Investigating the effects of three needling parameters (manipulation, retention time, insertion site) on needling sensation and pain profiles and regional pressure pain threshold: a study of eight deep needling interventions

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Certificate of original authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis

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Date:

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Abstract

Background: Since 1999, research studies on the effect of acupuncture on regional pressure pain threshold (PPT) have been carried out at the College of Traditional Chinese Medicine, University of Technology, Sydney. The current study extended the previous research by investigating three needling parameters (needle retention time, needle manipulation and site of needle insertion) on the strength and quality of needling sensation (*deqi*) and on the strength of needling pain.

Aims: To investigate the effect of three needling parameters (needle manipulation, needle retention time and site of needle insertion) on:

(a) the strength and quality of needling sensation reported,

(b) the strength of pain at the needling site and

(c) regional PPT measured at ten regional sites.

Methods: The design used in this study was a dual blind (subject and assessor) within subject experimental design with randomised repeated measures. Twenty-four healthy subjects (12 males and 12 females) completed eight interventions scheduled at least one week apart. In each intervention manual acupuncture to LI4 or to a designated nonacupoint (NAP) was applied on the hand. Real or simulated manipulation was applied every three minutes and the needle was retained for either one or 21 minutes. PPT measurements were completed before, during and following the 21-minute intervention period using an algometer at ten regional sites across the body. Intensities of needling sensation and pain were measured using a 100mm visual analogue scale (VAS) every three minutes and sensation qualities were reported post-intervention. The eight interventions comprised the following parameters:

Intervention	**Site**	**Retention time**	**Manipulation**
$LI4m^{+1}$	LI4	1 minute	present
$LI4m^{-1}$	LI4	1 minute	absent(simulated manipulation)
$LI4m^{+21}$	LI4	21 minutes	present
$LI4m^{-21}$	LI4	21 minutes	absent (simulated manipulation)
$NAPm^{+1}$	NAP	1 minute	present
$NAPm^{-1}$	NAP	1 minute	absent(simulated manipulation)
$NAPm^{+21}$	NAP	21 minutes	present
$NAPm^{-21}$	NAP	21 minutes	absent(simulated manipulation)

Results: Independent of the site of measurement (where the mean % PPT of all ten sites were combined for each intervention), the post-intervention mean % PPT were significantly elevated for all eight interventions. LI4m^{+21} produced the highest increase (9.1%) and LI4m^{-21} the lowest (3.7%). In terms of comparisons by site, the post-intervention mean % PPT were significantly elevated at all ten sites for the following interventions LI4m^{+21}, NAPm^{-21} and NAPm^{+1}; at nine sites for NAPm^{+21}, LI4m^{-1} and NAPm^{-1}, at seven sites for LI4m^{+1} and at only one site for LI4m^{-21}. No significant difference was found regarding the subjects' mean anxiety and tension levels and the acupuncturist's behaviour among the interventions. Immediately post-insertion, mean needle sensation and pain scores were similar for all eight interventions. At all other measurement intervals, irrespective of insertion site (LI4 or NAP), only the two interventions with needle manipulation every three minutes and with needle retention for 21 minutes maintained statistically significantly elevated needle sensation and pain scores.

Conclusions: The study did not find any clear relationship between the three needling parameters on regional PPT. However, it has shown that needle insertion is followed by an elevation in PPT above baseline levels that persists after needle removal. Presence or absence of needle manipulation and the duration of needle retention were important variables in terms of the intensity of needle sensation and pain. Similar needle sensation qualities and intensities were elicited at both the acupoint and the nonacupoint. This study also found that, irrespective of needling location, deep needling for 21 minutes with ongoing manipulation elicited and maintained elevated levels of needling pain and needling sensation. The study failed to provide findings that support the common Traditional Chinese Medicine (TCM) assumptions or assertions that *deqi* is necessary or essential for eliciting a physiological effect.

Supporting communications and publications

- Loyeung BYK, Cobbin DM. 2013 Investigating the effects of three needling parameters (manipulation, retention time, insertion site) on needling sensation and pain profiles: a study of eight deep needling interventions. Evidence-Based Complementary and Alternative Medicine. 2013: Article ID 136763, 12 pages, 2013. doi:10.1155/2013/136763.

Poster presentation:

- Loyeung BYK, Cobbin DM, Zaslawski C. 2012 Investigating the effects of the site of needle insertion, needle manipulation and retention time on *deqi* (needle sensation). 2012 Australian Acupuncture & Chinese Medicine Association conference, Brisbane.
- Loyeung BYK, Cobbin DM. 2013 Is needling retention time important? New Horizons conference, 18-20 November 2013, Royal North Shore Hospital, Sydney.

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Chapter I: Introduction

While acupuncture has its roots in antiquity, Richard Nixon's foreign policy initiatives to engage with China brought it to the attention of mainstream Western medical science (WMS). Perhaps most influential was the 1971 experience of a high profile columnist with the New York Times and associate of Henry Kissinger, who, while in China, required an emergency appendectomy. Receiving acupuncture to relieve post-operative pain, he wrote in glowing terms about its effectiveness. This engaged the interest of Nixon whose 1972 delegation to China observed an operation where the conscious patient received only acupuncture for surgery (Ford n.d., Jaffee 2009).

By the mid 1970s, the body's 'own opiates', as the endorphins (Woods 1994) were often dubbed, had been discovered and identified. This raised the possibility of a physiological mechanism for the pain-modulating effects of acupuncture. Pain modulation has continued to be an important focus perhaps unsurprisingly given pain is arguably the most common symptom reported by patients.

However, a broader interest was stimulated as evidenced by the burgeoning array of acupuncture-related clinical studies into its effectiveness or otherwise that has been published. Unfortunately, instead of investigating the validity of the basic acupuncture theories in the first place, most researchers have instead focussed in conducting clinical trials to show its efficacy. For example, to date no physical evidence for acupoints has been found or indeed whether acupoints are in fact at the locations detailed by TCM texts. The latter concern is complicated by the often quite disparate acupoint locations described in different acupuncture texts, as well as reliability issues concerning accuracy and precision of common traditional measurement procedures (Aird et al 2000, Coyle et al 2000, Aird et al 2002, Aird 2005).

Coyle's group (2000) used the traditional *cun* measurement system to investigate variation between the traditional measurements and the sample means for selected finger measurements, and for the forearm and lower leg lengths obtained from 50 volunteers from staff and students of the College of Traditional Chinese Medicine at the University of Technology, Sydney (UTS). Using the one *cun* measurement for the thumb as the standard the study reported significant differences between the traditional measurements and the sample means for almost all measurements in the sample. The

authors concluded that the *cun* measurement system failed to provide accurate measurements for contemporary Australian adults and recommended that point location should use methods that were less dependent upon *cun* measurements; that is proportional method measurements were regarded as preferable to directional method measurements.

However, Aird and colleagues' extensive evaluations of the accuracy and precision of both these traditional point location measurement systems found both to be poor performers in terms of being able to locate with reasonable accuracy a carefully defined location of a point. Aird and colleagues examined the relative precision of four methods of acupoint location by locating a fictitious acupoint operationally defined in terms of *cun* units from specific anatomical landmarks. The two traditional methods (directional and proportional) and two contemporary methods (elastic and ruler) were used in separate attempts by 72 subjects who were undergraduates of the Bachelor of Health Science in Acupuncture degree at UTS. The study found that both contemporary procedures were statistically significantly more precise than either traditional method. Interestingly, based on the varying scatter sizes obtained for the different methods, the authors calculated that for 95% of subjects to successfully locate the acupoint, the surface area of the acupoint would need to range from almost $13cm^2$ for the directional method compared with less than $3cm^2$ for the ruler method. The implications for acupuncture research are obvious. Inaccuracy in precisely locating the target acupoint in the treatment group may result in the subjects being needled at either a nonacupuncture point or a nearby acupoint, hence altering the desired treatment outcome. Similarly, subjects in a sham acupuncture group may be needled close to or exactly on an acupoint, resulting in a treatment effect when none is intended. The consequence of this inaccuracy in acupoint location is the likelihood of contributing to a Type II error, i.e., falsely retaining the null hypothesis and concluding that the treatment has no effect.

In regard to differences in published descriptions of acupoint locations, a survey by Aird (2005) of 151 research papers published in peer-reviewed and referenced journals between 1995 and 2000 identified the five most frequently used acupoints: ST36, LI4, SP6, GV20 and LR3. The point locations described for each of these five acupoints in seven well known acupuncture texts were then compared. Since LI4 is of central interest for the present study the definitions provided by the various texts have been included in Appendix VII. While the extent of variation in definitions varied widely among the five acupoints, and was greatest for GV20, all demonstrated the lack of consensus among texts. Since the dimensions or other makeup of any acupoint is still unknown, it is not possible to confirm whether even minor variations in location are clinically significant. In terms

of research, it again raises the unresolved problem of what location should be used when intending to needle a specific acupoint.

Agreement has been reached by consensus (WHO 2008) among experienced professionals in attempting to standardise the definitions of locations of every acupoint. While this may achieve reliable location (in spite of the inability of popular methods to reliably and accurately find the acupoint) of the same anatomical spot into which a needle may be inserted, it fails to provide any evidence to suggest they are in fact valid locations.

Since 1998 at UTS, experimental research into basic parameters of acupuncture has been undertaken in healthy subjects. The goal of the research program is to establish the veracity of many of the basic assertions of TCM encompassing pulse, tongue observation, point location and measurement systems and parameters of acupuncture needling. The present study represents the latest in a series of blinded controlled experimental studies into the effects of needling parameters and techniques on regional pressure pain threshold (PPT). All of these have been documented in successful MSc and PhD theses and in peer reviewed publications and conference presentations. All studies have maintained a similar protocol and operational definitions of procedures and variables and all have included one key intervention to facilitate comparisons among studies (deep needling of LI4, manual manipulation and 21-minute retention duration). A second common feature has been the involvement of the same two individuals to deliver the interventions and to record the regional PPT measurements. These features have been adopted to facilitate comparisons among results for the different programs. An important common finding was that deep needling of the acupoint LI4 with manipulation produced statistically significant increases in regional PPT in all studies (Yuan 2002, Zaslawski et al 2003, Szabo 2007, Li et al 2008). Importantly, Zaslawski and colleagues included an inactive laser at LI4 as a placebo control intervention and observed no significant change in PPT at any of the regional measurement sites. This finding indicated that minimal placebo effects on PPT were elicited, thereby providing justification for not including a placebo control group in subsequent studies following the same or similar protocols. In addition, it showed that the repeated application of pressure from the algometer to the regional measurement sites did not elicit an acupressure-like effect that could have been responsible for eliciting increases in regional PPT in interventions that involved acupuncture to LI4. That is, elevations in regional PPT in active acupuncture interventions could be attributed solely to the acupuncture interventions.

The effects on PPT following acupuncture do not to seem to be specific to acupoints. For example, significant increases in regional PPT were observed when a nonacupoint on the back of the hand was needled (Zaslawski et al 2003 and Yuan 2002). Indeed, one systematic review of 38 acupuncture clinical trials revealed that some forms of sham acupuncture were found to be as effective as 'true' acupuncture (Moffet 2009).

Another interesting finding in the UTS series of PPT studies is that the analgesic effects produced following deep needling of LI4 with manipulation were generalised across the body (Yuan 2002, Zaslawski et al 2003, Szabo 2007 and Li et al 2008). Therefore, neither neural segmental nor TCM channel theories were supported in terms of distribution of effects. For example, with respect to LI4, the generalised analgesic effects are at odds with acupuncture texts which described LI4 as an acupoint mainly used for conditions related to the head and face (Rogers and Rogers 1989 and Deadman et al 2001). These experimental findings, replicated in a series of studies, question the existence of acupoints, meridians and the various assumptions and assertions that underlie TCM acupuncture theories.

Another common assumption concerning acupuncture within the framework of TCM is that eliciting *deqi* during needling is important, possibly essential, for a therapeutic outcome (Benham et al 2010). *Deqi* is often described by acupuncture recipients as soreness, numbness, distension, aching or heaviness (Lai and Tong 2010). However, in spite of its assumed clinical relevance and importance, it is only in recent decades that research has been focussed on the nature of the *deqi* phenomena and the development of reliable instruments to measure and quantify it (Bovey 2006). The phenomenon of *deqi* remains poorly understood and operationally defined. Whether it is necessary for, or even contributes to, any specific clinical outcomes of therapeutic acupuncture is unknown and disputed in that while TCM-style acupuncture strives to elicit *deqi*, this is not the case with Japanese-style acupuncture.

While many authors have attempted to define the qualities that make up the *deqi* experience, few studies have evaluated the influence of needling parameters such as depth of needling, presence or absence of needle manipulation and duration of needle retention on the presence and maintenance of the *deqi* sensation (Benham et al 2010, Lin et al 1996). The present study examined three such needling parameters in relation to the reporting of *deqi* by healthy subjects. In addition, it reported the qualities of the needle sensation experienced and the intensity of pain at the needling site and effects on regional PPT elicited during and following the needling intervention. The three

parameters studied were site of needle insertion, needle manipulation and duration of needle retention.

This research protocol was divided into two distinct phases, both of which compared the effects of eight different acupuncture interventions. During the acupuncture intervention phase subjective perceptions of needling pain and sensation as well as of regional pressure pain threshold were recorded. Following the intervention, the effects on regional PPT were compared both within and between interventions. This study closely follows the design and protocols developed in 1999 at UTS and applied to related research into acupuncture and PPT in six previous postgraduate research programs (Li et al 2008, Zaslawski et al 2003).

Research Aims

To investigate the effect of three needling parameters: needle manipulation, needle retention time and site of needle insertion on:

- the strength and quality of needling sensation reported;
- the strength of pain at the needling site; and
- regional PPT measured at ten regional sites.

Outcomes were measured using quantitative VAS reporting of intensity of pain and of needle sensation and PPT measurements at ten regional sites.

Thesis format

Chapter II: Literature review

This chapter includes the evaluation of previous research in five areas: the effects of acupuncture as measured using PPT; the validity and reliability of algometry; a brief summary of the purported mechanisms relating to the pain-modulating effects of acupuncture especially relating to the neural mechanism known as Diffuse Noxious Inhibitory Control (DNIC); needle retention time; and needling sensation during acupuncture.

Chapter III: Methods

This chapter presents the study protocol and main statistical procedures used in the data analyses.

Chapter IV: Results

The study findings are presented in two parts. Part I focuses on the comparison on post-intervention changes in regional PPT from baseline values. This section involved extensive statistical analyses using the GLM and Tukey post hocs. To assist the flow of the text, the many analysis tables have been placed in Appendix VI and in general, only means and 95% confidence intervals or standard

errors appear in the chapter. Part II primarily presents findings relating to subjects' perceptions of needling sensation, needling pain and regional PPT reported during the 21 minute intervention period.

Chapter V: Discussion

In keeping with Chapter IV, the findings for the two parts of the study are discussed separately in this chapter.

Chapter VI: Conclusion.

This chapter summarises the main study outcomes and considers future directions.

Appendices

Appendix I: Information sheet for all participants.

Appendix II: Consent form for all participants.

Appendix III: The 100mm VAS sheet for recording each subject's beliefs in the effectiveness of acupuncture and willingness to receive acupuncture as a therapy.

Appendix IV: Table showing the type of needle sensation reported for each subject and intervention at different time interval during the intervention period.

Appendix V: Table showing the mean % PPT differences and p values between paired interventions (by site).

Appendix VI: Statistical analysis tables and scatterplots:

1. ANOVA tables and p values relevant to Part I of the study (Post-intervention changes in regional PPT).

2. ANOVA tables and p values relevant to Part II of the study (Needling pain, sensation and regional PPT profiles during the intervention phase) and scatterplots.

Appendix VII: An inter-text comparison of the operational definitions of the acupoint LI4.

Appendix VIII: Summary of the needling parameters used in the studies reviewed in Section 2.1.

Chapter II: Literature review

Five areas are reviewed in this chapter. These are (a) published studies that have evaluated the effects of acupuncture as measured using PPT; (b) published studies on the validity and reliability of algometry; (c) a brief summary of the purported mechanisms relating to the pain-modulating effects of acupuncture especially relating to the neural mechanism known as Diffuse Noxious Inhibitory Control (DNIC); (d) needle retention time and (e) the measurement of needling sensation during acupuncture.

2.1 Acupuncture and PPT

This review includes randomised controlled studies of acupuncture and its effects on PPT as measured by algometry. A final literature survey was conducted for studies in English in June 2013 using the electronic databases PUBMED and Science Direct. Search terms were: pressure pain threshold and acupuncture. After excluding studies that involved animals, dry needling or trigger point injection therapy, 14 papers were found. Two additional studies (Zaslawski et al 2003, Li et al 2008) were also excluded as these have been discussed in Chapter I. This left 12 studies which will be reviewed in this chapter.

A randomised double blind (subjects and assessor) placebo controlled study (Karst et al 2000) looked at the effects of acupuncture on chronic tension headache. Twenty one subjects received acupuncture at both LI4 and LV3 and a range of other points (GB14, GB21, GB41, BL2 BL10, BL60, LU7, TE5 ST8, ST36, ST44, GV20, EX1) depending on symptoms and tenderness at local muscular sites. The control group consisted of 18 subjects who each received noninvasive sham acupuncture at GB20 using blunt needles held on the skin surface with elastic foam. The verum acupuncture subjects received acupuncture twice a week for five weeks with each treatment lasted 30 minutes. PPT at both the left and right temporal regions of the head was measured with an algometer pre-intervention and six weeks post-intervention. While the authors reported a statistically significant increase in PPT for the verum but not the control group, they failed to report whether the effects elicited by the two interventions also differed significantly.

A recent single blind (subject) cross over design study (Choi et al 2013) looked at the effect of acupuncture with manual manipulation on PPT and needle sensation. Fifty three subjects were

randomised to receive each of these three needling conditions in a random order: superficial needling (0.3cm), deep needling (2cm) and needling with bi-directional rotation. There was a 48 hours interval between each session. The points needled were SP6, ST36, SP9 and GB39 on the left leg and each acupuncture session lasted five minutes. PPT was measured (pre and post-intervention) using an algometer at a site in the middle of the four acupoints but on the right lower leg. The mean percentage PPT change for the manipulation intervention was statistically significantly higher than for either the shallow needling or the deep needling conditions. The main weakness of this study is that baseline PPT was measured only once, the authors assuming that it would remain the same after a washout period of only 48 hours. Also, the depth of needle insertion for the intervention with manipulation was not reported.

The study by Mavrommatis and colleagues (2012) evaluated the use of acupuncture as an adjunctive therapy to pharmacological treatment in patients with chronic pain due to osteoarthritis of the knee. One hundred and twenty patients were randomly allocated to one of three groups: acupuncture combined with etoricoxib; sham acupuncture and etoricoxib; and etoricoxib alone. Retractable needles were used for the sham acupuncture group. Outcome measures included the WOMAC index, a pain VAS and PPT measured at a specific trigger point on the painful knee. The protocol for acupuncture was biweekly treatments for eight weeks at the following acupuncture sites: ST36, SP9, SP10, GB34, Ex-LE2, Ex-LE5, LI4, KI3, ST40 and SP6. From week three, electro stimulation was applied at the following acupoint pairs ST36-SP9 and GB34-SP10. By the end of week eight and again at week 12, patients treated with acupuncture plus etoricoxib experienced a statistically significantly higher pain threshold compare to the other two groups.

In a study (He et al 2004) investigating the effect of acupuncture treatment on chronic neck and shoulder pain in sedentary female workers, 14 subjects were randomly allocated to the verum acupuncture group and ten to the sham acupuncture group. The acupuncture treatment was a combination of body (LI11, GB31), ear (*shenmen*, cervical spine, shoulder, shoulder joint, shoulder back) and body electro (ExHN: *Jingjiaji*, GB12, BL12, GV14, SI15, SI14, LI4) acupuncture. Each subject received three treatments per week over three to four weeks, with a total of ten treatments. In the sham group, acupuncture was applied to points 10-40mm distal to actual body points and 4-6mm distal to auricular points. PPT were measured at baseline, on completion of the treatment phase and six months post-intervention at five shoulder muscles namely *trapezius, levator scapula, teres minor, sub occipital* and *supraspinatus* (description of the exact location was not given). For the *trapezius* and *levator scapula* muscles, there were statistically significant increases in PPT from

baseline both at completion of the treatment phase and at six months for the verum acupuncture group but not for the sham group. However, there was no statistically significant difference between the two groups at any time. For measurement at the *teres minor* muscle, there was a statistically significant increase from baseline at six months but not at the completion of the intervention phase for the verum acupuncture group while there were no significant changes for the sham group. For the *supraspinatus* and *sub occipital* muscles, there were no significant differences between groups and no significant increase of PPT from baseline for either intervention. However, the reliability of the study is questionable due to the small sample size and the locations of the acupoints used in the group were not precisely defined. The range of 10-40mm distal to actual body points was wide and the direction from actual body points was not given.

Another study (Nabeta and Kawakita 2002) investigated the effect of manual acupuncture to tender points on subjects with chronic neck and shoulder pain. In this randomised and subject blinded study, the 34 subjects were randomly and equally allocated to either the verum acupuncture group or the non-invasive sham acupuncture group. In the verum acupuncture group, acupuncture with manual manipulation was applied for five minutes at tender points located in the shoulder and neck regions. In the sham acupuncture group, blunt needles were used and the acupuncturist applied mock insertion, manipulation and removal of the blunt needles. PPT was measured using an algometer before and after the intervention. Since the acupuncturist located tender points on the shoulder and neck region and needling was done at these tender points. As a result, the number of needles per subject varied, depending on the number of tender points that the acupuncturist located. For the verum acupuncture group, the PPT values of all the trigger points statistically significantly increased from baseline but that was not the case for the sham acupuncture group. The authors did not report whether there was a significant difference between the two groups in terms of mean percentage PPT change.

A study by Itoh and colleagues (2011) looked the effect of the depth of acupuncture needle penetration on exercise-induced muscle pain (*extensor digitorum longus* muscle in the forearm). The 22 subjects were randomly assigned to four groups: control group, skin group, muscle group and the nonsegmental group. Participants in the control group rested supine on a standard treatment table with no intervention for 30 minutes. Participants in the skin and muscles group received acupuncture at the most tender point on the *extensor digitorum longus* muscle and the needle was retained for 30 minutes. Participants in the nonsegmental group received acupuncture on belly of the anterior *tibial* muscle approximately over the musculotendinous junction. PPT was measured

before exercise at a point 20mm distal to the maximum tender point and on two later occasions: two days following the exercise and immediately after acupuncture treatment. There was a statistically significant decrease in PPT values for all four groups two days after the exercise. However, after the acupuncture treatment statistically significant recovery (mean PPT values returning to baseline) was only observed in the skin group (3mm needle insertion) and in the muscle group (10mm needle insertion) but not in the control group. However, the reliability of the study is questionable due to the small sample size and the failure to blind the subjects. Furthermore, only the lowest PPT reading out of the three were recorded instead of using mean of those three values at each measurement time.

In a randomised and single blinded (subject blinding) crossover design study, Lang and colleagues (2010) investigated the effects of three different forms of acupuncture on PPT. The 24 subjects received the following interventions in a random order with one week washout between each intervention session: manual acupuncture with manipulation (MA); low frequency (2Hz) electroacupuncture (LF-EA); and high frequency (100Hz) electroacupuncture (HF-EA). Acupuncture was applied on the left leg at SP6, SP9, ST36 and GB39 and each acupuncture treatment lasted for 30 minutes. In the MA group, the needles were manually manipulated after insertion and 15 minutes post insertion. In both electroacupuncture groups, the needles were manually manipulated after insertion, and then EA was applied at all four acupoints using an electric device with a frequency of 2Hz for the LF-EA group and 100Hz for the HF-EA group and PPT was measured at baseline and after treatment. PPT was increased by all three interventions on both sides of the body, with individual PPT increases from baseline ranging from 25% to 52%. While the authors reported the significance level as $p<0.0001$ between the interventions, they failed to mention where that significant differences lies. In addition, the study does not report the location(s) at which the PPT measurements were recorded.

In a study conducted by Zhang and colleagues (2011) to determine the efficacy and specificity of the acupuncture in the treatment for *plantar fasciitis*, the subjects were randomly assigned to a treatment group (N=28) or control group (N=25). The treatment group received needling at the acupoint PC7 while the control group received needling at the acupoint LI4. Over a two week period, all subjects received ten daily treatment sessions of 30 minutes duration, with manual needle manipulation applied every five minutes. PPT at the most painful site on the painful foot was measured prior, then every day for nine days after the first treatment, then at one month following completion of the treatment phase. There was no significant change in PPT in either group up to day

nine. However, at the one month post, the mean PPT value for the treatment group (PC7) was statistically significantly higher than for the control group (LI4). The study did not report whether either increase was statistically significantly different from baseline values.

Another study (Hübscher et al 2008) investigated the effects of acupuncture on symptoms and muscle function in exercised-induced delayed-onset soreness (DOMS). Twenty two healthy subjects were randomly assigned to three treatment groups: real acupuncture (deep needling at classic acupoints and tender points, N=7), sham acupuncture (superficial needling at nonacupoints, N=8), and control (no needling, N=7). Participants in the acupuncture group received acupuncture at the following points: GB34, LU3, LU5, LI11, SP10 and *ashi* (tender) points. The needles were manually stimulated once at the beginning of each 15 minute session. The sham acupuncture participants received superficial needling with no manipulation at sites located 2 *cun* from the acupoints used in the acupuncture group. Treatments were applied 24 and 48 hours after DOMS was induced. In the control group, the participants lay on the treatment table for 15 minutes without any intervention. DOMS of the nondominant elbow-flexors was experimentally induced using a standardised exercise protocol and PPT was measured before and after every treatment session at seven equidistant points along a line joining the insertion of the biceps *brachii* on the radius and the acromion. PPT was measured before DOMS induction and at 24, 24 and 72 hours post DOMS induction. The results showed that there were no significant differences in mean PPT at any time interval. Participants in all three groups reached recovery 72 hours post DOMS induction, showing that acupuncture was no better than the control in relieving exercised-induced delayed-onset soreness.

In a study investigating the effectiveness of acupuncture for fibromyalgia (Targino et al 2008), 58 women with fibromyalgia were allocated randomly to receive either acupuncture together with standard care (N=34), or standard care alone (N=24). Patients in the acupuncture groups received acupuncture twice per week for ten weeks. Each session lasted 20 minutes and was administered at the following acupoints: Ex-HN-3, bilateral LR3, LI4, PC6, GB34 and SP6. The needles were not manipulated after insertion. Standard care consisted of 12.5-75 mg of tricyclic antidepressants per day, walking for 30 minutes twice a week at their own pace, deep breathing and mental relaxation exercises for another 30 minutes. PPT was measured at 18 fibromyalgia points across the body at baseline then at 3, 6, 12 and 24 months. The average PPT values of the 18 points were used in the analysis. There was no statistically significant increase of median PPT from baseline for the control group at any time. However, at the three and six month follow up period there were statistically

significant increases in median PPT from baseline for the acupuncture group. The median PPT value in the acupuncture group then decreased at 12 and 24 month follow ups and was not statistically significantly different from baseline. It was not mentioned whether there were any statistically significantly differences between the median PPT values of the two groups at any time. The validity of the study is questionable due to the small sample size and the failure to blind the subjects.

In a single blind (subject blinding) study, Shen and Goddard (2007) investigated the effects of acupuncture on myofascial pain patients after clenching. Fifteen chronic myofascial pain subjects were randomly assigned into two groups: acupuncture (N=9) and sham acupuncture (N=6). The participants in the acupuncture group received a single treatment with only one needle at LI4 to a depth 10-20mm and the needle was manually stimulated for five seconds after five minutes of needle retention which lasted for 15 minutes. Sham acupuncture consisted of the use of a blunt needle mock insertion one centimetre distal to LI4 and the needle was maintain in place using a foam pad. PPT was measured on the right masseter muscle before and after intervention. There were no statistically significant changes in mean PPT levels from baseline in either group. Again, the validity of the study is questionable due to the small sample size and only a single treatment which lasted for 15 minutes.

In a study conducted by Barlas and colleagues (2000) on the effect of acupuncture on exercised induced delayed onset muscle soreness (DOMS), 48 participants were randomly allocated to one of four groups: control (20 minutes rest), placebo (minimal needling at four nonacupoints between the biceps *brachii* and the humerus), treatment group one (acupuncture at classic acupoints (PC2, LI11, LU5 and LI4) and treatment group two (acupuncture at 'tender' points on the biceps *brachii*). In all three acupuncture groups, the needles were manipulated every five minutes for 15 seconds and each treatment lasted 20 minutes and treatment was delivered every day for five consecutive days. DOMS was induced in the biceps *brachii* muscle of the nondominant arm through a set of exercises with dumbbells and free weights. PPT measurements were performed at eight equidistant points on the biceps *brachii* of the nondominant arm pre-intervention and every day after each intervention. The mean PPT values of the eight measurement sites were used in the analysis. The mean PPT profiles for all four interventions were similar from day one to day five with the control group having higher mean PPT values compared with the other interventions but they were not statistically significant. Overall, there were no significant differences between the four interventions at any measurement time.

In summary the present evidence does suggest that some forms of manual acupuncture increase PPT in both healthy and chronic painful conditions. However, due to the diversity of needling parameters used (retention time, manipulation status, acupoints) and the lack of appropriate study designs, we do not know which of these parameters are contributing to an increase in PPT in those studies. Mavrammatis and colleagues (2012) used ten acupoints in their study while Nabeta and Kawakita (2002) used only one at a nondefined acupoint. Karsts and colleagues (2000) used LV3 and LI4 as mandatory acupoints and the acupuncturist could select from a further thirteen points additional acupoints depending on the subject's condition. Among the five studies, only three used LI4 in the verum acupuncture intervention (Karsts et al 2000, Mavrommatis et al 2012, He at al 2004). In terms of retention time, two studies left the needles in for five minutes (Choi et al 2013, Nabeta and Kawakita 2002), 15 to 20 minutes for four studies (Hübscher et al 2008, Targino et al 2008, Shen and Goddard 2007, Barlas et al 2000), 30 minutes for four studies (Karsts et al 2000, Itoh at al 2011, Lang et al 2010, Zhang et al 2011), 45 minutes for another study (He at al 2004) while Mavrammatis and colleagues (2012) failed to disclose the retention used in their study. Manual needle stimulation was applied in six studies (Choi et al 2013, Lang et al 2010, Zhang et al 2011, Hübscher et al 2008, Shen and Goddard 2007, Barlas et al 2000, Nabeta and Kawakita 2002), electro manipulation in another two studies (Mavrammatis at al 2012 and He at al 2004) while one study did not apply any needle stimulation (Karsts et al 2000). A table summarising the needling parameters used in these various studies has been included in Appendix VIII.

2.2 Measuring PPT

There are various methods that reportedly have good reliability, that are available for assessing pain perception (Thomson et al 2009). These include verbal or numerical rating scales and visual analogue scales (VAS) (Von Korff et al 2000) and pain questionnaires such as the McGill Melzack Pain Questionnaire (MMPQ) (Triano et al 1993). The measurement of PPT via pressure algometry is another method used to quantify a patient's perception of a painful experience (Thomson et al 2009) and has been shown to be a reliable and relatively inexpensive method of measuring soft tissue tenderness (Reeves et al 1986, Nussbaum and Downes 1998, Fischer 1987). The algometer is a spring loaded pressure gauge attached to a rubber plunger (Figure 2.1). The researcher holds the algometer against the skin of the subject and pressure is then applied gradually until the subject perceives the initial pressure change to a distinct sensation of unpleasantness or discomfort. At this point the pressure is terminated and is defined as the PPT (Li et al 2008).

Figure 2.1: An algometer showing the black rubber plunger (surface area of 1cm^2) connected to a spring loaded shaft. The scale indicates the force applied in kilogram (kg) per cm^2.

This technique has been studied widely and is being increasingly used not only as a diagnostic tool for many painful musculoskeletal conditions, but also in related clinical research (Fischer 1987, Ohrbach et al 1989, Brennum et al 1989, Antonaci et al 1992, Kosek et al 1993). Therefore, algometry is both a suitable and reliable method for measuring PPT under both experimental and clinical situation. Furthermore, reliability is improved if the same person completes all the measurements; if the initial measure in a series of trials is discarded; if adequate rest time between repeated trials is included; and if application of pressure is kept constant at a recommended rate of 1kg/sec (Nussbaum and Downes 1998). For these reasons, pressure algometry was chosen to measure pain threshold in the present study.

2.3 Analgesic effect of acupuncture

Acupuncture is widely used to treat different kind of acute and chronic pain conditions including osteoarthritis (Mavrommatis et al 2012), lower back pain (Leibing et al 2002), dysmenorrhoea (Witt et al 2002) and tension headache (Silva et al 2012). However, the mechanisms whereby acupuncture can modulate pain can only be partially explained and research studies have revealed that there are actually several hypotheses that can explain its mechanism for modulating acute and chronic pain. These hypotheses are: the release of endogenous opioids, modulation of the adrenergic system, modulation of the 5-hydroxytryptamine signalling system, modulation of N-methyl-D-aspartic acid/AMPA/ kainate signalling system, modulation of other neurotransmitter systems, the anti-inflammatory theory, modulation of long-term depression and long- term potentiation neural plasticity and the activation of the diffuse noxious inhibitory control (DNIC) system (Leung 2012).

The present research was not designed to provide findings that relate to or test any of these mechanisms. However, there is one suggested pain modulatory phenomenon that could possibly be explored because of the study design and that is the phenomenon of DNIC. This is justified by the

fact that a generalised pain modulatory effect ascribed to DNIC is known to be activated when a painful conditioning stimulus is applied at a single, limited area on the body. For example, experiments have shown that when a strong heat stimulus was applied at one site, this results in the dampening of pain experienced at other sites (Talbot et al 1987, Lautenbacher et al 2008). This phenomenon has, in fact, been recognised since ancient times as illustrated by the Hippocrates' aphorism: "*If a patient be subject to two pains arising in different parts of the body simultaneously, the stronger blunts the other*" (page 11 Le Bars and Willer 2002).

A recent acupuncture study reported that manipulation of the needles was followed by an increase in both needle sensation (*deqi*) and PPT (Choi et al 2013). It has been suggested that the painful stimulus provided by acupuncture needle manipulation is responsible for eliciting the analgesic effects of acupuncture (Le Bars and Willer 2002) via the pain modulatory processes. To investigate whether acupuncture analgesia is similar to cold induced DNIC, Schliessbach and colleagues (2011) compared the mean percentage change in PPT in healthy subjects at different time interval between three groups: acupuncture at LI4; cold induced DNIC; and sham acupuncture at LI4. The mean percentage PPT change for the verum acupuncture group represented a statistically significant increase from baseline, and remained relatively constant at two minutes and five minutes post needle insertion. By comparison, in the cold induced DNIC group the mean percentage PPT change had a marked, rapid increase post insertion (significantly higher than verum acupuncture group) and at two minutes post measurement. However by the five minute measuring period, the change in PPT had decreased to the same level reached by the verum acupuncture group. The authors concluded that their findings showed that the mean percentage PPT profile elicited by the acupuncture was different from that mediated by cold induced DNIC in healthy subjects. Unfortunately, due to multiple flaws in the study design the validity and reliability of their findings are highly questionable. For example, there was only a ten minute wash out interval between each intervention and no baseline PPT measurements were taken between each intervention.

Staud and colleagues (2003) compared the effect of heat induced DNIC (left hand in hot water bath) on thermal pain (repeated heat taps on the thenar surface of the right hand) on three subject groups: normal control (NCM) males; normal control (NCF) females; and fibromyalgia (FMS) females. They reported that DNIC significantly inhibited thermal pain in normal male subjects, but not for the NCF and FMS female patients, indicating that DNIC was gender specific and that women lacked this pain-inhibitory mechanism.

