary hyperalgesia, we then measured MPT with von Frey filaments using the method described as above. The measurement was conducted twice in each session of the experiment. MPT was measured within the areas of primary and secondary hyperalgesia, and on the non-capsaicin side. Data was recorded on a pain threshold recording sheet (Appendix 08)

3.5.2 Stimulus-response function

In Experiment 1, five von Frey monofilaments (4.93, 5.07, 5.46, 6.10, and 6.45 log10 mgforce) were used to test the stimulus-response function.

These five filaments were applied randomly to avoid any order effects. The random order was generated using Excel. They were applied twice on the skin of volar aspect of the capsaicin and non-capsaicin sides. Each filament was applied for 3 seconds with a 10-second interval during which subjects were asked to rate the stimulus on a modified 100-mm VAS (where 0=no sensation, 50mm= just painful, 100mm= worst pain possible) (Appendix 10). The mean VAS ratings to the repeated stimuli were then computed and used for statistical analyses.
3.5.3 Area of secondary hyperalgesia

The area of secondary hyperalgesia was measured by applying a von Frey filament (4.93) at a point 8 cm away from the center of the capsaicin area and then moving towards the center at approximately 1 cm intervals every 2 seconds. Subjects were provided with a written instruction about skin hypersensitivity (Appendix 09) and asked to report when the filament caused a definite increase in the magnitude of pricking sensation or pain. This point was marked on the skin using a colored fiber-tipped pen and this process was repeated in a pattern of eight radial lines from the center of the capsaicin site (Figure 3.1). The resulting eight points were connected to define the outline of secondary hyperalgesia, which was then transferred onto a transparent plastic sheet.

Figure 3.1 Illustration of measurement for the area of secondary hyperalgesia
The area of secondary hyperalgesia was then measured with a Digital Planimeter (Planix, Tamaya & Company Ltd., Japan) (Photo 3.4).

![Photo 3.4 Digital planimeter](image)

### 3.5.4 Heat pain threshold (HPT)

HPT is the lowest temperature that the subjects perceived as painful. In Experiment 2 it was measured on the forearm of both capsaicin and non-capsaicin sides using a 3×3 cm² thermode (Medoc TSA 2001). The test used was a modified Limits test with four continuous heat stimulations. The baseline temperature of the thermode was 32°C. After the test began, it increased at a rate of 1°C/s. Subjects indicated when the pain threshold (the point they began to feel the stimulation became painful) was reached by pressing the Yes mouse button. The temperature was automatically recorded by the computer and the thermode temperature immediately returned to baseline. The cut-off temperature was set at 50°C. The next stimulation was given 4 to 6 seconds after the restoration of the baseline. The HPT was calculated as the average of 4 measurements.

### 3.5.5 Painfulness of the long thermal stimulation

In Experiment 2, painfulness to the long thermal stimulation (40°C, 1 minute) was measured during baseline measurement and in the first minute during each rekindling, using a modified
VAS (Appendix 11). Subjects were instructed to rate their sensation at 5, 10, 15, 25, 35, 45 and 55 seconds to the stimulation.

### 3.6 Method of randomisation and double-blinding

Randomisation and blinding were used in Experiment 2. Before the acupuncture intervention, subjects drew a sealed envelope which contained a random number, indicating the assignment to either REA or SEA group. The random number sequence was generated with Microsoft Excel (Microsoft Office, Windows version) by an independent investigator who was involved in neither acupuncture intervention nor testing. The acupuncturist was the only person who knew the group assignment, and was blinded from the outcome measures. The investigator who performs the test was blinded from the group assignment and the process of the acupuncture intervention. During the acupuncture treatment, subjects lay on a treatment bed in supine posture and their vision to the site of the acupuncture was blocked. By the end of the treatment they were informed not to disclose any information about the nature of their treatment to the investigator who performed tests on them. During the data analysis stage, an independent investigator conducted the data analysis.

### 3.7 Acupuncture interventions

In Experiment two, the non-invasive sham electro-acupuncture was employed.

Acupuncture needles were eight 0.3×40 mm sterile single-use needles with guide tube (Huato, Suzhou Medical Appliance Company, China)

### 3.7.1 Selection of acupoints

Four acupoints on both sides were selected: Zusanli (ST36) and Fenglong (ST40), Hegu (LI4) and Shousanli (LI10). These points were commonly used for pain treatment (Chen 1994). The
following methods were used for locating these acupoints:

ST36: “With a flexed knee or in a supine position, ask the patient put the thumb on the middle of the patella with the remaining four fingers closed together and placed on the lateral side of the patella. The point is located at the tip of the middle finger. Alternative method: the point is located in the fossa one finger breadth lateral to the anterior margin of the tibia, and 3 inches inferior to Dubi (ST35).” Page 68 (Chen 1994)

ST40: “The point is located at the midpoint between the inferior margin of the patella and skin crease of the ankle joint, 1.5 inches lateral to the anterior margin of the tibia, and between the tibia and fibula; or 8 inches above the lateral malleolus and one finger’s breadth lateral to TiaoKuo (ST38).” Page 73 (Chen 1994)

LI4: “The point is located on the dorsum of the hand, between the first and second metacarpals at the midpoint of the radial margin of the second metacarpal bone. Alternative method: ask the patient to adduct the thumb and the index finger; the point is located at the highest spot of the first and second metacarpal muscles.” Page 37-38 (Chen 1994)

LI10: “With the elbow flexed, the point is located on the radial side of the elbow on the line between Quchi (LI11) and Yangxi (LI5), and 2 inches below Quchi (LI11); or clenching the hand firmly and flexing the elbow, the point is located in the fosa of the brachioradialis muscle.” Page 20 (Chen 1994)
3.7.2 REA

Needles were inserted into acupoints to a depth of 15-25 mm, and were manipulated to achieve De Qi sensations (described as soreness, numbness, or distension at the needling site). A bipolar electrical acupuncture stimulator (MEE 501, Australia) (photo 3.5) was connected to the eight acupoints on both sides of the body using four pairs of electrodes. The parameter for REA was dense-disperse (D-D) mode with alternating frequency between 5 and 15 Hz. The intensity was initially set to a strong but comfortable level with visible muscle contraction; and was further adjusted twice during the treatment to cater for the subject’s tolerance. The duration of EA treatment was 25 minutes.

Photo 3. 5 A modified electrical acupunture stimulator (MEE 501, Australia)
3.7.3 SEA

Non-invasive sham was used in this trial. First, an empty plastic guide tube was tapped at each acupoint to produce the discernible sensation; then bent needles with adhesive bandage (Photo 3.6) were taped to the dermal surface of each acupoint; and was connected to a mock electrical acupuncture stimulator without delivering electrical stimulation. The stimulator was placed within the subject’s sight, showing a continuously flashing light. The acupuncturist adjusted the stimulator twice. The treatment duration was 25 minutes during which bended needles were pressed to the skin three times to produce some discernible sensation. De Qi sensation was not intended to be produced during SEA.

Table 3.2 is a detailed comparison of REA and SEA procedure.

Photo 3.6 sham acupuncture
Table 3.2 A comparison of REA and SEA

<table>
<thead>
<tr>
<th></th>
<th>REA</th>
<th>SEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posture of subjects</td>
<td>Supine</td>
<td>Supine</td>
</tr>
<tr>
<td>Acupoints</td>
<td>Zusanli (ST36) and Fenglong (ST 40), Hegu (LI4) and Shousanli (LI10) on both sides of the body</td>
<td>Zusanli (ST36) and Fenglong (ST40), Hegu (LI4) and Shousanli (LI10) on both sides of the body</td>
</tr>
<tr>
<td>Insertion</td>
<td>15-25 mm</td>
<td>No insertion, tapped on acupoint</td>
</tr>
<tr>
<td>De Qi</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Duration</td>
<td>25 minutes</td>
<td>25 minutes</td>
</tr>
<tr>
<td>Schedule for adjusting the stimulation</td>
<td>Every 10 minutes</td>
<td>Every 10 minutes</td>
</tr>
<tr>
<td>Parameter</td>
<td>dense-disperse, 5-15 Hz</td>
<td>N/A</td>
</tr>
<tr>
<td>Intensity</td>
<td>Adjust to strong but comfortable level with muscle contraction</td>
<td>N/A</td>
</tr>
<tr>
<td>EA stimulator</td>
<td>Connected to the needles via alligator clips, delivering electrical pulse</td>
<td>Connected to the needles via alligator clips, without electrical stimulation, only showing flashing light within subject’s eyesight</td>
</tr>
</tbody>
</table>

3.8 Statistical analysis and sample size

3.8.1. Statistical analysis

For Experiment 1, ratings to von Frey filaments stimulation and the size of the area of secondary hyperalgesia were analyzed with two-way Analysis of Variance (ANOVA) with two repeated measures to test the effects of time and session. Mechanical pain threshold was analyzed with non-parametric Friedman tests. The responses within each group to topical application of capsaicin were correlated via Pearson’s correlation coefficient. SPSS software (Windows Version 16.0) was used.

For Experiment 2, data were summarized as means and standard deviations (SD) in the tables and means and standard error of mean (SEM) or percentage in the figures. The experiment was a two way (time point and intervention group) with one repeated measure (time point)
design. Two-way ANOVA was used to analyze the effect of intervention on the size of secondary hyperalgesia area, heat pain threshold and VAS rating to long thermal stimulation. A $p$-value of 0.05 (P<0.05) was considered statistically significant. *Independent-samples T test* was used to compare the age difference between groups. For the categorical data such as gender, hand dominance, sham procedure credibility and acupuncture perception, the *chi-squared test* was used.

In both experiments, a $p$-value of 0.05 was considered statistically significant.

Data analysis was conducted by an independent researcher who was blinded to the group assignment and tests. All of the statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS, Windows Version 16.0).

### 3.8.2. Sample size and power calculation

For Experiment 1, since no similar experiment was published, the sample size was estimated based on a study (Hughes, Macleod et al. 2002) in which 12 subjects were recruited to assess the reproducibility of intradermal capsaicin model.