2.4 Acupuncture and needle retention time

The earliest mention of needling retention time can be found centuries ago in one major TCM classical textbook - *The Systematic Classic of Acupuncture and Moxibustion*. This textbook was written by Huang –fu Mi in the *Jin* dynasty (265-420 CE) and was translated by Yang and Chase in 1994. The author recommended retaining the needle for only three exhalations for the acupoint LI4. Needling retention time was also mentioned in a later classical textbook - *The Great Compendium of Acupuncture and Moxibustion* written by Yang Jizhou and was published during the *Ming* dynasty in 1601. The needling retention time recommended by the author is six exhalations for the acupoint LI4. In a recent textbook on medical acupuncture (Jin et al 2006), the authors mentioned that studies in China observed that the threshold of pain tolerance first increased with a persistent needling stimulation, then reached its maximum after a certain period of time (called the induction period) in acupuncture anaesthesia. According to the authors, the induction period for analgesic effect is usually between 30 and 40 minutes.

Similarly duration of needle retention was found to not be a central concern of contemporary acupuncture clinical research publications. This was evidenced from various literature searches conducted as recently as June 2013 for studies in English using the electronic databases PUBMED, MEDLINE and Science Direct. The following combination of search terms were used: 'acupuncture' and 'needling retention time' or 'acupuncture' and 'treatment time' or 'acupuncture' and 'length of intervention'. None of the studies obtained specifically related to clinical studies on the effect of needling retention time on acupuncture treatment outcome. However, from general reading of clinical case studies and audits, it is clear that needle retention durations as brief as one or a few minutes are quite commonly employed, for example by medical acupuncturists working within the time constraints of the United Kingdom's National Health System (Downey 1995, Stellon 2001, Freedman 2002, Joseph 2002). These medical acupuncturists are medical practitioners who attended short training courses run by the British Medical Acupuncture Society (BAMS) (Joseph 2002). Given that the standard medical appointment in their general practice is only ten minutes, and acupuncture is reported to be found to be an effective treatment modality (Joseph 2002), the use of a brief needling duration is apparent. Indeed, audits of acupuncture practice in general medical practices are commonly completed and reported according to the editor in chief of the Acupuncture in Medicine journal (White 2005). From a search of the Acupuncture in Medicine online journal using the keywords 'acupuncture', 'audits' and 'pain', it was evident that the needling retention time reported by medical practitioners was typically between one and two minutes (Downer 1995). Further, clinically positive outcomes were commonly reported (Downey

1995, Stellon 2001, Freedman 2002, Joseph 2002). The validity of the outcomes, in terms of acupuncture being the causal agent for the reported clinical improvements in these audits is questionable due to the fact that they do not have the rigorous methodology of controlled trials. Absence of control groups, the selection by the practitioners of specific patients participating in the audit, as well as the evaluation of the patient's improvement; and failure to blind the patients are a few examples. However, these audits continue to report some successful outcomes in the treatment of a wide range of pain related conditions. Examples include: facial pain (Merchant 1995), chronic neck pain (Blossfeldt 2004), chemotherapy induced peripheral neuropathy (Donald et al 2011) and various types of musculoskeletal pain (Joseph 2002, Downey 1995).

Another form of therapy that makes use of a shorter needling retention time is dry needling. This is a technique commonly used by physiotherapists to treat pain related conditions (Kalichman et al 2010). Dry needling is a form of needling where myofascial trigger points (MTrPs) are needled instead of the traditional acupoints. According to Baldry (2002), there are two types of dry needling technique: superficial dry needling (SDN) and deep dry needling (DDN). For the former, Baldry recommended inserting an acupuncture needle into the tissues overlying each MTrP to depth of 5-10mm for 30 seconds to deactivate the MTrP and thereby relieve pain. If residual pain persists, the needle is reinserted for two to three minutes.

While the SDN technique is well documented, clinical studies showing its efficacy are rare. A search in PUBMED using the search word 'dry needling' and 'superficial' located a single clinical study. In a randomised and single blind (subject blinding) study, Edwards and Knowles (2003) tested the hypothesis that SDN combined with active stretching is more effective than stretching alone or no treatment in deactivating trigger points and reducing myofascial pain. Prior to needling, a physiotherapist identified up to six trigger points per subject, marking each on the skin with a pen. The 40 subjects were randomly allocated among three similar sized study groups that received: SDN and active stretching exercises, stretching exercises alone or no treatment control. The outcome measures were the MMPQ and PPT for which the algometer was applied directly over each trigger point. MMPQ and PPT were recorded at pre-intervention, post-intervention and at a three week follow up. At post-intervention there were no significant intergroup differences in both outcome measures. However, after three weeks the SDN with stretching exercises group had significantly less pain compare to the control group and significantly higher PPT compared with the stretching alone group.

Needling retention time is one acupuncture parameter that warrants investigation, not only because of the lack of information in traditional acupuncture texts but also because research has failed to focus on its importance. In the present research the effects of two different needle retention times (one and 21 minutes) on the analgesic effect of acupuncture as measured by PPT will be investigated.

2.5 Acupuncture and *deqi*

Many traditional Chinese acupuncturists consider the elicitation of *deqi* during needling as essential for a therapeutic outcome (Benham and Johnson 2010). *Deqi* is often described by acupuncture recipients as a constellation of sensations including soreness, numbness, distension, aching or heaviness (Lai and Tong 2010). However, it is only in recent decades that research has been undertaken to determine the nature of the *deqi* phenomena and develop reliable instruments to measure and quantify the sensations that arise during acupuncture (Bovey 2006).

Despite being mentioned in the Yellow Emperor's Internal Classic (*Huang Di Nei Jing*), in the 21st Century the phenomenon of *deqi* remains poorly understood and operationally defined. Whether it is necessary for, or even contributes to, any specific clinical outcomes of therapeutic acupuncture is unknown. Although TCM-style acupuncture strives to elicit *deqi*, this is not the case with some Japanese style acupuncture. This is in spite of a substantial number of studies that have attempted to identify and quantify the sensations that may make up *deqi*. Interestingly, on one hand, authors strive to differentiate those needle sensations that they regard as aspects of *deqi* from ones that reflect the acute pain associated with needle insertion and retention (MacPherson and Asghar 2006, White et al 2008). Yet on the other hand, the development of a variety of psychometric instruments to measure the qualities and often the intensities of the *deqi* sensations has its origin in the reliable and valid McGill Melzack Pain Questionnaire. This is not unexpected since Melzack (1973, 1975) and colleagues cast widely for descriptors (sensory, affective and evaluative) that people used to describe pain; then grouped them into categories of similar sensation; and within each grouping, ordered the terms from minimally discomforting or painful, through to the most intense.

In an early example, Vincent and colleagues (1989) adapted the MMPQ to create a new scale of 20 sensory descriptors to measure the sensations of acupuncture. Interestingly, in ~~a~~ this study with a sample of 65 volunteers, needling both at acupoints and nonacupoints provoked similar levels of needle sensation on the scale suggesting *deqi* was not exclusive to acupoints. This instrument, as with others that grew out of the MMPQ, has been criticised because it originated from a pain

questionnaire and consequently of potentially measuring pain in addition to the supposedly nonpainful sensations arising from acupuncture (Lundeberg 2012).

A range of psychometric instruments have subsequently been developed to measure *deqi*. Common modifications have been to select only a subset of the 20 categories included in the MMPQ to expand single descriptors from the MMPQ into a VAS or similar scale where the intensity of that one quality can be further refined; for example, ache, tingling and numbness, with each ranging from none to unbearable (Kong et al 2007). In some instruments, the descriptors have been sourced from subjects after receiving acupuncture. Others include terms selected by acupuncturists. For example, MacPherson and Asghar (2006) developed a classification of Needle Sensations Associated with *deqi* based on ratings by 20 TCM acupuncture experts. Two clusters of sensations were identified. One was linked with *deqi* (aching, dull, heavy, numb, radiating, spreading, tingling) while the other related to acute needling pain (burning, hot, hurting, pinching, pricking, sharp, shocking, stinging, tender). White and colleagues (2008), based on their qualitative interviews with patients, developed the 17 item Southampton Needle Sensation Questionnaire (SNSQ). Kong and colleagues (2007) developed the Massachusetts General Hospital Acupuncture Sensation Scale (MASS). The instrument uses 13 Likert scales for 12 sensory descriptors as well as a scale for other sensations, a mood scale and an acupuncture sensation spreading scale. It has been translated into the Chinese language for use in Asia (Yu et al 2012). In addition, visual analogue scales (VAS) have been used for recording and monitoring *deqi* sensations. Benham and colleagues (2010) used a modified single VAS while a German research group used several such scales for recording five *deqi* sensation variables (Kou et al 2007).

While many authors have attempted to define the qualities that make up the *deqi* experience, few studies have evaluated the influence of needling parameters such as depth of needling, presence or absence of needle manipulation and duration of needle retention on the presence and maintenance of the *deqi* sensation (Benham et al 2010, Lin et al 1996). The present study examined three such needling parameters in relation to the reporting of *deqi* by healthy subjects as measured by a single VAS. In addition, the present study reported the qualities of the needle sensation experienced and the intensity of pain at the needling site. The three parameters studied were site of needle insertion, needle manipulation and duration of needle retention.

Chapter III: Methods

3.1 Introduction

This study closely follows the design and protocols that were initially developed in 1999 at UTS and have been applied to related research into acupuncture and PPT in a series of postgraduate research programs. All of these have been documented in MSc or PhD theses and are available at the Closed Reserve section of the UTS library (Yuan 2002, Li 2005, Zaslawski 2006, Szabo 2007). A key feature of the studies has been the inclusion of a common intervention in each of them (deep needling of LI4, manual manipulation and 21-minute needle retention duration). A second has been the involvement of the same two individuals to deliver the interventions and to record the pressure pain thresholds. These features have been adopted to facilitate comparisons among results for the different studies.

Ethics approval was obtained from the UTS Human Research Ethics Committee prior to commencing the study (UTS HREC 2009-067A). Appendices I and II include copies of the information sheet and consent form given to the subjects.

3.2 Subjects

The 24 study subjects (12 men and 12 women) were volunteers from the broader university staff and student community, recruited via the UTS Faculty noticeboards and word of mouth. Study inclusion criteria were healthy adults with no medical history of chronic musculoskeletal disorder and aged between 18 and 45. Exclusion criteria included regular users of analgesic or other drugs that may dampen pain perception, haemophilia, and use of anticoagulant medication that may interfere with blood clotting. Participants were requested to abstain from analgesic medication on intervention days. Three volunteers were replaced during the study, each by a person of the same gender, for the following reasons: work commitments (completed one session), injury in a motorbike accident (completed two sessions), baseline PPT was at the maximum algometer scale value (completed three sessions). Subjects quantified their belief in acupuncture and whether they would be willing to receive acupuncture as a form of therapy using a 100mm VAS designated to range from no effect to certainly effective for the former and from never to anytime for the latter. A copy of the VAS recording sheet is found in Appendix III. For the 24 subjects, Table 3.1 presents

by gender and overall, mean height, age and weight, as well as handedness ratio and their responses to the questions shown in Appendix III.

	Male		Female		All subjects	
	Mean	sd	Mean	sd	Mean	sd
Age (years)	27.3	4.2	28.8	6.6	28.1	5.5
Height (cm)	177.3	7.1	163.9	6.7	170.6	9.6
Weight (kg)	71.6	7.3	59.8	6.5	65.7	9.1
BMI	22.7	1.7	22.2	1.9	22.5	1.8
Belief score (% VAS)	87.2	18.5	88.8	16.7	88.0	17.6
Willing to receive acupuncture score (%VAS)	86.0	18.5	94.8	6.0	90.4	12.3
Handedness (right:left)	12:0		11:1		23:1	
First acupuncture experience (yes:no)	7:5		3:9		10:14	

Table 3.1: Demographic data for the 24 subjects; sample standard deviation shown as (sd)

3.3 Methods

3.3.1 Design

The study comprised two arms: One examined needle sensations and pain at the site of needle insertion and involved a randomised single blind (subject) crossover design with repeated measures. For the other arm, which examined effects on regional PPT during and post needling intervention, both subject and assessors were blind to the intervention. All 24 subjects received all eight interventions in a randomly ordered sequence. All interventions were spaced at least seven days apart to avoid crossover effects from the previous intervention/session.

Intervention sequence allocation

A random sequencing of the eight interventions for each subject was achieved using an envelope method that was also stratified by gender to match as closely as possible the sequencing by gender. Each sequence was printed on a slip of paper and sealed into an individual envelope marked F or M. At the beginning of their first session, the subjects chose one of the available envelopes and this determined their unique sequence of interventions. Each subject completed eight intervention sessions spaced at least one week apart. The eight interventions were renamed for the individual session recording sheets (lily, rose, daisy, magnolia, honeysuckle, peony, sunflower and orchid) so that only the acupuncturist knew the actual intervention the subject received.

For each intervention session, a single 0.22mm x 30mm sterile stainless steel disposable needle (Viva USA) was inserted at either the acupoint LI4 or the nonacupoint and for either one or 21 minutes. Insertion on all occasions was perpendicular (90^0) to the skin and to a depth of 15-20mm, thereby not only puncturing the skin but also underlying structures such as muscle, fat and fascia. The intervention was applied unilaterally on the right arm. The needling parameters examined were site of insertion, needle manipulation and needle retention time. These are defined below:

Needle manipulation

- Manipulation present - needle manipulation involved rotating the needle for five seconds between the thumb and index finger through a large 540-720° angle in a bidirectional manner. This was applied just after needle insertion and at every three minutes.
- Manipulation absent – just after needle insertion and at every three minutes, the acupuncturist rested his hand in the same position as above and lightly moved his fingers on the back of the subject's hand to mimic movements that would accompany needle manipulation. This is referred to as 'simulated manipulation'.

Needle retention time

Duration of needle insertion was either one minute or 21 minutes. One minute retention is consistent with the recommendations from the earliest extant TCM textbooks *The Systematic Classic of Acupuncture and Moxibustion* and *The Great Compendium of Acupuncture and Moxibustion* (discussed in Section 2.4). Needle retentions of 20-30 minutes are common practice currently and 21 minutes is the same retention time used in previous studies with similar design as the current one (Yuan 2002, Li 2005, Zaslawski 2006, Szabo 2007). Note that for the one minute duration interventions, the needle was only present during the initial t=0-1 minute interval. However the acupuncturist applied simulated manipulation of the 'virtual' needle every three minutes throughout the 21-minute intervention period, as described above. At 21 minutes, the acupuncturist ensured the removal of the needle was evident to the subject.

Site of needling insertion

LI4: acupoint located at the highest point of the *adductor pollicis* muscle when the thumb is adducted (Rogers and Rogers 1989).

NAP: nonacupoint located within the same dermatome as LI4, on the dorsal aspect of the hand, midway along the medial side of the shaft of the second metacarpal bone. This point is equidistant between the two acupoints *luozhen* and *yao tong xue* (Zaslawski et al 2003). No reference to a classical acupoint at this site has been documented (Cheng 1987). Figure 3.1 shows the location of both LI4 and NAP.

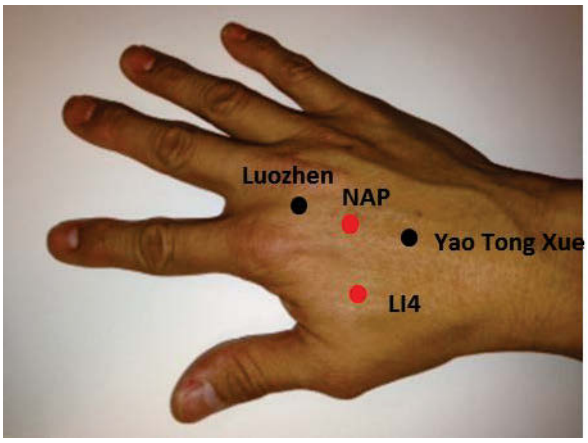


Figure 3.1: Location of LI4 and NAP in relation to the two extra acupoints *luozhen* and *yao tong xue.*

The eight interventions comprised the following parameters:

Intervention	Site	Retention time	Manipulation
LI4m^{+1}	LI4	1 minute	present
LI4m^{-1}	LI4	1 minute	absent(simulated manipulation)
LI4m^{+21}	LI4	21 minutes	present
LI4m^{-21}	LI4	21 minutes	absent (simulated manipulation)
NAPm^{+1}	NAP	1 minute	present
NAPm^{-1}	NAP	1 minute	absent(simulated manipulation)
NAPm^{+21}	NAP	21 minutes	present
NAPm^{-21}	NAP	21 minutes	absent(simulated manipulation)

3.3.2 Regional PPT measurement sites

All ten PPT measurement sites were marked with a felt pen to ensure consistent location throughout. These sites included acupoints and nonacupoints and are shown in Figure 3.2 and described in Table 3.2. The order of site measurement was KI3^{R}, 3^{R}, ST36^{R}, LI5^{L}, 1^{L}, PC6^{L}, 2^{L}, LI10^{L}, LI20^{R}, GB12^{R}. The same order of measurement was used throughout all pre and post-intervention cycles. This was to standardise the rest period between repeated measurements made at the same site.

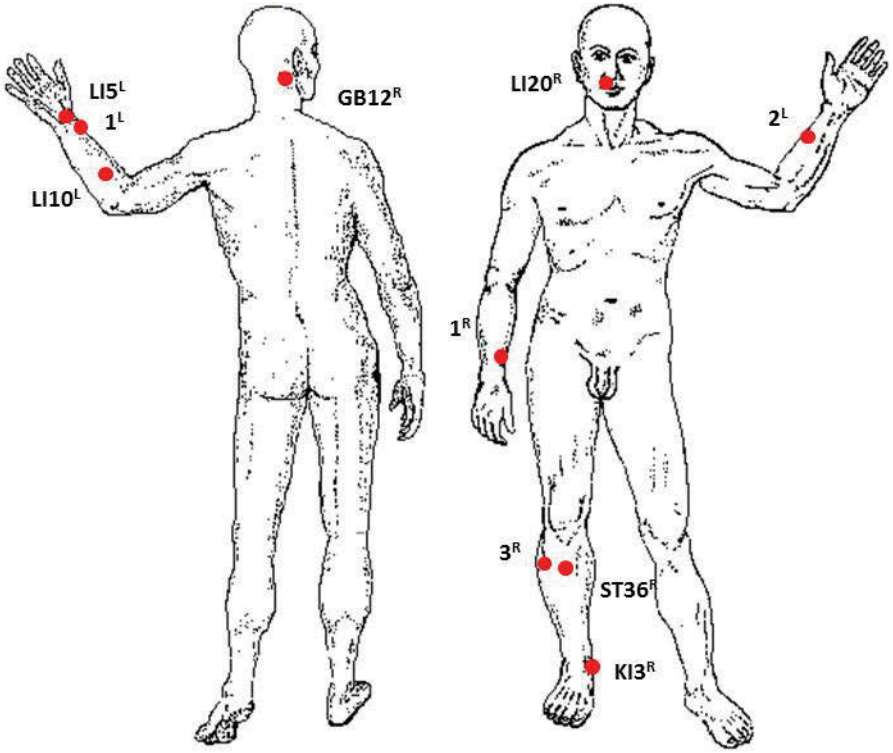


Figure 3.2: Anatomical location of the regional PPT measurement sites. The measurement sequence by site was: $KI3^{R}$, 3^{R}, $ST36^{R}$, $LI5^{L}$, 1^{L}, $PC6^{L}$, 2^{L}, $LI10^{L}$, $LI20^{R}$, $GB12^{R}$ (adapted from Rogers and Rogers 1989).

Site	Anatomical location	Location method (Aird et al 2002)	Channel / segmental regions (Chapple 2013, Marieb 2013)
(1) Acupoints			
KI3^{R}	Right foot, in the excavation between the medial malleolus and Achilles tendon, parallel to the medial malleolus	Anatomical landmark	Dermatome: distal segmental region of L4-S2 Channel: Kidney channel
ST36^{R}	Right leg 3*cun* from knee joint line approximately 2cm lateral to tibial shaft, level with the tibial tuberosity	Proportional (elastic method)	Dermatome: distal segmental region of L4,5 Channel: Stomach channel
LI5^{L}	Left arm, in the anatomical snuffbox at the wrist, which forms when the thumb is abducted	Anatomical landmark	Dermatome: same segmental region of C6 (as LI4) Channel:Large Intestine channel
GB12^{R}	Right side of the neck, in the depression inferior and slightly medial to the mastoid process	Anatomical landmark	Dermatome: distal segmental region of C2,3 Channel: Gall Bladder channel
LI20^{R}	Right side of the face, between the naso-labial groove and the midpoint of the lateral border of the nasal ala	Anatomical landmark	Dermatome: distal segmental region (maxillary branch of the trigeminal nerve) Channel:Large Intestine channel
PC6^{L}	Left arm, 2*cun* above the palmar wrist flexure between the tendons of the *flexor carpi radialis* and the *palmaris longus*	Proportional (elastic method)	Dermatome: same segmental region (T1, C7,8) as LI4 and NAP Channel: Pericardium channel
LI10^{L}	Left arm, 2*cun* below lateral end of the elbow flexure crease. Adjacent tissue includes the muscles bellies of the extensor carpi *radialis longus* and *brevis* and *brachioradialis*	Proportional (elastic method)	Dermatome: same segmental region (C6,7) as LI4 and NAP Channel:Large Intestine channel
(2) Nonacupoints			
1^{L}	Left arm, 2*cun* proximal to the wrist crease on the dorsal surface and on the medial border of the radius	Proportional (elastic method)	Dermatome: same segmental region (C6) as LI4 Channel: N/A
2^{L}	Left arm, midway between the medial side of the wrist joint and the medial epicondyle of the elbow, anterior to the ulna shaft	Proportional (elastic method)	Dermatome: adjacent segmental region (C8) to LI4 Channel: N/A
3^{R}	Left leg, 2*cun* distal to the fibula head, posterior to the fibula shaft	Proportional (elastic method)	Dermatome: distal segmental region L5 Channel: N/A

Table 3.2: Description of the anatomical location of the ten measurements sites, including their respective methods of location and their channel or segmental relationships to LI4 (Aird et al 2002, Chapple 2013, Marieb 2013, Rogers and Rogers 1989).

3.3.3 Location of ST36^{R}, PC6^{L}, LI10^{L}, 1^{L}, 2^{L} and 3^{L}

Some acupoints, including ST36, PC6 and LI10 can only be located by measuring a distance from an anatomical landmark (Rogers and Rogers 1989). While there are four methods commonly used to locate those acupoints Aird and colleagues (2002) have shown that two contemporary methods (the elastic and ruler methods) were significantly more precise than the traditional pair (directional and proportional methods). In this study the elastic method was chosen and applied as follows. A

length of 2cm wide elastic was marked with a series of horizontal lines at regular intervals. To measure a point on the body where (say) there are 12 division intervals (between the wrist crease and the elbow crease) the first mark on the elastic is placed on the wrist crease and the elastic stretched so that the 13th marking can be placed on the elbow crease. Then, for example, to locate a point reported to be sited 3*cun* from the wrist crease, this location would correspond with position on the arm of the fourth division marked on the elastic from the wrist crease.

ST36^{R}

Distance between landmarks of the lateral knee eye and the lateral extremity of the lateral malleolus on the right leg: 16*cun.*

Location of ST36^{R}: 3*cun* inferior to the lateral knee eye.

Elastic location: fourth marking on elastic with 17 equally spaced markings, stretched between the two landmarks.

PC6^{L}

Distance between landmarks of the wrist crease and the elbow crease on the left forearm: 12*cun.*

Location of PC6^{L}: 2*cun* superior to the wrist crease.

Elastic location: third marking on an elastic with 13 horizontal markings stretched from the wrist crease and between the tendons of the *flexor carpi radialis* and the *Palmaris longus*.

LI10^{L}

Distance between landmarks of the anatomical snuffbox at the wrist and the lateral end of the elbow flexure crease on the left forearm: 12*cun.*

Location of LI10^{L}: 2*cun* inferior to the latter landmark.

Elastic location: third marking on elastic with 13 horizontal markings from the lateral end of the elbow crease.

1^{L}

Distance between landmarks of the anatomical snuffbox at the wrist and the lateral end of the elbow flexure crease on the left forearm: 12*cun*

Location of 1^{L}: 2*cun* superior to the anatomical snuffbox landmark

Elastic location: third marking on elastic with 13 horizontal markings, from anatomical snuffbox landmark.

2^L

Distance between landmarks of the medial side of the wrist joint and the medial epicondyle of the elbow on the left forearm: 12*cun*

Location of 2^L: midway between the two landmarks

Elastic location: seventh marking on elastic with 13 horizontal markings, from the wrist joint.

3^L

Distance between landmarks of the head of the fibula and the lateral extremity of the lateral malleolus on the left leg: 13*cun*

Location of 3^L: 3*cun* inferior to the former landmark

Elastic location: fourth marking on elastic with 14 horizontal markings, from the head of the fibula.

3.3.4 Measurement of the subjects' perceptions of needle sensation and pain

Needling sensation was defined for the subjects as *any sensation other than needling pain*. Subjects quantified the intensity of the needle sensation using a 100mm VAS ranging from no sensation/pain to intense sensation/pain. For all interventions, every three minutes subjects reported in turn: needling sensation (*Do you feel any needling sensation at this point in time*) and pain intensity (*Are you experiencing pain at present*) on a 100mm VAS with a sliding scale (held up for them by the acupuncturist). Subjects were required to describe the sensations of needling and of the type of pain perceived and their responses (as well as their intensity reading) were recorded by the acupuncturist administering the intervention. At the completion of each session, subjects recorded global needle sensation and pain ratings, using a 100mm VAS. Where they recorded a needle sensation they included a written description of the sensations experienced.

There was a serious and significant breach of the study protocol with respect to this aspect of data collection. Because of the double blind nature of the research, the completed data sheets for each subject's experiment session were filed securely and no data were entered into Microsoft Excel or analysed until the end of the study, to avoid possible researcher bias effects.

As a consequence, it was not until after completion of the 18 months of data collection that it was discovered the acupuncturist had failed on about 30% of occasions to record the subjects' verbal descriptions of the sensations reported at three minute intervals during the 21-minute intervention period. The VAS scores however were all recorded. Examination of the data omissions revealed that they affected the reports of most of the subjects to varying degrees and so the entire data set

was discarded. Since they do include a considerable number of subjective reports of the types of sensation elicited by the various interventions, they have been included in Appendix IV as an item of interest.

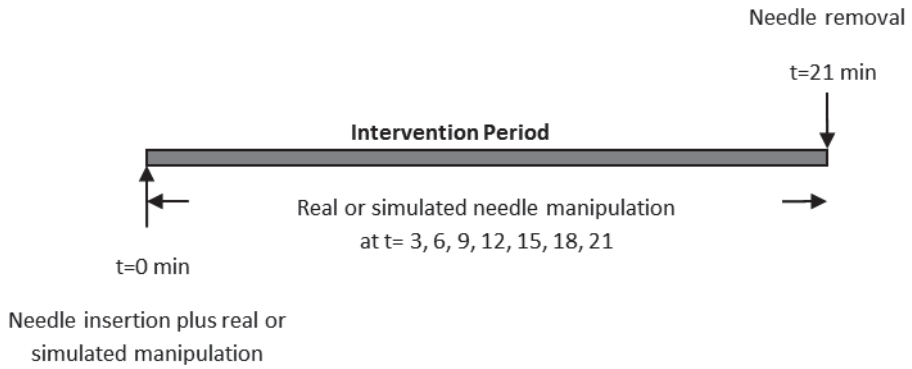
Fortuitously, the post-intervention questionnaire completed by subjects at the end of each experimental session also included a needle sensation VAS and required a written description of the needle sensation perceived for that intervention. While this failed to provide a temporally related profile of needling sensation (or pain), it did provide an overall perception by each subject of each needling intervention.

3.3.5 Measurement of regional PPT

All PPT measurements were taken by Researcher I (YKL) with an algometer (Activator Methods Phoenix USA) using the methods described by Fischer (1987) and used and detailed in all previous PPT studies at UTS. It was explained to subjects at each session that PPT was achieved when the pressure being applied by the algometer first becomes uncomfortable or the beginning of pain, not just pressure. They were asked to indicate as soon as this change in sensation was perceived. The algometer was then immediately lifted off the subject and handed to Researcher II (DC) who recorded the exact reading in kg/cm^2; reset the algometer to zero and returned the algometer to Researcher I for the next measurement site in the series.

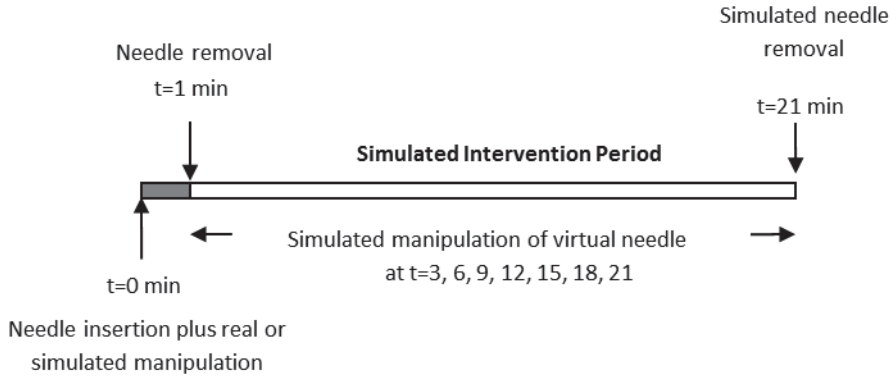
Intervention procedure

Throughout each session, the subject lay supine on the treatment table. Prior to receiving each intervention, as part of the broader research program, a standardised series of five baseline PPT measurements were recorded from the ten regional sites (see Table 3.2). Previous studies have reported that the first PPT reading in a series appears to be relatively unreliable, as the subject is becoming responsive to the procedure and in the present study the first reading obtained from each site was discarded (Nussbaum et al 1998, Kosek et al 1993). The following four PPT measurement cycles were retained as the pre-intervention baseline. The study's acupuncturist (with >35 years of clinical experience) then initiated the 21-minute intervention protocol described in Section 3.3.1 and summarised in the timelines shown below. Throughout the intervention period, a curtain was drawn between the subject's line of vision and the intervention site on their right hand, to blind them to the procedure and intervention sites. The timeline for the 21-minute interventions is shown in Figure 3.4 while the one for the one minute interventions is shown in Figure 3.5



- Real or simulated needle 'manipulation' at t=0, 3, 6, 9, 12, 15, 18 and 21
- VAS pain and needle sensation scores recorded at t=1, 4, 7, 10, 13, 16, 19 and 22

Figure 3.4: Timeline of the intervention sequencing for interventions with 21-minute needle retention.



- Real or simulated needle 'manipulation' at t=0
- Simulated 'needle' manipulation at t=3, 6, 9, 12, 15, 18 and 21
- VAS pain and needle sensation scores recorded at t=1, 4, 7, 10, 13, 16, 19 and 22

Figure 3.5: Timeline of the intervention sequencing for interventions with one minute needle retention.

PPT measurements were completed before, during and following the 21-minute intervention period. During the 21-minute acupuncture intervention period, PPT measurements were limited only to three sites that were distant from the needling site ($KI3^{R}$, $LI10^{L}$ and $GB12^{R}$) at t=4, t=10, t=16 minutes. At completion of the intervention period, four additional measurement cycles were completed on all ten regional sites. The sequencing of baseline, intervention period and post-intervention PPT measurements cycles are shown in Figure 3.6.

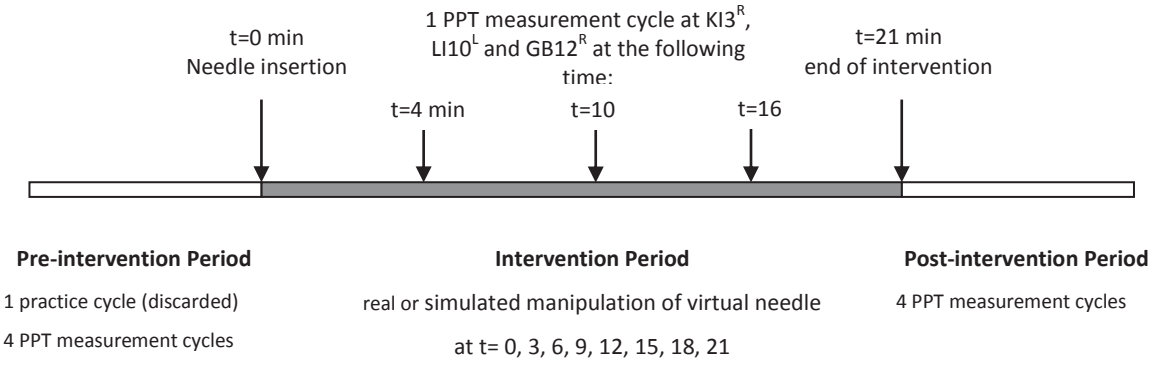


Figure 3.6: Timeline for all PPT measurement cycles.

3.3.6 Subjects' perceptions concerning each intervention period

In addition to the VAS reporting of intensity of needle pain and sensation every three minutes during the 21-minute intervention period at the completion of each session subjects recorded global needle sensation and pain ratings, anxiety prior to the intervention and ~~of~~ the level of tension during each intervention period using a 100mm VAS. Where they recorded a needle sensation they included a written description of the sensations experienced (Figure 3.3). In addition, for sessions two through to eight, subjects were requested to indicate whether they perceived changes in the acupuncturist's behaviour during different intervention sessions. Subjects who indicated there was a difference in behaviour were asked to specify the nature of the difference.

PLEASE INDICATE BY MARKING ON THE LINE YOUR ANSWER TO THE FOLLOWING QUESTIONS:

Question 1: How did you experience the needling today?

0 _____ 100

Absolutely painless — Extremely painful

Question 2: Did you have a special feeling during the needling?

0 _____ 100

No, nothing at all — Yes, intense feelings of… Please describe:

Question 3: How did you feel during the needling?

0 _____ 100

Completely calm and relaxed — Completely tense

Question 4: Were you anxious about feeling pain from the needling today?

0 _____ 100

No, not anxious at all — Yes, extremely anxious

Question 5: Did the acupuncturist behave differently today compared to the first session?

0 _____ 100

No, no difference at all — Yes, very differently

If yes, in what ways?

Figure 3.3: The 100mm VAS used to record subject's perceptions relating to the 21 minutes intervention period for each experimental session (after Roth et al 1997).

3.3.7 Blinding procedures

Throughout the intervention period, subjects were blind to the procedure and intervention sites. Researcher I who applied the algometer was blind to the intervention type and the algometer reading. Researcher II who recorded the algometer readings was blind to the intervention type and had no role in applying the algometer and also monitored the consistency of the rate at which Researcher I applied pressure with the algometer. The acupuncturist (CZ) who administered the intervention was blind to baseline and post-intervention PPT readings. Finally, no data were analysed during the data collection phase of the study to avoid possible biases related to researcher expectations.

3.4 Statistical analysis

3.4.1 PPT measurements

In each subject and intervention session, all PPT values were described as a percentage of the mean pre-intervention value. This was calculated using the following formula.

$$\text{PPT value as \%} = \frac{\text{Single post PPT value (g/cm}^2\text{) for the site}}{\text{Mean pre-intervention PPT (g/cm}^2\text{) for the site}} \times 100$$

This data transformation was applied in view of the range of baseline PPT measures encountered both between subjects and also with respect to the same subject across the ten regional measurement sites. Extensive checking of the appropriateness of both the transformation and the model tested in General Linear Model (GLM) was undertaken for the present research and has also been completed in related research studies at UTS. Further, it was reported in a related study that baseline PPT is not a useful predictor of the percentage change following an active intervention (Yuan 2002). Data were initially entered into Microsoft Excel with all statistical analyses completed using Minitab for Windows version 15. The main analyses involved various forms of analysis of variance using the GLM with Tukey post hoc analyses, Chi square goodness of fit and Pearson's product moment correlation. Analysis of variance used 95% confidence intervals (CI) among all pairwise comparisons testing significance of difference of means with p values. Comparisons were made both within each intervention across all ten sites, and between the eight interventions for each individual measurement site.

3.4.2 Needling sensation and pain intensity scores, anxiety, tension and acupuncturist's behaviour

Scores were measured (in mm) on the 100 mm VAS for each of these variables. Analyses included one way analysis of variance (ANOVA); Chi square goodness of fit and Pearson's product moment correlation coefficient.

Chapter IV: Results

4.1 Introduction

The study results are presented separately for the two distinct parts of the research. Findings for the post-intervention changes in regional PPT both within and between the eight interventions are presented in Part I. Those for the intervention period findings for needling sensation, pain and regional PPT elicited during the 21-minute intervention period are presented in Part II. Tables with associated analysis of variance are presented in Appendix VI.

Part I: Post-intervention changes in regional PPT

4.2 Within and between intervention comparisons (independent of site)

An initial analysis was completed, independent of measurement site (where the mean PPTs of all ten sites were combined for each intervention), to compare the effects on post-intervention PPT among the eight interventions. In Figure 4a.1 and Table 4a.1, the results are shown separately for the total 24 subjects and for the 12 men and 12 women.

Intervention	**Mean % increase in baseline PPT (95%CI)**		
	All subjects (N=24)	Women (N=12)	Men (N=12)
LI4m^{+1}	5.8 (3.8-7.7)	5.5 (2.7-8.3)	6.1 (3.3-8.8)
LI4m^{-1}	7.5 (5.6-9.4)	7.7 (4.8-10.5)	7.4 (4.7-10.1)
LI4m^{+21}	9.1 (7.1-11.0)	8.8 (5.9-11.6)	9.4 (6.7-12.1)
LI4m^{-21}	3.7 (1.8-5.7)	4.7 (1.8-7.5)	2.8 (0.1-5.1)
NAPm^{+1}	8.4 (6.5-10.4)	7.1 (4.2-9.9)	9.8 (7.1-12.5)
NAPm^{-1}	7.2 (5.3-9.2)	7.7 (4.9-10.1)	6.8 (4.1-9.5)
NAPm^{+21}	7.0 (5.1-9.0)	6.3 (3.5-9.1)	7.8 (5.1-10.5)
NAPm^{-21}	8.1 (6.2-10.1)	10.1 (7.3-13.0)	6.1 (3.4-8.9)

Table 4a.1: Mean percentage change in PPT from pre-intervention mean for the eight interventions. The 95% confidence intervals (95%CI) are shown.

In all three subject groupings and for all eight interventions, the post-intervention mean % increases in PPT were significantly elevated compared with baseline levels ($F_{7,15312}$ = 8.87 p<0.000, Tukey

post hocs p<0.000 in all cases). The only statistically significant differences within the total and the male subject groupings all involved the means for $LI4m^{-21}$. For the males, the $LI4m^{-21}$ mean (2.8%) was lower than the mean for $LI4m^{+21}$ (9.4%) and $NAPm^{+1}$ (9.8%). For the total subject group the $LI4m^{-21}$ mean (3.7%) was lower than the mean for $LI4m^{+21}$ (9.1%), $NAPm^{+1}$ (8.4%) and $NAPm^{-21}$ (8.1%).

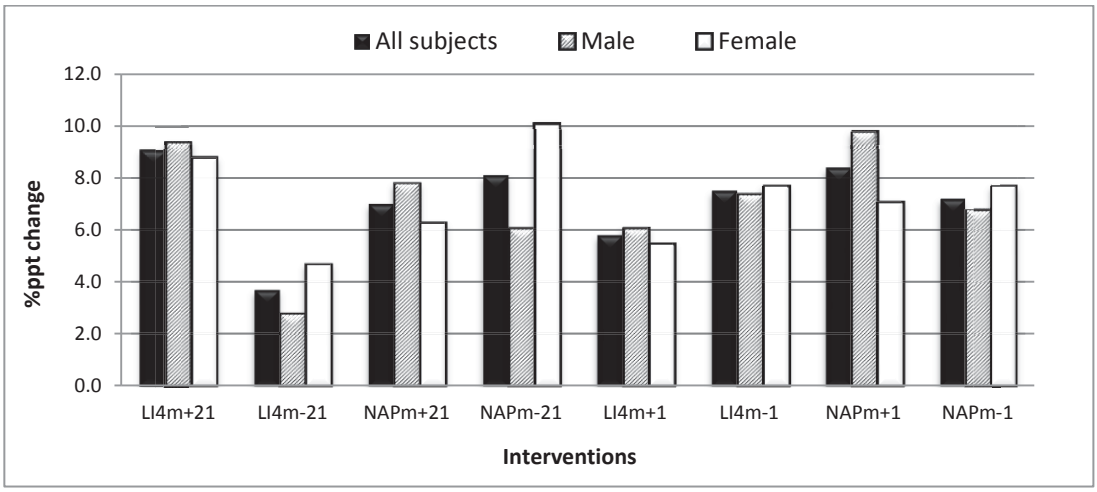


Figure 4a.1: Mean percentage change in PPT from pre-intervention mean for the eight interventions.

In view of the general lack of statistically significant differences found across the results for both genders for the eight interventions and the inclusion of equal numbers of men and women in all interventions, all subsequent analyses involve the total study sample of 24.

4.3 Within intervention comparisons (by site)

Table 4a.2 summarises post-intervention PPT mean % change from pre-intervention values and 95% confidence intervals within the eight interventions for the ten individual measurement sites. The content of the table presents 80 comparison cells has been simplified in Figure 4a.2 in order to assist comparison of statistically significant changes from pre-intervention means within each intervention.

Regional Site	LI4m^{+21}		LI4m^{-21}		NAPm^{+21}		NAPm^{-21}		LI4m^{+1}		LI4m^{-1}		NAPm^{+1}		NAPm^{-1}	
	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI
Acupoints																
KI3^{R}	**7.5****	**3.0 to 11.9**	2.7	-1.4 to 6.9	**8.9*****	**4.5 to 13.3**	**9.6*****	**5.4 to 13.7**	**6.9****	**1.9 to 11.8**	**5.1****	**1.2 to 9.0**	**7.0****	**2.1 to 11.9**	**8.5*****	**4.6 to 12.4**
ST36^{R}	**5.9****	**1.2 to 9.8**	2.1	-2.0 to 6.1	**4.9***	**0.6 to 9.2**	**6.5****	**2.4 to 10.6**	0.7	-3.4 to 4.9	3.4	-0.3 to 7.0	**7.2*****	**3.1 to 11.4**	1.2	-2.4 to 4.8
LI5^{L}	**10.1*****	**5.4 to 14.8**	4.5	-0.6 to 9.5	4.6	0.0 to 9.3	**6.0***	**0.9 to 11.1**	**6.2****	**1.4 to 11.0**	**8.4****	**3.3 to 13.5**	**7.3****	**2.5 to 12.1**	**9.3*****	**4.2 to 14.4**
PC6^{L}	**9.9*****	**5.5 to 14.4**	5	-1.0 to 10.9	**9.4*****	**4.9 to 13.8**	**10.6*****	**4.6 to 16.5**	**7.9****	**3.2 to 12.5**	**7.6****	**2.9 to 12.3**	**7.7****	**3.1 to 12.4**	**7.6****	**2.9 to 12.3**
LI10^{L}	**16.3*****	**11.2 to 21.6**	3.2	-1.3 to 7.7	**7.1****	**1.9 to 12.3**	**9.2*****	**4.7 to 13.7**	**5.9****	**1.2 to 10.6**	**7.9****	**2.9 to 12.9**	**11.8*****	**7.1 to 16.5**	**8.6****	**3.6 to 13.6**
LI20^{R}	**8.0*****	**4.2 to 11.8**	1.9	-1.7 to 5.5	**5.7****	**1.8 to 9.6**	**5.0****	**1.4 to 8.6**	3.2	-0.1 to 6.6	**5.9****	**2.3 to 9.6**	**6.9*****	**3.5 to 10.2**	**5.8****	**2.1 to 9.5**
GB12^{R}	**11.9*****	**8.2 to 15.6**	**8.7*****	**4.8 to 12.5**	**9.5*****	**5.9 to 13.2**	**11.5*****	**7.6 to 15.4**	**6.8*****	**3.4 to 10.2**	**11.9*****	**7.9 to 15.8**	**13.0*****	**9.6 to 16.5**	**9.6*****	**5.7 to 13.5**
Nonacupoints																
3^{R}	**5.3****	**1.3 to 9.3**	0.8	-3.8 to 5.4	**6.9****	**2.9 to 10.9**	**10.5*****	**5.9 to 15.1**	3.6	-0.6 to 7.8	**4.6***	**0.6 to 8.6**	**6.8****	**2.6 to 11.0**	**5.7****	**1.7 to 9.7**
1^{L}	**8.5****	**3.2 to 13.9**	4.9	-0.2 to 9.9	**8.0****	**2.7 to 13.3**	**6.8****	**1.8 to 12.0**	**7.0****	**1.8 to 12.1**	**13.6*****	**7.8 to 19.4**	**9.6*****	**4.5 to 14.8**	**9.7****	**3.8 to 15.5**
2^{L}	**7.8*****	**3.6 to 11.9**	3.8	-0.5 to 8.1	**5.3****	**1.1 to 9.5**	**5.6****	**1.4 to 10.0**	**9.7*****	**4.5 to 14.8**	**6.8****	**2.4 to 11.3**	**6.9****	**1.8 to 12.1**	**6.5****	**2.0 to 11.0**

Table 4a.2: Mean % change in PPT from pre-intervention values and 95% confidence intervals for the eight interventions for the ten individual measurement sites. Adjusted significance levels are also shown: * ($p<0.05$), ** ($p<0.01$), *** ($p<0.0001$).

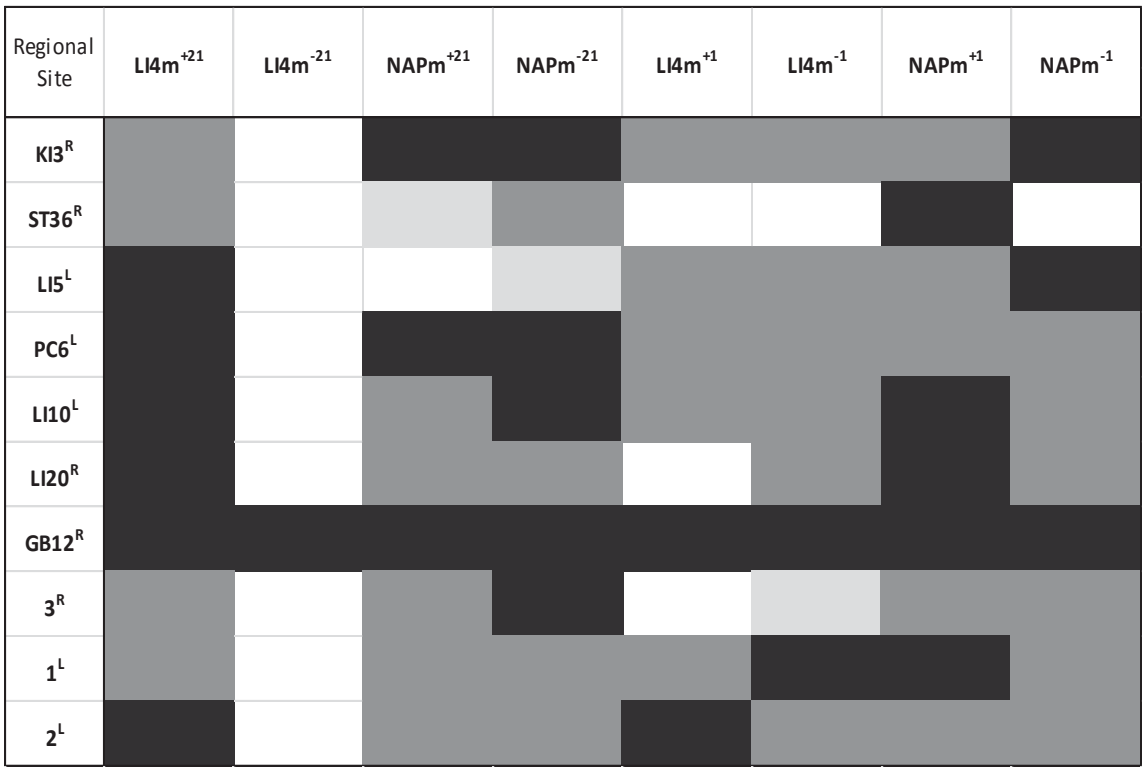


Figure 4a.2: The 80 comparison cells shown in Table 4.2 colour coded with respect to the levels of statistical significance of mean % changes in regional PPT from pre-intervention levels. Each mean percentage change in PPT from baseline value is colour coded with respect to whether the mean change is statistically significant at $p<0.0001$ (black), $p<0.01$ (dark grey), $p< 0.05$ (light grey) or not significant $p>0.05$ (white).

In Figure 4a.2 an obvious feature of the pattern of response cells is that the most common response to each intervention elicited at each of the ten regional measurement sites was a statistically significant increase in mean % PPT compared with baseline values. Among the 80 response cells available, for 27 of them such changes were significant at $p<0.0001$; for 35 at $p<0.01$ and for three at $p<0.05$. For the remaining 15 cells, the changes were not statistically significant ($p>0.05$) and nine of these were for the one intervention ($LI4m^{-21}$), three for $LI4m^{+1}$ and one each for $NAPm^{+21}$, $LI4m^{-1}$ and $NAPm^{-1}$. Among the ten regional sites measurement, $GB12^{R}$ was the only one for which a statistically significant increase was achieved for all eight interventions and in each case, at $p<0.0001$. The least responsive site was $ST36^{R}$ with only four significant changes; one at $p<0.05$, two at $p<0.01$ and one at $p<0.0001$. Only three of the eight interventions elicited statistically significant increases in PPT at all ten sites. These were: $LI4m^{+21}$ (six sites at $p<0.0001$; four at $p<0.01$), $NAPm^{-21}$ (five sites at $p<0.0001$; four at $p<0.01$); one at $p<0.05$) and $NAPm^{+1}$ (five sites each at $p<0.0001$and at $p<0.01$).

Table 4a.2 includes all the post-intervention mean % increases in regional measurement site by intervention. Among the 27 PPT outcomes where $p<0.0001$ was achieved, the typical spread of mean increases was between 8.6% and 10.6% with median increase of 9.6%, while the range

extended from 16.3%, at site $LI10^{L}$ (for $LI4m^{+21}$) and 13.6% at 1^{L} (for $LI4m^{-1}$) to 6.9% at $LI20^{R}$ (for $NAPm^{+1}$) and 6.8% at $GB12^{R}$ (for $LI4m^{+1}$).

Among the 35 increases significant at $p<0.01$, means ranged from 9.7% at 1^{L} ($NAPm^{-1}$) and 8.4% at $LI5^{L}$ ($LI4m^{-1}$) and 5.1% at $KI3^{R}$ (for $LI4m^{-1}$) to 5.0% at $LI20^{R}$ ($NAPm^{-21}$). Most means lay between 7.6% and 5.9% with the median being 6.9%.

The three increases significant at $p<0.05$ were 4.9% at $ST36^{R}$ (for $NAPm^{+21}$), 6.0% at $LI5^{L}$ ($NAPm^{-21}$) and 4.6% at 3^{R} ($LI4m^{-1}$).

The 15 mean increases that were not statistically significant at $p<0.05$ ranged from values of 0.7% to 5% with a median of 3.2%. These only related to five interventions: $LI4m^{-21}$ (nine sites); $LI4m^{+1}$ (three sites) and one site each for $NAPm^{+21}$, $LI4m^{-1}$ and $NAPm^{-1}$.

In summary, the typical PPT response to the needling interventions recorded at the ten regional measurement sites was an increase from pre-intervention mean values. Notably, while such changes were not all statistically different from baseline means, there was no occasion where the post-intervention means were lower than the baseline values.

4.4 Between intervention comparisons (by site)

This section initially examines between intervention effects on PPT at the ten regional measurement sites in a similar sequence to the above within intervention comparisons. Further examination of the post-intervention changes is then presented in relation to the three needling parameters under investigation: presence or absence of manipulation; duration of needle insertion; site of needle insertion. Comparisons are presented between pairs of interventions for which the values of the remaining two parameters were held constant. The complete table of between intervention comparisons by site showing means, and associated p values is included in Appendix V. Table 4a.3 includes only the sites and interventions where there were statistically significant differences between a pair of PPT means. Response cells are colour coded with respect to the level of statistical significance of the difference between the pair of means.

Comparison		KI3^{R}	ST36^{R}	LI5^{L}	PC6^{L}	LI10^{L}	LI20^{R}	GB12^{R}	3^{R}	1^{L}	2^{L}
LI4m^{+21}	LI4m^{-21}	+ 4.8		+ 5.6	+ 4.9	+ 13.2	+ 6.1				
	NAPm^{+21}			+ 5.5		+ 9.2					
	LI4m^{+1}		+ 4.8			+ 10.5	+ 4.8	+ 5.1			
LI4m^{-21}	NAPm^{-21}	- 6.9	- 4.4			- 6.0			- 9.7		
	LI4m^{-1}						- 4.0			- 8.7	
NAPm^{+21}	NAPm^{-21}										
	NAPm^{+1}					- 4.7					
NAPm^{-21}	NAPm^{-1}		+ 5.3						+ 4.8		
LI4m^{+1}	LI4m^{-1}							- 5.1		- 6.6	
	NAPm^{+1}		- 6.5			- 5.9	- 3.7	- 6.2			
LI4m^{-1}	NAPm^{-1}										
NAPm^{+1}	NAPm^{-1}		+ 6.0								

Table 4a.3: Between intervention comparisons by site showing only the sites and interventions where there were statistically significant differences between a pair of PPT means. Cells are colour coded with respect to whether the difference in the two means was significant at $p<0.0001$ (black), $p<0.01$ (dark grey), $p< 0.05$ (light grey) or not significant $p>0.05$ (white). A + sign in front of the mean % PPT difference indicates that the first comparison intervention (shown on the far left) is higher than the second; a - sign indicates it is lower than the second member of the comparison pair.

Among the relevant intervention comparisons for this study (with a focus on the three parameters of needle manipulation, insertion duration and insertion site), there were 27 comparison pairs for which there were statistically significant differences between the mean % elevations elicited at the same measurement site. There were patterns of differences with respect to measurement site, the intervention pair involved and the statistical significance level of the differences.

Significant differences by site

Eighteen of the significant comparisons involved four sites: these comprised LI10^{L} (N=6), ST36^{R} (N=5), LI20^{R} (N=4) and GB12^{R} (N=3). There were none at 2^{L}, one at PC6^{L} and two at each of the remaining four sites (3^{R}, 1^{L}, KI3^{R} and LI5^{L}).

Statistical significance level

Only five differences were significant at $p<0.0001$: three involved LI10^{L} and one each at 3^{R} and GB12^{R}. The 12 differences that were significant at $p<0.01$ comprised four at ST36^{L}, two at each of LI10^{L}, LI20^{R} and GB12^{R} and one at KI3^{L} and 1^{L}. The ten differences significant at $p<0.05$ were distributed across all sites except GB12^{R} and 2^{L}.

Comparison pair

Some comparison pairs were consistently similar, for example there were no significant differences involving either the pair of one minute interventions without manipulation ($LI4m^{-1}$ and $NAPm^{-1}$), or the pair of 21-minute NAP interventions (NAP^{+21} and NAP^{-21}). The four comparison pairs most frequently showing significant differences at sites included: the pair of $LI4^{21}$ interventions (N=5); the pair of m^{-21} interventions (N=4) and the pair of m^{+1} interventions (N=4) (that is, comparing the two insertion sites LI4 and NAP).

Some interventions were more frequently involved either because their mean PPT increase was higher than other interventions for that measurement site or conversely, because their effect on PPT was the weakest elicited. For example, the former case applied to $LI4m^{+21}$ at 11 comparison cells while for $LI4m^{-21}$ the latter was the case with respect to six additional comparison pairs (that is, not involving $LI4m^{+21}$).

The following sections examine between- intervention comparison pairs in relation to the three needling parameters that are the focus of this research study: needling site, duration and manipulation status.

4.5 Examination of the effect of duration of needle retention on change in mean PPT from baseline (1 minute vs 21 minutes)

4.5.1 Comparison pair: site LI4, manipulation present

$LI4m^{+}$

Site	1min	21min	p (bet)
$KI3^{R}$	**6.9**	**7.5**	0.9823
$ST36^{R\#}$	0.7	**5.5**	**0.0085**
$LI5^{L}$	**6.2**	**10.1**	0.1916
$PC6^{L}$	**7.9**	**9.9**	0.6632
$LI10^{L\#}$	**5.9**	**16.4**	**0.0000**
$LI20^{R\#}$	3.2	**8.0**	**0.0041**
$GB12^{R\#}$	**6.8**	**11.9**	**0.0006**
3^{R}	3.6	**5.3**	0.6962
1^{L}	**7.0**	**8.5**	0.8638
2^{L}	**9.7**	**7.8**	0.7447

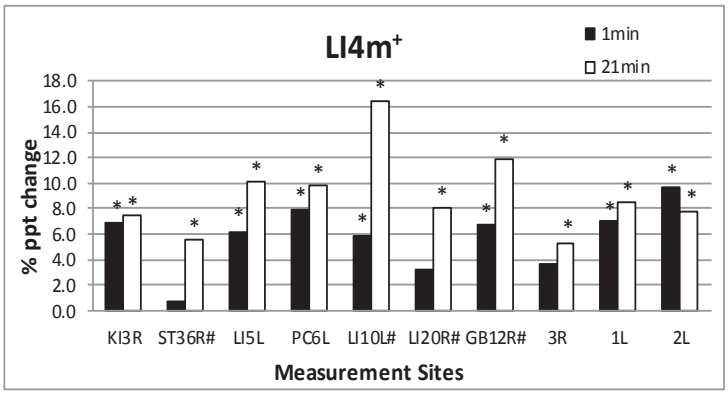


Figure 4a.3: Mean % change in PPT from pre-intervention mean for the ten regional measurement sites, following the pair of LI4 interventions with manipulation present and with needle retention times of either one minute or 21 minutes. Statistically significant increases from pre-intervention means are marked in bold in the list of means in the table and on the bar chart (*). The p values (shown in bold) on the table, identify significant differences in between pair comparisons.

From the within intervention comparisons at LI4 when manipulation was present, with baseline PPT means there are both differences and similarities in the response patterns for the one and 21-minute interventions. While at all sites a statistically significant increase in mean PPT was

elicited with 21-minute needle retention this did not occur at three sites for the one minute retention. There were four sites for which the increase in mean PPT was significantly greater for the 21-minute insertion (in all cases p was at least<0.01). For six sites, the pairs of means did not differ statistically significantly and for five of these sites, the mean values were very similar (p values were at least >0.66). These were KI3^{R}, PC6^{L}, 3^{R}, 1^{L} and 2^{L}.

By comparison the remaining four sites were very different. Most notable was LI10^{L} for which the 21-minute mean increase was the highest recorded across all 80 comparisons involved in the entire study. This mean increase (16.4%) is a strong contrast with that for the one minute retention (5.9%) at the same site (p=0.0000).

4.5.2 Comparison pair: site LI4, manipulation absent

LI4m^{-}

Site	1min	21min	p (bet)
KI3^{R}	**5.1**	2.7	0.4296
ST36^{R}	3.4	2.0	0.8059
LI5^{L}	**8.4**	4.5	0.1768
PC6^{L}	**7.6**	**5.0**	0.4068
LI10^{L}	**7.9**	3.2	0.0569
LI20$^{R\#}$	**5.9**	1.9	**0.0281**
GB12^{R}	**11.9**	**8.7**	0.1877
3^{R}	**4.6**	0.8	0.1289
1$^{L\#}$	**13.6**	4.9	**0.0006**
2^{L}	**6.8**	3.8	0.2237

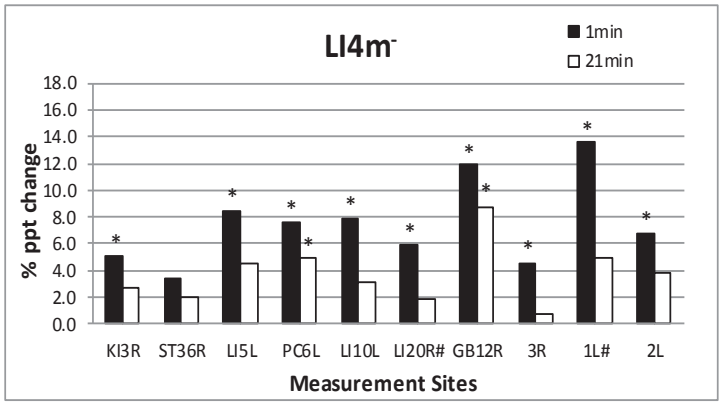


Figure 4a.4: Mean % change in PPT from pre-intervention mean for the ten regional measurement sites, following the pair of LI4 interventions without manipulation and with needle retention times of either one minute or 21 minutes. Statistically significant increases from pre-intervention means are marked in bold in the list of means in the table and on the bar chart (*). The p values shown in bold on the table identify significant differences in between pair comparisons.

The LI4 comparison pair without manipulation included eight sites where the 21-minute insertion failed to elicit a statistically significant increase in mean % PPT from baseline compared with only one for the one minute retention. However, the between intervention comparisons only reached statistical significance for two sites and for both the one minute mean was the higher value. These were LI20^{R} (5.9% cf. 1.9%, p= 0.028) and 1^{L} (13.6% cf. 4.9%, p=0.0006). It is worth noting that the means for the one minute intervention at each site were greater than for the paired 21-minute intervention mean (although not necessarily in a statistically significant sense).

4.5.3 Comparison pair: site NAP, manipulation present

NAPm^{+}

Site	1min	21min	p (bet)
KI3^{R}	**7.0**	**8.9**	0.7892
ST36^{R}	**7.2**	**4.9**	0.5068
LI5^{L}	**7.3**	**4.6**	0.4114
PC6^{L}	**7.7**	**9.4**	0.7643
LI10$^{L\#}$	**11.8**	**7.1**	**0.0457**
LI20^{R}	**6.9**	**5.7**	0.6845
GB12^{R}	**13.0**	**9.5**	0.0776
3^{R}	**6.8**	**6.9**	1.0000
1^{L}	**9.6**	**8.0**	0.8568
2^{L}	**6.9**	**5.3**	0.8024

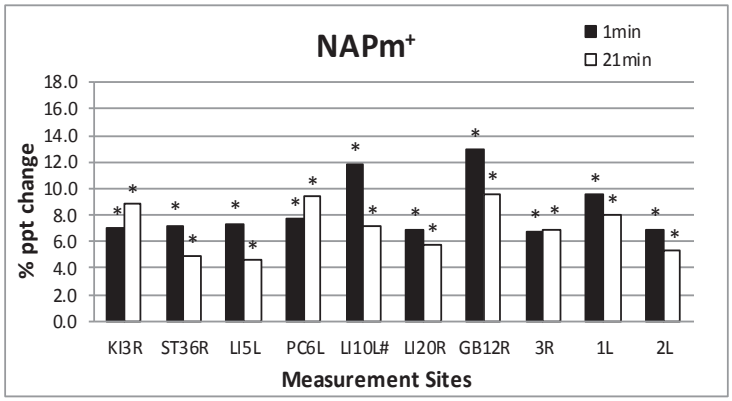


Figure 4a.5: Mean % change in PPT from pre-intervention mean for the ten regional measurement sites, following the pair of NAP interventions with manipulation present and with needle retention times of either one minute or 21 minutes. Statistically significant increases from pre-intervention means are marked in bold in the list of means in the table and on the bar chart (*). The p values shown in bold on the table identify significant differences in between pair comparisons.

Comparison of the two durations for the NAP with manipulation showed similar patterns in that all site means were statistically significantly increased from pre-intervention levels. At only one site was there a statistically significant difference between the two means. At LI10^{L} the one minute mean (11.8%) was borderline significantly higher than the 21-minute mean of 7.1% (p=0.0457). Thus duration appeared to have a minimal effect on PPT response in this set of comparison pairs.

4.5.4 Comparison pair: site NAP, manipulation absent

NAPm^{-}

Site	1min	21min	p (bet)
KI3^{R}	**8.5**	**9.6**	0.8888
ST36$^{R\#}$	1.2	**6.5**	**0.0022**
LI5^{L}	**9.3**	**6.0**	0.3997
PC6^{L}	**7.6**	**10.6**	0.5971
LI10^{L}	**8.6**	**9.2**	0.9844
LI20^{R}	**5.8**	**5.0**	0.9457
GB12^{R}	**9.6**	**11.5**	0.5598
3$^{R\#}$	**5.7**	**10.5**	**0.0292**
1^{L}	**9.7**	**6.8**	0.4806
2^{L}	**6.5**	**5.6**	0.9587

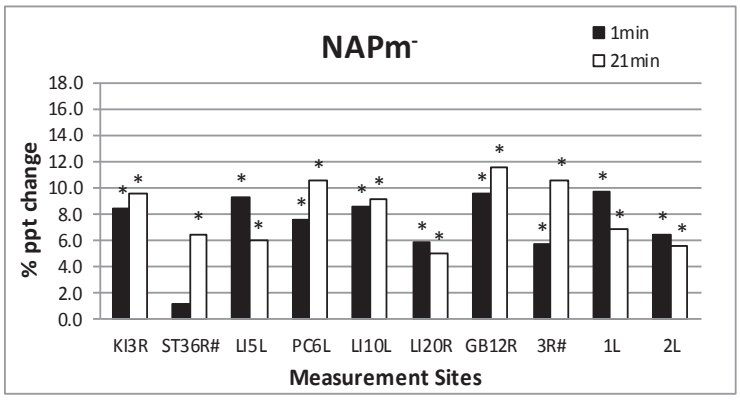


Figure 4a.6: Mean % change in PPT from pre-intervention mean for the ten regional measurement sites, following the pair of NAP interventions with manipulation absent and with needle retention times of either one minute or 21 minutes. Statistically significant increases from pre-intervention means are marked in bold in the list of means in the table and on the bar chart (*). The p values shown in bold on the table identify significant differences in between pair comparisons.

Only ST36^{R} failed to show a significant increase in mean PPT from baseline for the one minute duration intervention and the changes in PPT across sites and both durations were in general quite similar. The two sites that differed significantly were ST36^{R} and 3^{R}. In both cases, the one minute mean was lower than that for 21 minutes (1.2% cf. 6.5%, p=0.0022, and 5.7% cf. 10.5%, p=0.0292, respectively).

4.5.5 Summary: one minute or 21 minutes retention time

- Site: LI4 manipulation present: at four sites the 21-minute mean PPT was significantly greater than the one minute insertion (in all cases p was at least<0.01).
- Site: LI4 manipulation absent: the between intervention comparisons only reached statistical significance for two sites and in both cases, the one minute mean was the higher value (in both cases p was at least <0.05). .
- Site: NAP manipulation present: at one site (LI10^{L}) the mean for one minute (11.8%) was borderline significantly (p=0.0457) higher than for 21 minutes (7.1%).
- Site: NAP manipulation absent: at two sites the one minute mean was significantly lower than that that for 21 minutes (in both cases p was at least <0.05).

4.6 Examination of the effect of site of needle insertion on change in mean PPT from baseline (LI4 vs NAP)

4.6.1 Comparison pair: duration 21 minutes, manipulation present

m^{+21}

Site	LI4	NAP	p (bet)
KI3^{R}	**7.5**	**8.9**	0.8439
ST36^{R}	**5.9**	**4.9**	0.9835
LI5$^{L\#}$	**10.1**	4.6	**0.0153**
PC6^{L}	**9.9**	**9.4**	0.9876
LI10$^{L\#}$	**16.3**	**7.1**	**0.0000**
LI20^{R}	**8.0**	**5.7**	0.3238
GB12^{R}	**11.9**	**9.5**	0.3541
3^{R}	**5.3**	**6.9**	0.7194
1^{L}	**8.5**	**8.0**	0.9940
2^{L}	**7.8**	**5.3**	0.4202

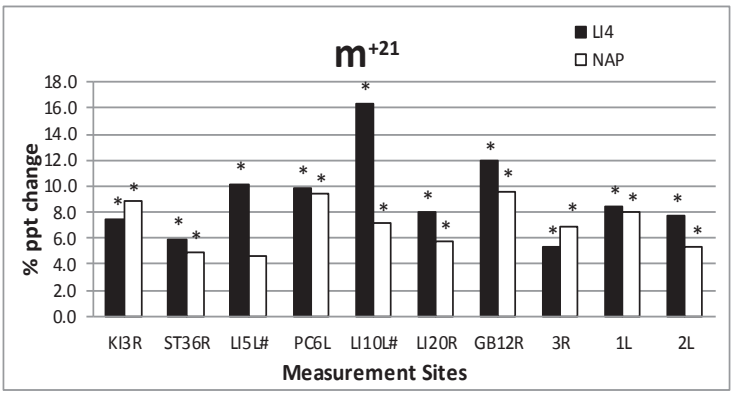


Figure 4a.7: Mean % change in PPT from pre-intervention mean for the ten regional measurement sites, following the pair of 21-minute needle retention interventions with manipulation present and at either needling site LI4 or NAP. Statistically significant increases from pre-intervention means are marked in bold in the list of means in the table and on the bar chart (*). The p values shown in bold on the table identify significant differences in between pair comparisons.

For all ten regional measurement sites apart from the mean at LI5^{L} for the NAP intervention, both interventions elicited significant elevations in mean % PPT from baseline. There were only two sites where the pairs of means differed statistically significantly and in both the LI4 mean

were higher. These were for $LI10^{L}$ (where the mean increase for the LI4 intervention of 16.3% was the largest increase encountered among all 80 response cells recorded in the total study) and for $LI5^{L}$.

4.6.2 Comparison pair: duration 21 minutes, manipulation absent

m^{-21}

Site	LI4	NAP	p (bet)
$KI3^{R\#}$	2.7	**9.6**	**0.0001**
$ST36^{R\#}$	2.1	**6.5**	**0.0227**
$LI5^{L}$	4.5	**6.0**	0.8633
$PC6^{L}$	5.0	**10.6**	0.0748
$LI10^{L\#}$	3.2	**9.2**	**0.0032**
$LI20^{R}$	1.9	**5.0**	0.1065
$GB12^{R}$	**8.7**	**11.5**	0.2365
$3^{R\#}$	0.8	**10.5**	**0.0000**
1^{L}	4.9	**6.8**	0.7533
2^{L}	3.8	**5.6**	0.6924

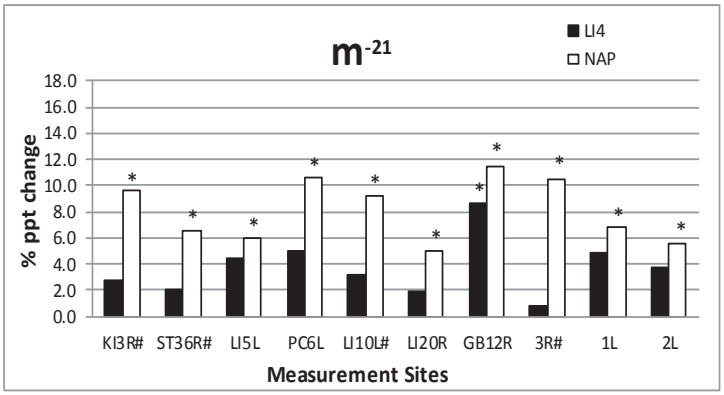


Figure 4a.8: Mean % change in PPT from pre-intervention mean for the ten regional measurement sites, following the pair of 21-minute needle retention interventions with manipulation absent and at either needling site LI4 or NAP. Statistically significant increases from pre-intervention means are marked in bold in the list of means in the table and on the bar chart (*). The p values shown in bold on the table identify significant differences in between pair comparisons.

The effects on mean PPT for the 21-minute manipulation absent pair of interventions for the two sites contrast sharply with the above presented profiles where manipulation was present. For the NAP intervention, there were statistically significant elevations from baseline means at all ten sites, compared with only one ($GB12^{R}$) for the LI4 intervention. These differences were reflected in the four comparison pairs where the NAP intervention response was significantly larger than that elicited for the LI4 intervention. These were $KI3^{R}$ (9.6% cf. 2.7% $p<0.0001$), $ST36^{R}$ (6.5% cf. 2.1% $p=0.0227$), $LI10^{L}$ (9.2% cf. 3.2% $p=0.0032$) and 3^{R} (10.5% cf. 0.8% $p=0.0000$).