For Experiment 2, as this is the first study assessing the anti-hyperalgesia effect in human subject, no previous data was available for the sample size calculation. The sample size was calculated based on the data from a drug study (Petersen, Jones et al. 2001) using heat/capsaicin model. In this study, the mean area of secondary hyperalgesia reduced from $142 \pm 60 \text{ cm}^2$ to $34 \pm 17 \text{ cm}^2$ during the drugs infusion, with a sample size of 14 subjects. Based on an online sample size calculator (http://home.clara.net/sisa/samsize.htm), the recommended sample size was 29 per group to achieve 80% power.
By the end of the Experiment 2, post-hoc power analysis and sample size calculations were done using G*Power (Version 3.0.10).
Chapter 4 Experiment 1: the reproducibility of hyperalgesia in the topical capsaicin model

4.1 Introduction
In order to evaluate the effect of EA on hyperalgesia in human subjects, we need a hyperalgesia model that can be reliably reproduced on the same subject over a period of time. Capsaicin is the most commonly used hyperalgesia due to its unique features. It can be applied either topically or intradermally. It is found that the intradermal capsaicin injection produces relatively reliable areas of allodynia and hyperalgesia within a subject over a period of one hour (Hughes, Macleod et al. 2002). However intradermal injection of capsaicin also elicits severe pain that cannot be accepted by all subjects. Therefore, we chose topical capsaicin model for the current research. The topical capsaicin model has been widely used in hyperalgesia research for decades. However, its reproducibility has not been examined systematically. This pilot study aimed to evaluate the between-session, within-subject reproducibility of this hyperalgesia model over a period of one month. Mechanical hyperalgesia will be assessed as it is an indication of central sensitisation and could be used to test the central effect of acupuncture.

4.2 Aims
The aim of this pilot study was to examine the reproducibility of several key parameters of this model, including the area of mechanical hyperalgesia, mechanical pain threshold, ratings to sub- and supra-threshold mechanical stimulation and rating to spontaneous pain in healthy volunteers at one month interval.

4.3 Design
This experiment is a two-session trial conducted on 12 healthy volunteers (7 male, 5 female). In each session, capsaicin solution (0.1 ml, 1mg/ml) was applied topically onto the non-
dominant forearm for 45 minutes. A series of tests were carried out to determine the time-course of the hyperalgesia in these subjects. The two sessions were separated by an interval of one month. The data from the two sessions was compared to determine the reproducibility of this model.

4.3.1 Procedure

Table 4.1 demonstrates the detailed procedure of the experiment.

Before the experiment, subjects were instructed not to drink coffee on the day of the experiment. On the day of the experiment, the eligible subjects were first asked to be familiarised with the procedure and tests of the experiment. A series of tests was then conducted, including measuring mechanical pain threshold and stimulus response function to mechanical stimulation. Refer to “Methods” chapter for detailed description of these tests. Immediately after the baseline measurements, a capsaicin patch (1×1 cm²) saturated with capsaicin solution (0.1 ml, 1mg/ml) was applied topically onto the skin in the middle of the non-dominant forearm. Forty-five minutes later the patch was removed and residue capsaicin was cleaned. After a 10-minute rest, the subjects went through the first session of post-capsaicin measurements. Sixty minutes after the removal of the capsaicin patch, the second session of post-capsaicin measurement was conducted. The whole session took approximately three hours, and was repeated one month later.
Table 4.1 Procedure and time points of the experiment

<table>
<thead>
<tr>
<th>Time point</th>
<th>Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20 min</td>
<td>Preparation: eligible subjects were familiarised with the procedure and instruments of the study. The consent form was signed before the commencement of the trial.</td>
</tr>
<tr>
<td>-15 min</td>
<td>Pre-capsaicin (baseline) measurements: (1) mechanical pain threshold (2) stimulus-response function (VAS)</td>
</tr>
<tr>
<td>0-45 min</td>
<td>Application of the capsaicin patch immediately after the baseline measurements.</td>
</tr>
<tr>
<td>45 min</td>
<td>Remove the capsaicin patch</td>
</tr>
<tr>
<td>45-55 min</td>
<td>Rest</td>
</tr>
<tr>
<td>55-75 min</td>
<td>post-capsaicin measurement 1: (1) area of the secondary hyperalgesia; (2) mechanical pain threshold within the secondary hyperalgesia area primary hyperalgesia area; (3) stimulus-response function (VAS) within the secondary hyperalgesia area.</td>
</tr>
<tr>
<td>75-115 min</td>
<td>Rest</td>
</tr>
<tr>
<td>115-135 min</td>
<td>post-capsaicin measurements 2: (1) area of the secondary hyperalgesia; (2) mechanical pain threshold within the secondary hyperalgesia area primary hyperalgesia area; (4) stimulus-response function (VAS) within the secondary hyperalgesia area.</td>
</tr>
</tbody>
</table>

Please refer to Methodology (Chapter 3) for detailed description regarding screening of subjects, producing the topical capsaicin model, outcome measurements, and statistical methods.
4.4 Results

4.4.1 General information of subject

The experiment was conducted from July to October 2007. In total, 12 subjects were recruited based on the inclusion and exclusion criteria. All subjects signed the consent form (Appendix 07) before starting the experiment and completed the two-session trial. No side effects were reported.

The average age of subjects was 30.50 ± 4.72 (Mean ± SD), ranging from 25 to 38 years. There were 7 male and 5 female, all of whom were right-handed.
4.4.2 Development of hyperalgesia sensitisation

After the topical capsaicin application, hyperalgesia developed in both sessions.

Table 4.2 shows the area of mechanical hyperalgesia in the two sessions. Immediately after capsaicin application, the mean area was 33 cm² in session one and 29 cm² in session two, and dropped significantly to 25 cm² and 22 cm² immediately and one hour after the removal of capsaicin patch, respectively (time effect: F (1, 11) = 57.978; p < 0.001). There was no main effect of sessions (F (1, 11) = 0.363, p = 0.199) (Figure 4.1).

Table 4.2 Area of secondary hyperalgesia in session one and two

<table>
<thead>
<tr>
<th>Session No. (n = 12)</th>
<th>Post-capsaicin 1 (immediately after capsaicin) (Mean ± SD)</th>
<th>Post-capsaicin 2 (one hour after capsaicin) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33 ± 12 cm²</td>
<td>25 ± 10 cm²</td>
</tr>
<tr>
<td>2</td>
<td>29 ± 6 cm²</td>
<td>22 ± 8 cm²</td>
</tr>
</tbody>
</table>

Figure 4.1 Area of secondary hyperalgesia to a von Frey filament in first and second session
Data are expressed as mean ± S.E.M. post-capsaicin 1 = immediately after capsaicin; post-capsaicin 2 = one hour after capsaicin
Table 4.3 and figure 4.3 show the VAS rating to von Frey filaments stimulation during each session. Rating to five von Frey filaments increased as the pressure increased (significant bar effect: session 1, $F(4, 44) = 71.998, p < 0.001$; session 2, $F(4, 44) = 88.202, p < 0.001$). The overall rating of each bar increased significantly after capsaicin application (time effect: session 1, $F(2, 22) = 10.658, p = 0.001$; session 2, $F(2, 22) = 18.222, p < 0.001$), demonstrating a left-shift phenomenon. Before capsaicin, stimulation delivered with Bars 4 and 5 were considered to be painful. After capsaicin, Bar 3 was also considered painful.
<table>
<thead>
<tr>
<th>Session No.</th>
<th>Stage</th>
<th>Von Frey bar</th>
<th>VAS rating (Mean ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pre-capsaicin (Before capsaicin)</td>
<td>Bar 1</td>
<td>1.7 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 2</td>
<td>2.1 ± 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 3</td>
<td>3.1 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 4</td>
<td>5.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 5</td>
<td>7.1 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Post-capsaicin 1 (Immediately after capsaicin)</td>
<td>Bar 1</td>
<td>3.7 ± 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 2</td>
<td>3.5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 3</td>
<td>5.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 4</td>
<td>6.9 ± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 5</td>
<td>8.0 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Post-capsaicin 2 (One hour after capsaicin)</td>
<td>Bar 1</td>
<td>3.4 ± 2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 2</td>
<td>3.6 ± 2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 3</td>
<td>5.0 ± 2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 4</td>
<td>6.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 5</td>
<td>7.7 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>Pre-capsaicin (Before capsaicin)</td>
<td>Bar 1</td>
<td>1.2 ± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 2</td>
<td>1.6 ± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 3</td>
<td>2.4 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 4</td>
<td>5.9 ± 1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 5</td>
<td>7.3 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Post-capsaicin 1 (Immediately after capsaicin)</td>
<td>Bar 1</td>
<td>4.0 ± 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 2</td>
<td>4.4 ± 2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 3</td>
<td>4.8 ± 2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 4</td>
<td>7.1 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 5</td>
<td>8.5 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Post-capsaicin 2 (One hour after capsaicin)</td>
<td>Bar 1</td>
<td>3.5 ± 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 2</td>
<td>3.9 ± 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 3</td>
<td>4.7 ± 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 4</td>
<td>6.8 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bar 5</td>
<td>8.3 ± 1.0</td>
</tr>
</tbody>
</table>
On the non-capsaicin side, rating to five von Frey filaments also increased as the pressure increased (significant bar effect: session 1, $F(4, 44) = 85.334, p < 0.001$; session 2, $F(4, 44) = 135.954, p < 0.001$), indicating the subjects differentiated the sensation induced by different bars successfully. Time effect was not significant (time effect: session 1, $F(2, 22) = 0.429, p = 0.657$; session 2, $F(2, 22) = 0.883, p = 0.428$), indicating hyperalgesia was not induced on the non-capsaicin side.
Table 4.4 and Figure 4.4 show the mechanical pain thresholds in primary and secondary hyperalgesia area in both sessions. There was a significant decrease in the mechanical pain threshold after the application of capsaicin in both sessions within the areas of primary hyperalgesia and secondary hyperalgesia, respectively. The pain threshold was measured as actual forces (gram/cm²) and then each converted into a \( \log_{10} \) scale measurement. *Friedman’s* non-parametric ANOVA was used to assess for the differences among different stages. Friedman’s test produced a chi-squared value, which was assessed for statistical significance via a \( p \)-value.

<table>
<thead>
<tr>
<th></th>
<th>Pre-capsaicin</th>
<th>Post-capsaicin 1</th>
<th>Post-capsaicin 2</th>
<th>Friedman test ( \chi^2(df) )</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Session 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT (capsaicin side, primary hyperalgesia area) ( (mean \pm SD) )</td>
<td>5.77 ± 0.26</td>
<td>4.98 ± 0.34</td>
<td>5.04 ± 0.30</td>
<td>18.766(2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PT (capsaicin side, secondary hyperalgesia area) ( (mean \pm SD) )</td>
<td>5.77 ± 0.26</td>
<td>4.86 ± 0.34</td>
<td>4.86 ± 0.43</td>
<td>18.426(2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PT (non-capsaicin side) ( (mean \pm SD) )</td>
<td>5.70 ± 0.33</td>
<td>5.60 ± 0.42</td>
<td>5.49 ± 0.52</td>
<td>4.526(2)</td>
<td>0.104</td>
</tr>
<tr>
<td><strong>Session 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT (capsaicin side, primary hyperalgesia area) ( (mean \pm SD) )</td>
<td>5.85 ± 0.12</td>
<td>5.14 ± 0.39</td>
<td>5.16 ± 0.39</td>
<td>16.650(2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PT (capsaicin side, secondary hyperalgesia area) ( (mean \pm SD) )</td>
<td>5.85 ± 0.12</td>
<td>4.99 ± 0.30</td>
<td>5.04 ± 0.44</td>
<td>17.149(2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PT (non-capsaicin side) ( (mean \pm SD) )</td>
<td>5.85 ± 0.12</td>
<td>5.75 ± 0.23</td>
<td>5.59 ± 0.34</td>
<td>4.222(2)</td>
<td>0.121</td>
</tr>
</tbody>
</table>
Figure 4. 3 Mechanical pain thresholds to von Frey filaments within the primary and secondary hyperalgesia in both secessions
Data are expressed as mean ± S.E.M.
Pre-capsaicin = before capsaicin; post-capsaicin 1 = immediately after capsaicin; post-capsaicin 2 = one hour after capsaicin;
4.4.3 Reproducibility of area of secondary hyperalgesia area

As shown in the Table 4.5, the area of secondary hyperalgesia in the two sessions showed significant correlation immediately after the removal of capsaicin, but not one hour after.

Table 4.5 Correlation of hyperalgesia area in session one and two

<table>
<thead>
<tr>
<th>Stage</th>
<th>Sample size</th>
<th>Pearson Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-capsaicin 1</td>
<td>12</td>
<td>0.643</td>
<td>0.024*</td>
</tr>
<tr>
<td>Post-capsaicin 2</td>
<td>12</td>
<td>0.456</td>
<td>0.136</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed)
4.4.4 Reproducibility of the pain threshold

Table 4.6 shows the correlation of pain threshold of both sessions in each stage of the experiment. There was no significant correlation detected on the non-capsaicin (right) side. On the capsaicin (left) side, correlation was significant in the final stage of the experiment, which was one hour after the removal of capsaicin.

Table 4.6 Correlation of mechanical pain threshold measured in session one and two

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Sample size</th>
<th>Pearson Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-capsaicin: baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain threshold on the non-capsaicin side</td>
<td>12</td>
<td>0.234</td>
<td>0.464</td>
</tr>
<tr>
<td>Pain threshold on the capsaicin side</td>
<td>12</td>
<td>-0.133</td>
<td>0.680</td>
</tr>
<tr>
<td>Post-capsaicin 1: immediately after capsaicin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain threshold on the non-capsaicin side</td>
<td>12</td>
<td>0.017</td>
<td>0.959</td>
</tr>
<tr>
<td>Pain threshold in the primary hyperalgesia area</td>
<td>12</td>
<td>0.553</td>
<td>0.062</td>
</tr>
<tr>
<td>Pain threshold in the secondary hyperalgesia area</td>
<td>12</td>
<td>0.429</td>
<td>0.164</td>
</tr>
<tr>
<td>Post-capsaicin 2: one hour after capsaicin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain threshold on the non-capsaicin side</td>
<td>12</td>
<td>0.531</td>
<td>0.076</td>
</tr>
<tr>
<td>Pain threshold in the primary hyperalgesia area</td>
<td>12</td>
<td>0.745</td>
<td>0.005**</td>
</tr>
<tr>
<td>Pain threshold in the secondary hyperalgesia area</td>
<td>12</td>
<td>0.644</td>
<td>0.024*</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)
4.4.5 Reproducibility of the VAS rating

Tables 4.7 and 4.8 show the correlation of VAS ratings to mechanical stimulation applied on both sides. On the non-capsaicin side, there was moderate, non-significant correlation at baseline, with one significant correlation ($p < 0.05$); the correlation became more obvious immediately and one hour after the capsaicin, three out of five rating were found to be significantly correlated ($p < 0.05$ and $p < 0.01$).

Similarly, correlation on the capsaicin side was moderate and significant at the baseline stage (two bars, $p < 0.05$ and $p < 0.01$), and improved after capsaicin. Four out five rating were found significantly correlated immediately after capsaicin ($p < .05$ and $p < 0.01$), and all five rating correlated significantly one hour after capsaicin removal ($p < 0.01$).

In general, the number of significant correlations increased from baseline to immediately after capsaicin and one hour after capsaicin on both sides of the forearm. However, the number of significant correlations obtained was more on the capsaicin side compared to the non-capsaicin side.
Table 4.7  Correlations of VAS rating side in session one and two on the non-capsaicin

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Sample size</th>
<th>Pearson Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS rating 1</td>
<td>12</td>
<td>0.546</td>
<td>0.066</td>
</tr>
<tr>
<td>VAS rating 2</td>
<td>12</td>
<td>0.660</td>
<td>0.020*</td>
</tr>
<tr>
<td>VAS rating 3</td>
<td>12</td>
<td>0.503</td>
<td>0.096</td>
</tr>
<tr>
<td>VAS rating 4</td>
<td>12</td>
<td>0.360</td>
<td>0.250</td>
</tr>
<tr>
<td>VAS rating 5</td>
<td>12</td>
<td>0.431</td>
<td>0.162</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Sample size</th>
<th>Pearson Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS rating 1</td>
<td>12</td>
<td>0.517</td>
<td>0.085</td>
</tr>
<tr>
<td>VAS rating 2</td>
<td>12</td>
<td>0.782</td>
<td>0.003**</td>
</tr>
<tr>
<td>VAS rating 3</td>
<td>12</td>
<td>0.659</td>
<td>0.020*</td>
</tr>
<tr>
<td>VAS rating 4</td>
<td>12</td>
<td>0.774</td>
<td>0.003**</td>
</tr>
<tr>
<td>VAS rating 5</td>
<td>12</td>
<td>0.459</td>
<td>0.133</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Sample size</th>
<th>Pearson Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS rating 1</td>
<td>12</td>
<td>0.737</td>
<td>0.006**</td>
</tr>
<tr>
<td>VAS rating 2</td>
<td>12</td>
<td>0.758</td>
<td>0.04*</td>
</tr>
<tr>
<td>VAS rating 3</td>
<td>12</td>
<td>0.328</td>
<td>0.298</td>
</tr>
<tr>
<td>VAS rating 4</td>
<td>12</td>
<td>0.429</td>
<td>0.164</td>
</tr>
<tr>
<td>VAS rating 5</td>
<td>12</td>
<td>0.707</td>
<td>0.010*</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)
Table 4.8 Correlations of VAS rating in session one and two on the capsaicin side

<table>
<thead>
<tr>
<th>Pre-capsaicin: baseline</th>
<th>Measurements</th>
<th>Sample size</th>
<th>Pearson Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS rating 1</td>
<td>12</td>
<td>0.248</td>
<td>0.437</td>
<td></td>
</tr>
<tr>
<td>VAS rating 2</td>
<td>12</td>
<td>0.692</td>
<td>0.013*</td>
<td></td>
</tr>
<tr>
<td>VAS rating 3</td>
<td>12</td>
<td>0.396</td>
<td>0.202</td>
<td></td>
</tr>
<tr>
<td>VAS rating 4</td>
<td>12</td>
<td>0.551</td>
<td>0.064</td>
<td></td>
</tr>
<tr>
<td>VAS rating 5</td>
<td>12</td>
<td>0.786</td>
<td>0.002**</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Post-capsaicin: immediately after capsaicin</th>
<th>Measurements</th>
<th>Sample size</th>
<th>Pearson Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS rating 1</td>
<td>12</td>
<td>0.673</td>
<td>0.017*</td>
<td></td>
</tr>
<tr>
<td>VAS rating 2</td>
<td>12</td>
<td>0.654</td>
<td>0.021*</td>
<td></td>
</tr>
<tr>
<td>VAS rating 3</td>
<td>12</td>
<td>0.847</td>
<td>0.001**</td>
<td></td>
</tr>
<tr>
<td>VAS rating 4</td>
<td>12</td>
<td>0.567</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td>VAS rating 5</td>
<td>12</td>
<td>0.731</td>
<td>0.007**</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Post-capsaicin: one hour after capsaicin</th>
<th>Measurements</th>
<th>Sample size</th>
<th>Pearson Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS rating 1</td>
<td>12</td>
<td>0.888</td>
<td>&lt; 0.001**</td>
<td></td>
</tr>
<tr>
<td>VAS rating 2</td>
<td>12</td>
<td>0.881</td>
<td>&lt; 0.001**</td>
<td></td>
</tr>
<tr>
<td>VAS rating 3</td>
<td>12</td>
<td>0.797</td>
<td>0.002**</td>
<td></td>
</tr>
<tr>
<td>VAS rating 4</td>
<td>12</td>
<td>0.840</td>
<td>0.001**</td>
<td></td>
</tr>
<tr>
<td>VAS rating 5</td>
<td>12</td>
<td>0.865</td>
<td>&lt; 0.001**</td>
<td></td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)
4.5 Conclusion

Topical capsaicin application (0.1 ml, 1 mg/ml, 1×1cm²) successfully induced hyperalgesia in both sessions. The significant change in the area of secondary punctate hyperalgesia, mechanical pain threshold and VAS rating to von Frey filaments stimulation indicated the development of skin sensitivity. On the non-capsaicin side, VAS rating and pain threshold remains unchanged, indicating capsaicin did not sensitise the skin on the opposite of the body.

The statistical analysis showed that the correlations of area of secondary hyperalgesia and mechanical pain threshold between the two sessions were poor, suggesting between-day within-subject reproducibility was poor to justify their use in the acupuncture study. However, the VAS rating on the capsaicin side of the two sessions was found correlated with each other, especially after the capsaicin sensitisation. This suggests that VAS rating might be a reliable measurement for the evaluation of intervention. However, these results alone does not justify the use of topical capsaicin model in evaluating the acupuncture effect on hyperalgesia, as the primary outcome measure, which is the hyperalgesia area, was not reliable for the evaluation of acupuncture’s effect on central sensitisation.
Chapter 5: Experiment 2-the anti-hyperalgesia effect of EA in healthy humans

5.1 Introduction

Experiment 1 demonstrated that hyperalgesia induced by using the topical capsaicin model was reduced over time. Furthermore, its between-day reproducibility of area of secondary hyperalgesia and mechanical pain threshold were poor. These two weaknesses do not allow us to evaluate the effect of acupuncture on the same subject over a period of one month. In this experiment a more stable model called heat/capsaicin model was used to assess the effect of electroacupuncture (EA) on hyperalgesia as compared to a sham procedure. This was made possible after the purchase of the heat simulator Medoc TSA 2001 (Refer to Methodology, Chapter 3) by the Chinese Medicine Division. The heat/capsaicin model has been successfully used in a number of drug studies to assess their anti-hyperalgesia effect (Refer to Review Chapter 2). In previous drug studies, cross-over designs were widely used. However, in the acupuncture study, it is not appropriate to use this design as subjects can easily distinguish the difference between real and sham electroacupuncture.

5.2 Aims

The primary aim of this experiment was:

1. To compare the effect of real electroacupuncture (REA) and sham electroacupuncture (SEA) on mechanical hyperalgesia in a heat/capsaicin model.