4.6.3 Comparison pair: duration one minute, manipulation present

m^{+1}

Site	LI4	NAP	p (bet)
$KI3^{R}$	**6.9**	**7.0**	0.9998
$ST36^{R\#}$	0.7	**7.2**	**0.0003**
$LI5^{L}$	**6.2**	**7.3**	0.9350
$PC6^{L}$	**7.9**	**7.7**	0.9998
$LI10^{L\#}$	**5.9**	**11.8**	**0.0074**
$LI20^{R\#}$	3.2	**6.9**	**0.0269**
$GB12^{R\#}$	**6.8**	**13.0**	**0.0000**
3^{R}	3.6	**6.8**	0.1730
1^{L}	**7.0**	**9.6**	0.5544
2^{L}	**9.7**	**6.9**	0.5091

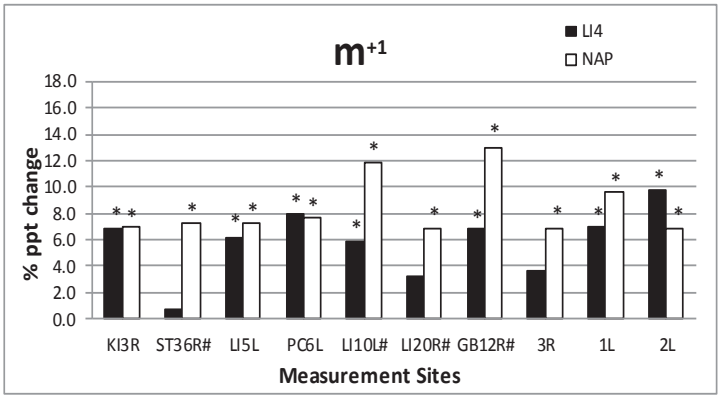


Figure 4a.9: Mean % change in PPT from pre-intervention mean for the ten regional measurement sites, following the pair of one minute needle retention interventions with manipulation present and at either needling site LI4 or NAP. Statistically significant increases from pre-intervention means are marked in bold in the list of means in the table and on the bar chart (*). The p values shown in bold on the table identify significant differences in between pair comparisons.

At all ten regional sites the one minute NAP intervention with manipulation elicited statistically significant increases in mean % PPT from the baseline values. For the LI4 intervention this did not occur at three sites ($ST36^{R}$, $LI20^{R}$ and 3^{R}). While at five regional sites, the mean changes were very similar for both interventions and all represented significant elevations from baseline means, there were four sites where the NAP intervention elicited mean increases superior to those following the LI4 intervention. These were $ST36^{R}$ (7.2% cf. 0.7% p=0.0003), $LI10^{L}$ (11.8% cf. 5.9% p=0.0074), $LI20^{R}$ (6.9% cf. 3.2% p=0.0269) and $GB12^{R}$ (13.0% cf. 6.8% p=0.0000).

4.6.4 Comparison pair: duration one minute, manipulation absent

m^{-1}

Site	LI4	NAP	p (bet)
$KI3^{R}$	**5.1**	**8.5**	0.1199
$ST36^{R}$	3.4	1.2	0.4145
$LI5^{L}$	**8.4**	**9.3**	0.9701
$PC6^{L}$	**7.6**	**7.6**	1.0000
$LI10^{L}$	**7.9**	**8.6**	0.9855
$LI20^{R}$	**5.9**	**5.8**	0.9998
$GB12^{R}$	**11.9**	**9.6**	0.4500
3^{R}	**4.6**	**5.7**	0.9022
1^{L}	**13.6**	**9.7**	0.3095
2^{L}	**6.8**	**6.5**	0.9988

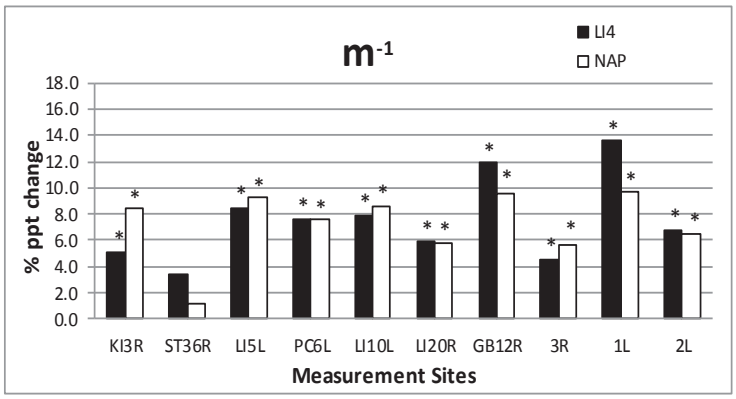


Figure 4a.10: Mean % change in PPT from pre-intervention mean for the ten regional measurement sites, following the pair of one minute needle retention interventions with manipulation absent and at either needling site LI4 or NAP. Statistically significant increases from pre-intervention means are marked in bold in the list of means in the table and on the bar chart (*). The p values shown in bold on the table identify significant differences in between pair comparisons.

In the one minute manipulation absent pair of interventions, there were no between intervention statistically significant differences in % PPT mean changes. In addition, within interventions showed similar profiles including the same nine sites where increases were statistically significant and the same site ($ST36^{R}$) where the change was not significant.

4.6.5 Summary: LI4 or NAP

- Duration: 21 minutes, manipulation present: at two sites LI4 mean was higher. These were for $LI10^{L}$ (where the mean increase for the LI4 intervention of 16.3% was the largest increase encountered among all 80 response cells recorded in the total study) and $LI5^{L}$. For all ten regional measurement sites apart from the mean at $LI5^{L}$ for the NAP intervention, both interventions elicited significant elevations in PPT from baseline.
- Duration: 21 minutes, manipulation absent: at four sites NAP intervention response was significantly larger than that elicited for the LI4 intervention. These were $KI3^{R}$ (9.6% cf. 2.7% $p<0.0001$), $ST36^{R}$ (6.5% cf. 2.1% $p=0.0227$), $LI10^{L}$ (9.2% cf. 3.2% $p=0.0032$) and 3^{R} (10.5% cf. 0.8% $p=0.0000$). Note that for the NAP intervention, there were statistically significant elevations from baseline means at all ten sites, compared with only one ($GB12^{R}$) for the LI4 intervention.
- Duration: one minute, manipulation present: at four sites the NAP intervention elicited mean increases superior to those following the LI4 intervention: $ST36^{R}$ (7.2% cf. 0.7% $p=0.0003$), $LI10^{L}$ (11.8% cf. 5.9% $p=0.0074$), $LI20^{R}$ (6.9% cf. 3.2% $p=0.0269$) and $GB12^{R}$ (13.0% cf. 6.8% $p=0.0000$).

- Duration: one minute, manipulation absent: there were no between intervention differences.

4.7 Examination of the effect of needle manipulation on change in mean PPT from baseline (manipulation vs no manipulation)

4.7.1 Comparison pair: site LI4, duration 21 minutes

$LI4^{21}$

Site	m+	m-	p (bet)
$KI3^{R\#}$	**7.5**	2.7	**0.0286**
$ST36^{R}$	**5.5**	2.1	0.1171
$LI5^{L\#}$	**10.1**	4.5	**0.0274**
$PC6^{L\#}$	**9.9**	**5.0**	**0.0325**
$LI10^{L\#}$	**16.4**	3.2	**0.0000**
$LI20^{R\#}$	**8.0**	1.9	**0.0007**
$GB12^{R}$	**11.9**	**8.7**	0.1362
3^{R}	**5.3**	0.8	0.0517
1^{L}	**8.5**	4.9	0.2744
2^{L}	**7.8**	3.8	0.0848

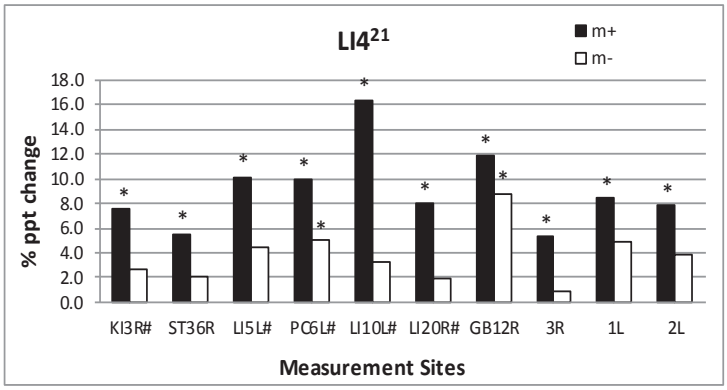


Figure 4a.11: Mean % change in PPT from pre-intervention mean for the ten regional measurement sites, following the pair of LI4 interventions with needle retention times of 21 minutes and with manipulation either present or absent. Statistically significant increases from pre-intervention means are marked in bold in the list of means in the table and on the bar chart (*). The p values shown in bold on the table identify significant differences in between pair comparisons.

This set of comparison pairs includes the most exceptional comparisons encountered in this study. There is a clear visual pattern evident at all ten sites with the intervention with manipulation present having a higher mean than the manipulation absent intervention. This visual difference was statistically significant at five sites and on the cusp (p=0.0517) at a sixth. The set of comparisons also includes the highest mean change in PPT elicited during the study (16.4% at $LI10^{L}$ which contrasts obviously with the manipulation absent partner of 3.2% p=0.0000. Further, it includes the second lowest (0.8%) change in the study (3^{R}).

The increases in mean PPT for the manipulation absent intervention were only statistically significant from baseline at two sites. For one of these ($PC6^{L}$) the increase was significantly lower than for the manipulation present condition (5.0% cf. 9.9%, p=0.0325). This set of comparisons suggests that for needling the site LI4, with 21-minute needle retention, the presence of manipulation had a significant effect on the strength of PPT change elicited.

4.7.2 Comparison pair: site LI4, duration one minute

LI4[1]

Site	m+	m-	p (bet)
KI3[R]	**6.9**	**5.1**	0.6639
ST36[R]	0.7	3.4	0.2683
LI5[L]	**6.2**	**8.4**	0.6606
PC6[L]	**7.9**	**7.6**	0.9988
LI10[L]	**5.9**	**7.9**	0.7472
LI20[R]	3.2	**5.9**	0.1879
GB12[R#]	**6.8**	**11.9**	**0.0018**
3[R]	3.6	**4.6**	0.9007
1[L#]	**7.0**	**13.6**	**0.0174**
2[L]	**9.7**	**6.8**	0.3987

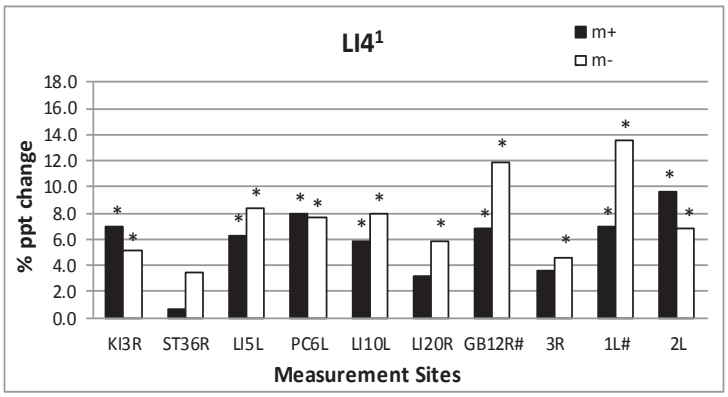


Figure 4a.12: Mean % change in PPT from pre-intervention mean for the ten regional measurement sites, following the pair of LI4 interventions with needle retention times of one minute and with manipulation either present or absent. Statistically significant increases from pre-intervention means are marked in bold in the list of means in the table and on the bar chart (*). The p values shown in bold on the table identify significant differences in between pair comparisons.

The results for the LI4 interventions with one minute duration show a very different set of comparison pairs. The intervention with manipulation absent elicited statistically significant increases in mean PPT at nine sites, including two of the greatest increases in the study (13.6% at 1^{L} and 11.9% at $GB12^{R}$). With both these regional sites, the intervention achieved a significantly higher elevation than the equivalent intervention with manipulation present (7.0%, p=0.0174 and 6.8%, p=0.0018 respectively). The manipulation present intervention did elicit increases in mean PPT that were significantly higher than baseline but on no occasions were they superior to those recorded for the manipulation absent intervention.

4.7.3 Comparison pair: site NAP, duration 21 minutes

NAP[21]

Site	m+	m-	p (bet)
KI3[R]	**8.9**	**9.6**	0.9801
ST36[R]	**4.9**	**6.5**	0.7663
LI5[L]	4.6	**6.0**	0.8765
PC6[L]	**9.4**	**10.6**	0.9506
LI10[L]	**7.1**	**9.2**	0.6595
LI20[R]	**5.7**	**5.0**	0.9803
GB12[R]	**9.6**	**11.5**	0.5246
3[R]	**6.9**	**10.5**	0.1431
1[L]	**8.0**	**6.8**	0.9334
2[L]	**5.3**	**5.6**	0.9972

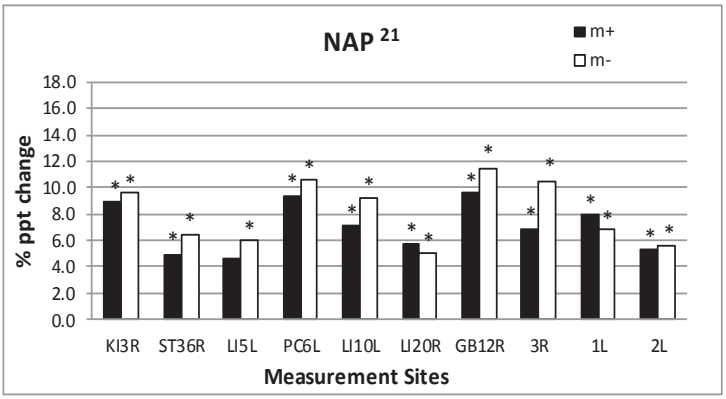


Figure 4a.13: Mean % change in PPT from pre-intervention mean for the ten regional measurement sites, following the pair of NAP interventions with needle retention times of 21 minutes and with manipulation either present or absent. Statistically significant increases from pre-intervention means are marked in bold in the list of means in the table and on the bar chart (*). The p values shown in bold on the table identify significant differences in between pair comparisons.

With the exception of the site LI5^{L} with manipulation present, whether or not manipulation was present, a statistically significant increase was elicited compared with baseline. None of the pairs differed statistically significantly.

4.7.4 Comparison pair: site NAP, duration one minute

NAP^{1}

Site	m+	m-	p (bet)
KI3^{R}	**8.4**	**7.2**	0.1526
ST36$^{R\#}$	**7.2**	1.2	**0.0005**
LI5^{L}	**7.3**	**9.2**	0.7468
PC6^{L}	**7.7**	**7.6**	1.0000
LI10^{L}	**11.8**	**8.6**	0.2794
LI20^{R}	**6.9**	**5.8**	0.8852
GB12^{R}	**13.0**	**9.6**	0.0867
3^{R}	**6.8**	**5.7**	0.8813
1^{L}	**9.6**	**9.7**	1.0000
2^{L}	**6.9**	**6.5**	0.9976

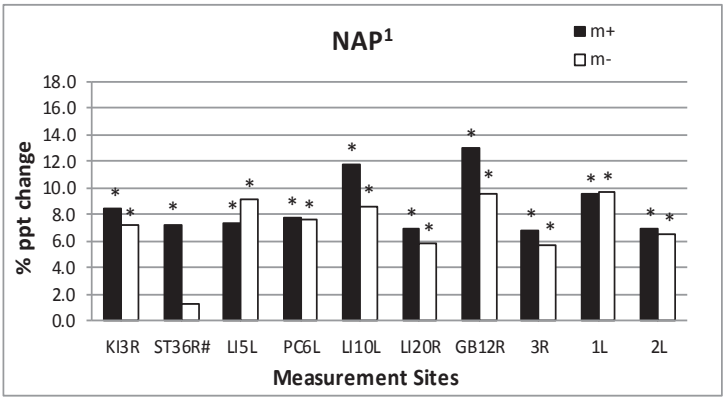


Figure 4a.14: Mean % change in PPT from pre-intervention mean for the ten regional measurement sites, following the pair of NAP interventions with needle retention times of one minute and with manipulation either present or absent. Statistically significant increases from pre-intervention means are marked in bold in the list of means in the table and on the bar chart (*). The p values shown in bold on the table identify significant differences in between pair comparisons.

Again, all but one measurement cell showed statistically significant increases in mean PPT from baseline values. With the exception of ST36^{R} the post-intervention PPT mean was close to baseline (1.2% increase) for the manipulation absent intervention and was significantly lower ($p<0.0005$) than the mean when manipulation was present (7.2%). This very low value contrasted strongly with the range of values recorded for the other sites.

4.7.5 Summary: manipulation present or absent

- Site: LI4, duration 21 minutes: at five sites the manipulation present mean PPT was statistically significantly greater than for the manipulation absent intervention, and was on the cusp ($p=0.0517$) at a sixth site. These sites included the greatest mean change in PPT elicited in the study (16.4% at LI10^{L}) which contrasts sharply ($p=0.0000$) with the nonmanipulated partner's mean of 3.2%. The nonmanipulated responses included the second lowest (0.8%) change observed (3^{R}).
- Site: LI4, duration one minute: at two sites the manipulation absent intervention elicited statistically significantly higher elevations in mean PPT. These are also two of the greatest increases in the study (13.6% at 1^{L} and 11.9% at GB12^{R}).

- Site: NAP, duration 21 minutes: None of the response pairs differed statistically significantly. With the sole exception of site $LI5^{L}$ with manipulation present, statistically significant increases from the baseline means were elicited.
- Site: NAP, duration one minute: the only between interventions difference that was statistically significant involved the $ST36^{R}$ response pair, where the manipulation absent mean remained close to baseline (1.2% increase). All other pairs of response cells showed similar significant increases from baseline.

4.8 Overall summary of significant differences identified in the above sets of comparison pairs

Table 4a.4 shows the number and direction of statistically significant differences for the various comparison pairs presented in the preceding sections. The cells show the number of regional sites involved for each comparison pair. Direction of the difference is denoted by (+) or (-) showing whether the relevant mean for the intervention listed in the left hand column was more (+) or less (-) that the mean increase elicited by the second pair member, shown in the second column of the table.

Intervention pair		**Number and direction of statistically significant differences for various comparison pairs**
$LI4m^{+21}$	$LI4m^{+1}$	moderate (4+)
	$NAPm^{+21}$	minor (2+)
	$LI4m^{-21}$	moderate (5+)
$NAPm^{-1}$	$NAPm^{+1}$	none/minimal (1+)
	$LI4m^{-1}$	none/minimal (0)
	$NAPm^{-21}$	minor (2+)
$NAPm^{+1}$	$LI4m^{+1}$	moderate (4-)
	$NAPm^{+21}$	none/minimal (1-)
$LI4m^{-1}$	$LI4m^{+1}$	minor (2-)
	$LI4m^{-21}$	minor (2-)
$NAPm^{-21}$	$NAPm^{+21}$	none/minimal (0)
	$LI4m^{-21}$	moderate (4-)

Table 4a.4: The number and direction of statistically significant differences for the various study comparison pairs. The cells show the number of regional sites involved for each comparison pair and shows whether the mean for the intervention listed in the left hand column was more (+) or less (-) that the mean increase elicited by the second pair member, shown in the second column of the table.

Based on the number of significant differences in PPT means at individual regional measurement sites obtained for intervention comparison pairs, extent of overall differences in

intervention effects on regional PPT has been designated as none/minimal (pair differences either 0 or 1) minor (N=2) or moderate (N≥4). Among the 12 comparison pairs, four had either none or one significant difference between means. A further four showed only two significant differences. The remaining four intervention pairs showed either four or five significant differences and each comparison pair included an LI4 intervention, compared with only two that involved NAP interventions. $LI4m^{+21}$ was significantly superior to $LI4m^{-21}$ or $LI4m^{+1}$. However $LI4m^{+1}$ was inferior to $NAPm^{+1}$ and $LI4m^{-21}$ was inferior to $NAPm^{-21}$.

4.9 Comparison of subjects' perceptions of the acupuncture experience among interventions

Table 4a.5 and Figure 4a.15 summarises the results for the eight interventions for the subjects' VAS reporting of mean levels of pain, needle sensation, tension, anxiety and changes in acupuncturist's behaviour across sessions.

	$LI4m^{+21}$ mean (sem)	$LI4m^{-21}$ mean (sem)	$NAPm^{+21}$ mean (sem)	$NAPm^{-21}$ mean (sem)	$LI4m^{+1}$ mean (sem)	$LI4m^{-1}$ mean (sem)	$NAPm^{+1}$ mean (sem)	$NAPm^{-1}$ mean (sem)	p value	F
Pain	26.0 (5.27)	11.5 (4.07)	26.4 (5.90)	6.9 (1.83)	16.9 (4.17)	8.9 (3.69)	19.6 (5.44)	10.3 (4.58)	0.000	$F_{7, 161} = 4.35$
Needle sensation	30.0 (5.94)	15.9 (4.81)	26.3 (5.72)	13.1 (3.39)	25.3 (5.19)	15.7 (4.58)	18.0 (5.37)	14.0 (4.59)	0.014	$F_{7, 161} = 2.62$
Tension	14.0 (3.72)	5.8 (3.01)	10.7 (3.32)	8.0 (3.7)	9.0 (3.42)	4.3 (2.25)	7.0 (2.69)	3.2 (2.12)	0.050	$F_{7, 161} = 2.07$
Anxiety	3.9 (1.47)	4.3 (1.78)	9.0 (3.40)	3.9 (1.73)	6.3 (3.03)	2.7 (1.42)	4.8 (1.95)	2.1 (1.23)	0.236	$F_{7, 161} = 1.34$
Acupuncturist's Behaviour	5.1 (4.07)	1.2 (0.73)	1.1 (0.87)	0.0 (0.14)	1.0 (0.44)	2.1 (0.79)	0.8 (0.59)	0.8 (0.66)	0.433	$F_{7, 137} = 1.00$

Table 4a.5: Comparison of mean percentage scores for pain, needle sensation, tension, anxiety and changes in acupuncturist's behaviour, each recorded on 100mm VAS by subjects for the 21 minutes of each intervention (sem=standard error of the mean).

There were only six statistically significant differences between intervention VAS mean scores with respect to the five variables. Four involved pain scores where means for both $NAPm^{+21}$ (26.4) and $LI4m^{+21}$ (26.0) were statistically significantly higher than the means for $NAPm^{-21}$ (6.9) and $LI4m^{-1}$ (8.9). The mean needle sensation score for $LI4m^{+21}$ (30.0) was higher than for $NAPm^{-21}$ (13.1); and mean tension reported for $LI4m^{+21}$ was greater than for $NAPm^{-1}$ (14.0 cf. 3.2). For both anxiety and acupuncturist's behaviour, the mean values were all minimal or very low and no statistically significant differences were found.

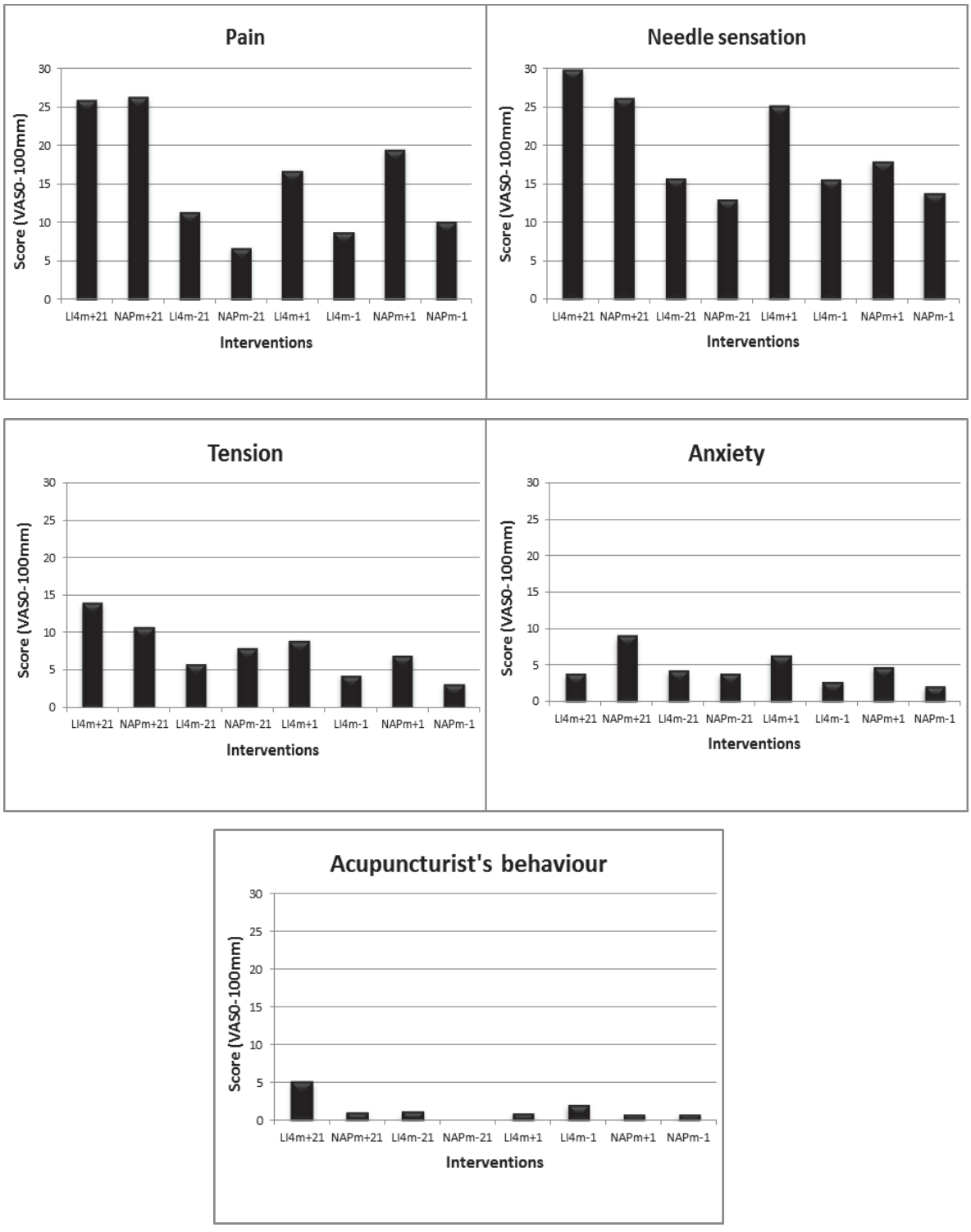


Figure 4a.15: Comparison of the pain experienced, needle sensation, feeling of tension, anxiety and acupuncturist's behaviour change between interventions (the vertical axis indicates the mean percentage VAS score and the horizontal axis indicates the eight interventions).

Subjects' responses to the post experimental session questionnaires were examined for possible relationships between pairs of subjective variables associated with needling that may have impacted on the PPT findings. Table 4a.6 includes both the Pearson r and the coefficient of determination r^2 for the VAS scores for pain, needle sensation, tension and anxiety, recorded

for the 21-minute intervention period for each intervention. Since r^2 values are indicative of the extent of covariation for a variable pair, r^2 values >0.5 were used as a basis for judging a relationship's strength, from mild to moderate (0.5 – 0.7) to very strong and very high (>0.8). Note that all correlations were examined to determine whether the relationship was linear and therefore appropriate for analysis using Pearson r and coefficients of determination. The table only includes relationships with $r^2 \geq 0.5$.

Variables	Intervention	r	r^2
Pain and Sensation	$NAPm^{+21}$	0.77	0.60
	$NAPm^{-21}$	0.72	0.51
	$LI4m^{-21}$	0.94	0.88
	$LI4m^{+1}$	0.79	0.62
	$LI4m^{-1}$	0.90	0.82
	$NAPm^{-1}$	0.94	0.88
Pain and Tension	$LI4m^{+21}$	0.77	0.59
	$LI4m^{-21}$	0.74	0.54
Anxiety and Tension	$NAPm^{+21}$	0.90	0.81

Table 4a.6: Pearson product moment correlation coefficient r and r^2 for the VAS scores for pain, needle sensation, tension, anxiety, recorded for the 21-minutes intervention period for the eight interventions.

There were nine correlations where at least 50% of the variance was accounted for by covariance. Eight of these included pain; six linked with needle sensation and two with tension. The remaining relationship linked tension with anxiety ($NAPm^{+21}$). The strongest relationships were between pain and sensation for $NAPm^{-1}$ (r^2=0.88), $LI4m^{-1}$ (r^2=0.82) and $LI4m^{-21}$ (r^2=0.88); and between anxiety and tension for $NAPm^{+21}$ (r^2=0.81). Notably, while for six interventions, pain and needle sensation had moderate to strong relationships, this was not the case for $LI4m^{+21}$.

The weakest (accounting for ≤60% of the variance) were between pain and tension for $LI4m^{-21}$ (r^2=0.54), $LI4m^{+21}$ (r^2=0.59) and pain and sensation for $NAPm^{-21}$ (r^2=0.51) and $NAPm^{+21}$ (r^2=0.60). The two interventions for which pain and needle sensation were not strongly related were $LI4m^{+21}$ and $NAPm^{+1}$. No strong relationships among variable pairs were found for $NAPm^{+1}$.

4.10 Comparison of distribution of regional PPT effects by intervention

Table 4a.7 summarises the observed and predicted distribution of significant effects on regional PPT that would be predicted from neural segment theory or (in the case of LI4 interventions) by TCM channel theory.

Site	Observed and predicted distribution of effect	Measurement site									
		KI3^{R}	ST36^{R}	LI5^{L}	PC6^{L}	LI10^{L}	LI20^{R}	GB12^{R}	3^{R}	1^{L}	2^{L}
LI4	Observed m^{+21}	√	√	√	√	√	√	√	√	√	√
	Observed m^{-21}							√			
	Observed m^{+1}	√		√	√	√		√		√	√
	Observed m^{-1}	√		√	√	√	√	√	√	√	√
	Neural segment theory (P)										
	TCM channel theory (P)										
NAP	Observed m^{+21}	√	√		√	√	√	√	√	√	√
	Observed m^{-21}	√	√	√	√	√	√	√	√	√	√
	Observed m^{+1}	√	√	√	√	√	√	√	√	√	√
	Observed m^{-1}	√		√	√	√	√	√	√	√	√
	Neural segment theory (P)										

Table 4a.7: Summary of observed distribution of statistically significant changes to regional PPT by intervention and the distribution that would be predicted (shaded in grey) by neural segment theory or by TCM channel (in the case of LI4 interventions).

From examination of the regional response pattern of significant PPT affects elicited by the various interventions it is clear the effects on PPT were generalised. With the exception of LI4m^{-21} which showed minimal significant change from baseline, they typically occurred at sites the length and breadth of the body. Therefore they were not consistent with either TCM channel theory (with respect to the four LI4 variants) nor for neural segmental theory. The results suggest a generalised effect on PPT; one that is consistently a statistically significant increase in threshold from pre-intervention levels.

Part II: Needling pain, sensation and regional PPT profiles during the intervention phase

4.11 Introduction

The aim of this part of the research was to compare the eight combinations of three needling parameters in relation to the reporting of needling sensation and needling pain in terms of: the reporting of the qualities of the needle sensation experienced and the intensity of pain at the needling site. The mean percentages changes in PPT from pre-intervention baseline at three regional measurement sites during and post 21-minute intervention phase were also compared.

4.12 Needle sensation and pain intensity profiles during the intervention period

Figure 4b.1 displays (left) mean sensation intensity scores and mean pain intensity scores (right) for (% of 100mm VAS) for the eight interventions at three minute intervals during the 21-minute intervention period. Standard error bars with standard error of the mean (sem) are also shown in the graphs. The same colour key applies to both graphs.

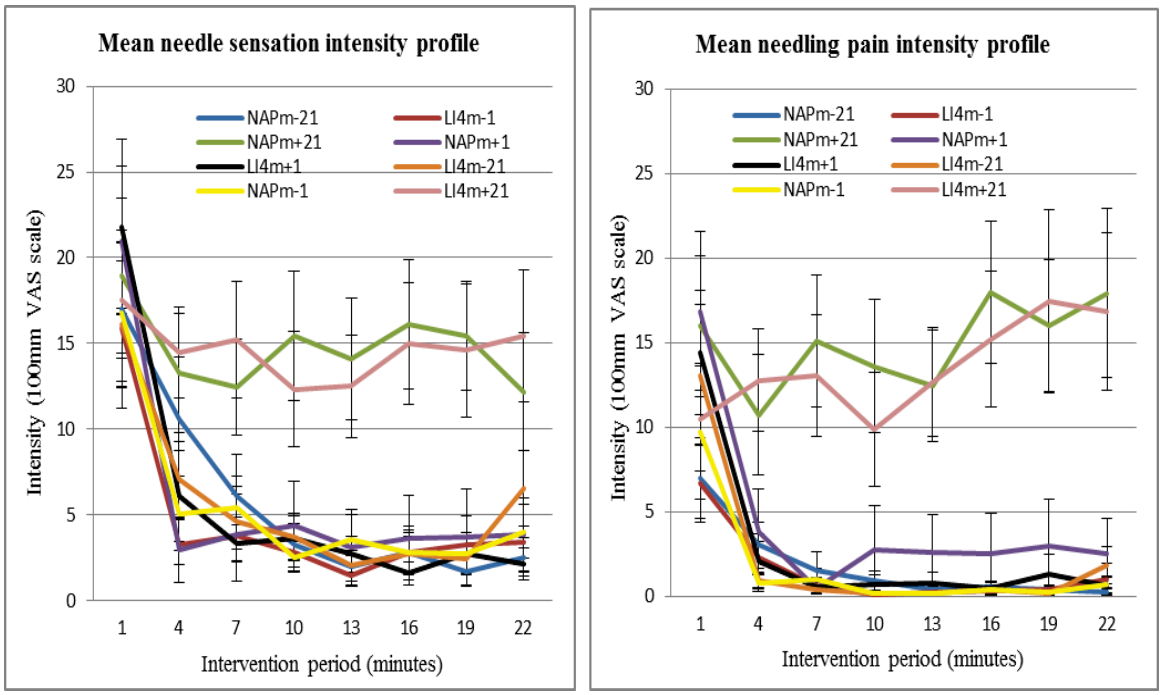


Figure 4b.1: The mean sensation intensity scores (left hand graph) and the mean pain intensity scores (right hand graph) for the eight interventions at three minute intervals during the 21-minute intervention period. Standard error bars (±1sem) are also shown in the graphs.

At time t=1, there was no statistically significant difference between the interventions for both the pain VAS mean scores (ANOVA $F_{7,161}$=1.74, p=0.103) and for the needle sensation VAS scores (ANOVA $F_{7,161}$=0.48, p=0.85). At all other time intervals (t=4, t=7, t=10, t=13, t=16,

t=19 and t=22), there were multiple statistically significant differences among the means for pairs of interventions for both needle pain (ANOVA F statistics lay between 5.33 and 12.54, p=0.000 in all cases) and needle sensation means (ANOVA F statistics lay between 4.07 and 9.19, p=0.000 in all cases). Table 5 and Table 6 in Appendix VI summarise the findings for the ANOVA and Tukey post hoc analyses for each time interval.

These analyses revealed that, with respect to both sets of profiles, similar patterns were evident. These included the following main features. Initially, as noted above, all eight interventions showed similar mean levels of needle pain and of needle sensation at t=1. Mean values ranged from 6.7% to 16.9% for pain and 15.9% to 21.8% for needle sensation. However, at the subsequent time intervals, among the interventions, these elevated levels were either maintained or fell away rapidly. The two interventions that involved 21 minutes needle retention and ongoing manipulation maintained similarly elevated mean VAS scores that did not differ statistically significantly from each other at each measurement period. In all cases for both pain and needle sensation the values of the ANOVA F statistics lay between 1.48 and 0.0 with associated p values of between 0.24 and 0.95. The remaining six interventions in general all followed similar rates of decrease in mean VAS scores for pain and for needle sensation to low values that did not differ statistically significantly from each other. In all cases for both pain and needle sensation the values of ANOVA F statistics for the individual measurement times lay between 1.94 and 0.27 with associated p values of between 0.09 and 0.93.

With respect to pain, post hoc analyses (Tukey post hocs, in all cases statistical significance $p<0.05$) showed that the $LI4m^{+21}$ intervention mean VAS levels were typically statistically significantly higher than those for the six interventions that did not involve 21 minutes and manipulation for all time intervals from t=4 to t=22, with the single exception of $NAPm^{+1}$ at t=10. For the $NAPm^{+21}$, the mean VAS scores were also statistically significantly higher than all other interventions from t=7 to t=22 and also for $LI4m^{-21}$ and $NAPm^{-1}$ at t=4.