The secondary aim was:

2. To compare the effect of REA and SEA on Heat Pain Threshold (HPT), and pain rating on the capsaicin-treated and untreated (contralateral) sides of the body.
5.3 Design

The current experiment is a one-session, double-blind, randomised, sham acupuncture-controlled study in healthy human subjects.

Twenty young volunteers who responded to the advertisements were screened according to the inclusion and exclusion criteria. All eligible volunteers who signed the consent form (Appendix 07) before participating in the experiment were recruited.

5.3.1 Procedure

Table 5.1 illustrates the detailed procedure used in the experiment. Full experimental procedure details were explained to subjects. Subjects were familiarised with the HPT test and the use of VAS. During the baseline (pre-capsaicin) measurement, HPT in both forearms, and rating to the long thermal stimulation (40°C for 1 minute) was measured. Following this, hyperalgesia was produced on the non-dominant forearm of subject, referred to as capsaicin side, and dominant forearm, referred to as non-capsaicin side, by heating an area of 3×3 cm² to 45°C in the middle of the forearm for 5 minutes, followed by the application of a thick layer of capsaicin cream (0.075%) in the sensitised site for 30 minutes. Subjects were allowed to rest for 35 minutes following the removal of the capsaicin cream. The sensitised area was then rekindled with heat stimulation at 40°C for 5 minutes. Subjects was asked to rate the stimulation every 10 seconds during the first minute. The area of the secondary hyperalgesia was measured with a von Frey filament immediately after the rekindling and the previous measurements at baseline (pre-capsaicin) were repeated. Subjects were then randomly allocated to receive a 25-minute intervention of either REA or SEA delivered by an acupuncturist who was blinded to the group allocation. Forty minutes after the first rekindling, the second rekindling was given and the same measurements were taken. The third rekindling was given 40 minutes after the second rekindling, and no measurements were taken.
after this rekindling. The last rekindling was given 40 minutes after the third one, and the previous measurements (area of hyperalgesia, HPT) were repeated. The whole session took about four and half hours.
Table 5. 1 Procedure and time points of the experiment

<table>
<thead>
<tr>
<th>Time point</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30 min</td>
<td>Explain the procedure to the subjects; subjects completed the forms and tasks to familiarize themselves with the tests.</td>
</tr>
<tr>
<td>-15 min</td>
<td>Locate and mark the heat/capsaicin site</td>
</tr>
<tr>
<td>-10 min</td>
<td><strong>Baseline (pre-capsaicin) measurement</strong></td>
</tr>
<tr>
<td>-10 min</td>
<td>Measure Heat Pain Threshold (HPT) on the non-capsaicin side</td>
</tr>
<tr>
<td>-10 min</td>
<td>Measure HPT on the capsaicin side</td>
</tr>
<tr>
<td>-10 min</td>
<td>Subjects rate the long thermal stimulation (40°C, 1 min) applied to the capsaicin side using VAS.</td>
</tr>
<tr>
<td>0 min</td>
<td>Apply thermal stimulation (45°C) on the capsaicin side for 5 min to sensitised the skin</td>
</tr>
<tr>
<td>5 min</td>
<td>Application of topical capsaicin cream for 30 min</td>
</tr>
<tr>
<td>35 min</td>
<td>Remove capsaicin cream</td>
</tr>
<tr>
<td>35-70 min</td>
<td>Rest 1</td>
</tr>
<tr>
<td>70-75 min</td>
<td>Rekindle (rekindling 1) the sensitised area (40°C for 5 min), and subjects rate the first minute using VAS</td>
</tr>
<tr>
<td>75-120 min</td>
<td><strong>Measurement 1 (rekindling 1)</strong></td>
</tr>
<tr>
<td>75-120 min</td>
<td>Measure Area of the secondary hyperalgesia</td>
</tr>
<tr>
<td>75-120 min</td>
<td>Measure HPT on the non-capsaicin side</td>
</tr>
<tr>
<td>75-120 min</td>
<td>Measure HPT on the capsaicin side</td>
</tr>
<tr>
<td>75-120 min</td>
<td><strong>Intervention</strong></td>
</tr>
<tr>
<td>75-120 min</td>
<td>Real or sham EA</td>
</tr>
<tr>
<td>120-125 min</td>
<td>Rekindle (rekindling 2) the sensitised area (40°C for 5 min)</td>
</tr>
<tr>
<td>125-165 min</td>
<td><strong>Measurement 2 (rekindling 2)</strong></td>
</tr>
<tr>
<td>125-165 min</td>
<td>Area of the secondary hyperalgesia</td>
</tr>
<tr>
<td>125-165 min</td>
<td>Measure HPT on the non-capsaicin side</td>
</tr>
<tr>
<td>125-165 min</td>
<td>Measure HPT on the capsaicin side</td>
</tr>
<tr>
<td>125-165 min</td>
<td><strong>Rest 2</strong></td>
</tr>
<tr>
<td>165-170 min</td>
<td>Rekindle (rekindling 3) the sensitized area (40°C for 5 min)</td>
</tr>
<tr>
<td>170-210 min</td>
<td>Rest 3</td>
</tr>
<tr>
<td>210-215 min</td>
<td>Rekindle (rekindling 4) the sensitised area (40°C for 5 min), and subjects rated the first minute of the stimulation using VAS</td>
</tr>
<tr>
<td>215-230 min</td>
<td><strong>Measurement 3 (rekindling 4)</strong></td>
</tr>
<tr>
<td>215-230 min</td>
<td>Area of the secondary hyperalgesia</td>
</tr>
<tr>
<td>215-230 min</td>
<td>Measure HPT on the non-capsaicin side</td>
</tr>
<tr>
<td>215-230 min</td>
<td>Measure HPT on the capsaicin side</td>
</tr>
<tr>
<td>230 min</td>
<td>Subjects completed Acupuncture treatment questionnaire.</td>
</tr>
</tbody>
</table>

Please refer to methodology chapter 3 for a detailed description of subject screening, producing of the heat/capsaicin model, outcome measurements, and statistical method.
5.4 Results

5.4.1 General information on the subjects

The experiment was conducted from April to July in 2008. A total 20 subjects were recruited according to the inclusion and exclusion criteria. All 20 subjects completed the experiment, and no serious side effects were reported.

Of the 20 subjects, 12 were male, and 8 were female. The average age was 24.90 ± 4.12 (Mean ± SD), ranging from 18 to 32. One subject was left handed, the rest were right handed. Table 5.2 shows the demographic data of the subjects. No significant difference in age, gender or hand dominance were found between the two groups.

Table 5.2 Demographic data of subjects

<table>
<thead>
<tr>
<th></th>
<th>EA (n=10)</th>
<th>SEA (n=10)</th>
<th>Statistical tests</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean ± SD)</td>
<td>23.20 ± 4.02</td>
<td>26.60 ± 3.63</td>
<td>t-value = 1.985</td>
<td>0.063*</td>
</tr>
<tr>
<td>Gender (Male : Female)</td>
<td>6 : 4</td>
<td>6 : 4</td>
<td>$\chi^2$ (df=1) &gt; 0.000 *</td>
<td>1.000*</td>
</tr>
<tr>
<td>Hand dominance (Right : Left)</td>
<td>10 : 0</td>
<td>9 : 1</td>
<td>$\chi^2$ (df=1) = 1.053 b</td>
<td>0.305*</td>
</tr>
</tbody>
</table>

$\chi^2$: chi-square value

* 2 cells (50.0%) have expected count less than 5. The minimum expected count is 4.00

b 2 cells (50.0%) have expected count less than 5. The minimum expected count is 0.50

* The significance level for the above chi-square calculations was at $p < 0.05$

5.4.2 Heat/capsaicin sensitisation

All subjects developed mechanical and thermal hyperalgesia, following heat/capsaicin sensitisation (Table 5.3). The area of secondary hyperalgesia was 57.29 ± 19.24 cm² in the REA group and 57.96 ± 21.00 cm² in the SEA group.

The first VAS rating (at the 5th second) increased from 2.00 ± 1.83 to 6.34 ± 1.22 in the SEA
group and from 2.29 ± 2.02 to 6.47 ± 1.95 in the REA group. However, rating to heat stimulation reduced progressively over the next 55 seconds (Figure 5.1). There was a significant change within the 1-minute measurement (time effect: F (5, 14) = 2.284, p = 0.01). For this reason, only the rating to the first 5 seconds was used for future analysis.

![Figure 5.1: VAS rating to long thermal stimulation in rekindling 1 in REA and SEA group](image)

Data are expressed as mean ± S.E.M. VAS 1 = rating at the 5th second, VAS 2 = rating at the 15th second, VAS 3 = rating at the 25th second, VAS 4 = rating at the 35th second, VAS 5 = rating at the 45th second, VAS 6 = rating at the 55th second.

HPT was also slightly reduced from 41.96 ± 3.30 to 41.53 ± 1.97 in the SEA group (t = 0.553, \( p = 0.593 \)) and from 42.84 ± 3.23 to 42.54 ± 2.08 in REA group (t = 0.451, \( p = 0.663 \)) on the capsaicin side. One the non-capsaicin side, HPT changed from 43.90 ± 3.26 to 43.30 ± 2.65 in the SEA group (t = 0.991, \( p = 0.348 \)), and from 44.25 ± 3.29 to 43.72 ± 2.66 in the REA group (t = 0.782, \( p = 0.454 \)). However, there was no time effect for these changes.

### 5.4.3 Comparison of pre-treatment data between groups

As shown in Table 5.3, there was no significant difference between two groups in pre-capsaicin and rekindling in 1 stage, in terms of HPT, VAS rating to long thermal stimulation
and the area of secondary hyperalgesia.