A similar pattern was evident for the needle sensation profiles at the various time intervals. In the case of $LI4m^{+21}$ the mean needle sensation was statistically significantly greater than for the same six interventions as above with the following exceptions: at t=4 for $LI4m^{+1}$, $LI4m^{-21}$, $NAPm^{-1}$ and $NAPm^{-21}$; at t=7 and t=10 for $NAPm^{+1}$; and at t=22 with $LI4m^{-21}$. There were similarities between $NAPm^{+21}$ and $LI4m^{+21}$ profiles in that both did not differ statistically significantly from $LI4m^{+1}$, $NAPm^{-1}$, $LI4m^{-21}$ and $NAPm^{-21}$ at t=4; from $NAPm^{+1}$ at t=7; or from $LI4m^{-21}$ at t=22. However, the means for $NAPm^{+21}$ did not differ statistically significantly from four other interventions at t=7 ($NAPm^{-21}$, $LI4m^{-21}$, $NAPm^{+1}$ and $NAPm^{-1}$) and from five at t=22 (that is, all except $NAPm^{-21}$).

In summary, the continued application of needle manipulation and retention of the needle were important for maintaining a constant intensity of needle sensation. The strength of needle sensations experienced was independent of insertion site.

4.13 Relationship between pain and needle sensation perceptions

In the following four graphs, the eight interventions are compared with respect to the number of subjects at each three minute recording interval who had: neither pain nor needle sensation; both pain and needle sensation; only pain; or only needle sensation.

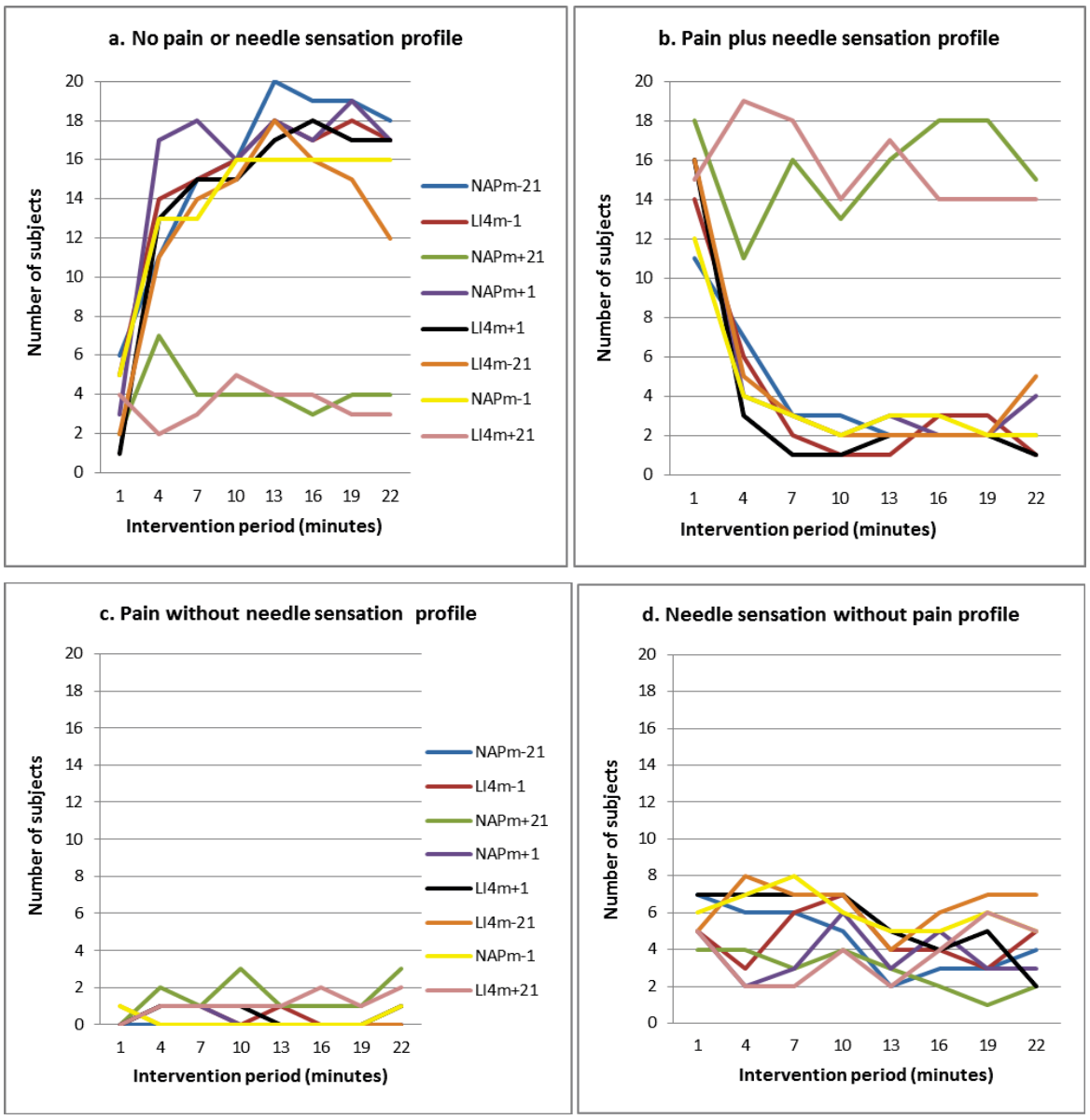


Figure 4b.2: Comparison of the eight interventions with respect to the number of subjects at each three minute recording interval who had: neither pain nor needle sensation (3a); both pain and needle sensation (3b); only pain (3c); or only needle sensation (3d). Total subjects = 24.

From the frequencies of subjects among interventions reporting neither pain nor needle sensation (Figure 4b.2a) or both pain and needle sensation (Figure 4b.2b), the profiles for both

interventions that included needle retention and ongoing manipulation during the 21-minute period are distinctly different from the remaining six interventions. With the former pair, very few subjects did not experience both pain and needle sensation at each interval. These frequencies were similar across the 21-minute intervention period. Indeed, the profiles for pain plus needle sensation (Figure 4b.2b) are strikingly similar to the profiles shown in Figure 4b.1 for both the mean needle sensation intensity and the mean pain intensity.

By contrast, virtually no subjects reported pain alone (Figure 4b.2c) for any intervention or time interval. Similarly, needle sensation in the absence of pain (Figure 4b.2d) was only experienced by a small proportion of subjects at any time interval. Interestingly, although four of the interventions involved only one minute of needling, there were still several reports of pain and or needle sensation throughout the entire 21-minute reporting period.

In summary, the varying experiences of needle sensation and pain associated with the different interventions were independent of the site of needling but were closely linked with whether or not the needle was retained and whether ongoing manipulation was applied.

From the pain and needle sensation profiles for individual interventions shown below in Figure 4b.3, all interventions had two common features. At t=1 (that is, when all had the common experience of a needle being inserted and retained for one minute) significantly more subjects reported the presence of both pain and needle sensation than other possibilities ($p<0.05$ for all eight interventions, Chi square I). The reporting of pain alone was absent at either most of ($LI4m^{-21}$, $NAPm^{+1}$, $NAPm^{-1}$) or all of the three minute measurement periods ($NAPm^{-21}$).

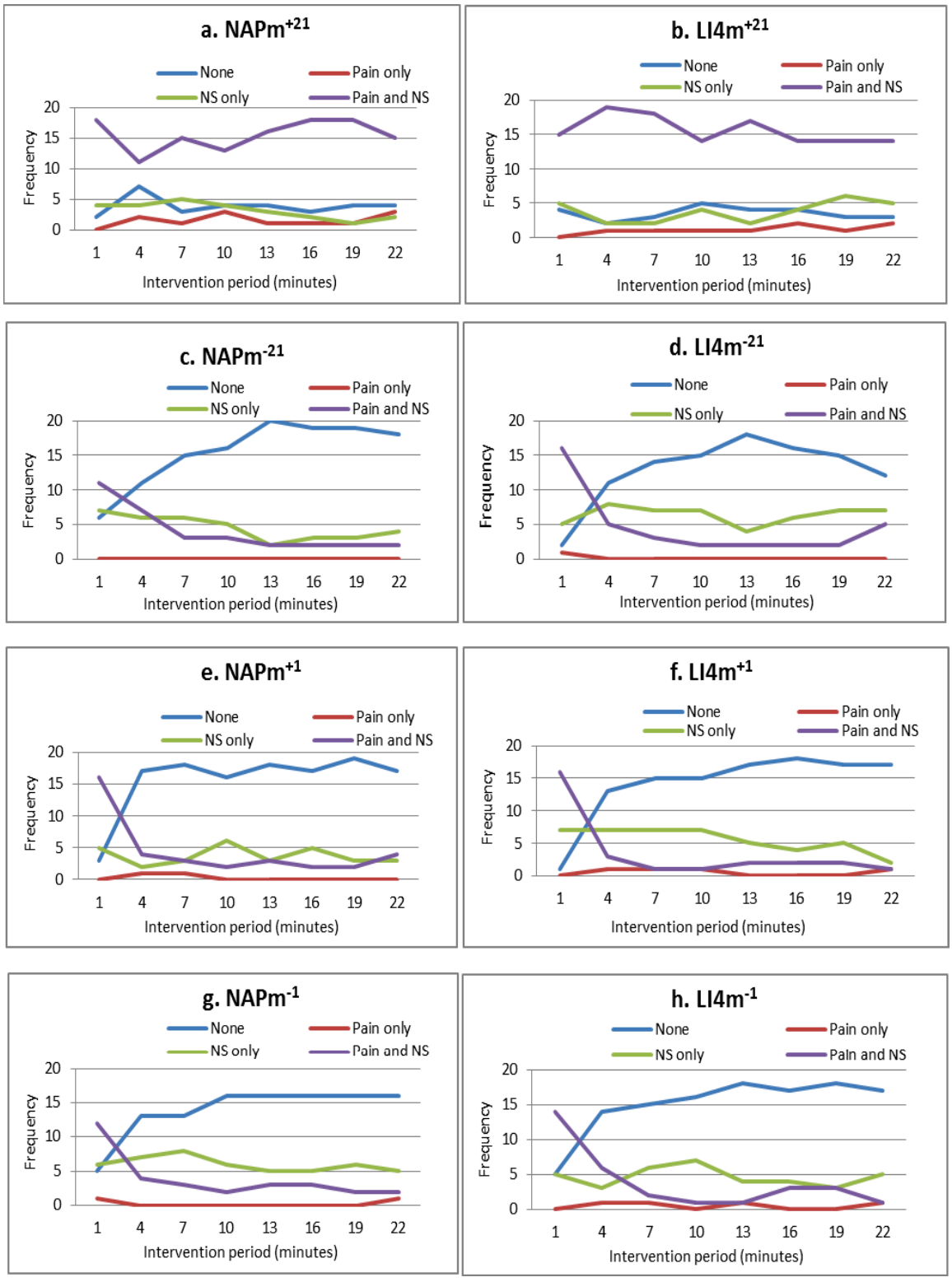


Figure 4b.3: Comparison within each of the eight interventions, of the number of subjects at each three minute recording interval who had neither pain nor needle sensation; both pain and needle sensation; only pain; or only needle sensation. Total number of subjects = 24.

The eight profiles clustered into two distinct response patterns: one shared by the two 21-minute interventions with regular manipulation, and the other by the remaining six interventions.

Pattern 1: For all measurement intervals including t=1, significantly more subjects reported the presence of both pain and needle sensation ($p<0.05$, Chi square I).

Pattern 2: Here, for all measurement intervals except t=1, significantly more subjects reported absence of both pain and needle sensation ($p<0.05$, Chi square I). This applied whether the needles were retained for one or 21 minutes and with the one minute retentions, whether or not manipulation was applied. Therefore, again, the distinguishing parameter values were needle retention and application of manipulation but not site of insertion.

4.14 Qualities of needle sensation: needling sensation descriptors

At the end of each session, subjects reported the needling sensations they had experienced during the intervention. Note that subjects were not limited to a single descriptor. The unsolicited descriptors provided by subjects were found to be strikingly concordant with ones from the MMPQ and so have been grouped according to MMPQ categories (Melzack 1973, 1975). This system addresses both quality and intensity of a descriptor so that interventions could be compared in terms of both the number and the intensity of descriptors used both within individual MMPQ descriptor categories and overall. Three minor additional categories were created for unrepresented terms: warm (since the MMPQ category commences with 'hot'); 'electricity'; and 'can't describe'.

The categories that contain descriptors provided by subjects are listed below:

Category	Descriptors (in order of intensity rank)
1	Flickering, Pulsing, Quivering, Throbbing, Beating, Pounding
3	Pricking, Boring, Drilling, Stabbing
4	Sharp, Cutting, Lacerating
5	Pinching, Pressing, Gnawing, Cramping, Crushing
6	Tugging, Pulling, Wrenching
7	Hot, Burning, Scalding, Searing
8	Tingling, Itchy, Smarting, Stinging
9	Dull, Sore, Hurting, Aching, Heavy
17	Spreading, Radiating, Penetrating, Piercing
18	Tight, Numb, Squeezing, Drawing, Tearing
19	Cool, Cold, Freezing
21	Electricity
22	Warm
23	Indescribable

Study subjects' results are summarised in Table 4b.1 and Figure 4b.4. Among the eight interventions, obviously more descriptors (41 and 43) have been reported for the two 21-minute interventions with manipulation compared with the other six interventions. Further, for these six interventions, the lower number of descriptors reported was very similar, ranging from 22 to 29

and is not explained by differing numbers of reports of no sensation among the eight interventions. For both one minute interventions without manipulation, the number and intensity scores are identical and similar to the one minute NAP intervention with manipulation ($NAPm^{+1}$). The intensity and number of descriptors for the one minute ($LI4m^{+1}$) with manipulation closely resembles the findings for the 21-minute LI4 without manipulation intervention ($LI4m^{-21}$).

Intervention	$NAPm^{+}$		$NAPm^{-}$		$LI4m^{+}$		$LI4m^{-}$	
	I	N	I	N	I	N	I	N
21 minutes	81	41(2)	33	22(7)	87	43(2)	53	27(6)
1 minute	43	27(3)	41	24(7)	55	29(6)	41	24(7)

Table 4b.1: Total Number (N) and intensity (I) of sensory descriptors reported for the eight interventions. (No sensation responses are shown in parentheses).

The MMPQ-based descriptor intensity profiles are shown below for the eight interventions in Figure 4b.4(a-h). The MMPQ categories relevant to these results are listed in the caption together with the three ungrouped additions of electricity, warm and indescribable.

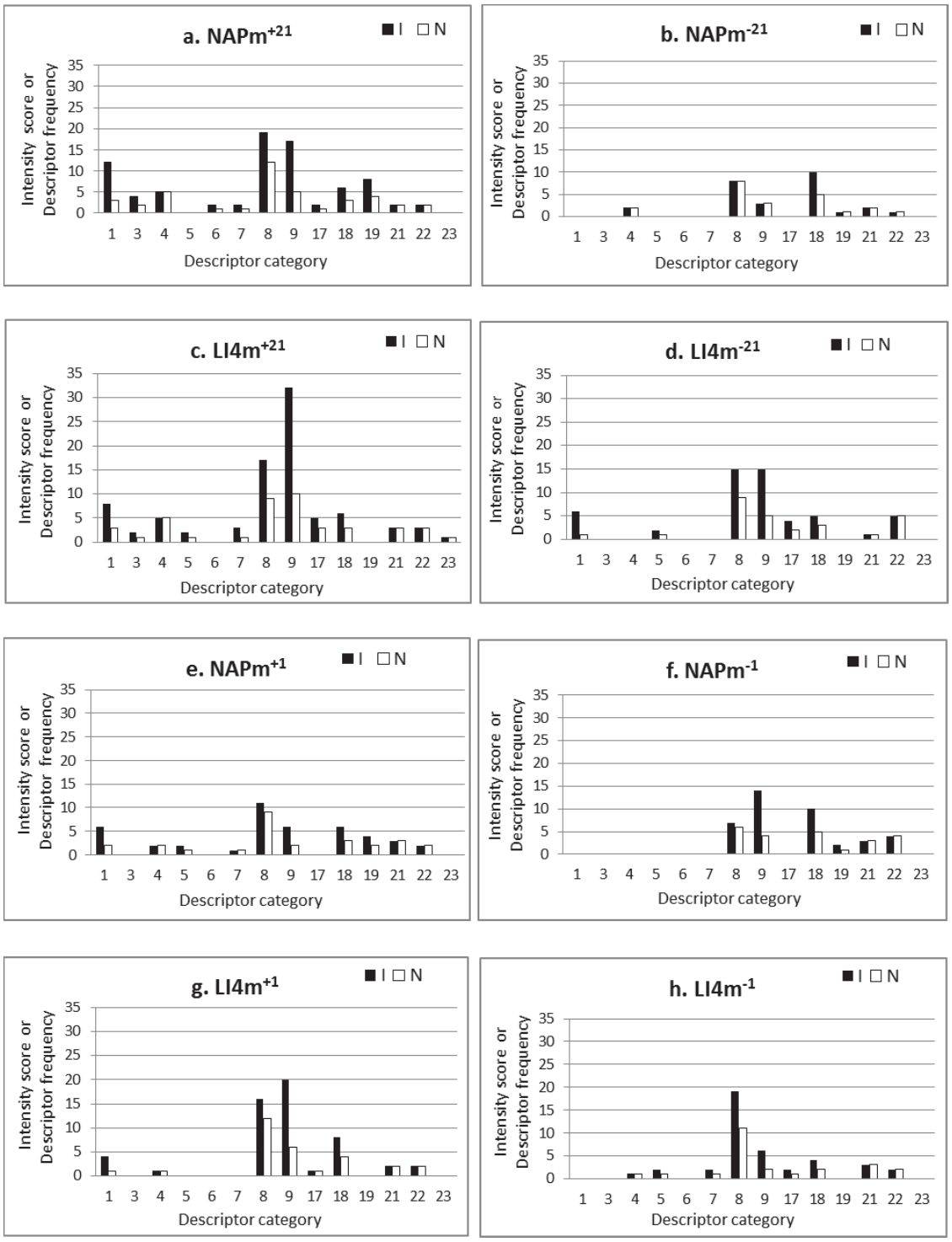


Figure 4b.4: Profiles for each intervention for the frequency (N) and intensity (I) of sensory descriptors reported from each descriptor category list.

Category list

1 Flickering, Pulsing, Quivering, Throbbing, Beating, Pounding
3 Pricking, Boring, Drilling, Stabbing
4 Sharp, Cutting, Lacerating
5 Pinching, Pressing, Gnawing, Cramping, Crushing
6 Tugging, Pulling, Wrenching
7 Hot, Burning, Scalding, Searing
8 Tingling, Itchy, Smarting, Stinging
9 Dull, Sore, Hurting, Aching, Heavy
17 Spreading, Radiating, Penetrating, Piercing
18 Tight, Numb, Squeezing, Drawing, Tearing
19 Cool, Cold, Freezing
21 Electricity
22 Warm
23 Indescribable

For all eight interventions, descriptors were reported from the same five descriptor categories, comprising 8, 9, 18 and the additional 21 (electricity) and 22 (warm). The most frequently reported descriptors were from category 8, and included some form or intensity of tingle, sting or itch. The second most frequent terms were from category 18 (typically numbness). Less frequent but reported for all interventions were category 9 terms (dull ache). Far less frequent, were the ungrouped terms 'warm' and 'electricity'.

Comparison of the descriptor profiles among the interventions found there was little to distinguish LI4 from NAP descriptor profiles. The only minor exception was the reporting of category 9 more frequently among the LI4 interventions. The two LI4 interventions involving manipulation had the highest intensity scores among all interventions for category 9 terms and for the four LI4 interventions, category 9 terms were reported 21 times compared with 12 times for the four NAP interventions. However these reports only involved a minority of subjects for the four interrentions at either site (20 reports cf 12).

4.15 Regional PPT profiles during the intervention period

The above findings have examined differences in needling pain and needle sensation profiles among the eight different needling parameter combinations during the intervention phase. This section is concerned with identifying possible effects of the different parameter combinations on regional PPT during the intervention phase. This was regarded as important in view of the two different actual needling durations involved (one and 21 minutes) since possibly short term changes in PPT elicited by the one minute needling interventions would not be detected at the post-intervention PPT measurement cycles which occurred over 20 minutes later.

At times 4, 10 and 16 minutes during the 21-minute intervention phase, three cycles of PPT measurements were taken at three sites ($KI3^{R}$, $LI10^{L}$ and $GB12^{R}$) that were distant from the needling locations. The PPT profiles at these sites are shown separately in Figure 4b.5a-h for each intervention. The mean post-intervention changes are also included for each site. Note that since the post-intervention mean drew on four sequential cycles of readings over approximately four to five minutes, these are presented as midway between the start and finish times for this period on the graphs (that is, 22 to 27 minutes).

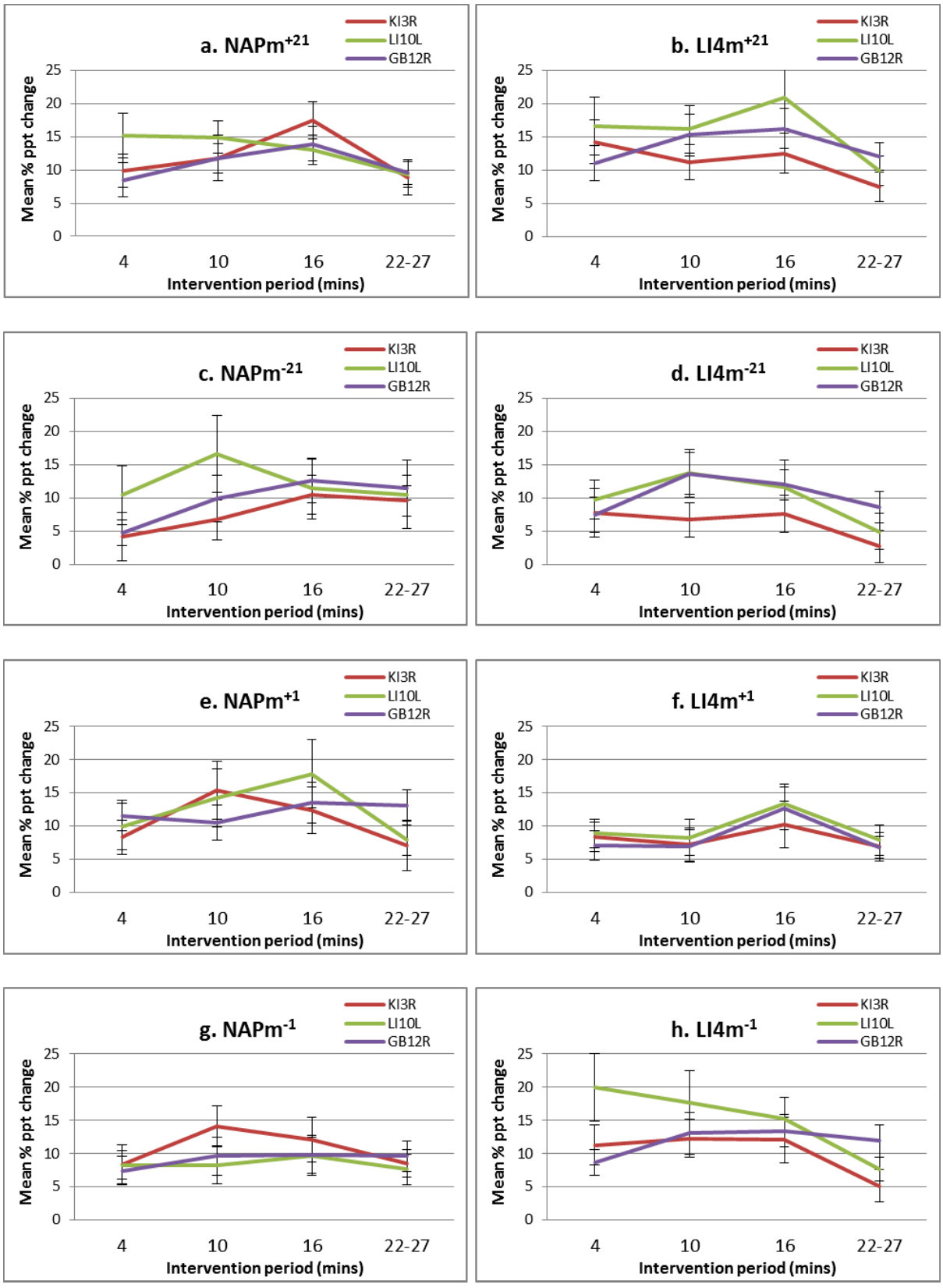


Figure 4b.5: Profiles for the mean % increase in regional PPT for the three regional sites measured during and post-intervention, shown separately for the eight interventions. Standard error bars (±1sem) are also shown in the graphs.

Since analysis of variance revealed that among the interventions and time intervals the profiles for the three sites did not differ statistically significantly (see Appendix VI), the mean changes from baseline were examined for the combined site data for each intervention. Figure 4b.5 shows the profiles for the three sites individually for the measurement times for the eight interventions. Table 4b.2 and Figure 4b.6 show the combined mean percentage change in PPT by intervention and time interval. While for each of the time intervals all interventions elicited mean % increases in PPT that were statistically significantly elevated above the baseline, there was only one statistically significant different between interventions for the same time interval: at t=4, $F_{7,545}$=2.41 p=0.019 $NAPm^{-21}$ (6.5%) less than $LI4m^{+21}$ (14.0%). The lack of statistically significant differences in % change in PPT between interventions during this 21-minute phase of the study is likely in part, to relate to the limited number of PPT measurements involved in these comparisons, that comprised 24 values at each site for each time interval, compared with 96 and 120 for pre or post-intervention comparisons.

Time	$NAPm^{-21}$	$LI4m^{-1}$	$NAPm^{+21}$	$NAPm^{+1}$	$LI4m^{+1}$	$LI4m^{-21}$	$NAPm^{-1}$	$LI4m^{+21}$	p value
4	6.47	13.25*	11.20*	9.93*	8.07*	8.36*	8.02*	14.01*	0.02#
10	11.09*	14.31*	12.83*	13.36*	7.47*	11.38*	10.62*	14.23*	0.16
16	11.50*	13.53*	14.82*	14.54*	12.06*	10.43*	10.48*	16.57*	0.18
22-27	10.54*	8.20*	9.25*	9.32*	7.17*	5.45*	8.57*	9.87*	0.18

Table 4b.2: Mean percentage change in PPT from pre-intervention mean for the combined three regional measurement sites, measured at intervals during the 21-minute intervention phase. The post-intervention (22-27 minutes) means are also shown (p=between intervention significance level, based on ANOVA and Tukey post hoc analyses). Significance level of increases from pre-intervention means are indicated as follows * $p<0.05$.

Figure 4b.6 shows the PPT profiles for the eight interventions over the intervention period. Also shown is Figure 4b.1 for comparison, which shows the needling pain and sensation profiles for the same colour coded interventions.

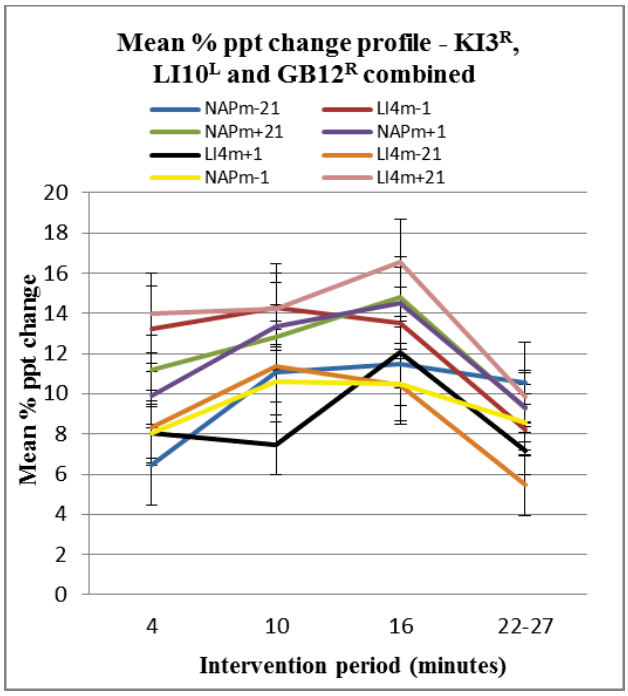


Figure 4b.6: Profiles for the mean % increase in regional PPT for the combined regional site measurements during and post-intervention, shown for the eight interventions. The post-intervention (22 to 27 minutes) means are also shown. Standard error bars (±1sem) are also shown in the graphs.

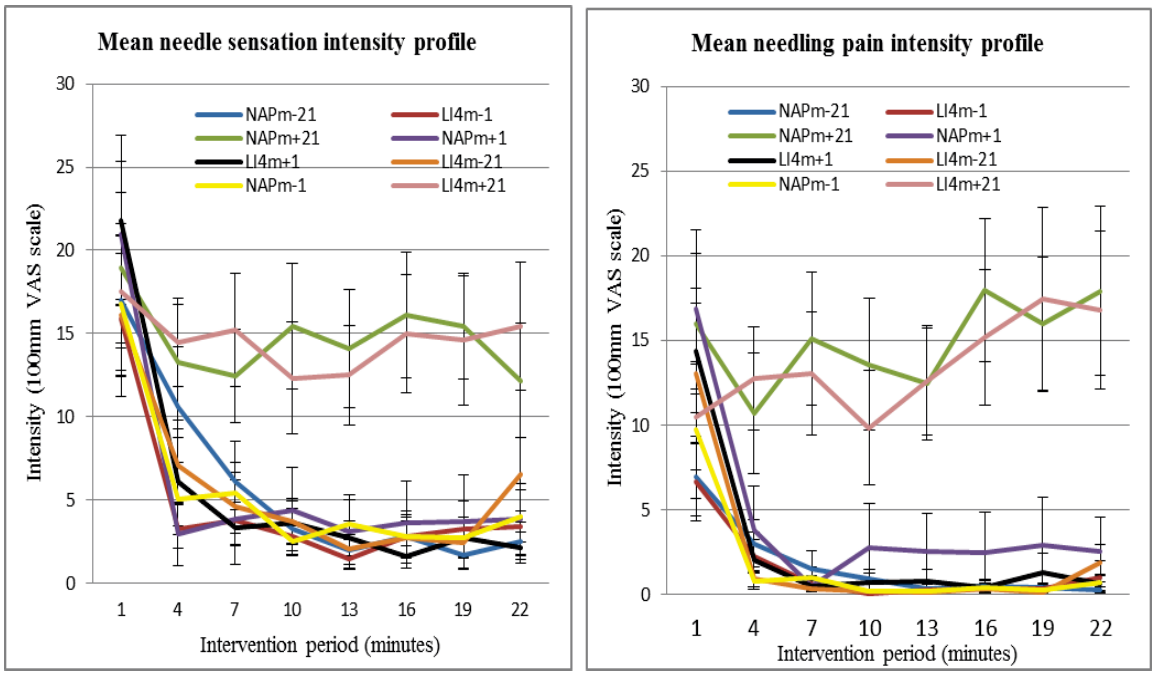


Figure 4b.1: The mean sensation intensity scores (left hand graph) and the mean pain intensity scores (right hand graph) for the eight interventions at three minute intervals during the 21 minute intervention period. Standard error bars (±1sem) are also shown in the graphs.

Unlike the latter two sets of profiles, where there are distinct differences in the profile patterns among the interventions, there is no obvious discrimination among the PPT profiles. Rather, the visual appearance is generally similar for all eight. The graphs show a general trend (*note: statistical significance is not being inferred*) with an immediate increase in mean PPT from

baseline at t=4; and a further increase up to t=16 followed by, at post-intervention, a general decline to a level still above the pre-intervention mean. The results do not indicate a relationship between needling pain and or needling sensation intensity and changes elicited in regional PPT during the 21-minute intervention phase.

4.16 Summary of results

This section of the research examined three needling parameters (site of insertion, manipulation and retention time) in relation to the outcome measures of intensity of pain or needle sensation and qualitative descriptors of the needle sensation. Results showed that while the levels of needle sensation and pain were similarly intense following needle insertion for all interventions, initial intensity levels faded away rapidly unless the needle was both retained and manipulation repeated. Neither the eliciting nor maintaining of needle sensation or pain was restricted to a designated acupoint, with similar outcomes obtained at both LI4 and NAP. Typically, both pain and needle sensation were present (or absent) together and very few subjects reported pain or needle sensation in isolation. The parameters, manipulation and needle retention time, were important for maintaining an elevated intensity of both needle sensation and needle pain. In contrast, the strength of needle sensations experienced was independent of insertion site. The needle sensation descriptors spontaneously reported by subjects were congruent with terms included in the MMPQ categories of pain descriptors. Based on the MMPQ categories, the descriptors reported by subjects did not differentiate between the two insertion sites in terms of either quality or the intensity of the terms used. However, they did discriminate between the two 21-minute interventions with manipulation compared with the other six interventions, with the former reporting more descriptors and greater intensity scores compared with the latter six interventions which reported very similar, lower number of descriptors and intensity scores.

During the 21-minute acupuncture administration period, the interventions did elicit increases in regional PPT. However, there was no indication that measurement site influenced the extent of this effect, since a similar pattern of increase was reported for all three sites measured at the same time interval. No relation could be seen between the very clear differences in pain and needle sensation presented by different interventions, and their effects on PPT during or following the intervention phase.

Chapter V: Discussion

Part I: Post-intervention changes in regional PPT

It is common practice for thesis discussions to start by focussing on the research findings and gradually expand out to link findings with the broader research literature. In the present case it is less appropriate for several reasons which reflect the project's lineage. That is, it represents an extension of a series of closely related research programs completed at UTS all of which share important intervention commonalities and virtually identical protocols. In particular, they have all included at least one identical intervention: deep needling at LI4 for 21 minutes with repeated application of manual manipulation. In addition, many of the regional measurement sites were included in several of the studies and Table 5.1 displays the mean % increase in PPT from baseline at each of the regional sites used in each study with statistically significant elevations marked in bold.

Measurement site	Mean % increase in PPT from baseline				
	Zaslawski et al (2003)	Yuan (2002)	Li (2005)	Szabo (2007)	This study (2013)
Acupoint					
$LI5^{R}$	**22.3**	**18.3**	**10.9**	**7.5**	
$LI10^{R}$	**18.3**				
$LI20^{R}$	**15.7**	**14.2**	**8.3**	**10.3**	**8.0**
$SI3^{R}$	**13.3**				
$PC6^{R}$	**15.2**			**8.8**	
$PC6^{L}$					**9.9**
CV12	**19.3**	**16.3**			
$ST36^{R}$	**13.6**	**11.2**	**6.5**		**5.9**
$LI5^{L}$		9.6	**7.9**		**10.1**
$ST36^{L}$		**12.4**	**7.2**		
$KI3^{L}$			**12.2**		
$KI3^{R}$			**10.4**	**10.5**	**7.5**
$GB20^{R}$			**14.3**		
$LI10^{L}$				5.8	**16.3**
$SP6^{R}$				**8.4**	
$GB12^{R}$				**8.8**	**11.9**
Nonacupoint					
1^{R}	**18.5**	**10.1**	**9.0**	**7.4**	
2^{R}	**15.7**	**14.7**			
3^{R}	**12.7**	9.2			**5.3**
2^{L}		**10.8**			**7.8**
1^{L}			6.1		**8.5**
NAP-Ulna				**9.1**	
NAP-Foot				**5.5**	
Proportion of sites achieving a statistically significant increase	10/10	8/10	9/10	9/10	10/10

Table 5.1: Mean percentage increase in PPT from baseline elicited following the same LI4 intervention (21 minutes deep needling with manipulation) at each of the regional sites used in each study. Statistically significant increases (p at least<0.05) are marked in bold.

What is striking from the table is that among the 50 response sets there were only four that did not involve a statistically significant increase in mean % PPT from pre-intervention levels. In each case, the PPT increase in one or two of the other related studies was statistically significant. Therefore, the studies together support and strengthen each other's findings that this intervention reliably elicits an increase in regional PPT that is quite general across the body at measurement sites located variously on the sole of the foot, both arms and legs, the torso and the face and head. Each study included from 20 to 40 'healthy' volunteers with equal gender ratios (in repeated measures within subjects experimental designs), numbers that may be regarded as relatively small samples. However, the consistency in PPT findings for this common LI4 intervention across all subject groups is strong support for the reliability of the findings; not just for this present study, but for those of each of the other studies.

A second important relationship between the present research and two of the previous studies involves the inclusion of the same nonacupoint (NAP) in a comparable intervention to the 'gold standard' for LI4 (that is 21 minutes, deep needling with regular manipulation). Table 5.2 displays the mean % increase in PPT from baseline elicited at each of the regional sites used in the three studies, with statistically significant elevations marked in bold.

Measurement site	Mean % increase in PPT from baseline		
	Zaslawski et al (2003)	Yuan (2002)	This study (2013)
Acupoint			
$LI5^{R}$	4.3	**13.0**	
$LI10^{R}$	5.3		
$LI20^{R}$	**6.9**	**12.6**	**5.7**
$SI3^{R}$	0.5		
$PC6^{R}$	5.4		
$PC6^{L}$			**9.4**
CV12	**10.6**	**10.8**	
$ST36^{R}$	4.0	**15.2**	**4.9**
$LI5^{L}$		**13.6**	4.6
$ST36^{L}$		6.7	
$KI3^{R}$			**8.9**
$LI10^{L}$			**7.1**
$GB12^{R}$			**9.5**
Nonacupoint			
1^{R}	**8.3**	6.7	
2^{R}	4.0	**17.0**	
3^{R}	3.2	**15.6**	**6.9**
2^{L}		**12.7**	**5.3**
1^{L}			**8.0**
Proportion of sites achieving a statistically significant increase	3/10	8/10	9/10

Table 5.2: Mean percentage increase in PPT from baseline elicited following the same NAP intervention (21 minutes deep needling with manipulation) at each of the regional sites for the three studies that included this intervention. Statistically significant increases (p at least<0.05) are marked in bold.

Among the 30 response cells, 20 represented statistically significant increases in mean % PPT from baseline. Note that the Zaslawski study elicited only three such elevations, compared with eight and nine for Yuan and the present study respectively. Comparisons are limited since there are only 12 occasions where pairs of studies included the same site and only six of these both achieved statistical significance. Only five of the regional sites for the present study were common to one or both the previous studies and at one site ($LI5^{L}$) it failed to agree with Yuan's significant increase. Although the available data sets are more limited and less uniform in terms of outcome, with 20 out of 30 regional site responses showing significant increases in mean PPT, again the findings suggest that needling the NAP results in a generalised increase in regional PPT.

Another consistent finding was the similar paucity of significant changes in regional PPT elicited following deep needling to LI4 for 21 minutes in the absence of ongoing needle manipulation that was observed in the present study and in the initial study (Zaslawski et al 2003) which also included this intervention. While there were only three regional measurement sites common to both studies, in view of the agreement in findings for all three, it is likely that similar relationships might apply to the 14 other measurement sites that were not shared by the studies.

These commonalities have been presented at the beginning of the discussion in order to establish that, where there have been comparable interventions among studies, the results have been consistent. That is, they show that the present findings provide reliable replications and extensions of those from previous research. In turn, it may be argued that they support the interpretation of findings in the present research for the remaining interventions (that is, for those for which there is no previous similar research on which to draw) as being similarly reliable and sound and not perhaps, aberrant outcomes resulting from an atypical set of subjects. The latter case is always possible in spite of thorough stratified randomisation since this is the nature of the sampling beast where neither type I nor type II errors can be totally expunged.

Another common consideration concerns possible 'placebo' effects. The likelihood of changes in regional PPT being attributed to placebo influences become unlikely when such similar effects on PPT were obtained in spite of such diverse groups of subjects being involved in the five different studies. For example, subjects in the initial program (Zaslawski et al 2003) were virtually all either TCM undergraduates or staff in the TCM division at UTS. Subsequent studies increasingly drew on volunteers from the broader community and the present study advertised across the staff at UTS; and included staff members, their friends from the general community, postgraduate students from nonacupuncture disciplines and several TCM

undergraduates. In addition, the initial study included a noninvasive control intervention in the form of an inactive laser as well as an active laser intervention). Neither intervention elicited any significant changes in regional PPT at any sites, with individual site mean % changes ranging from -0.8 to 7.6% (active laser) and -1.1 to 4.7% (inactive laser).

Another similarity across studies has been the limited number of statistically significant difference in effects on regional PPT elicited between interventions. This is perhaps hardly surprising, given the generous spread of most of the 95% confidence intervals (a reflection of both the wide variation in effects obtained for individual subjects and the sample size) associated with the mean percentage increases in PPT. Typically the changes in PPT from baseline elicited at most regional sites by the different interventions were sufficiently large to achieve significance of at least $p<0.05$. However, this was not the case with the comparisons involving the mean differences in changes to PPT of interventions since the mean differences were obviously smaller than the total mean elevations in PPT.

As with the previous studies, only a subset of the intervention and sites achieved effects that were statistically significantly different from any other intervention. In this respect, it is likely that the consistency between studies is a reflection of the impact of small PPT differences and inadequate sample numbers.

However, it should be noted that the lack of significant difference in mean changes between the majority of pairs of comparison by site and intervention does suggest that even if a type II error has been made, the difference in effects *is clearly relatively small*. The alternative is that the results are a correct reflection of the different interventions effects being very similar or the same.

What is notable with respect to the present and previous studies is the lack of findings where the post-intervention PPT levels were significantly lower than the baseline levels. For example, as shown in the present study figures, at all sites and all interventions, the post-intervention mean value for the change in PPT from baseline was always elevated above the baseline level. There were three occasions where there was virtually no change in mean % change in PPT. Two were at the site $ST36^{R}$ (-0.7% and 1.2% for the interventions $LI4m^{+1}$ and $NAPm^{-1}$ respectively). The third one was at the site 3^{R} with a mean % PPT change of 0.8% for the $LI4m^{-21}$ intervention. This study, by virtue of the large number of interventions was able to identify regional sites patterns of responses: for example, sites that were more likely to show different PPT outcomes following the different interventions, or sites at which significant PPT changes was more or less likely to be elicited.

From Table 4a.2 the pattern of increases shows that there were two unusual patterns: one with respect to a single intervention; and one with respect to a single site. Among interventions of the 15 response cells where nonsignificant ($p>0.05$) effects on PPT were observed, nine involved a single intervention ($LI4m^{-21}$). By contrast $LI4m^{+21}$ was the strongest performer with all ten sites showing significant increases in mean % PPT. Indeed, only three of the eight interventions elicited statistically significant increases in PPT at all ten sites and both of the others involved NAP interventions: $NAPm^{-21}$ and $NAPm^{+1}$. Among the ten regional sites measurement, $GB12^{R}$ was the only one for which a statistically significant increase was achieved for all eight interventions and in each case, at $p<0.0001$. This contrasts with the least responsive site, which was $ST36^{R}$ at which there were four nonsignificant changes. An alternative view might be that the latter site provided a better discrimination between the eight interventions: only one LI4 intervention compared with three NAP interventions elicited significant changes.

The needling parameters of interest to the present research were needling site, duration, and manipulation status. The findings do not provide many consistent patterns of linked with various combinations and levels of these parameters.

The main similarities or differences included:

(i) two comparison pairs that showed no statistically significant differences. These were the two m^{-1} interventions (site had no effect) and the pair of NAP^{21} interventions (manipulation had no effect).

(ii) three comparison pairs that most frequently showing significant differences at measurement sites. These comprised the $LI4^{21}$ interventions, with five significant differences (manipulation had an effect); the pair of m^{-21} interventions, and the two m^{+1} interventions, each with four such differences and both indicating that site had an effect.

The findings for duration were more complex. The only significant difference among pairs was a highly significant difference between the LI4 pair with $LI4m^{+}$ where the 21-minute duration was superior to one minute. However there was no difference for the NAP^{+} pair or for the one minute same site comparison pairs. It is noteworthy that the very low pattern of responses observed for $LI4m^{-21}$ is similar to the findings for the same intervention reported in the initial study in the series by Zaslawski and colleagues (2003).

The number and direction of statistically significant differences for the 12 relevant comparison pairs were summarised in Table 4a.3. Four pairs had either none or one significant difference between regional PPT means; four showed only two significant differences; and four pairs showed either four or five significant differences, with each comparison pair involving an LI4

intervention, compared with only two that involved NAP interventions. Intervention $LI4m^{+21}$ was significantly superior to $LI4m^{-21}$ or $LI4m^{+1}$. However $LI4m^{+1}$ was inferior to $NAPm^{+1}$ and $LI4m^{-21}$ was inferior to $NAPm^{-21}$.

The comparison of differences suggests that the PPT responses elicited by the two needling sites were not influenced similarly by either the duration of needle insertion nor the presence or absence of manipulation. Whether this reflects the sites being a designated acupoint and a non acupoint; or whether they reflect simply the different responses of different bodily locations is not known.

When examining the regional response pattern of significant PPT effects elicited by the various interventions with respect to the combinations of site, manipulation and needle duration, the obvious conclusion is that the effects on PPT were generalised. With the exception of $LI4m^{-21}$ which showed minimal change from baseline, they typically occurred at regional measurement sites the length and breadth of the body. This was not consistent with the limited distribution predicted by either TCM channel theory (with respect to the four LI4 variants) or for all eight interventions for neural segmental theory. The results suggest a generalised effect on PPT; one that is consistently an increase in threshold which for the majority of measurement sites and occasions represented a statistically significant increase over baseline.

One interpretation of this is that it perhaps represents a nonspecific response to the intrusion of a needle through the skin and into underlying tissues. The phenomenon described by Le Bars and colleagues (1992), termed diffuse noxious inhibitory controls (DNIC) has been suggested as being implicated (discussed below). An alternative suggestion might be that it represents a highly specific local bodily response to the clear threat to normal functioning obvious from the skin or related tissues detecting the invasion by a foreign threat. It would be somewhat surprising and a less than effective survival mechanism were the protective surface layers of the body only capable of responding to insertion of a foreign object at specific sites such as acupoints. Effective defence must draw on a holistic and alert, well integrated system or series of systems.

The study raises a puzzling consideration: why should invasive interventions such as deep needling of tissues for 21 minutes with regular manipulation (irrespective of whether an acupoint or not) trigger a response such as increasing pain threshold? That is, why dampen the warning, or alarm system's response to an ongoing breach of the body's intact skin. More puzzling is why an acupoint should elicit such a response, given the assumption that

acupuncture is concerned with restoring balance. DNIC, functioning as part of the body's normal integrated central modulating systems for pain, has been demonstrated to elicit generalised pain attenuation (and thereby increasing pain threshold) in certain specific stimulus conditions. According to Le Bars et al (1992), the application of a strong nociceptive stimulus to any area of the body, will lead to the strong inhibition of certain neurones in the dorsal horn of the spinal cord. The effect endures for some minutes after removal of the 'conditioning' nociceptive stimulus. The type of painful conditioning stimulus may vary (thermal, mechanical, chemical) with the common finding that the more intense the stimulus, the greater the degree of pain inhibition.

The attraction of DNIC with respect to acupuncture (particularly deep needling with vigorous manipulation) is that it demonstrates that a strong nociceptive stimulus applied in one area of the body has been reliably shown to be accompanied by attenuation of pain in remote areas. Indeed Schliessbach and colleagues (2011) compared the analgesic effects elicited by needling LI4 with the effects attributable to DNIC, by application of the cold pressor and with a nonpenetrating sham control. Their dependent variable was changed in PPT measured on the second toe pre and post for each intervention. Unfortunately as discussed in Chapter II, the study design rendered the findings inadequate in terms of both reliability and validity.

However, the design of the present research does permit some consideration of the findings with respect to a possible DNIC modulated influence. In particular, in Part II of the research, needling pain, needle sensation (see Figure 4b.1) and PPT (see Figure 4b.5 and Figure 4b.6) profiles were compiled during the active intervention phase. The needling pain profiles for the set of interventions were similar to their needle sensation profiles during the 21 minute intervention period. For the initial one minute of needle retention common to all interventions, the pain intensities reported did not differ statistically significantly among interventions. However, for both needle sensation and pain profiles, the scores for the six interventions that did not combine both needle retention and manipulation, fell away rapidly and remained at minimal values. Both the LI4 and NAP interventions with manipulation and 21 minutes needle retention maintained similar elevations ($p>0.05$) of mean needle sensation and pain levels during the 21 minutes of up to 16 to 18%, showing that ongoing needle manipulation and retention but not site of needle insertion were important for producing and maintaining both needling sensation and needling pain.

With respect to possible DNIC influence, both interventions involving 21-minute needle retention and application of manipulation fill the role of a significant and enduring nociceptive stimulus. By comparison, the remaining six interventions at best provoked a short effect on

perceived pain (or needle sensation). DNIC has been reported to endure for 'several minutes' after removal of the painful conditioning stimulus. Therefore, with the four interventions involving one minute insertion, any induced pain inhibition mediated by DNIC could be expected to have faded by the later PPT measurement cycles of t=10 and t=16 minutes and the post-intervention cycles between 22 and 26 minutes. However the findings presented in Figure 4b.5 and Figure 4b.6 show no evidence of such differences among the PPT profiles.

Unlike the needle sensation and pain of profiles, where there are distinct differences in the profile patterns among the interventions, there is no obvious discrimination among the PPT profiles. Rather, the visual appearance is generally similar for all eight: a pattern where the PPT means show an immediate increase from baseline at t=4; and a further increase up to t=16 followed at post-intervention, by a general decline to a level still above the pre-intervention mean. That is, the PPT profiles do not indicate a relationship between needling pain and or needling sensation intensity and changes elicited in regional PPT during the 21 minute intervention phase.

From the post-intervention PPT means the findings are not consistent with the DNIC phenomenon. Given the assumption that DNIC effects are short lived, only the 21-minute interventions are considered here. The pairs of same site 21-minute interventions either with or without manipulation show two quite different relationships. For the LI4 pair, the generally significantly higher PPT means for the manipulated intervention (which also produced stronger painful stimulation during intervention, shown in Figure 4b.1) compared with the nonmanipulated intervention do support a possible DNIC role.

However, for the two NAP 21-minute duration interventions (Figure 4a.13), at nine of the ten measurement sites (m^+) and for all ten sites (m^-) statistically significant increases from baseline were elicited although none of the pairs differed statistically significantly. This is in spite of the elevated pain profile for the m^+ condition compared to m^- shown during the intervention phase (Figure 4b.1).

A departure by this research from the previous related studies at UTS has been the inclusion of four interventions for which the duration of needle retention was only one minute and not 21 minutes. This posed difficulties for the timing and comparison of post-intervention PPT measurements.

The standard protocol involves five cycles of regional PPT measurements following removal of the needles at 21 minutes. However, for the one minute retention interventions, this resulted in a delay of 20 minutes between needle removal and post-intervention PPT measurements.

An alternative approach would have been to complete the post-intervention cycles immediately after removal of the needles (after one minute). However this departure would significantly alter the time related intervention experiences between the different duration interventions.

It is possible that effects on PPT elicited by the one minute duration interventions may have partially or completely dissipated by the time of the standard post-intervention measurement cycles. This was partially addressed by completing regional PPT measurements at three minute intervals for all interventions during the 21-minute needling phase. It was not feasible to complete cycles on all ten sites given that during the three minute time interval available, subjects were also required to report VAS responses for needling pain and sensation and to describe their perceptions.

Therefore measurements were taken at three regional sites and a global mean percentage change reported for each time interval and PPT profiles compared. No significant differences were evident in these PPT profiles between any interventions (including both one and 21-minute needle retentions). This suggests that the effects of needling are enduring through the experimental session.

Further support for this conclusion was provided by the two initial PPT studies at UTS (Zaslawski 2006 and Yuan 2002). In these early studies it was not known whether effects on PPT would persist following removal of the needles after 21 minutes. Therefore the post-intervention PPT cycles were conducted over two time periods. Three initial cycles were commenced immediately following needle removal. After a delay of ten minutes, a further series of cycles was recorded. No statistically significant differences in the mean changes across the two time periods were reported for any site or either study. While the ten minute delay prior to completion of the second series of PPT measurements is less than the 21 minutes in the current research, there was no trend evident in the Zaslawski findings that suggested the mean elevations in PPT were fading by the end of the second series of cycles.

Part II: Needling pain, sensation and regional PPT profiles during the intervention phase

The findings for both needle sensation and pain among the eight interventions are strikingly consistent in terms of providing both positive and negative instances, all of which support the conclusion that needle manipulation and needle retention time are important for producing and maintaining an elevation in needle sensation and pain. By contrast, no additional or differential effect was shown for the site of needling insertion although one was an acupoint (LI4) and the other was not (NAP). These findings related to both quantitative VAS scores as well as the qualitative descriptors spontaneously reported by subjects and discussed later in this section: that is, needle sensation was not restricted to the acupoint intervention.

Another clear relationship among the findings was that needling pain and needle sensation overwhelmingly were present or absent together and may relate to the typical role of acute pain. The function of acute pain is to help protect the body. It is one of warning, alerting, and protecting the conscious organism about a noxious or potentially harmful sensory stimulus. This is demonstrated by the similar role of pain in relation to all our varied sensory systems, from touch, sound, light, taste and so on. Therefore, piercing the intact skin and underlying tissues with a needle represents an invasive threat and should activate appropriate sensory mechanisms. Deep piercing and with needle manipulation, causing more stimulation would augment the sensory input and be expected to produce a more intense sensory perception of discomfort and pain. Since '*deqi*' or needle sensation is encouraged by mechanical manipulation and deep needle insertion (as opposed to shallow insertion without manipulation) the simultaneous elicitation of pain would be expected. Therefore, pain or discomfort would be a likely accompaniment to '*deqi*'. Is it possible, for example, that the original concept of *deqi* embraced the whole range of sensations elicited by needling, including acute pain? Further, what is not known from the early literature is whether originally, *deqi* was demonstrated - *as opposed to being assumed* - to be restricted solely to acupoints, rather than an experience associated with needling living tissues more generally.

The qualitative descriptor terms used by the subjects in this study were not solicited: no list was provided for them to select among; they were the subjects' own individually provided terms. Therefore it is noteworthy first, that the profiles for qualities of needle sensation were similar for NAP and LI4 and secondly, that the terms fitted almost perfectly into the category groupings developed for the well validated MMPQ (Melzack 1973, 1975). Moreover, the intensities of the

descriptors used for the two interventions that produced the significantly higher needle sensation and pain scores during the interventions ($LI4m^{+21}$ and $NAPm^{+21}$) were higher than for the remaining six interventions, as well as more numerous. These two findings again do not discriminate between acupoint and nonacupoint locations and again, link pain with needle sensation.

The MMPQ has served as a basis for several instruments developed to measure '*deqi*', for example, that by Vincent and colleagues (1989). One criticism of such an instrument has been that the developed from a pain questionnaire and as a result, measures pain in addition to the peculiar nonpainful sensations arising from acupuncture (Lundberg et al 2012). The present study findings do not support this interpretation. In the study, the subjects were not given any list of descriptors; they described the needle sensation experienced in their own words. Interestingly these matched the MMPQ terms closely (Figure 4b.4). The only descriptors reported that were not included in the MMPQ lists were low frequencies of 'warm' and 'electricity'.

MacPherson and Asghar (2006) developed a qualitative and quantitative classification of needle sensations associated with *deqi* based on ratings by 20 TCM acupuncture experts. Two clusters of sensations were identified. One cluster was linked with *deqi* and comprised seven sensations: aching, dull, heavy, numb, radiating, spreading, and tingling. The second cluster relating to acute needling pain included nine sensations: burning, hot, hurting, pinching, pricking, sharp, shocking, stinging, and tender. In the present study, it is noteworthy that the needle sensation descriptors primarily fell into these authors' *deqi* descriptor cluster. All eight interventions reported descriptors from five descriptor categories, comprising 8, 9, 18 and the ungrouped additional 'warm' and 'electric'. The most frequently reported descriptors among interventions were from category 8, and included some form or intensity of tingle, sting or itch. The second most frequent terms were from category 18 (typically numbness). Less frequent but reported for all interventions were category 9 terms (dull ache). Far less frequent, were the ungrouped terms 'warm' and 'electricity'. These findings do support the notion that subjects were, at least primarily, discriminating and reporting on needle sensation, rather than pain.

The study also showed that needle sensation was produced and maintained only when the needle was both retained and received ongoing manipulation (Figure 4b.1). A plausible explanation is that mechanical manipulation causes injury to the tissues around the needle and one of the body's reactions to this injury is pain (needle sensation or *deqi*). This may contribute to activation of the body's defensive system by increasing blood flow to the site of insertion

(Sandberg et al 2005) which in turn modifies delivery of oxygen, neurohumoral and anti-inflammatory mediators to the site (Zhang et al 2012).

Subjects spontaneously provided needle sensation descriptors that also describe pain: qualitatively and quantitatively and relevant here, is the concept of 'pain threshold'. That is, the intensity of a nonpainful sensory stimulus when it begins to take on *the beginnings of discomfort, the beginnings of pain.* Obviously the stimulus quality prior to this level was not perceived as painful. An individual's pain threshold is not constant and experimental studies have confirmed the enhancing effect of anxiety on ratings of pain intensity (Al Absi and Rokke 1991), unpleasantness (Weisenberg et al 1984) and pain threshold (Rhudy and Meagher 2000). In response to experimental cold pressor pain stimulation, McCaul and Haugtvedt (1982) found that distraction is a better coping strategy than attention to sensations when subjects are asked to report pain threshold and tolerance. Wagner and colleagues (2009) reported that induced sad affect leads to reduced heat pain thresholds in healthy subjects. This was regarded as probably due to altered lateral thalamic activity, which is potentially associated with changed attentional processes.

The descriptors in the MMPQ are not the sole preserve of pain. They are merely descriptors of sensory experiences, in terms of quality and intensity and may not necessarily be describing something that is unpleasant or potentially noxious. Even some of the more intense descriptors in some sensory experiences may reflect positive and very pleasurable sensations in healthy individuals, in certain settings, as for example with the pressure of deep, strong massage or the spreading and radiating heat from a heat lamp.

Pain may contribute to '*deqi*' with respect to clinical effects associated with needling, given the linking of pain with endogenous endorphin system activation. Certainly it has been extensively demonstrated that pain induces the synthesis of beta endorphins by the pituitary gland and when released, these affect the central and peripheral nervous system and relieve pain by binding to specific opioid receptors in these areas (Sprouse-Blum et al 2010)

Vincent and colleagues' early study (1989), using a modified MMPQ found that similar levels of needle sensation were produced at both acupoints and nonacupoints, suggesting *deqi* was not exclusive to acupoints. The present study strongly supports these findings. For both acupoint and nonacupoint with manipulation and a needle retention time of 21 minutes ($LI4m^{+21}$ and $NAPm^{+21}$), the level of needle sensation remained constant at around 15% compare to the other interventions which dropped below 5% (Figure 4b.1). Furthermore, these findings were evident both quantitatively, from VAS sensation intensity scores during the needling period, and

qualitatively, from the sensation descriptors provided by subjects (Figure 4b.4a and 4b.4c and Table 4b.1).

Typically, and necessarily, studies of needle sensation have involved healthy study subjects. This is perhaps incongruous, given that the intent of clinical acupuncture interventions is to restore balance or health when there is some imbalance or illness. That is, is it appropriate to assume that needle sensation may be linked in either a causative or a correlative manner with a specific, measurable physiological response; and if so, what clinical response(s) could be regarded as being appropriate to examine in relation to presence or levels of needle sensation in a healthy subject? Pain threshold has been a common choice here. Not only can it be quantified with VAS and MMPQ style instruments, but measurement is neither invasive nor injurious to tissues. However, it may be regarded as counter intuitive that acupuncture, a process hypothesised to restore bodily functional balance, should modify the resting pain threshold in a healthy individual. On the other hand, if needle sensation is regarded as simply part of the sensory system's alerting of the presence of an invasive, potentially noxious insult to the tissues, then the recruitment of defences would be typical and expected.

In the present study, there was little evidence of a significant placebo effect in that in general, subjects did not report further pain or needle sensation post needle removal in the one minute retention interventions. The sensations reported post needling for these interventions typically included numbness and tickling/tingling and Figure 4.3 shows that needling sensation and pain were reported by a small minority of these subjects throughout the 21-minute intervention period. A possible explanation for ongoing numbness or tingling following the removal of the needle may be in part, neither an actual effect of the needling, nor a placebo response, but an sensory perception due to the arm and hand being left immobile for 21 minutes: that is, it was not a placebo effect but an actual physiological response to this unnatural inactivity. The UTS research group had previously encountered a similar phenomenon in a study where subjects received, as the control intervention, inactive laser, with some subjects reporting feelings of heaviness, numbness and tickling/itching (Zaslawski 2006) These findings suggest that studies may need to take such factors into account, not only when considering 'placebo' responses, but also with respect to subjects' perceived responses to potentially real interventions.

Chapter VI: Conclusion

19200 PPT measurements later - was it all worth it?

Looking back though the research design, implementation and outcomes with hindsight's unsettling mix of wisdom and regret, this chapter considers the central question of what has been the contribution to knowledge? What has the research achieved?

In general, this study has demonstrated a reliable protocol for studying regional PPT in acupuncture oriented experimental study in a double blinded experimental research program. It has also demonstrated that possible placebo effects on PPT, needle pain and sensation within the protocol are minimal and also, given the lack of changes in baseline PPT following some interventions and sites, that algometry does not elicit an acupressure like effect to which the increases in regional PPT could be attributed.

In terms of needle sensation and needling pain, the study showed that while the levels of needle sensation and pain were similarly intense following initial needle insertion for all interventions, these levels diminished rapidly unless the needle was both retained and manipulation repeated. Neither eliciting nor maintaining needle sensation or pain was restricted to a designated acupoint, with similar outcomes obtained at both LI4 and NAP. Typically, both pain and needle sensations were present (or absent) together with very few subjects reported pain or needle sensation in isolation. In summary: manipulation and continued needle retention were important for maintaining an elevated intensity of both needle sensation and needle pain. By contrast, the strength of needle sensations experienced was independent of insertion site.

The needle sensation descriptors spontaneously reported by subjects were congruent with terms included in the MMPQ categories of pain descriptors. Based on the MMPQ categories, the descriptors reported by subjects did not differentiate between the two sites in terms of either quality or the intensity of the terms used. However, they did indicate differences with respect to the two 21-minute interventions that involved ongoing manipulation compared with the other six interventions. For the former pair, more descriptors and greater intensity scores were reported, than for the latter six interventions.

In terms of the effects of the three needling parameters on regional PPT, the following points emerged:

$LI4m^{+21}$ has been shown to be a reliable intervention with respect to eliciting significant elevations in regional PPT and similar outcomes were observed in four previous studies (Zaslawski 2006, Yuan 2002, Li 2005, Szabo 2007). This consistency among the five studies makes $LI4m^{+21}$ a reliable comparison intervention to be used in future PPT studies.

For both $LI4m^{+21}$ and $NAPm^{+21}$ interventions, the outcomes were not specific to a particular acupuncture channel or part of the body. This study has thus replicated and extended previous findings that increase in regional PPT elicited following deep needling of either an acupoint or nonacupoint are generalised and is not predicted by channel theory (for LI4) or neural segment theory.

The study has failed to show any clear relationship between the three parameters central to the research and regional PPT. This appears to reflect the complex nature of the relationships. However it has shown that in general, needle insertion is followed by an elevation in PPT above baseline levels that persists after needle removal. In view of the 20 minute delay until post-intervention measurements it was obviously the case that for the one minute needle retention interventions, the effects on PPT continued to be present 25 minutes after needle removal.

The sole exception here was the very clear and highly significant effect on regional PPT elicited by $LI4m^{+21}$ compared with the virtually ineffective $LI4m^{-21}$. This appears to support the TCM assumption that deep needling with vigorous manipulation and eliciting of *deqi* are important for eliciting a clinical outcome, and was also found in the Zaslawski et al study. However it is being considered out of context of the rest of the intervention outcomes which do not provide similar support.

Nevertheless, since the same unusual finding was evident in the Zaslawski et al study, this pair of interventions may warrant further investigation: the poor performance by $LI4m^{-21}$, compared with the NAP interventions or the one minute LI4 interventions is quite intriguing and definitely a stand alone finding.

While this study has shown the close relationships between maintenance of needling pain and needling sensation associated with needle retention with deep needling and ongoing presence of manipulation, it has failed to provide findings that support the common TCM assumptions or assertions that *deqi* is necessary or essential for eliciting a physiological effect (in this case, analgesia).

It has failed to demonstrate significant differences in PPT effects elicited between the various interventions, not because they may not exist, but because of the limitations of sample size and relative difference between the PPT effects elicited by different interventions. As a result, it is recommended that studies designed to detect such differences must use an increased sample size. It may also be advantageous to complete the same interventions on two occasions with each subject, the within subjects experimental design assisting to decrease the error component.

Interestingly, Schliessbach and colleagues (2011), in their study that involved acupuncture to LI4 and effects on regional PPT, based their calculations of an appropriate sample size on the findings from the Zaslawski et al (2003) study. They estimated that the Zaslawski study found an elevation in regional PPT of approximately 8% over baseline values and, assuming a standard deviation of 18%, they calculated that a sample of 43 subjects would provide 80% power to detect a difference at $p<0.05$. The aim of the within subjects repeated measures design study was to detect possible different effects on regional PPT elicited by their three experimental conditions, one of which was needling LI4.

The sample size they used (45 subjects) is nearly double that used in the present research and is of the order probably required to detect statistically significant differences between interventions that all elicit some degree of effect on regional PPT, as for example in the present research. Unfortunately, as discussed in Chapter II, the poor study design used by Schliessbach et al, rendered the study findings of little or no value and made it impossible to determine whether their sample size was appropriate.

The results have shown that for all four NAP interventions, there were statistically significant increases in the mean % PPT from baseline, ranging from 7.0% ($NAPm^{+21}$) to 8.4% ($NAPm^{+1}$) and the increase was generalised across all regional measurement sites. The NAP used was located between two acupoints, *luozhen* and *yao tong xue*, which are commonly used to treat stiff neck and lower back pain respectively. There is a possibility that the NAP is actually a potential pain relief point that needs future investigations. Since *luozhen*, the NAP, *yao tong xue* and LI4 are all located in close vicinity on the dorsal part of the hand, this raises another question: what are the likely dimensions of an acupoint?

One important recommendation from this research is based on the actual experience of conducting the program. As noted in Chapter III, the study protocol required strict blinding of the individual researchers with respect to viewing each other's data recordings during and after each experimental session; together with the deliberate delay in computer entry and analysis of

data until all subjects' participation was completed. This was to minimise potential bias or other distortion of outcomes that might be generated by informed researcher expectations.

However, this led to the unexpected and unfortunate situation where an intermittent and serious breach of data recording by the acupuncturist not only occurred but remained undetected throughout the 18 months of experimental sessions. As a consequence, an important and extensive set of data involving all interventions and all subjects was discarded.

Clearly, this would have been avoided had an independent scrutiniser been included in the group of researchers, one whose sole role was to check on the completeness and quality of the record sheets completed by each of the researchers for each session. It is recommended that a person performing this function be included in studies where more than one researcher is involved in concurrent data collection.

The study design does require modification if it is to be used in studies that include variable durations of needle insertion. Again, with hindsight, it was probably an unfortunate decision to include all eight interventions into the (one size fits all) standard protocol timeline. It may have been more appropriate to have treated the research as two separate studies: one involving the four 21-minute needle retention interventions and the other the four that involved one minute retentions. With the latter study, repeated cycles of post-intervention regional PPT would commence immediately after needle removal. In addition, further cycles could also have been included at (say) 21 minutes. These various sets of PPT data would clarify both the initial magnitude and persistence of the one minute duration effects on PPT.

So, returning to the question of: 19200 PPT measurements later - was it all worth it?
As any politicians worth their salt (including the late Richard Nixon) know: pressing the flesh is a very important activity to be encouraged with vigour and enthusiasm,

the answer is:

Yes.

References

Aird M. 2005 Variability in the Precision of Acupoint Location Methods. PhD thesis, Faculty of Science, University of Technology, Sydney.

Aird M, Cobbin D, Rogers C. 2002 A study of the comparative precision of traditional and contemporary methods of locating acupoints. Journal of Alternative and Complementary Therapies. 2002; 8(5): 635-642.

Aird M, Coyle M, Cobbin D, Zaslawski C. 2000 A study of the comparative accuracy of two methods of locating acupuncture points. Acupuncture in Medicine. 18(1):15-21.

Al Absi M, Rokke PD. 1991 Can anxiety help us tolerate pain? Pain. 46(1):43–51.

Antonaci F, Bovim G, Fasano M, Bonamico L, Shen JM. 1992 Pain Threshold in Humans: A Study with the Pressure Algometer. Functional Neurology. 7(4):282-288.

Baker K, Deadman PA. 2000 Manual of Acupuncture. East Sussex: Journal of Chinese Medicine Publications.

Baldy, P. 2002 Management of Myofascial Trigger Point Pain. Acupuncture in Medicine. 20(1):2-10.

Barlas P, Robinson J, Allen J, Baxter GD. 2000 Lack of effect of acupuncture upon signs and symptoms of delayed onset muscle soreness. Clinical Physiology. 20(6):449-56.

Benham A, Phillips G, Johnson MI. 2010 An experimental study on the self-report of acupuncture needle sensation during deep needling with bi-directional rotation. Acupuncture in Medicine. 28(1):16-20.

Blossfeldt P. 2004 Acupuncture for chronic neck pain – a cohort study in an NHS pain clinic. Acupuncture in Medicine. 22(3):146-151.

Bovey M. 2006 Deqi. Journal of Chinese Medicine. 81:18-29.

Brennum J, Kjeldsen M, Jensen TS. 1989 Measurements of Human Pressure Pain Threshold on Fingers and Toes. Pain. 38(2):211-217.

Chapple W. 2013 A Proposed Catalog of the Neuroanatomy and Stratified Anatomy for the 361 Acupuncture Points of the 14 Channels. Journal of Acupuncture and Meridian Studies. http://dx.doi.org.ezproxy.lib.uts.edu.au/10.1016/j.jams.2013.01.001.

Cheng XN. 1987 Chinese Acupuncture and Moxibustion. Beijing: Foreign Language Press.

Choi Y-J, Lee J-E, Moon, WK, Cho SH. 2013 Does the effect of acupuncture depend on needling sensation and manipulation? Complementary Therapies in Medicine. 21(3):207-214.

Coyle M, Aird M, Cobbin D, Zaslawski C. 2000 The cun measurement system: an investigation into its suitability in current practice. Acupuncture in Medicine. 18(1):10-14.

Deadman P, Al-Khafaji M, Baker K. 2001 A Manual of Acupuncture. Eastland Press, California.

Donald GK, Tobin I, Stringer J. 2011 Evaluation of acupuncture in the management of chemotherapy-induced peripheral neuropathy. Acupuncture in Medicine. 29(3):230-233.

Downey P. 1995 Acupuncture in the normal general practice consultation: an assessment of clinical and cost effectiveness. Acupuncture in Medicine.13(1):45-47.

Edwards J, Knowles N. 2003 Superficial dry needling and active stretching in the treatment of myofascial pain – a randomised controlled trial. Acupuncture in Medicine. 21(3):80-86.

Ellis A, Wiseman N, Boss K. 1989 Grasping the Wind. Brookline: Paradigm Publications.

Fischer AA. 1987 Pressure algometry over normal muscles. Standard values, validity and reproducibility of pressure threshold. Pain. 30(1):115–126.

Ford R. n.d. Travelling to China.
http://www.rayfordacupuncture.com.au/documents/Acupuncture_Transmission_To_The_West.pdf.

Freedman J. 2002 An audit of 500 acupuncture patients in general practice. Acupuncture in Medicine. 20(1):30-34.

He D, Veiersted KB, Høstmark AT, Medbø JI. 2004 Effect of acupuncture treatment on chronic neck and shoulder pain in sedentary female workers: a 6-month and 3-year follow-up. Pain. 109(3):299-307.

Hübscher M, Vogt L, Bernhörster M, Rosenhagen A, Banzer W. 2008 Effects of acupuncture on symptoms and muscle function in delayed-onset muscle soreness. Journal of Alternative and Complementary Medicine. 14(8):1011-16.

Itoh K, Minakawa Y, Kitakoji H. 2011 Effect of acupuncture depth on muscle pain. Chinese Medicine. 6(1):6-24.