Table 5.3  A comparison of the baseline (pre-capsaicin) and pre-acupuncture (Rekindling 1) values of all measurements between REA and SEA group

<table>
<thead>
<tr>
<th>Stage</th>
<th>Measurement (Mean ± SD)</th>
<th>n</th>
<th>df</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-capsaicin</td>
<td>HPT non-capsaicin side</td>
<td>SEA: 44.25±3.29</td>
<td>10</td>
<td>18</td>
<td>-0.239</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 43.90±3.26</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPT capsaicin side</td>
<td>SEA: 41.96±3.29</td>
<td>10</td>
<td>18</td>
<td>-0.603</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 42.84±3.23</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rekindling 1</td>
<td>HPT non-capsaicin side</td>
<td>SEA: 43.30±2.65</td>
<td>10</td>
<td>18</td>
<td>-0.353</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 43.72±2.66</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPT capsaicin side</td>
<td>SEA: 41.53±1.97</td>
<td>10</td>
<td>18</td>
<td>-1.114</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 42.54±2.08</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-capsaicin</td>
<td>VAS 1</td>
<td>SEA: 2.00±1.83</td>
<td>10</td>
<td>18</td>
<td>-0.336</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 2.29±2.02</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAS 2</td>
<td>SEA: 1.90±1.70</td>
<td>10</td>
<td>18</td>
<td>-0.547</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 2.37±2.12</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAS 3</td>
<td>SEA: 1.82±1.57</td>
<td>10</td>
<td>18</td>
<td>-0.664</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 2.38±2.16</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAS 4</td>
<td>SEA: 1.97±1.68</td>
<td>10</td>
<td>18</td>
<td>-0.092</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 2.04±1.71</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAS 5</td>
<td>SEA: 2.11±1.84</td>
<td>10</td>
<td>18</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 2.07±1.90</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAS 6</td>
<td>SEA: 2.12±1.80</td>
<td>10</td>
<td>18</td>
<td>0.153</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 1.99±1.99</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rekindling 1</td>
<td>VAS 1</td>
<td>SEA: 6.34±1.21</td>
<td>10</td>
<td>18</td>
<td>-0.179</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 6.47±1.95</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAS 2</td>
<td>SEA: 5.83±1.24</td>
<td>10</td>
<td>18</td>
<td>-0.017</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 5.84±1.45</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAS 3</td>
<td>SEA: 5.90±1.11</td>
<td>10</td>
<td>18</td>
<td>0.435</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 5.60±1.87</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAS 4</td>
<td>SEA: 5.76±1.25</td>
<td>10</td>
<td>18</td>
<td>0.602</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 5.19±2.72</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAS 5</td>
<td>SEA: 5.43±1.02</td>
<td>10</td>
<td>18</td>
<td>0.321</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 5.12±2.88</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAS 6</td>
<td>SEA: 5.47±1.34</td>
<td>10</td>
<td>18</td>
<td>0.639</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REA: 4.82±2.92</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Area of mechanical</td>
<td>SEA: 57.96±21.00</td>
<td>10</td>
<td>18</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>hyperalgesia</td>
<td>REA: 57.29±19.24</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.4.4 The effect of EA on the area of secondary hyperalgesia

Table 5.4 shows data corresponding to the area of secondary hyperalgesia at each point of measurement. The pre-treatment area of hyperalgesia was 57.96 ± 21.00 cm² in the SEA group, and 57.29 ± 19.24 cm² in REA group. Ninety minutes post intervention, the area decreased significantly by 30% in REA group and 24% in the SEA group (Figure 5.2). Two-way ANOVA shows there was a statistical significant time effect ($F(2, 36) = 10.209, p < 0.001$) but no treatment group by time interaction ($F(2, 36) = 2.146, p = 0.988$), indicating both REA and SEA reduced the area of secondary hyperalgesia in a similar manner.

Table 5.4 Area of secondary hyperalgesia at each point of measurement

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Stage</th>
<th>Area of secondary hyperalgesia ($Mean \pm SD$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham EA</td>
<td>10</td>
<td>Rekindling 1</td>
<td>57.96 ± 21.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rekindling 2</td>
<td>49.24 ± 16.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rekindling 4</td>
<td>39.07 ± 14.06</td>
</tr>
<tr>
<td>EA</td>
<td>10</td>
<td>Rekindling 1</td>
<td>57.29 ± 19.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rekindling 2</td>
<td>47.26 ± 24.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rekindling 4</td>
<td>37.72 ± 18.65</td>
</tr>
</tbody>
</table>
Figure 5.2  Effect of REA and SEA on the area of secondary hyperalgesia immediately (rekindling 2) and 90 minutes (rekindling 4) after intervention. Data are expressed as mean ± S.E.M.
5.4.5 The effect of EA on the heat pain threshold

Table 5.5 shows the effect of acupuncture on heat pain threshold in both REA and SEA groups. HPT increased to or above the baseline (pre-capsaicin) values in both groups after the intervention (rekindling 2). After rekindling 4, there was a reduction in the HPT in SEA group, while the HPT in REA group remained relatively unchanged. A two-way ANOVA showed that after rekindling 1, there was a statistically significant time effect ($F (2, 36) = 4.342, p = 0.02$) but no significant group by time interaction ($F (2, 36) = 2.569, p = 0.091$), indicating the changes in HPT changed in both REA and SEA were similar (Figure 5.3).
Table 5. 5  HPT during baseline (pre-capsaicin) and each rekindling

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Stage</th>
<th>HPT (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEA</td>
<td>10</td>
<td>Pre-capsaicin</td>
<td>41.96 ± 3.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rekindling 1</td>
<td>41.53 ± 1.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rekindling 2</td>
<td>42.50 ± 2.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rekindling 4</td>
<td>41.18 ± 2.50</td>
</tr>
<tr>
<td>REA</td>
<td>10</td>
<td>Pre-capsaicin</td>
<td>42.84 ± 3.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rekindling 1</td>
<td>42.54 ± 2.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rekindling 2</td>
<td>42.93 ± 1.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rekindling 4</td>
<td>42.84 ± 1.72</td>
</tr>
</tbody>
</table>

Figure 5. 3  Effect of REA and SEA on the heat pain threshold on capsaicin side
Data are expressed as mean ± S.E.M

On the non-capsaicin side, two-way ANOVA showed, after rekindling 1, there was no statistically significant time effect ($F(2, 36) = 1.308, p = 0.283$) and group by time interaction ($F(2, 36) = 0.339, p = 0.715$), indicating HPT in both REA and SEA on non-capsaicin side remained stable (Figure 5.4).
5.4.6 The effect of EA on the pain rating to long thermal stimulation

Figure 5.5 shows the effect of acupuncture on the first pain rating assessed at the 5\textsuperscript{th} second of the 1 minute thermal stimulation. The pain at this point was the strongest during the one minute test; the sensation would gradually decrease due to adaptation. Compared to the baseline (pre-capsaicin), pain rating increased significantly ($p < 0.01$) after the heat/capsaicin sensitisation.

After rekindling 1, two-way ANOVA showed there was a statistically significant time effect ($F (2, 36) = 26.994, p < 0.001$) but no significant treatment group by time interaction ($F (2, 36) = 2.256, p = 0.119$), indicating both REA and SEA significantly reduced the VAS rating to long thermal stimulation in a similar manner.
Figure 5.5  Effect of acupuncture on pain rating to long thermal stimulation. Data are expressed as mean ± S.E.M.

Table 5.6  First pain rating (at the 5th second) during baseline (pre-capsaicin) and each rekindling

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Measurement</th>
<th>Stage</th>
<th>VAS rating (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEA</td>
<td>10</td>
<td>VAS1</td>
<td>Pre-capsaic</td>
<td>2.00 ± 1.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rekindling 1</td>
<td>6.34 ± 1.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rekindling 2</td>
<td>4.20 ± 2.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rekindling 4</td>
<td>5.48 ± 1.17</td>
</tr>
<tr>
<td>REA</td>
<td>10</td>
<td>VAS1</td>
<td>Pre-capsaic</td>
<td>2.29 ± 2.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rekindling 1</td>
<td>6.47 ± 1.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rekindling 2</td>
<td>2.75 ± 1.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rekindling 4</td>
<td>4.29 ± 2.59</td>
</tr>
</tbody>
</table>
5.4.7 Credibility of sham acupuncture

At the end of the experiment, a questionnaire was used to assess the credibility of sham acupuncture. All 20 subjects completed this questionnaire (appendix 12). There was no difference between the REA and SEA groups on the subject guessing which group they were assigned to, indicating the blinding procedure was successful (Table 5.7).

Table 5. 7  Subject’s perception of treatment

<table>
<thead>
<tr>
<th>Subject’s answer</th>
<th>Frequency of answer in each group (number)</th>
<th>Statistical test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>REA (n)</td>
<td>SEA (n)</td>
</tr>
<tr>
<td>I had Real acupuncture</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>I had placebo/sham acupuncture</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Don’t know</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

* 4 cells (66.7%) have expected count less than 5. The minimum expected count is 1.5.
** Significance for the above chi-square calculations at p<0.05.

$df$: degrees of freedom.
$\chi^2$: chi-square value

5.4.8 Perception of acupuncture stimulation and side effects

At the end of the experiment, a questionnaire (Appendix 12) was used to assess subjects’ perception of acupuncture stimulation (Table 5.8). Four choices were given: no pain, slight pain, moderate pain and severe pain.

None of the subjects reported experiencing severe pain during the intervention. The majority of REA groups (70%) reported slight pain compared to only 30% in the SEA groups. While 60% of SEA groups considered the intervention to be painless, only one subject (10%) in the REA group rated it so. Moderate pain was experienced by 20% of subjects in the REA group and 10% in the SEA group. Although there was a trend to show group difference in the perception of acupuncture stimulation, it was not significant ($p = 0.064$).
None of the subjects reported any side effects such as nausea or dizziness during or after the procedure.

Table 5.8 Rating to acupuncture stimulation

<table>
<thead>
<tr>
<th>Rating to acupuncture stimulation</th>
<th>No pain; n (%)</th>
<th>Slight/mild pain; n (%)</th>
<th>Moderate pain; n (%)</th>
<th>Severe pain; n (%)</th>
<th>$\chi^2 (df)$</th>
<th>$p$-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA ($n=10, 100%$)</td>
<td>1(10%)</td>
<td>7(70%)</td>
<td>2(20%)</td>
<td>0</td>
<td>5.505* (2)</td>
<td>0.064</td>
</tr>
<tr>
<td>Sham EA ($n=10, 100%$)</td>
<td>6(60%)</td>
<td>3(30%)</td>
<td>1(10%)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 4 cells (66.7\%) have expected count less than 5. The minimum expected count is 1.5

** Significance for the above chi-square calculations at $p<0.05$.

df: degrees of freedom

$\chi^2$: chi-square value
5.4.9 Post-hoc power analysis and sample size calculations

The current study was underpowered. Table 5.9 shows the effective power of the statistical tests with the existing sample size and required sample size for 80% power for comparison of REA and SEA.

Table 5.9 Post-hoc power analyses and required sample size for comparison of REA and SEA

<table>
<thead>
<tr>
<th>Measurements</th>
<th>SEA (Mean ± SD)</th>
<th>REA (Mean ± SD)</th>
<th>Effect size</th>
<th>Power</th>
<th>Sample size per group to achieve 80% power</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS rating (first 5th, second)</td>
<td>4.20 ± 2.10</td>
<td>2.75 ± 1.65</td>
<td>0.177</td>
<td>37%</td>
<td>28</td>
</tr>
<tr>
<td>HPT</td>
<td>42.50 ± 2.30</td>
<td>42.93 ± 1.35</td>
<td>0.23</td>
<td>8%</td>
<td>303</td>
</tr>
<tr>
<td>Area of secondary hyperalgesia</td>
<td>39.07 ± 14.06</td>
<td>37.72 ± 18.65</td>
<td>0.08</td>
<td>0.05%</td>
<td>2351</td>
</tr>
</tbody>
</table>
5.5 Summary

In this experiment, capsaicin combined with repeated heat stimulation successfully produced mechanical and heat hyperalgesia state in both REA and SEA groups. The area of secondary hyperalgesia, HPT, and VAS rating before acupuncture was comparable between the two groups. Thus, the randomisation procedure was successful.

After REA or SEA intervention, area of secondary hyperalgesia was significantly reduced in both groups, however, no statistically significant difference was detected between them. There were some fluctuations in the HPT and rating to heat stimulation during the experiment, however no group difference was detected.

In general, hyperalgesia was reduced in both REA and SEA groups to a similar degree. Potential factors underlying these findings are discussed in Chapter Six.
Chapter 6 Discussion and conclusion

6.1 Summary of results
The main purpose of the current project was to use a validated hyperalgesia model to assess the anti-hyperalgesia effect of acupuncture and compare it with a sham procedure.

In the first part of the literature review, hyperalgesia was reviewed and two promising candidates of hyperalgesia model were identified, i.e., topical capsaicin model and heat/capsaicin model. In the second part, the review focused on the animal studies which assessed the anti-hyperalgesia effect of acupuncture. It is concluded that electroacupuncture reduces hyperalgesia in both inflammatory and neuropathic models. No human studies were identified.

The literature review was followed by two experiments in healthy humans. In Experiment 1, we examined the reproducibility of the topical capsaicin model on 12 healthy subjects. This experiment is the first study examining its reproducibility of this model over a period of one month. It was found that in the same subject the between-day reproducibility of the area of secondary hyperalgesia and the mechanical pain threshold was poor, thus not appropriate for the evaluation of anti-hyperalgesia effect of acupuncture.

Experiment 2 is the first human study to examine the anti-hyperalgesia effect of acupuncture. We used the heat/capsaicin model to compare the effect of REA with SEA. It was found that REA and SEA significantly reduced both the area of secondary punctate hyperalgesia and the VAS rating compared to thermal stimulation. However, there was no statistically significant difference between the two groups.

6.2 Strengths of the study
The entire project follows a logical design. Firstly, the parameters of acupuncture used in animal research of hyperalgesia were identified. Then, the reproducibility of a hyperalgesia model was assessed. Finally, a validated model was used to evaluate the anti-hyperalgesia effect of acupuncture on humans.

Both mechanical and thermal hyperalgesia were assessed with well-accepted methods. Where possible, each type of hyperalgesia was assessed with two methods of measurements. For instance, mechanical hyperalgesia was assessed with the area of mechanical hyperalgesia, mechanical pain threshold, and VAS rating to suprathreshold stimulation. All of these methods have been used in other hyperalgesia studies (Park, Max et al. 1995; Zheng, Gibson et al. 2000). For heat hyperalgesia, both HPT and rating to long thermal stimulation were adopted. These measures have been used to examine the anti-hyperalgesia effects of analgesics (Burns, Hill et al. 2006; Frymoyer, Rowbotham et al. 2007; Mattia and Coluzzi 2007).

To control the placebo effect, acupuncture naïve and pain free healthy subjects were recruited and randomly allocated to either the REA or SEA groups. We used a validated, non-invasive sham procedure that has been used in both animal (Lao, Zhang et al. 2004) and human (Feng 2007) studies. This model successfully blinds subjects from the nature of the treatment they receive, as shown by the acupuncture credibility questionnaire.

Acupuncture intervention was carried out by a registered acupuncturist, and the evaluator was blinded from treatment allocation. This blinding procedure ensures that the performance bias of both subjects and investigator was well-controlled. Before starting the experiment, subjects were also given an oral explanation and training session, so as to be familiar with the experimental procedure.
To minimise the impact of room temperature on pain perception (Pertovaara, Kauppila et al. 1996), both experiments were conducted in a temperature-controlled room (20-22 degrees Celsius).

### 6.3 Limitations

The major limitation of this study is the relatively small sample size and a lack of a non-treatment group. As this experiment is the first study assessing the anti-hyperalgesia effect of acupuncture in healthy human, it was difficult to determine the proper sample size. The calculation of the sample size in this study was based on the data reported in a previous drug study using the heat/capsaicin model (Petersen, Jones et al. 2001). According to the calculation, a total number of 58 subjects were needed with 29 subjects in each group. However, only 20 subjects with 10 in each group participated in the experiment.

Several factors limited the recruitment of participants. Firstly, subjects were required to be acupuncture naïve. Secondly, subjects were required to remain in the lab for 4 to 5 hour duration. Thirdly, the experiment involved possible needling, thus the potential of induced pain. It was understood that this aspect may have intimidated a group of people who were initially interested in participating in the study. Finally, the student conducting the research of this project was due to complete the thesis, thus there was insufficient time for further rounds of recruitment. As a result, the relatively small sample size limited the power of the statistical analysis.

The small number of participants may have impacted on conclusions drawn regarding heat hyperalgesia, as the study had only one third of the required sample of 56 subjects, indicated by our post-hoc sample size calculation. However, it is understood that this should not impact
on the outcome of the area of mechanical hyperalgesia. The sample size required for detecting one eighth of a standard deviation difference with 80% power is 4702 in total.

Although other researchers have shown that hyperalgesia is stable in the heat/capsaicin model, without a third non-treatment group in the current study, we cannot be sure that the area of mechanical hyperalgesia will not naturally reduce, as seen in Experiment 1. Furthermore we did not include a second measure of mechanical hyperalgesia in Experiment 2 making it even harder to know its resolution.

In Experiment 1, we found that rating to suprathreshold mechanical stimulation was a reliable measurement. However, due to the time limit during Experiment 2, this outcome measure was not taken. In addition, the area of secondary hyperalgesia is the recommended method and has been successfully used in other studies for evaluation of the anti-hyperalgesia effect of various medications (refer to review chapter 2). Suprathreshold mechanical stimulation was not used in preliminary studies testing the heat/capsaicin model (Petersen and Rowbotham 1999; Dirks, Petersen et al. 2003), however it does have an application to determine hyperalgesia and could be used in future studies. Results suggest that it may be important to include a 2nd measure of mechanical hyperalgesia.

There is also a limitation in the HPT measurement due to the modified Method of Limits test having four measurements. This produced relatively less sensitive results when compared with an alternative test, Staircase tests. However, the Limits test had also been used in other drug studies using the heat/capsaicin model (Petersen, Maloney et al. 2003; Burns, Hill et al. 2006; Frymoyer, Rowbotham et al. 2007). Furthermore, due to the time limit between various measurements and rekindling of hyperalgesia, the Limits test (approximately 8 minutes) was the only choice we had within the given time for measurements (20 minutes) of the
heat/capsaicin model. Staircase tests require considerable time, approximately 20 minutes, to complete. Thus, due to the time constraints, this test was not appropriate to be used in this model.

6.4 Interpretation of the findings

In the 32 animal studies reviewed, 32 studies employed the use of SEA and REA, however REA is found to be consistently better than SEA. Furthermore, no universal optimum EA parameter was identified from the review. Two studies identified different optimum EA parameters using different animal models (Lao, Zhang et al. 2004; Taguchi and Taguchi 2007). It is concluded that it may be likely that there is a set of optimum EA parameters for specific hyperalgesia model.

In Lao’s study, an optimum parameter for EA stimulation, 10 Hz, 3 mA, 0.1 ms, 20 minutes, was found (Lao, Zhang et al. 2004). In the present study, we used a similar parameter in order to achieve optimum results. However, there were not any group differences to be observed.

This result, though was not what we expect to find, was not surprising. In the clinical studies conducted, the effect of REA compared with SEA was also controversial. For instance, in one osteoarthritis study, both EA and manual acupuncture was significantly superior to non-penetrating sham acupuncture in relieving osteoarthritic knee pain (Jubb, Tukmachi et al. 2008). However, in a migraine study, sham acupuncture had a similar effect when compared with manual acupuncture (Alecrim-Andrade, Maciel-Junior et al. 2008). A review has shown that the evidence supporting acupuncture in treating chronic pain when compared with sham acupuncture is inconclusive (Itoh and Kitakoji 2007). It is also possible that the magnitude of hyperalgesia in the heat/capsaicin model was not strong enough to detect the difference between REA and SEA. The average VAS rating to thermal stimulation was approximate 6.5
immediately after the establishment of the model in both groups. This rating is a relatively 
low given in a modified 0-10 VAS scale 5 is defined as just painful and 10 as worst pain 
possible. Lastly, the EA parameter may also account for the insignificant result, as only low 
frequency were used. In the inflammatory animal model it was found that EA with high 
frequency (100 Hz) produces shorter lasting, within 1 day, but high potent anti-hyperalgesia 
effects, whilst low frequency, 10 Hz, showed prolonged anti-hyperalgesia effects, 
approximately 1 to 7 days, but less potent effect on PWL (Lao, Zhang et al. 2004).

Experiment 2 found that HPT on the non-capsaicin side was not affected by either REA or 
SEA. This result is contradictory to Feng’s finding (Feng 2007) in which EA (2 /100 Hz 
alternating) significantly increased the pain threshold to single electrical stimulation mostly at 
the site of EA stimulation. This discrepancy may be due to the difference in the nature of 
stimulation (heat vs. electrical) or EA frequency. Furthermore, as previous evidence suggests, 
compared with non-invasive sham, EA with high frequency (120 Hz) significantly increases 
heat pain tolerance threshold (Berlin, Bartlett et al. 1975), whilst EA with low frequency (2.5 
Hz) does not significantly increase heat pain threshold or heat pain tolerance threshold 
(Stewart, Thomson et al. 1977).

An additional explanation is the possible time effect. In Feng’s study, the anti-temporal 
summation effect of EA became more evident post 24 hours. The current model only lasted 
for 4 hours, thus does not allow sufficient time for assessment of the time profile of 
acupuncture.

The results from experiment 2 are contradictory to the finding in the animal studies. The 
animal studies demonstrated that acupuncture always outperformed sham acupuncture and 
furthermore, the non-invasive sham procedure consistently showed no anti-hyperalgesia effect. 
This discrepancy may be due to several factors. Firstly, the magnitude of hyperalgesia in
animal model is much higher compared with the human model. It is possible that at this level of severity, the sham procedure is unable to produce any significant non-specific effect. Secondly, the intensity of EA stimulation is stronger than that used in the human studies. It is clear from the animal studies that the higher the intensity, the stronger the effect of EA. Thirdly, level of expectancy may also play a significant role. There is evidence suggesting that expectancy contributes positively to the clinical outcome in patients with low back pain treated with acupuncture (Kalauokalani, Cherkin et al. 2001). It is unlikely that animals have any expectancy from the treatment, thus do not develop any expectancy-related placebo effect. However, the level of expectancy was not assessed in the current study.