Jaffee L. 2009 Richard Nixon, Appendicitis, and Chinese Medicine.
http://acupuncturetwincities.com/2009/05/richard-nixon-appendicitis-and-chinese-medicine/.

Jin G-Y, Jin J-J, Jin LL. 2006 Contemporary Medical Acupuncture - A Systems Approach. Higher Education Press, Beijing.

Joseph, P. 2002 A survey of acupuncture treatments in general practice. Acupuncture in Medicine. 20(2-3):100-101.

Kalichman L, Vulfsons S. 2010 Dry needling in the management of musculoskeletal pain. Journal of American Board of Family Practice. 23(5): 640-646.

Karsts M, Rollnik JD, Fink M, Reinhard M, Piepenbrock S. 2000 Pressure pain threshold and needle acupuncture in chronic tension-type headache – a between placebo-controlled study. Pain. 88(2):199-203.

Kong J, Gollub R, Huang T, Polich G, Napadow V, Hui K, Vangel M, Rosen B, Kaptchuk TJ. 2007 Acupuncture de qi, from qualitative history to quantitative measurement. Journal of Alternative and Complementary Medicine. 13(10):1059-70.

Kosek E, Ekholm J, Nordemar R. 1993 A Comparison of Pressure Pain Thresholds in Different Tissues and Body Regions. Scandinavian Journal of Rehabilitation Medicine. 25(3):117-124.

Kou W, Gareus I, Bell JD, Goebel MU, Spahn G, Pacheco-López G, Bäcker M, Schedlowski M, Dobos GJ. 2007 Quantification of DeQi sensation by visual analog scales in healthy humans after immunostimulating acupuncture treatment. American Journal of Chinese Medicine. 35(5):753-65.

Lade A. 1989 Acupuncture Points: Images and Functions. Seattle: Eastland Press Inc.

Lai XS, Tong Z. 2010 A study on the Classification and the 'Catching' of the 'Arrived Qi' in Acupuncture. Journal of Traditional Chinese Medicine. 30(1):3-8.

Lang P, Stoer J, Schober GM, Audette JF, Irnich D. 2010 Bilateral acupuncture analgesia observed by quantitative sensory testing in healthy volunteers. Anesthesia and Analgesia. 110(5):1448-56.

Lautenbacher S, Kunz M, Burkhardt S. 2008 The effects of DNIC-type inhibition on temporal summation compared to single pulse processing: Does sex matter? Pain. 140(3):429-435.

Le Bars D, Villanueva L, Bouhassira D, Willer JC. 1992 Diffuse noxious inhibitory controls (DNIC) in animals and in man. Patologicheskaya Fiziologiya i EksperimentalnayaTerapiya. 4:55–65.

Le Bars D, Willer CJ. 2002 Pain modulation triggered by high-intensity stimulation: implication for acupuncture analgesia? International Congress Series. 1238:11-29.

Leibing E, Leonhardt U, Köster G, Goerlitz A, Rosenfeldt JA, Hilgers R, Ramadori G. 2002 Acupuncture treatment of chronic low-back pain – a randomized, blinded, placebo-controlled trial with 9-month follow-up. Pain. 96(1–2):189-196.

Leung L. 2012 Neurophysiological Basis of Acupuncture-induced Analgesia - An Updated Review. Journal of Acupuncture and Meridian Studies. 5(6):261–270.

Li SH. 1976 In MD Broffman (Ed.), Points 2001: a comprehensive textbook/manual of the 20th Century. Hundreds Talents Press: Taipei.

Li W. Comparison of the effects of deep manual acupuncture and acupressure on regional pressure pain threshold. PhD Thesis: University of Technology, Sydney.

Li W, Cobbin D, Zaslawski C. 2008 A comparison of effects on regional pressure pain threshold produced by deep needling of LI4 and LI11, individually and in combination. Complementary Therapies in Medicine. 16(5):278-87.

Lin GJ, Wang CF, Huang CT, Chen CF. 1996 A survey of the depth for deqi in acupuncture therapy. Chinese Journal of Integrative Medicine. 2(2):101-103.

Lundberg T, Lund I, Naslund J. 2012 The needling sensation: A factor contributing to the specific effects of acupuncture? Acupuncture and Related Therapies. 1(1):2-4.

Marieb EN, Hoehn K. 2013 Human anatomy and physiology. 9th edition. Pearson Education, Boston.

Mavrommatis CI, Argyra E, Vadalouka A, Vasilakos DG. 2012 Acupuncture as an adjunctive therapy to pharmacological treatment in patients with chronic pain due to osteoarthritis of the knee: A 3-armed, randomized, placebo-controlled trial. Pain. 153(8):1720-1726.

McCaul KD, Haugtvedt C. 1982 Attention, distraction, and cold-pressor pain. Journal of Personality and Social Psychology. 43(1):154-162.

McPherson H, Asghar A. 2006 Acupuncture needle sensations associated with de qi: a classification based on experts' ratings. Journal of Alternative and Complementary Medicine. 12(7):633-637.

Melzack R. 1973 The Puzzle of Pain. Basic Books: New York.

Melzack R. 1975 The McGill Pain Questionnaire: Major properties and scoring methods. Pain. 1(3):277-299.

Merchant M. 1995 Facial pain: a review of 200 cases treated with acupuncture. Acupuncture in Medicine. 13(2): 110-111.

Mi HF. (trans. Yang SZ, Chace C). 1994 The Systematic Classic of Acupuncture and Moxibustion. Boulder: Blue Poppy Press.

Moffet HH. 2009 Sham acupuncture may be as efficacious as true acupuncture: a systematic review of clinical trials. The Journal of Alternative and Complementary Medicine. 15(3):213-216.

Nabeta T, Kawakita K. 2002 Relief of chronic neck and shoulder pain by manual acupuncture to tender points—a sham-controlled randomized trial. Complementary Therapies in Medicine. 10(4):217-222.

Nussbaum EL, Downes L. 1998 Reliability of clinical pressure-pain algometric measurements obtained on consecutive days. Physical Therapy. 78(2):160–169.

O'Connor J, Bensky D (trans.). 1981 Acupuncture: A Comprehensive Text. Seattle: Eastland Press, Chicago.

Reeves JL, Jaeger B, Graff-Radford SB. 1986 Reliability of the pressure Algometer as a measure of Myofascial Trigger Point Sensitivity. Pain. 24(3):313-321.

Rhudy JL, Meagher MW. 2000 Fear and anxiety: divergent effects on human pain thresholds. Pain. 84:65-75.

Rogers CJ, Rogers C. 1989 Acupuncture Point Location and Point Dynamics Manual. Third Edition. Acupuncture Colleges Press: Sydney.

Roth LU, Maret-Maric A, Adler RH, Neuenschwander BE. 1997 Acupuncture points have subjective (needling sensation) and objective (serum cortisol increase) specificity. Acupuncture in Medicine. 15(1):2-5.

Sandberg M, Larsson B, Lindberg L-G, Gerdle B. 2005 Different patterns of blood flow response in the trapezius muscle following needle stimulation (acupuncture) between healthy subjects and patients with fibromyalgia and work-related trapezius myalgia. European Journal of Pain. 9(5):497-510.

Schliessbach J, van der Klift E, Siegenthaler A, Arendt-Nielsen L, Curatolo M, Streitberger K. 2011 Does Acupuncture Needling Induce Analgesic Effects Comparable to Diffuse Noxious Inhibitory Controls? Evidence-Based Complementary and Alternative Medicine. 2012: Article ID 785613, 5 pages, doi:10.1155/2012/785613.

Shandong Medical College. 1988 Anatomical Atlas of Chinese Acupuncture Points. Jinan: Shandong Science and Technology Press.

Shen YF, Goddard G. 2007 The short-term effects of acupuncture on myofascial pain patients after clenching. Pain Practice. 7(3):256-64.

Silva JBG, Nakamura, MU, Codeiro, JA, Kullay, L. 2012 Acupuncture for tension-type headache in pregnancy: A prospective, randomized, controlled study. European Journal of Integrative Medicine. 4(4):e366-e370.
Sprouse-Blum AS, Smith G, Sugai D, Parsa FD. 2010 Understanding endorphins and their importance in pain management. Hawaii Medical Journal. 69(3):70-71.

Staud R, Robinson ME, Vierck CJJr, Price DD. 2003 Diffuse noxious inhibitory controls (DNIC) attenuate temporal summation of second pain in normal males but not in normal females or fibromyalgia patients. Pain. 101(1–2):167-174.

Stellon A. 2001 An audit of acupuncture in a single-handed general practice over one year. Acupuncture in Medicine.19(3):36-42.

Szabo S. 2007 Comparison of the effects of manual acupuncture, electroacupuncture and TENS on regional pressure pain threshold readings. PhD thesis, Faculty of Science, University of Technology, Sydney.

Talbot JD, Duncan GH, Bushnell MC, Boyer M. 1987 Diffuse noxious inhibitory controls (DNICs): psychophysical evidence in man for intersegmental suppression of noxious heat perception by cold pressor pain. Pain. 30(2):221-232.

Targino RS, Imamura M, Kaziyama HHS, Souza LPM, Hsing WT, Furlan AD, Imamura ST, Neto RSA. 2008 A randomized controlled trial of acupuncture added to usual treatment for fibromyalgia. Journal of Rehabilitation Medicine. 40(7):582-8.

Thomson O, Haig L, Mansfield H. 2009 The effects of high-velocity low-amplitude thrust manipulation and mobilisation techniques on pressure pain threshold in the lumbar spine. International Journal of Osteopathic Medicine. 12(2):56-62.

Triano JJ, McGregor M, Emde DL. 1993 A Comparison of Outcome Measures for Use with Back Pain Patients: results of a feasibility study. Journal of Manipulative and Physiological Therapeutics. 16(2):67-73.

Vincent CA, Richardson PH, Black, JJ, Pithers CE. 1989 The significance of needle placement site in acupuncture. Journal of Psychosomatic Research. 33(4):489-496.

Von Korff M, Jensen M, Karoly P. 2000 Assessing Global Pain Severity by Self-Report in Clinical and Health Services Research. Spine. 25(4):3140-3151.

Wagner G, Koschke M, Leuf T, Schlösser R, Bär K. 2009 Reduced heat pain thresholds after sad-mood induction are associated with changes in thalamic activity. Neuropsychologia. 47(4):980–987.

Weisenberg M, Aviram O, Wolf Y, Raphaeli N. 1984 Relevant and irrelevant anxiety in the reaction to pain. Pain. 20(4):371-383.

White A. 2005 Conducting and reporting case series and audits - author guidelines for acupuncture in medicine. Acupuncture in Medicine. 23(4):181-187.

White P, Bishop F, Hardy H, Abdollahian S, White A, Park J, Kaptchuk TJ, Lewith GT. 2008 Southampton needle sensation questionnaire: development and validation of a measure to gauge acupuncture needle sensation. Journal of Alternative and Complementary Medicine . 14(4):373-9.

Witt CM, Reinhold T, Brinkhaus B, Roll S, Jena S, Willich SN. 2008 Acupuncture in patients with dysmenorrhea: a randomized study on clinical effectiveness and cost-effectiveness in usual care. American Journal of Obstetrics and Gynecology, 198(2):166.e161-166.e168.

Woods J. 1994 The Discovery of Endorphins. From Methadone Treatment Works: A Compendium For Methadone Maintenance Treatment. NYS Office of Alcoholism and Substance Abuse Services. Chemical Dependency Research Working Group.
World Health Organisation Regional Office for the Western Pacific. 2008 WHO Standard Acupuncture Point Locations in the Western Pacific Region. World Health Organisation: Geneva.

Yu DT, Jones AY, Pany MY. 2012 Development and validation of the Chinese version of the Massachusetts General Hospital Acupuncture Sensation Scale: an exploratory and methodological study. Acupuncture in Medicine. 30(3):214-21.

Yuan XY. 2002 A study into the effects of deep manual acupuncture on pain pressure threshold MSc Thesis, Faculty of Science, University of Technology, Sydney.

Zaslawski CJ. 2006 The relevance of needling parameters and participant experience for acupuncture research. PhD Thesis: University of Technology, Sydney.

Zaslawski CJ, Cobbin D. Lidums E, Petocz P. 2003 The impact of site specificity and needle manipulation on changes to pain pressure threshold following manual acupuncture: a controlled study. Complementary Therapies in Medicine. 11(1): 11-21.

Zhang SP, Yip TP, Li QS. 2011 Acupuncture treatment for plantar fasciitis: a randomized trial with six months follow up. Evidenced-Based Complementary and Alternative Medicine. 2011: Article ID 154108, doi:10.1093/ecam/nep186.

Zhang ZJ, Wang XM, McAlonan GM. 2012 Neural Acupuncture Unit: A New Concept for Interpreting Effects and Mechanisms of Acupuncture. Evidence-Based Complementary and Alternative Medicine. 2012 Article ID 429412, 23 pages doi:10.1155/2012/429412.

Appendix I: Information sheet for all participants

UTS
UNIVERSITY
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SYDNEY

UNIVERSITY OF TECHNOLOGY, SYDNEY
SUBJECT/PARTICIPANT INFORMATION SHEET- STUDENT RESEARCH

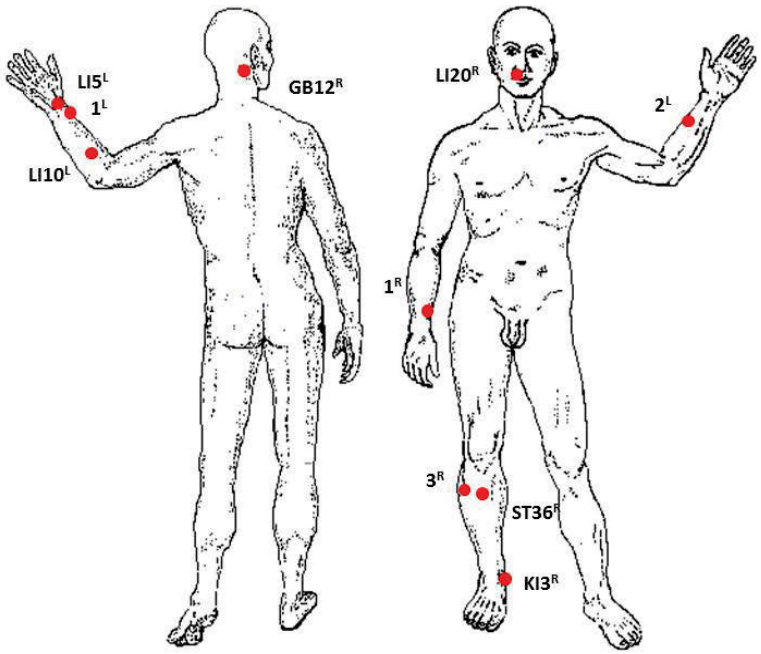
The aim of this project is to evaluate the effect of acupuncture on pressure pain threshold. You will be required to attend for eight sessions each taking approximately one hour. Each session will involve one acupuncture needle that will puncture the skin in the area on your hand and will remain in place for up to 21 minutes. A practitioner of 30 years experience will provide the acupuncture. Before needling and at different time intervals, an algometer will be applied to a number of regions on your body (see figure below) to measure the pressure pain threshold. Pressure pain threshold refers to discomfort produced by pressing on your skin or muscles. This pressure is produced by an instrument called an algometer which is a spring loaded pressure (force) gauge that is attached to a rubber plunger with a 0.5 cm diameter (see photograph below). It does not puncture the skin.

You will be asked to expose some areas of the body during algometric measurement and these are only located on the lower arm, lower leg and side of the head. Therefore, you will be required to remove your shoes and socks and be able to pull up your shirt sleeves up to the elbow. There are some minor risks associated with acupuncture, for example possible bruising at the needling site. All care will be taken by the acupuncturist to minimise such possibilities.

You will also be asked to record your perceptions of sensations associated with each needling intervention and to record the acupuncturist's consistency of clinical approach at the completion of each session.

Your identity will be confidential since all data will be identified by a code and not by name. To thank you for your participation a pecuniary of $10 per session has been allocated to cover travel cost.

If at any stage you wish to withdraw from the study, please inform the researchers immediately.



Points on your body at which the algometer will be applied

Picture of an algometer that will be used in the study

Appendix II: Consent form for all participants

UNIVERSITY OF TECHNOLOGY SYDNEY

UNIVERSITY OF TECHNOLOGY, SYDNEY
CONSENT FORM - STUDENT RESEARCH

I _____ *(participant's name)* agree to participate in the research project "Acupuncture and Pressure Pain Threshold (PPT): how important is needle retention time?" being conducted by PhD student Bertrand Y.K Loyeung, Room 3.44 Building 4 Broadway University of Technology, Sydney. Bertrand can be contacted either by phone (0406 080 255) or e-mail (YewKian.Loyeung-1@uts.edu.au). This research is financially supported by a UTS scholarship and is being conducted in the UTS TCM Clinic which is covered by UTS insurance and indemnity policy. The researchers, who are all staff members of the Department of Medical and Molecular Biosciences at UTS, have declared that it does not involve any conflict of interest.

I understand that the purpose of this study is to investigate the analgesic effects of acupuncture, measured in terms of PPT (Pressure pain threshold) which is defined as the minimum pressure which induces discomfort at the site of application.

I understand that my participation in this research will involve approximately eight hours of my time for which I will receive a pecuniary of $10 per session with eight sessions planned. I have read the subject/participant information sheet and understand that there may be some risks associated with the procedure such as a bruise or slight discomfort/pain. If at any time during the procedure should I wish to discontinue the procedure the process will be stopped immediately and acupuncture needle withdrawn.

I am aware that I can contact Mr Yew Kian (Bertrand) Loyeung or his supervisor Dr Deirdre Cobbin (ph: 9514-2231), if I have any concerns about the research. I also understand that I am free to withdraw my participation from this research project at any time I wish and without giving a reason. I understand that I am allowed to keep any pecuniary already paid prior to withdrawal and that if I am currently a UTS student, the withdrawal from the research will not prejudice my academic progress.

I have read the subject/participant information sheet and I agree that Yew Kian (Bertrand) Loyeung has answered all my questions fully and clearly. I agree that I have correctly confirmed my health status and that I do not have a medical history of chronic musculoskeletal disorder, or of haemophilia; nor am I taking anticoagulant medication or other medication that interferes with blood clotting; or am I regularly using analgesic or other drugs that may dampen pressure perception.

I understand that I will be required to record my perceptions of sensations associated with each needling intervention and to record the acupuncturist's consistency of clinical approach. I agree that the research data gathered from this project may be published in a form that does not identify me in any way.

_____ ____/____/____
Signed by

_____ ____/____/____
Witnessed by

NOTE:
This study has been approved by the University of Technology, Sydney Human Research Ethics Committee. If you have any complaints or reservations about any aspect of your participation in this research which you cannot resolve with the researcher, you may contact the Ethics Committee through the Research Ethics Officer (ph: +61 2 9514 9772 Research.Ethics@uts.edu.au) and the UTS HREC reference number is 2009-067A. Any complaint you make will be treated in confidence and investigated fully and you will be informed of the outcome.

Appendix III: The 100mm VAS sheet for recording the subject's beliefs in the effectiveness of acupuncture and willingness to receive acupuncture as a therapy.

PLEASE INDICATE BY MARKING SOMEWHERE ON THE LINE YOUR ANSWER TO THE FOLLOWING:

Question 1: Do you believe in acupuncture?

0 _____ 100

It has no effect — It is certainly effective

Question 2: Would you be willing to receive acupuncture as a form of therapy?

0 _____ 100

Never — Anytime

Appendix IV: Table showing the type of needle sensation reported by each subject and intervention at different time interval during the intervention period (blank cell = no sensation reported; 'not defined'= sensation not recorded by the acupuncturist)

		NAPm^{-21}	LI4m^{-1}	NAPm^{+21}	NAPm^{+1}	LI4m^{+1}	LI4m^{-21}	NAPm^{-1}	LI4m^{+21}
Subject#	t=1	Electric	Electricity, Sharp	Dull ache	Stinging	Dull ache	Stinging	Pressure	Penetrating
	t=4	Electric	Scratch	Numb		Not defined	splinter	Not defined	Not defined
	t=7			Hot					Prickling, sharp
	t=10		Numb	Sharp				Not defined	Not defined
	t=13			Sharp					Not defined
	t=16			Sharp					Sharp
	t=19			Warm				Not defined	Sharp
	t=22			Hot					Not defined
Subject #	t=1		Prickling, Stinging	Stinging	Wriggling	Prickling	Itchy	Prickling	Sharp, prickling
	t=4								Stinging
	t=7			Stinging				Warm	Not defined
	t=10			Not defined					Sharp, dull ache
	t=13			Stinging					Dull ache
	t=16			Stinging, Sharp					Dull ache
	t=19			Stinging					Sharp
	t=22			Not defined			Not defined		Dull ache
Subject #	t=1		Warm	Not defined	Sharp, warm	Tingling	Tingling, warm		Tingling
	t=4		Warm	Not defined		Tingling, numbness			Not defined
	t=7			Not defined					Sharp
	t=10			Not defined		Tingling			Not defined
	t=13			Not defined					Not defined
	t=16			Not defined					Sharp, thick
	t=19			Not defined					Sharp, intense
	t=22			Not defined			Numbness, tingling		Not defined
Subject #	t=1		Pulsing, stinging		Cold	Stinging		Felt needle coming out	Pulsing
	t=4								Not defined
	t=7		Twisting				Tickling		
	t=10								
	t=13								
	t=16								
	t=19								Tingling
	t=22								Twisting

		NAPm^{-21}	LI4m^{-1}	NAPm^{+21}	NAPm^{+1}	LI4m^{+1}	LI4m^{-21}	NAPm^{-1}	LI4m^{+21}
Subject #[redacted]	t=1			Sharp		Pulsing			
	t=4			Sharp					
	t=7			Sharp					
	t=10			Not defined		Pulsing			Not defined
	t=13			Not defined					Not defined
	t=16			Sharp, twisting					
	t=19			Twisting					Not defined
	t=22			Twisting					
Subject #[redacted]	t=1	Presence of needle, dull ache		Dull ache	Numbness	Numbness, tingling	Sharp, piercing, spreading	Numb	
	t=4	Dull ache		Dull ache		Numbness	Numbness	Numb	Sharp
	t=7	Presence of needle		Dull ache		Not defined	Numbness		Sharp
	t=10			Not defined		Not defined	Numbness		Not defined
	t=13			Dull ache			Numbness		Not defined
	t=16			Dull ache			Numbness		Sharp
	t=19			Dull ache			Numbness		Sharp
	t=22			Not defined			Not defined		Not defined
Subject #[redacted]	t=1		Itchy, prickling	Throbbing	Not defined	Stinging, hot, radiating	Hot, pounding	Hot	Throbbing
	t=4	Tingling		Hot, cold, throbbing		Not defined	Not defined		Throbbing
	t=7			Hot			Hot, pounding		Not defined
	t=10		Hot		Not defined		Hot, pounding		Throbbing
	t=13				Tingling			Pulsing	Not defined
	t=16			Throbbing	Not defined		Not defined	Throbbing, warm	Throbbing
	t=19			Throbbing, hot	Not defined		Not defined	Pulsing	Not defined
	t=22	Throbbing		Throbbing	Numb, hot		Not defined		Throbbing
Subject #[redacted]	t=1	Tingling, dull ache	Electricity	Tingling	Tingling	Electricity, tingling	Electricity, tingling	Dull ache, hot	Warm
	t=4	Numbness		Dull ache, tingling, spreading	Numbness		Numbness	Dull ache, spreading, numbness	Numbness, radiating
	t=7	Not defined		Spreading			Stinging	Not defined	Dull ache, stinging
	t=10			Dull ache, tingling				Not defined	Dull ache, radiating
	t=13			Not defined				Not defined	Deep, dull ache
	t=16	Numbness		Cool			Stinging		Deep, radiating
	t=19	Numbness		Cool			Stinging		Radiating, deep, dull ache
	t=22			Dull ache					Not defined

		NAPm^{-21}	LI4m^{-1}	NAPm^{+21}	NAPm^{+1}	LI4m^{+1}	LI4m^{-21}	NAPm^{-1}	LI4m^{+21}
Subject #[redacted]	t=1	Dull, pulsing, tingling	Tingling	Pressure, pulsing, radiating	Heavy, squeezing	Pointed	Contraction of skin, tense	Calming	Pressure
	t=4	Prickling		Pressure, pulsing, radiating		Pressure	Not defined		Pressure
	t=7	Dull, pulsing, tingling	Warm	Not defined		Pressure	Tense	Tingling	Pressure
	t=10	Feel tip of needle	Warm	Not defined			Tightness	Tingling	Pressure
	t=13	Feel tip of needle	Warm	Sharp		Pressure	Tightness	Tingling	Pulsing
	t=16	Tingling	Warm	Dull, pulsing		Pressure	Tightness	Not defined	Not defined
	t=19	Tingling		Tingling, numbness			Not defined	Not defined	Not defined
	t=22	Tingling		Not defined		Not defined	Not defined	Not defined	Pressure
Subject #[redacted]	t=1	Sharp	Electric	Flowing, cool		Electric, sharp	Tingling		Dull ache
	t=4			Not defined				Dull ache	Dull ache
	t=7			Not defined					Dull ache
	t=10			Dull ache					Not defined
	t=13			Not defined					Sharp
	t=16			Dull ache					Sharp
	t=19			Electricity					Sharp
	t=22			Not defined					Not defined
Subject #[redacted]	t=1	Not defined	Dull ache	Pin prick	Tingling	Prickling	Not defined	Pin prick	Tingling
	t=4	Not defined	Not defined	Tingling, dull ache	Not defined	Not defined	Dull ache	Not defined	Tingling
	t=7	Cool	Not defined	Not defined	Not defined	Not defined	Not defined	Not defined	Not defined
	t=10	Not defined	Not defined	Jabbing	Not defined	Not defined	Not defined	Not defined	Dull ache, prickling
	t=13	Not defined	Not defined	Not defined	Not defined	Not defined	Not defined	Not defined	Not defined
	t=16	Not defined	Numbness	Tingling	Not defined	Not defined	Not defined	Not defined	Jabbing
	t=19	Not defined	Numbness	Jabbing	Not defined	Not defined	Not defined	Not defined	Jabbing
	t=22	Not defined	Numbness	Tingling	Not defined	Not defined	Not defined	Not defined	Not defined
Subject #[redacted]	t=1								
	t=4								
	t=7								
	t=10								
	t=13								
	t=16								
	t=19								
	t=22								

		NAPm^{-21}	LI4m^{-1}	NAPm^{+21}	NAPm^{+1}	LI4m^{+1}	LI4m^{-21}	NAPm^{-1}	LI4m^{+21}
Subject #[redacted]	t=1	Itchy	Warm, itchy, propagated	Itchy	Itchy	Itchy	Numbness, tingling	Tingling, warm	Itchy, tingling, radiating
	t=4		Itchy					Fluttering, faint	Itchy, dull ache, radiating
	t=7		Not defined	Wooden				Not defined	Dull ache, itchy
	t=10								Itchy
	t=13	Dull ache, sharp		Itchy					Itchy
	t=16	Wooden							Itchy
	t=19	Numbness							Tingling
	t=22								Tingling
Subject #[redacted]	t=1	Tingling, sharp	Sharp, tingling	Dull ache	Dull ache, throbbing	Dull, throbbing	Heavy, dull ache	Tingling	Throbbing, dull
	t=4	Sharp, tingling	Numbness	Sharp			Itchy		Tingling
	t=7	Tingling	Not defined	Sharp			Not defined	Numbness	Dull ache, throbbing
	t=10	Tingling		Not defined	Not defined	Not defined	Itchy	Numbness, cold	
	t=13		Not defined	Sharp		Not defined		Not defined	Tingling
	t=16		Not defined	Sharp		Not defined	Itchy	Not defined	Throbbing
	t=19		Not defined	Not defined		Not defined	Not defined	Not defined	Throbbing
	t=22		Not defined	Not defined	Not defined		Not defined	Not defined	Not defined
Subject #[redacted]	t=1	Not defined		Sharp	Sharp	Sharp	Dull ache		Diffusing
	t=4	Not defined	Dull ache						Dull ache, heavy
	t=7								Dull ache, heavy, throbbing
	t=10			Not defined					Not defined
	t=13			Not defined					Throbbing, deep, heavy
	t=16			Sharp, cool					Throbbing, deep, heavy
	t=19			Not defined					
	t=22			Not defined					
Subject #[redacted]	t=1	Dull ache	Itchy	Tingling, itchy	Dull ache	Tingling, electric	Tingling, itchy	Itchy	Sharp, numb
	t=4	Not defined	Not defined	Not defined	Not defined		Tingling, itchy	Not defined	Not defined
	t=7		Not defined	Not defined	Dull ache, sharp			Not defined	Not defined
	t=10	Not defined	Not defined	Dull ache, numbness	Dull ache	Not defined	Not defined	Not defined	Not defined
	t=13			Dulla ache	Not defined	Not defined		Not defined	Not defined
	t=16		Not defined	Not defined	Not defined	Not defined		Not defined	Not defined
	t=19	Not defined	Not defined	Not defined	Not defined	Not defined		Not defined	Not defined
	t=22	Not defined	Not defined	Not defined	Not defined		Not defined	Not defined	Not defined

		NAPm^{-21}	LI4m^{-1}	NAPm^{+21}	NAPm^{+1}	LI4m^{+1}	LI4m^{-21}	NAPm^{-1}	LI4m^{+21}
Subject #[redacted]	t=1	Numbness	Numbness	Numbness	Tingling	Not defined	Tingling	Numbness	Numbness, tingling
	t=4					Numbness		Sharp	Numbness, tingling
	t=7					Not defined		Tingling	
	t=10		Not defined	Tingling					
	t=13								Not defined
	t=16		Not defined	Not defined					
	t=19			Not defined	Not defined				
	t=22				Not defined		Not defined	Not defined	Numbness
Subject #[redacted]	t=1	Tickling	Tingling	Numbness	Sharp	Tingling	Prickling	Tingling	Dull ache
	t=4		Not defined	Not defined	Tickling	Tickling			Not defined
	t=7	Tickling	Not defined	Tickling	Not defined	Not defined		Not defined	Not defined
	t=10		Not defined	Not defined	Not defined	Not defined		Not defined	Prickling
	t=13		Not defined	Tingling	Not defined	Not defined		Not defined	Not defined
	t=16		Not defined	Not defined	Not defined	Not defined		Not defined	Prickling, dull ache
	t=19		Not defined	Not defined	Not defined	Not defined		Not defined	Not defined
	t=22	Not defined	Not defined	Not defined	Not defined	Not defined		Not defined	Not defined
Subject #[redacted]	t=1	Numbness	Pressure	Itchy	Itchy	Heavy	Pressure	Not defined	Numb
	t=4	Numbness	Pressure	Itchy	Not defined	Not defined	Pressure	Not defined	Tingling
	t=7	Not defined	Not defined	Not defined	Not defined	Not defined	Pressure	Not defined	Numb
	t=10	Numbness	Pressure	Itchy	Not defined	Not defined	Not defined	Not defined	Numb
	t=13	Not defined	Not defined	Not defined	Not defined	Not defined	Not defined	Not defined	Numb
	t=16	Not defined	Not defined	Spasm	Not defined	Not defined	Not defined	Not defined	Numb
	t=19	Numbness, tikling	Not defined	Tingling	Not defined	Not defined	Not defined	Not defined	Not defined
	t=22	Not defined	Not defined	Not defined	Not defined	Not defined	Not defined	Not defined	Numb
Subject #[redacted]	t=1	Tingling, warm	Tingling	Dull ache, numbness	Warmth	Not defined	Warm	Tingling	Not defined
	t=4	Numbness		Twisting			Not defined		Tingling, numbness
	t=7	Numbness	Tingling	Twisting		Tingling	Not defined	Warm	Not defined
	t=10	Not defined	Not defined				Not defined		Not defined
	t=13			Not defined		Tingling			Not defined
	t=16	Not defined		Not defined	Cold				Not defined
	t=19			Twisting, numbness		Tingling	Not defined		Not defined
	t=22	Tingling				Tingling	Not defined		Not defined

		NAPm^{-21}	LI4m^{-1}	NAPm^{+21}	NAPm^{+1}	LI4m^{+1}	LI4m^{-21}	NAPm^{-1}	LI4m^{+21}
Subject #[redacted]	t=1	Electric	Electric, burning	Electric, burning	Electric	Electric	Electric	Electric	Electric, burning
	t=4								Burning
	t=7			Burning					Burning
	t=10								
	t=13								
	t=16								
	t=19								Burning
	t=22								Burning
Subject #[redacted]	t=1			Cold, sharp	Warm, tingling	Sharp, tingling	Dull ache		
	t=4			Dulla che	Tickling	Throbbing	Dull ache		Sharp
	t=7			Dull ache, radiating	Not defined				
	t=10			Dull ache, radiating	Tingling				Dull ache
	t=13			Hot	Not defined				
	t=16			Dull ache, warm	Not defined			Not defined	Not defined
	t=19			Dull ache, warm					Dull ache
	t=22			Not defined		Dull ache			
Subject #[redacted]	t=1	Warm	Tingling	Warm, tickling, spreading	Warm	Tingling	Tingling	Tingling	Warm, tingling
	t=4	Warm					Tingling		Not defined
	t=7								Warm
	t=10	Warm							
	t=13								
	t=16								
	t=19								Tingling
	t=22								
Subject #[redacted]	t=1	Fuzzy, pleasant	Tingling	Throbbing	Numbness, throbbing	Dull ache, swelling	Dull, fuzzy		Throbbing, numbness
	t=4	Not defined	Not defined	Throbbing	Numbness	Warm, swelling	Swollen, full	Not defined	Warm, throbbing, numbness
	t=7	Dull ache		Dull ache, throbbing	Numbness	Not defined	Swollen, throbbing, full		Numbness
	t=10	Not defined		Throbbing	Numbness	Hot	Dull ache, radiating		Numbness, hot
	t=13			Not defined			Fullness		Numbness
	t=16			Not defined			Not defined		Numbness, throbbing
	t=19			Not defined			Not defined		Tingling, pressure
	t=22			Not defined			Not defined		Numbness, hot

Appendix V: Table showing the mean % PPT differences and p values between paired interventions, by site (statistically significant differences between each pair are shown in bold).