6.5 Implication for future study

In future studies, the suitability of the heat/capsaicin mode requires to be adjusted for acupuncture studies. The time intervals between outcome measures, rekindling and acupuncture treatments may need to be re-arranged so that more measures can be included. Furthermore, a hyperalgesia model that lasts for more than 24 hours would be ideal, such as a burn injury (Pedersen 2000).

The current study shows a trend that REA may be better than SEA in reducing VAS rating to sub-threshold thermal stimulation. The potential disparity in acupuncture effect on mechanical and heat hyperalgesia may have indicated it has a different impact on peripheral and central sensitisation. In future studies, EA’s effect on the response to sub-threshold and supra-threshold thermal or mechanical stimulation should be tested.

Further studies to determine a possible impact of different EA parameters could provide significant understanding to support clinical application of EA. Such studies to establish the level of frequency on anti-hyperalgesic effect using alternating frequencies, such as 2/100 Hz,
could be useful, as this parameter has been shown to be effective in reducing temporal summation (Feng 2007). Furthermore, subjects’ expectancy for acupuncture should also be assessed in healthy human studies to determine its impact.


Mattia, C. and F. Coluzzi (2007). "Indantadol, a novel NMDA antagonist and nonselective


Appendix 1 Ethics approval letter

Appendix

18 June 2007

Linghan Bai
10 Colby Avenue
BUNDOORA VIC 3083

Dear Linghan

Project No 13/07: The effect of electro-acupuncture on capsaicin-induced hyperalgesia in healthy volunteers: a randomised and sham acupuncture controlled study

Further to my earlier email I would like to formally advise that the project is now approved.

This project is approved from the date of this letter until 31 December 2007. This approval is conditional on the submission of an annual report. 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/research/hipec). 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.

All data should normally be stored on University Network systems. These systems provide high levels of manageable security and data integrity, can provide secure remote access, are backed on a regular basis and can provide Disaster Recover processes should a large scale incident occur. The use of portable devices such as CDs and memory sticks is valid for archiving, data transport where necessary and some works in progress. The
authoritative copy of all current data should reside on appropriate network systems; 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.

If you anticipate any problems in meeting this requirement please contact me to discuss
an alternative secure data storage arrangement.

We wish you well with your research.

Yours sincerely

Peter Burke
Ethics Executive Officer
RMIT Human Research Ethics Committee

cc: Julie Barnett
    Zhou Zheng
Appendix 2  Advertisement for the experiment one

RMIT University
School of Health Sciences
The Chinese Medicine Research Group

Make a contribution to acupuncture research
Volunteer needed!

Are you eligible for the research?
If you are between 18-40 years old, healthy and do not have pain currently, we welcome you to this experiment.

What is this research about?
We want to find out whether electro-acupuncture can reduce tenderness, called hyperalgesia, a very important feature of chronic pain, in healthy humans.

What will you be asked to do?
We will place a patch saturated with chili pepper solution, called capsaicin, on one of your forearms for about 45 minutes. The application will induce mild to moderate hot or burning sensation, and induce temporary tenderness on your forearm. This sensation will peak within one hour of application, and gradually reduce to no pain within the next three to four hours.

We then use nylon filaments to test your pain threshold and your rating to mechanical stimuli applied around the area of the capsaicin application. The stimulation from the nylon filaments may cause transient pain of a few seconds. You are required to evaluate the sensation produced by these mechanical stimuli, whether it is painful or not, and how strong it is. Then the tests will be repeated once in the following one hour. In one month, you will be asked to come back and the procedure will be repeated.

How long does this experiment take?
The whole experiment consists of two sessions with one month interval, and each takes about three hours. Totally, the experiment takes about six hours.

Although your participation in the study might not benefit you directly, it will help us advance our knowledge of the mechanism of electro-acupuncture and lead us to better clinical applications for pain management.

Will you get paid for taking part in the study?
You will not get paid for taking part in the study.

If you agree to take part in this experiment, please contact:

Linghan Bai
Day time: 9925 7176
Mobile: 0409131670 (Telstra)
Email: s3148731@student.rmit.edu.au

This project has been reviewed and approved by the Human Research Ethics Committee of RMIT University
Appendix 3 Advertisement for the experiment two

School of Health Sciences
The Chinese Medicine Research Group

Make a contribution to acupuncture research
Volunteer needed!

Are you eligible for the research?
If you are between 18-50 years old, pain free and have not had acupuncture in the past one year, we welcome you to this experiment.

What is this research about?
We want to find out whether electro-acupuncture can reduce tenderness, called hyperalgesia, a very important feature of chronic pain, in healthy humans.

What will you be asked to do?
We will apply a layer of capsaicin (chili pepper) cream and moderate heat stimulation to your non-dominant forearm for 35 minutes in total. The application will induce mild to moderate hot or burning sensation and temporary tenderness on your forearm. This sensation will peak within one hour of application, and gradually reduce to no pain within the next three to four hours.

We then use nylon filaments to test the tender area around the capsaicin site. The stimulation from the nylon filaments may cause transient pain for a few seconds. After this a heat stimulator will be attached to the sensitized skin, and you are required to evaluate whether the heat sensation produced is painful or not and rate it on a visual analogues scale. These tests will be also conducted on the untreated skin on the other arm. Then these tests will be repeated after reheating the same site.

According to your group assignment, you might receive either real or fake electro-acupuncture stimulation for half an hour after the removal of the capsaicin patch. You may experience pricking sensation caused by needle insertion. The sensations of numbness or heaviness may also be perceived at the acupuncture sites.

How long does this experiment take?
The whole experiment takes about 4 hours.

Will you get paid for taking part in the study?
You will not get paid for taking part in the study.

Although your participation in the study might not benefit you directly, it will help us advance our knowledge of the mechanism of electro-acupuncture and lead us to better clinical applications for pain management.

If you would like to take part in this experiment, please contact:
Linghan Bai
Day time: 9925 7176
Mobile: 0409131670 (Telstra)
Email: s3148731@student.rmit.edu.au

This project has been reviewed and approved by the Human Research Ethics Committee of
RMIT University
Appendix 4 Plain language statement for experiment one

School of Health Sciences
The Chinese Medicine Research Group

Plain Language Statement

Information about the pilot study of capsaicin-induced hyperalgesia model

PROJECT TITLE: The effect of electro-acupuncture on capsaicin-induced hyperalgesia in healthy volunteers: a randomised and sham acupuncture controlled study

INVESTIGATOR: Linghan Bai, Masters Candidate

Dear Volunteer,

I am Linghan Bai, a master’s student at the division of Chinese Medicine Research Group, RMIT University. My current project is under the supervision of Dr. Zhen Zheng and Prof. Charlie Xue, A/Prof Chunguang Li (RMIT, Chinese Medicine Research Group). In this pilot study, I will use capsaicin, chili pepper solution, to produce a pain model in healthy humans to test the reproducibility of the capsaicin-induced tenderness, i.e. hyperalgesia. This is to provide you with relevant information about my study.

1. Purpose of this study

This is the first step of a bigger study, and aims to test the reproducibility of capsaicin-induced hyperalgesia. Will capsaicin produce the same level of hyperalgesia in the same subject after a period of one month? We will find it out after this study.

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

If you agree to participate in the experiment, which consists of two sessions with one month interval, and each session takes about three hours. You will go through the following procedure in each session:

1) Complete the questionnaire about your current health condition (only in the 1st session);
2) Take part in the pre-capsaicin test. We will use nylon filaments to test your pain threshold and your rating to mechanical stimulation. You will be asked to evaluate the sensation produced by these mechanical stimuli, and report whether the sensation painful or not, and how strong it is.
3) Receive capsaicin treatment. We will put a piece of fillet paper absorbed with chili pepper solution, called capsaicin, on one of your forearms for 45 minutes;
4) Take part in the 1st post-capsaicin test. At the end of the 45 minutes, the filter paper will be removed, we will then repeat step 2 to test your sensitivity to mechanical stimulation. At this stage, your skin will become tender and sensitive to mechanical stimulation, which indicates the status of hyperalgesia.
5) Take part in the 2nd post-capsaicin test. One hour after step 4, step 2 will be repeated again. Your sensitivity to mechanical stimulation may or may not change at this stage.

3. What kind of pain you will experience?
In this pilot study, you might experience two types of pain: burning pain induced by capsaicin, evoked pain when testing hyperalgesia.

In this study the majority of the painful sensation is caused by the topical application of capsaicin. This chemical compound will be dissolved and absorbed into a small patch of filter paper. And then this capsaicin patch will be applied onto the forearm. Within the area of the patch, you will feel a kind of mild burning sensation that peaks at about 20 to 40 minutes after the application and slowly diminishes after about one hour. Around the patch there will be an area of tenderness, which will disappear in 24 hours.

During the test, we will use some mechanical device called von Frey filaments to elicit slightly painful pinprick sensation in order to determine the boundary of the tender area and the pain thresholds. All the induced painful sensations are temporary, mild to moderate in intensity, lasting up to 10 seconds, and will not cause any injury to the skin.

4. Safety issue and potential discomfort of capsaicin

Capsaicin is natural, processed vegetable matter that has been part of the human diet for many years. It is unlikely that it will pose a significant threat to human health. Excessive exposure to capsaicin may cause some slight eye and skin irritation. However in this experiment, only a tiny dose (0.1 ml) of capsaicin solution will be applied topically on the skin of one of the forearm. It will produce some minor burning sensation and hyper sensitivity within and around the area of application as required by the experiment. The tenderness will gradually wane and disappear within 24 hours. The sensitivity of the capsaicin treated site will return to the normal status after 24 hours. However the site might become less responsive to capsaicin than untreated area, and this de-sensitisation will resolve completely within one month.

If you are allergic to chili, you should not take part in this study.

5. 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.

6. 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. Authorized auditors may inspect your records. You will have access to your records through the investigator. The result of this project might be published. Only group data or coded data will appear in any publication and all personal details and identifiable information will not be published. A summary of the results will be sent to you upon the completion of the study.

7. Benefit of your participation

Hyperalgesia is an important feature underlying clinical pain. Your participation will benefit human pain studies and improve our understanding of analgesic effect of acupuncture in treating clinic pain. There is no direct benefit to you.

8. 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.

If you have any questions about the experiment, please contact me (Tel: 9925 7176, E-mail: s3148731@student.rmit.edu.au) or my supervisor Dr. Zhen Zheng (Tel: 9925 7167)

For any complain about this experiment please contact the HREC committee (Tel: 9925 1745, GPO Box 2476V, Melbourne, 3001)
Appendix 5  Plain language statement for experiment two

School of Health Sciences
The Chinese Medicine Research Group

Plain Language Statement

Information about acupuncture and pain study

PROJECT TITLE: The effect of electro-acupuncture on hyperalgesia associated with heat/capsaicin model in healthy volunteers: a randomised sham acupuncture controlled study

INVESTIGATOR: Linghan Bai, Masters Candidate

Dear Volunteer,

I am Linghan Bai, a master’s student at the Division of Chinese Medicine Research Group, RMIT University. My current project is under the supervision of Dr. Zhen Zheng and Prof. Charlie Xue, and A/Prof Chunguang Li (RMIT, Chinese Medicine Research Group). In this research, I will use capsaicin (chili pepper solution) combined with moderate heat stimulation to produce a pain model in healthy humans and then test the effect of electro-acupuncture on this model. This is to provide you with relevant information about my study.

1. Purpose of this study

This study aims to test the effect of electro-acupuncture on the tenderness, called hyperalgesia, induced by capsaicin and heat. Will electro-acupuncture reduce or enhance hyperalgesia or simply has no effect on it? We will find it out after this research. The outcome will further our understanding of the anti-pain mechanism of acupuncture; therefore improve our clinic practice of acupuncture.

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

If you agree to participate in the experiment, which takes about 4 hours, you will go though the following procedure.

1) Complete the questionnaire about your current health condition and sign the consent form;
2) Take part in the baseline test. We will use a computer-controlled device to test your heat pain threshold. A short-lasting, mild heat stimulation will be applied to both of your forearms and you will be asked to evaluate whether the sensation is painful or not by using a mouse.
3) Then you will receive heat and capsaicin treatment. We will attach the same computer-controlled device to the middle of your non dominant forearm to deliver mild heat stimulation and then apply a layer of capsaicin cream on the heated area for 30 minutes.
4) After removal of the capsaicin cream, you will be asked to rest quietly in the lab for 35 minutes.
5) We will test your pain sensitivity again. We will put the same computer-controlled device to the same area to deliver mild heat stimulation for 5 minutes. We then use a nylon hair to test the area of the sensitized skin. After this, the previous test in step 2 will be repeated.
6) At this stage you will receive electro-acupuncture treatment for 30 minutes while you rest on a
7) Repeat step 5 twice.

3. What kind of pain you will experience?

In this study, you might experience three types of pain: mild burning pain induced by capsaicin, evoked pain when testing hyperalgesia, and pain on acupuncture.

In this study the majority of the painful sensation is caused by the topical application of capsaicin and heat stimulation. Capsaicin cream will be applied onto the forearm. Within the application area, you will feel a kind of mild burning sensation that peaks at about 20 to 40 minutes after the application and slowly diminishes after one hour. Around the patch there will be an area of tenderness, which will disappear in 24 hours. The heat stimulation causes mild to moderate pain at 45°C, and mild pain at 40°C.

In the experiment, we will use a mechanical device called von Frey filaments to elicit slightly painful pinprick sensation in order to determine the boundary of the tender area. All the induced painful sensations are temporary, mild to moderate in intensity, lasting up to 10 seconds, and will not cause any injury to the skin.

During acupuncture, needles will be inserted into eight acupuncture points on your arms and legs. You might experience minor transient sharp sensation caused by the penetration. During the treatment, you might also feel mild soreness, vibration, numbness or distention at the needle sites. These sensations will disappear once the needles are withdrawn.

4. The real or placebo treatment

It is necessary to have an inactive treatment group who will receive fake acupuncture, so that the true effect of electro-acupuncture treatment can be demonstrated. Fake electro-acupuncture is a form of placebo treatment with minimal effect on your body. It is used to show whether real treatment has a true effect. Once you have met the inclusion and exclusion criteria, you will be allocated randomly into one of the two groups (using real or sham acupuncture). Please note that you will have a 50% chance of being placed in an inactive treatment group.

In real electro-acupuncture (REA) treatment groups, very fine needles will be inserted into your skin, and electrical current will be delivered to the needles in REA group.

5. Safety issue and potential discomfort of capsaicin

Capsaicin is natural, processed vegetable matter that has been part of the human diet for many years. It is unlikely that it will pose a significant threat to human health. Excessive exposure to capsaicin may cause some slight eye and skin irritation. However in this experiment, only a small dose of capsaicin cream (0.075%) will be applied topically on the skin of one of the forearm. It will produce some minor burning sensation and hypersensitivity within and around the area of application as required by the experiment. The tenderness will gradually wane and disappear within 24 hours. The sensitivity of the capsaicin treated site will return to the normal status after 24 hours. However the site might become less responsive to capsaicin than untreated area, and this de-sensitisation will resolve completely within one month.

If you are allergic to chili, you should let the researcher know and should not take part in this study.
6. Safety issue and potential discomfort of electro-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.

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

Some people may experience minor pricking sensations during the early phase of acupuncture. This normally subsides after a few seconds. The sensation of soreness, numbness or distension may be perceived at the acupuncture sites.

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.

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. Authorized auditors may inspect your records. You will have access to your records through the investigator. The result of this project might be published. Only group data or coded data will appear in any publication and all personal details and identifiable information will not be published. A summary of the results will be sent to you upon the completion of the study.

10. Your participation in other research projects

Hyperalgesia is an important feature underlying clinical pain. Your participation will benefit human pain studies and improve our understanding of analgesic effect of acupuncture in treating clinic pain. There is no direct benefit to you.

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

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

If you have any questions about the experiment, please contact Linghan Bai (Tel: 9925 7176, E-mail: s3148731@student.rmit.edu.au) or my supervisor Dr. Zhen Zheng (Tel: 9925 7167)

For any complain about this experiment please contact the HREC committee (Tel: 9925 1745, GPO Box 2476V, Melbourne, 3001)
Appendix 6  General Information and Screening Questionnaire

General information and questionnaire

General Information

<table>
<thead>
<tr>
<th>Name:</th>
<th>Gender: Male / Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of birth (d/m/y):</td>
<td>/ /</td>
</tr>
<tr>
<td>Address:</td>
<td>Postcode:</td>
</tr>
<tr>
<td>Contact No.:</td>
<td></td>
</tr>
</tbody>
</table>

Screening Questionnaire

1. Did you receive any acupuncture treatment in the last one year? Yes / No

2. Are you allergic to chili? Yes / No

3. Are you currently taking any following medication at moment?

   - Analgesics (Medication for relieving pain) Yes / No
   - Anti-inflammatory agents (Medication for reducing inflammation) Yes / No
   - Anti-anxiety agents (Medication for reducing anxiety) Yes / No
   - Anti-depressants (Medication for reducing depression) Yes / No
   - Anti-psychotic agents (Medication for psychosis) Yes / No

4. Do you have any history of the following conditions?

   - Stoke Yes / No
   - Epilepsy Yes / No
   - Diabetes Yes / No
   - Severe Alcoholism Yes / No
   - Peripheral Vascular Disease Yes / No
   - Peripheral Neuropathy Yes / No
   - Psychosis Yes / No
   - Heart disease Yes / No
   - Impaired circulation in hands or feet Yes / No
   - Wearing a cardiac pacemaker Yes / No
   - Pregnancy Yes / No
   - Have metal implant Yes / No

5. Do you suffer from any chronic pain conditions? Yes / No

   - Location
     - Head
     - Neck
     - Back
     - Arm: L R
     - Leg: L R
   - Duration
     - Continuous
     - Intermittent
   - Degree
     - Weak
     - Mild
     - Moderate
     - Strong
     - Severe

6. Are you having any pain at moment? Yes / No

   - Location
     - Head
     - Neck
     - Back
     - Arm: L R
     - Leg: L R
   - Degree
     - Weak
     - Mild
     - Moderate
     - Strong
     - Severe
Appendix 7  Consent form

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

PORTFOLIO OF  
SCHOOL OF  
Science, Engineering and Technology  
Health Sciences

Name of participant: 
Project Title: 

The effect of electro-acupuncture on hyperalgesia associated with the heat/capsaicin model in healthy volunteers: a randomised sham acupuncture controlled study

Name(s) of investigators:  
(1) Linghan Bai  
Phone: 9925 7176

(2) Dr. Zhen Zheng  
Phone: 9925 7167

(3) Prof. Charlie Xue  
Phone: 9925 7745

(4) A/Prof. Chenguang Li  
Phone: 9925 7635

(5) Meredith O'Loughlan  
Phone: 9925 7176

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

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

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

5. 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)

Where participant is under 18 years of age:

I consent to the participation of ___________________________ in the above project.

Signature: ___________________________  ___________________________  
(date)  (Signatures of parents or guardians)

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 8  Pain threshold recording sheet

#### Pain Threshold

<table>
<thead>
<tr>
<th></th>
<th>Dominant arm ( )</th>
<th>Non-dominant arm (Capsaicin) ( )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-cap 1</td>
</tr>
<tr>
<td>PT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT(1st HA)</td>
<td></td>
<td></td>
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<tr>
<td>PT(2nd HA)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 9  Instructions for testing skin hypersensitivity

Instructions for testing skin hypersensitivity

Hypersensitivity to mechanical stimulation—definite stronger sensation

Mechanical stimulation will be delivered with a nylon filament. When the stimulus is moved towards the chili pepper site your response to the stimulation might be changed and you might feel a definite increase in its intensity. Please indicate this point as soon as you feel it.
Appendix 10  VAS for mechanical stimulation

<table>
<thead>
<tr>
<th>Name</th>
<th>Group</th>
<th>Date</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
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# VAS Recording Sheet

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Appendix 12  Acupuncture treatment questionnaire

**Acupuncture treatment questionnaire**

Name:                                             Number:                                                  Date:     /     / 2008

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**

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)
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<th>Abbreviation List</th>
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<tr>
<td>ANOVA: analysis of variance</td>
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<td>AWR: Abdominal withdrawal reflex</td>
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<td>CCI: chronic constrictive injury</td>
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<td>CFA: complete Freund’s adjuvant</td>
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<td>CNS: central nervous system</td>
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<td>CRD: colorectal distention</td>
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<td>D-D: dense-disperse</td>
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<tr>
<td>EA: electroacupuncture</td>
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<td>EMG: electromyogram</td>
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<tr>
<td>IBS: irritable bowel syndrome</td>
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<td>ICTR: inferior caudal trunk resection</td>
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<td>NMDA: N-methyl-D-aspartic</td>
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<td>PPT: paw pressure threshold</td>
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<td>PTP: pain threshold pressure</td>
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<td>PWL: paw withdrawal threshold</td>
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<td>REA: real electroacupuncture</td>
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<td>SD: standard deviation</td>
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<tr>
<td>SEA: sham electroacupuncture</td>
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<tr>
<td>SEM: standard error of mean</td>
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<tr>
<td>SPSS: statistical package for the social sciences</td>
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<td>SNL: spinal nerve ligation</td>
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<td>VAS: visual analogue scale</td>
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<td>WAS: water-avoidance stress</td>
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<td>WHO: World Health Organization</td>
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