Comparison		KI3^{R}	ST36^{R}	LI5^{L}	PC6^{L}	LI10^{L}	LI20^{R}	GB12^{R}	3^{R}	1^{L}	2^{L}
LI4m^{+21}	LI4m^{-21}	**p = 0.0286**	p = 0.1171	**p = 0.0274**	**p = 0.0325**	**p = 0.0000**	**p = 0.0007**	p = 0.1362	p = 0.0517	p = 0.2744	p = 0.0848
		4.8	3.4	**5.6**	**4.9**	**13.2**	**6.1**	3.2	4.5	3.6	4.0
		Tx8>Tx6	N.S	**Tx8>Tx6**	**Tx8>Tx6**	**Tx8>Tx6**	**Tx8>Tx6**	N.S	N.S	N.S	N.S
	NAPm^{+21}	p = 0.8439	p = 0.9835	**p = 0.0153**	p = 0.9876	**p = 0.0000**	p = 0.3238	p = 0.3541	p = 0.7194	p = 0.994	p = 0.4202
		-1.4	1.0	**5.5**	0.5	**9.2**	2.3	2.4	-1.6	0.5	2.5
		N.S	N.S	**Tx8>Tx3**	N.S	**Tx8>Tx3**	N.S	N.S	N.S	N.S	N.S
	LI4m^{+1}	0.9823	**0.0085**	0.1916	0.6632	**0.0000**	**0.0041**	**0.0006**	0.6962	0.8638	0.7447
		0.6	**4.8**	3.9	2.0	**10.5**	**4.8**	**5.1**	1.7	1.5	-1.9
		N.S	**Tx8>Tx1**	N.S	N.S	**Tx8>Tx1**	**Tx8>Tx1**	**Tx8>Tx1**	N.S	N.S	N.S
LI4m^{-21}	NAPm^{-21}	**0.0001**	**0.0227**	0.8633	0.0748	**0.0032**	0.1065	0.2365	**0.0000**	0.7533	0.6924
		-6.9	**-4.4**	-1.5	-5.6	**-6.0**	-3.1	-2.8	**-9.7**	-1.9	-1.8
		Tx6<Tx1	**Tx6<Tx1**	N.S	N.S	**Tx6<Tx1**	N.S	N.S	**Tx6<Tx1**	N.S	N.S
	LI4m^{-1}	0.4296	0.8059	0.1768	0.4068	0.0569	**0.0281**	0.1877	0.1289	**0.0006**	0.2237
		-2.4	-1.4	-3.9	-2.6	-4.7	**-4.0**	-3.2	-3.8	**-8.7**	-3.0
		N.S	N.S	N.S	N.S	N.S	**Tx6<Tx2**	N.S	N.S	**Tx6<Tx2**	N.S
NAPm^{+21}	NAPm^{-21}	0.9801	0.7663	0.8765	0.9506	0.6595	0.9803	0.5246	0.1431	0.9334	0.9972
		-0.7	-1.6	-1.4	-1.2	-2.1	0.7	-1.9	-3.6	1.2	-0.3
		N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
	NAPm^{+1}	0.7892	0.5068	0.4114	0.7643	**0.0457**	0.6845	0.0776	1.0000	0.8568	0.8024
		1.9	-2.3	-2.7	1.7	**-4.7**	-1.2	-3.5	0.1	-1.6	-1.6
		N.S	N.S	N.S	N.S	**Tx3<Tx4**	N.S	N.S	N.S	N.S	N.S
NAPm^{-21}	NAPm^{-1}	0.8888	**0.0022**	0.3997	0.5971	0.9844	0.9457	0.5598	**0.0292**	0.4806	0.9587
		1.1	**5.3**	-3.3	3.0	0.6	-0.8	1.9	**4.8**	-2.9	-0.9
		N.S	**Tx1>Tx7**	N.S	N.S	N.S	N.S	N.S	**Tx1>Tx7**	N.S	N.S
LI4m^{+1}	LI4m^{-1}	0.6639	0.2683	0.6606	0.9988	0.7472	0.1879	**0.0018**	0.9007	**0.0174**	0.3987
		1.8	-2.7	-2.2	0.3	-2.0	-2.7	**-5.1**	-1.0	**-6.6**	2.9
		N.S	N.S	N.S	N.S	N.S	N.S	**Tx5<Tx2**	N.S	**Tx5<Tx2**	N.S
	NAPm^{+1}	0.9998	**0.0003**	0.9350	0.9998	**0.0074**	**0.0269**	**0.0000**	0.1730	0.5544	0.5091
		-0.1	**-6.5**	-1.1	0.2	**-5.9**	**-3.7**	**-6.2**	-3.2	-2.6	2.8
		N.S	**Tx5<Tx4**	N.S	N.S	**Tx5<Tx4**	**Tx5<Tx4**	**Tx5<Tx4**	N.S	N.S	N.S
LI4m^{-1}	NAPm^{-1}	0.1199	0.4145	0.9701	1.0000	0.9855	0.9998	0.4500	0.9022	0.3095	0.9988
		-3.4	2.2	-0.9	0.0	-0.7	0.1	2.3	-1.1	3.9	0.3
		N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
NAPm^{+1}	NAPm^{-1}	0.1526	**0.0005**	0.7468	1.0000	0.2794	0.8852	0.0867	0.8813	1.0000	0.9976
		1.2	**6.0**	-1.9	0.1	3.2	1.1	3.4	1.1	-0.1	0.4
		N.S	**Tx4>Tx7**	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

Legends:

Tx1: NAPm^{-21}
Tx2: LI4m^{-1}
Tx3: NAPm^{+21}
Tx4: NAPm^{+1}
Tx5: LI4m^{+1}
Tx6: LI4m^{-21}
Tx7: NAPm^{-1}
Tx8: LI4m^{+21}

Appendix VI: Statistical analysis tables and scatterplots

Part I: Post-intervention changes in regional PPT

1. Within and between intervention comparisons, independent of site (Table 4a.1)

Both gender combined:

```
Factor           Type    Levels  Values
Tx No            fixed        8  1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)  fixed       16  1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source              DF     Seq SS     Adj SS   Adj MS       F      P
Tx No                7     9650.3     9668.2   1381.2    8.87  0.000
pre/post(Tx No)      8   204153.3   204164.2  25520.5  163.91  0.000
Subject             23    84030.1    84030.1   3653.5   23.46  0.000
Error            15312  2384108.9  2384108.9    155.7
Total            15350  2681942.6
```

Male only:

```
Factor           Type    Levels  Values
Tx No            fixed        8  1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)  fixed       16  1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2
Subject          random      12  4, 5, 9, 10, 11, 17, 18, 19, 20, 21, 22, 24


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source              DF     Seq SS     Adj SS   Adj MS      F      P
Tx No                7     8123.3     8127.8   1161.1   7.65  0.000
pre/post(Tx No)      8   102461.5   102469.6  12808.7  84.44  0.000
Subject             11    46949.3    46949.3   4268.1  28.14  0.000
Error             7644  1159511.3  1159511.3    151.7
Total             7670  1317045.4
```

Female only:

```
Factor           Type    Levels  Values
Tx No            fixed        8  1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)  fixed       16  1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2
Subject          random      12  1, 2, 3, 6, 7, 8, 12, 13, 14, 15, 16, 23


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source              DF     Seq SS     Adj SS   Adj MS      F      P
Tx No                7     5201.3     5201.3    743.0   4.67  0.000
pre/post(Tx No)      8   105367.6   105367.6  13170.9  82.81  0.000
Subject             11    37028.0    37028.0   3366.2  21.16  0.000
Error             7653  1217263.4  1217263.4    159.1
Total             7679  1364860.2
```

2. Within intervention comparisons, per site (Table 4a.2)

KI3^{R}

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	8	1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)	fixed	16	1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	12070.0	12070.0	524.8	3.56	0.000
Tx No	7	1656.3	1656.3	236.6	1.61	0.129
pre/post(Tx No)	8	20532.1	20532.1	2566.5	17.43	0.000
Error	1497	220462.1	220462.1	147.3		
Total	1535	254720.5				

3^{R}

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	8	1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)	fixed	16	1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	12168.0	12168.0	529.0	3.79	0.000
Tx No	7	2687.9	2687.9	384.0	2.75	0.008
pre/post(Tx No)	8	14323.0	14323.0	1790.4	12.83	0.000
Error	1497	208850.4	208850.4	139.5		
Total	1535	238029.3				

ST36^{R}

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	8	1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)	fixed	16	1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	12498.1	12514.3	544.1	4.44	0.000
Tx No	7	2051.4	2053.4	293.3	2.39	0.020
pre/post(Tx No)	8	7967.6	7967.6	995.9	8.13	0.000
Error	1496	183341.9	183341.9	122.6		
Total	1534	205859.0				

LI5^{L}

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	8	1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)	fixed	16	1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	17735.3	17735.3	771.1	4.18	0.000
Tx No	7	1453.9	1453.9	207.7	1.13	0.344
pre/post(Tx No)	8	20471.1	20471.1	2558.9	13.87	0.000
Error	1497	276104.7	276104.7	184.4		
Total	1535	315765.1				

1[L]

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	8	1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)	fixed	16	1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	11288.9	11288.9	490.8	2.30	0.000
Tx No	7	2262.6	2262.6	323.2	1.52	0.157
pre/post(Tx No)	8	30084.0	30084.0	3760.5	17.65	0.000
Error	1497	319009.1	319009.1	213.1		
Total	1535	362644.5				

PC6[L]

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	8	1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)	fixed	16	1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	21562.1	21562.1	937.5	5.07	0.000
Tx No	7	1025.9	1025.9	146.6	0.79	0.593
pre/post(Tx No)	8	26839.9	26839.9	3355.0	18.15	0.000
Error	1497	276743.0	276743.0	184.9		
Total	1535	326171.0				

2[L]

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	8	1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)	fixed	16	1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	14683.1	14683.1	638.4	4.07	0.000
Tx No	7	1032.0	1032.0	147.4	0.94	0.475
pre/post(Tx No)	8	17535.4	17535.4	2191.9	13.97	0.000
Error	1497	234940.3	234940.3	156.9		
Total	1535	268190.8				

LI10[L]

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	8	1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)	fixed	16	1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	10311.2	10311.2	448.3	2.54	0.000
Tx No	7	5271.6	5271.6	753.1	4.26	0.000
pre/post(Tx No)	8	34766.8	34766.8	4345.9	24.58	0.000
Error	1497	264662.2	264662.2	176.8		
Total	1535	315011.9				

LI20^{R}

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	8	1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)	fixed	16	1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	8698.85	8691.56	377.89	3.81	0.000
Tx No	7	1269.98	1269.98	181.43	1.83	0.078
pre/post(Tx No)	8	12005.70	12005.70	1500.71	15.14	0.000
Error	1489	147550.79	147550.79	99.09		
Total	1527	169525.32				

GB12^{R}

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	8	1, 2, 3, 4, 5, 6, 7, 8
pre/post(Tx No)	fixed	16	1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	11297.4	11297.4	491.2	4.65	0.000
Tx No	7	1443.4	1443.4	206.2	1.95	0.058
pre/post(Tx No)	8	42709.6	42709.6	5338.7	50.57	0.000
Error	1497	158051.0	158051.0	105.6		
Total	1535	213501.3				

3. Between intervention comparisons, per site (Table 4a.3, Appendix V and fig. 4a3-14)

(a) Does manipulation has an effect?

KI3^{R}

Tx5 (LI4m^{+1}) and Tx2 (LI4m^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   4272.9   4272.9   185.8   1.63  0.035
Tx No              1     74.2     74.2    74.2   0.65  0.420
pre/post(Tx No)    2   3515.9   3515.9  1758.0  15.43  0.000
Error            357  40678.0  40678.0   113.9
Total            383  48541.0
```

Tx8 (LI4m^{+21}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  6, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6056.5   6056.5   263.3   1.86  0.010
Tx No              1    544.5    544.5   544.5   3.84  0.051
pre/post(Tx No)    2   3032.2   3032.2  1516.1  10.68  0.000
Error            357  50655.1  50655.1   141.9
Total            383  60288.3
```

Tx4 (NAPm^{+1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source             DF    Seq SS    Adj SS   Adj MS       F      P
Subject            23   52521.4   52521.4   2283.5   14.64  0.000
Tx No               1     354.0     354.0    354.0    2.27  0.132
pre/post(Tx No)     2   59268.6   59268.6  29634.3  189.95  0.000
Error            3813  594876.5  594876.5    156.0
Total            3839  707020.6
```

Tx3 (NAPm^{+21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 3
pre/post(Tx No)  fixed        4  1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5108.4   5108.4   222.1   1.57  0.046
Tx No              1     10.7     10.7    10.7   0.08  0.784
pre/post(Tx No)    2   8181.1   8181.1  4090.6  29.00  0.000
Error            357  50357.2  50357.2   141.1
Total            383  63657.4
```

3^R

Tx5 ($LI4m^{+1}$) and Tx2 ($LI4m^{-1}$)

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   4732.7   4732.7   205.8  1.98  0.005
Tx No              1     24.8     24.8    24.8  0.24  0.626
pre/post(Tx No)    2   1647.2   1647.2   823.6  7.93  0.000
Error            357  37061.1  37061.1   103.8
Total            383  43465.9
```

Tx8 ($LI4m^{+21}$) and Tx6 ($LI4m^{-21}$)

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  6, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   9156.2   9156.2   398.1  2.68  0.000
Tx No              1    485.0    485.0   485.0  3.27  0.072
pre/post(Tx No)    2   1361.4   1361.4   680.7  4.59  0.011
Error            357  52991.6  52991.6   148.4
Total            383  63994.3
```

Tx4 ($NAPm^{+1}$) and Tx7 ($NAPm^{-1}$)

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9459.4   9459.4   411.3   2.94  0.000
Tx No              1     48.9     48.9    48.9   0.35  0.555
pre/post(Tx No)    2   3772.3   3772.3  1886.1  13.48  0.000
Error            357  49942.3  49942.3   139.9
Total            383  63222.8
```

Tx3 ($NAPm^{+21}$) and Tx1 ($NAPm^{-21}$)

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 3
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23  10108.2  10108.2   439.5   3.30  0.000
Tx No              1    302.7    302.7   302.7   2.27  0.133
pre/post(Tx No)    2   7542.0   7542.0  3771.0  28.30  0.000
Error            357  47566.9  47566.9   133.2
Total            383  65519.8
```

ST36^{R}

Tx5 (LI4m^{+1}) and Tx2 (LI4m^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   2749.4   2749.4   119.5  1.19  0.253
Tx No              1    165.0    165.0   165.0  1.64  0.201
pre/post(Tx No)    2    567.7    567.7   283.8  2.82  0.061
Error            357  35956.7  35956.7   100.7
Total            383  39438.9
```

Tx8 (LI4m^{+21}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  6, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   9830.6   9857.0   428.6  3.63  0.000
Tx No              1    301.5    303.5   303.5  2.57  0.110
pre/post(Tx No)    2   1648.5   1648.5   824.2  6.98  0.001
Error            356  42064.3  42064.3   118.2
Total            382  53844.9
```

Tx4 (NAPm^{+1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   7382.3   7382.3   321.0   2.80  0.000
Tx No              1    875.7    875.7   875.7   7.64  0.006
pre/post(Tx No)    2   2582.2   2582.2  1291.1  11.27  0.000
Error            357  40901.0  40901.0   114.6
Total            383  51741.2
```

Tx3 (NAPm^{+21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 3
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   8373.8   8373.8   364.1   2.68  0.000
Tx No              1     64.1     64.1    64.1   0.47  0.493
pre/post(Tx No)    2   3175.2   3175.2  1587.6  11.67  0.000
Error            357  48571.7  48571.7   136.1
Total            383  60184.8
```

LI5^{L}

Tx5 (LI4m^{+1}) and Tx2 (LI4m^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9410.0   9410.0   409.1   2.29  0.001
Tx No              1    117.5    117.5   117.5   0.66  0.418
pre/post(Tx No)    2   5198.9   5198.9  2599.4  14.54  0.000
Error            357  63842.0  63842.0   178.8
Total            383  78568.3
```

Tx8 (LI4m^{+21}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  6, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9579.7   9579.7   416.5   2.13  0.002
Tx No              1    757.0    757.0   757.0   3.88  0.050
pre/post(Tx No)    2   5838.6   5838.6  2919.3  14.96  0.000
Error            357  69661.2  69661.2   195.1
Total            383  85836.5
```

Tx4 (NAPm^{+1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23  11424.6  11424.6   496.7   2.62  0.000
Tx No              1     95.6     95.6    95.6   0.50  0.478
pre/post(Tx No)    2   6667.2   6667.2  3333.6  17.60  0.000
Error            357  67629.2  67629.2   189.4
Total            383  85816.6
```

Tx3 (NAPm^{+21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 3
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5759.8   5759.8   250.4  1.58  0.045
Tx No              1     44.6     44.6    44.6  0.28  0.596
pre/post(Tx No)    2   2766.4   2766.4  1383.2  8.73  0.000
Error            357  56533.5  56533.5   158.4
Total            383  65104.4
```

1[L]

Tx5 (LI4m+1) and Tx2 (LI4m-1)

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS   Adj SS  Adj MS      F      P
Subject           23    9184.5   9184.5   399.3   1.64  0.033
Tx No              1    1050.3   1050.3  1050.3   4.32  0.038
pre/post(Tx No)    2   11211.0  11211.0  5605.5  23.04  0.000
Error            357   86864.2  86864.2   243.3
Total            383  108310.1
```

Tx8 (LI4m^{+21}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  6, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   7062.9   7062.9   307.1   1.53  0.058
Tx No              1    324.6    324.6   324.6   1.62  0.204
pre/post(Tx No)    2   4637.5   4637.5  2318.7  11.54  0.000
Error            357  71706.2  71706.2   200.9
Total            383  83731.1
```

Tx4 (NAPm^{+1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23  11839.3  11839.3   514.8   2.67  0.000
Tx No              1      0.1      0.1     0.1   0.00  0.984
pre/post(Tx No)    2   8919.9   8919.9  4460.0  23.12  0.000
Error            357  68873.0  68873.0   192.9
Total            383  89632.4
```

Tx3 (NAPm^{+21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 3
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   7175.9   7175.9   312.0   1.65  0.032
Tx No              1     33.6     33.6    33.6   0.18  0.674
pre/post(Tx No)    2   5315.5   5315.5  2657.8  14.04  0.000
Error            357  67591.9  67591.9   189.3
Total            383  80117.0
```

PC6^{L}

Tx5 (LI4m^{+1}) and Tx2 (LI4m^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   3365.8   3365.8   146.3   1.05  0.407
Tx No              1      1.6      1.6     1.6   0.01  0.916
pre/post(Tx No)    2   5734.4   5734.4  2867.2  20.48  0.000
Error            357  49968.7  49968.7   140.0
Total            383  59070.5
```

Tx8 (LI4m^{+21}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  6, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   8354.0   8354.0   363.2   2.28  0.001
Tx No              1    590.1    590.1   590.1   3.71  0.055
pre/post(Tx No)    2   5910.2   5910.2  2955.1  18.56  0.000
Error            357  56831.7  56831.7   159.2
Total            383  71686.1
```

Tx4 (NAPm^{+1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9938.6   9938.6   432.1   2.44  0.000
Tx No              1      0.2      0.2     0.2   0.00  0.971
pre/post(Tx No)    2   5646.0   5646.0  2823.0  15.95  0.000
Error            357  63199.1  63199.1   177.0
Total            383  78783.9
```

Tx3 (NAPm^{+21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 3
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS   Adj SS  Adj MS      F      P
Subject           23   19457.1  19457.1   846.0   3.46  0.000
Tx No              1      34.9     34.9    34.9   0.14  0.706
pre/post(Tx No)    2    9549.2   9549.2  4774.6  19.55  0.000
Error            357   87190.1  87190.1   244.2
Total            383  116231.4
```

2^L

Tx5 (LI4m^{+1}) and Tx2 (LI4m^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9340.5   9340.5   406.1   2.57  0.000
Tx No              1    193.4    193.4   193.4   1.22  0.269
pre/post(Tx No)    2   6740.9   6740.9  3370.4  21.34  0.000
Error            357  56373.3  56373.3   157.9
Total            383  72648.1
```

Tx8 (LI4m^{+21}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  6, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5002.2   5002.2   217.5   1.60  0.040
Tx No              1    378.0    378.0   378.0   2.79  0.096
pre/post(Tx No)    2   3595.1   3595.1  1797.6  13.25  0.000
Error            357  48432.8  48432.8   135.7
Total            383  57408.2
```

Tx4 (NAPm^{+1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6692.8   6692.8   291.0   1.55  0.053
Tx No              1      3.4      3.4     3.4   0.02  0.894
pre/post(Tx No)    2   4331.0   4331.0  2165.5  11.52  0.000
Error            357  67092.3  67092.3   187.9
Total            383  78119.5
```

Tx3 (NAPm^{+21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 3
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   8993.2   8993.2   391.0   2.93  0.000
Tx No              1      2.4      2.4     2.4   0.02  0.893
pre/post(Tx No)    2   2868.3   2868.3  1434.2  10.73  0.000
Error            357  47696.3  47696.3   133.6
Total            383  59560.2
```

LI10^{L}

Tx5 (LI4m^{+1}) and Tx2 (LI4m^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   7469.3   7469.3   324.8   1.71  0.023
Tx No              1     95.6     95.6    95.6   0.50  0.478
pre/post(Tx No)    2   4672.0   4672.0  2336.0  12.32  0.000
Error            357  67713.1  67713.1   189.7
Total            383  79949.9
```

Tx8 (LI4m^{+21}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  6, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   8444.2   8444.2   367.1   1.97  0.006
Tx No              1   4164.0   4164.0  4164.0  22.29  0.000
pre/post(Tx No)    2  13339.0  13339.0  6669.5  35.70  0.000
Error            357  66690.3  66690.3   186.8
Total            383  92637.5
```

Tx4 (NAPm^{+1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   8460.8   8460.8   367.9   2.36  0.000
Tx No              1    248.5    248.5   248.5   1.60  0.207
pre/post(Tx No)    2  10218.9  10218.9  5109.4  32.84  0.000
Error            357  55545.4  55545.4   155.6
Total            383  74473.5
```

Tx3 (NAPm^{+21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 3
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   3498.4   3498.4   152.1   0.95  0.531
Tx No              1    105.5    105.5   105.5   0.66  0.417
pre/post(Tx No)    2   6537.0   6537.0  3268.5  20.42  0.000
Error            357  57152.0  57152.0   160.1
Total            383  67292.9
```

LI20^{R}

Tx5 (LI4m^{+1}) and Tx2 (LI4m^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS    Adj SS   Adj MS      F      P
Subject           23   2734.00   2734.00   118.87   1.34  0.135
Tx No              1    176.86    176.86   176.86   2.00  0.158
pre/post(Tx No)    2   2186.80   2186.80  1093.40  12.37  0.000
Error            357  31565.74  31565.74    88.42
Total            383  36663.40
```

Tx8 (LI4m^{+21}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  6, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   4291.2   4291.2   186.6   1.55  0.053
Tx No              1    897.8    897.8   897.8   7.45  0.007
pre/post(Tx No)    2   3231.2   3231.2  1615.6  13.41  0.000
Error            357  43006.1  43006.1   120.5
Total            383  51426.3
```

Tx4 (NAPm^{+1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS    Adj SS   Adj MS      F      P
Subject           23   3340.80   3340.80   145.25   1.47  0.078
Tx No              1     26.36     26.36    26.36   0.27  0.606
pre/post(Tx No)    2   3878.40   3878.40  1939.20  19.59  0.000
Error            357  35337.11  35337.11    98.98
Total            383  42582.67
```

Tx3 (NAPm^{+21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 3
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS    Adj SS   Adj MS      F      P
Subject           23   5225.82   5220.24   226.97   2.58  0.000
Tx No              1      4.12      4.12     4.12   0.05  0.829
pre/post(Tx No)    2   2709.26   2709.26  1354.63  15.38  0.000
Error            349  30747.15  30747.15    88.10
Total            375  38686.35
```

GB12^{R}

Tx5 (LI4m^{+1}) and Tx2 (LI4m^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS    Adj SS   Adj MS      F      P
Subject           23   6029.41   6029.41   262.15   2.73  0.000
Tx No              1    620.89    620.89   620.89   6.46  0.011
pre/post(Tx No)    2   8977.04   8977.04  4488.52  46.73  0.000
Error            357  34292.12  34292.12    96.06
Total            383  49919.46
```

Tx8 (LI4m^{+21}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  6, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6789.1   6789.1   295.2   2.71  0.000
Tx No              1    253.0    253.0   253.0   2.32  0.129
pre/post(Tx No)    2  10411.0  10411.0  5205.5  47.74  0.000
Error            357  38927.3  38927.3   109.0
Total            383  56380.5
```

Tx4 (NAPm^{+1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   8003.2   8003.2   348.0   3.39  0.000
Tx No              1    284.0    284.0   284.0   2.76  0.097
pre/post(Tx No)    2  12603.6  12603.6  6301.8  61.35  0.000
Error            357  36672.7  36672.7   102.7
Total            383  57563.4
```

Tx3 (NAPm^{+21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 3
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS    Adj SS   Adj MS      F      P
Subject           23   3654.84   3654.84   158.91   1.62  0.037
Tx No              1     90.61     90.61    90.61   0.92  0.337
pre/post(Tx No)    2  10717.95  10717.95  5358.98  54.69  0.000
Error            357  34979.68  34979.68    97.98
Total            383  49443.08
```

(b) Does duration of needling retention time has an effect?

KI3^{R}

Tx5 (LI4m^{+1}) and Tx8 (LI4m^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  5, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5998.6   5998.6   260.8   2.06  0.003
Tx No              1      8.8      8.8     8.8   0.07  0.792
pre/post(Tx No)    2   4943.3   4943.3  2471.7  19.54  0.000
Error            357  45147.5  45147.5   126.5
Total            383  56098.2
```

Tx2 (LI4m^{-1}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   7396.5   7396.5   321.6  2.66  0.000
Tx No              1    138.2    138.2   138.2  1.14  0.286
pre/post(Tx No)    2   1604.8   1604.8   802.4  6.64  0.001
Error            357  43119.8  43119.8   120.8
Total            383  52259.3
```

Tx4 (NAPm^{+1}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 4
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23  15141.0  15141.0   658.3   3.37  0.000
Tx No              1     84.4     84.4    84.4   0.43  0.512
pre/post(Tx No)    2   6158.9   6158.9  3079.4  15.75  0.000
Error            357  69812.5  69812.5   195.6
Total            383  91196.8
```

Tx7 (NAPm^{-1}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6470.3   6470.3   281.3   2.55  0.000
Tx No              1     28.7     28.7    28.7   0.26  0.611
pre/post(Tx No)    2   7825.2   7825.2  3912.6  35.41  0.000
Error            357  39445.9  39445.9   110.5
Total            383  53770.0
```

3[R]

Tx5 (LI4m[+1]) and Tx8 (LI4m[+21])

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  5, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   3487.1   3487.1   151.6  1.35  0.132
Tx No              1     66.6     66.6    66.6  0.59  0.442
pre/post(Tx No)    2   1955.9   1955.9   977.9  8.71  0.000
Error            357  40098.5  40098.5   112.3
Total            383  45608.1
```

Tx2 (LI4m[-1]) and Tx6 (LI4m[-21])

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   7018.7   7018.7   305.2  2.04  0.004
Tx No              1    354.9    354.9   354.9  2.38  0.124
pre/post(Tx No)    2   1052.8   1052.8   526.4  3.52  0.031
Error            357  53337.4  53337.4   149.4
Total            383  61763.7
```

Tx4 (NAPm[+1]) and Tx3 (NAPm[+21])

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 4
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   8135.9   8135.9   353.7   2.56  0.000
Tx No              1      1.1      1.1     1.1   0.01  0.928
pre/post(Tx No)    2   4505.3   4505.3  2252.6  16.30  0.000
Error            357  49325.9  49325.9   138.2
Total            383  61968.2
```

Tx7 (NAPm[-1]) and Tx1 (NAPm[-21])

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   8733.8   8733.8   379.7   2.66  0.000
Tx No              1    544.4    544.4   544.4   3.82  0.051
pre/post(Tx No)    2   6809.0   6809.0  3404.5  23.89  0.000
Error            357  50881.2  50881.2   142.5
Total            383  66968.4
```

ST36^{R}

Tx5 (LI4m^{+1}) and Tx8 (LI4m^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  5, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   7465.5   7465.5   324.6  3.00  0.000
Tx No              1    541.0    541.0   541.0  5.00  0.026
pre/post(Tx No)    2   1469.9   1469.9   734.9  6.80  0.001
Error            357  38593.6  38593.6   108.1
Total            383  48070.0
```

Tx2 (LI4m^{-1}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   5225.3   5246.3   228.1  2.07  0.003
Tx No              1     49.1     49.9    49.9  0.45  0.502
pre/post(Tx No)    2    747.8    747.8   373.9  3.39  0.035
Error            356  39321.9  39321.9   110.5
Total            382  45344.0
```

Tx4 (NAPm^{+1}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 4
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23  12268.9  12268.9   533.4   3.84  0.000
Tx No              1    133.7    133.7   133.7   0.96  0.327
pre/post(Tx No)    2   3654.7   3654.7  1827.3  13.16  0.000
Error            357  49555.7  49555.7   138.8
Total            383  65613.0
```

Tx7 (NAPm^{-1}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   4888.1   4888.1   212.5  1.97  0.005
Tx No              1    677.7    677.7   677.7  6.28  0.013
pre/post(Tx No)    2   2102.7   2102.7  1051.3  9.74  0.000
Error            357  38516.1  38516.1   107.9
Total            383  46184.6
```

LI5^{L}

Tx5 (LI4m^{+1}) and Tx8 (LI4m^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  5, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23  15841.8  15841.8   688.8   3.71  0.000
Tx No              1    367.8    367.8   367.8   1.98  0.160
pre/post(Tx No)    2   6707.3   6707.3  3353.6  18.06  0.000
Error            357  66305.7  66305.7   185.7
Total            383  89222.6
```

Tx2 (LI4m^{-1}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6654.9   6654.9   289.3   1.62  0.037
Tx No              1    367.8    367.8   367.8   2.06  0.152
pre/post(Tx No)    2   4330.2   4330.2  2165.1  12.14  0.000
Error            357  63690.5  63690.5   178.4
Total            383  75043.3
```

Tx4 (NAPm^{+1}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 4
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6992.3   6992.3   304.0   2.18  0.002
Tx No              1    166.4    166.4   166.4   1.19  0.276
pre/post(Tx No)    2   3576.0   3576.0  1788.0  12.80  0.000
Error            357  49869.1  49869.1   139.7
Total            383  60603.8
```

Tx7 (NAPm^{-1}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9742.8   9742.8   423.6   2.02  0.004
Tx No              1    255.9    255.9   255.9   1.22  0.270
pre/post(Tx No)    2   5857.7   5857.7  2928.8  13.99  0.000
Error            357  74743.0  74743.0   209.4
Total            383  90599.3
```

1[L]

Tx5 (LI4m[+1]) and Tx8 (LI4m[+21])

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	2	5, 8
pre/post(Tx No)	fixed	4	1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	7802.8	7802.8	339.3	1.76	0.018
Tx No	1	58.5	58.5	58.5	0.30	0.582
pre/post(Tx No)	2	5840.6	5840.6	2920.3	15.16	0.000
Error	357	68761.5	68761.5	192.6		
Total	383	82463.5				

Tx2 (LI4m[-1]) and Tx6 (LI4m[-21])

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	2	2, 6
pre/post(Tx No)	fixed	4	1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	11435.7	11435.7	497.2	2.04	0.003
Tx No	1	1829.8	1829.8	1829.8	7.52	0.006
pre/post(Tx No)	2	10007.9	10007.9	5003.9	20.58	0.000
Error	357	86817.7	86817.7	243.2		
Total	383	110091.1				

Tx4 (NAPm[+1]) and Tx3 (NAPm[+21])

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	2	3, 4
pre/post(Tx No)	fixed	4	1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	9219.7	9219.7	400.9	2.06	0.003
Tx No	1	61.6	61.6	61.6	0.32	0.574
pre/post(Tx No)	2	7512.5	7512.5	3756.2	19.27	0.000
Error	357	69582.5	69582.5	194.9		
Total	383	86376.3				

Tx7 (NAPm[-1]) and Tx1 (NAPm[-21])

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	2	1, 7
pre/post(Tx No)	fixed	4	1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	8990.1	8990.1	390.9	2.06	0.003
Tx No	1	193.8	193.8	193.8	1.02	0.313
pre/post(Tx No)	2	6723.0	6723.0	3361.5	17.73	0.000
Error	357	67687.9	67687.9	189.6		
Total	383	83594.8				

PC6^{L}

Tx5 (LI4m^{+1}) and Tx8 (LI4m^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  5, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6238.1   6238.1   271.2   1.72  0.022
Tx No              1    102.7    102.7   102.7   0.65  0.420
pre/post(Tx No)    2   7688.7   7688.7  3844.3  24.42  0.000
Error            357  56190.8  56190.8   157.4
Total            383  70220.2
```

Tx2 (LI4m^{-1}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6638.5   6638.5   288.6   2.08  0.003
Tx No              1    166.7    166.7   166.7   1.20  0.273
pre/post(Tx No)    2   3956.0   3956.0  1978.0  14.28  0.000
Error            357  49452.8  49452.8   138.5
Total            383  60214.1
```

Tx4 (NAPm^{+1}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 4
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   7265.2   7265.2   315.9   2.34  0.001
Tx No              1     64.1     64.1    64.1   0.47  0.491
pre/post(Tx No)    2   7056.9   7056.9  3528.5  26.10  0.000
Error            357  48271.0  48271.0   135.2
Total            383  62657.2
```

Tx7 (NAPm^{-1}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS   Adj SS  Adj MS      F      P
Subject           23   29056.0  29056.0  1263.3   4.74  0.000
Tx No              1     207.2    207.2   207.2   0.78  0.379
pre/post(Tx No)    2    8138.3   8138.3  4069.1  15.26  0.000
Error            357   95192.9  95192.9   266.6
Total            383  132594.3
```

2[L]

Tx5 (LI4m[+1]) and Tx8 (LI4m[+21])

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  5, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9572.7   9572.7   416.2   2.43  0.000
Tx No              1     87.1     87.1    87.1   0.51  0.476
pre/post(Tx No)    2   7396.1   7396.1  3698.1  21.58  0.000
Error            357  61164.7  61164.7   171.3
Total            383  78220.6
```

Tx2 (LI4m[-1]) and Tx6 (LI4m[-21])

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5157.0   5157.0   224.2   1.85  0.011
Tx No              1    221.0    221.0   221.0   1.82  0.178
pre/post(Tx No)    2   2939.9   2939.9  1469.9  12.13  0.000
Error            357  43254.3  43254.3   121.2
Total            383  51572.2
```

Tx4 (NAPm[+1]) and Tx3 (NAPm[+21])

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 4
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23  10611.0  10611.0   461.3   3.03  0.000
Tx No              1     62.3     62.3    62.3   0.41  0.523
pre/post(Tx No)    2   3629.7   3629.7  1814.9  11.92  0.000
Error            357  54348.6  54348.6   152.2
Total            383  68651.7
```

Tx7 (NAPm[-1]) and Tx1 (NAPm[-21])

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9818.6   9818.6   426.9   2.74  0.000
Tx No              1     20.3     20.3    20.3   0.13  0.719
pre/post(Tx No)    2   3569.6   3569.6  1784.8  11.44  0.000
Error            357  55696.3  55696.3   156.0
Total            383  69104.9
```

LI10^{L}

Tx5 (LI4m^{+1}) and Tx8 (LI4m^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  5, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS   Adj SS  Adj MS      F      P
Subject           23    6606.3   6606.3   287.2   1.34  0.139
Tx No              1    2623.6   2623.6  2623.6  12.22  0.001
pre/post(Tx No)    2   14525.1  14525.1  7262.6  33.83  0.000
Error            357   76640.1  76640.1   214.7
Total            383  100395.1
```

Tx2 (LI4m^{-1}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   7160.4   7160.4   311.3   1.86  0.010
Tx No              1    532.9    532.9   532.9   3.18  0.076
pre/post(Tx No)    2   3485.8   3485.8  1742.9  10.39  0.000
Error            357  59910.2  59910.2   167.8
Total            383  71089.2
```

Tx4 (NAPm^{+1}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 4
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5471.0   5471.0   237.9   1.54  0.054
Tx No              1    522.0    522.0   522.0   3.39  0.067
pre/post(Tx No)    2   9128.1   9128.1  4564.1  29.62  0.000
Error            357  55017.6  55017.6   154.1
Total            383  70138.7
```

Tx7 (NAPm^{-1}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   7133.8   7133.8   310.2   1.94  0.006
Tx No              1     10.2     10.2    10.2   0.06  0.801
pre/post(Tx No)    2   7627.7   7627.7  3813.9  23.87  0.000
Error            357  57034.2  57034.2   159.8
Total            383  71805.9
```

LI20^{R}

Tx5 (LI4m^{+1}) and Tx8 (LI4m^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  5, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS    Adj SS   Adj MS      F      P
Subject           23   6755.46   6755.46   293.72   3.06  0.000
Tx No              1    546.05    546.05   546.05   5.69  0.018
pre/post(Tx No)    2   3560.20   3560.20  1780.10  18.55  0.000
Error            357  34258.24  34258.24    95.96
Total            383  45119.95
```

Tx2 (LI4m^{-1}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   3927.4   3927.4   170.8  1.66  0.030
Tx No              1    395.8    395.8   395.8  3.85  0.050
pre/post(Tx No)    2   1857.8   1857.8   928.9  9.05  0.000
Error            357  36656.0  36656.0   102.7
Total            383  42837.0
```

Tx4 (NAPm^{+1}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 4
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS    Adj SS   Adj MS      F      P
Subject           23   5619.03   5665.68   246.33   2.85  0.000
Tx No              1     78.88     78.88    78.88   0.91  0.340
pre/post(Tx No)    2   3745.68   3745.68  1872.84  21.66  0.000
Error            349  30180.12  30180.12    86.48
Total            375  39623.71
```

Tx7 (NAPm^{-1}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS    Adj SS   Adj MS      F      P
Subject           23   5931.15   5931.15   257.88   2.80  0.000
Tx No              1     14.08     14.08    14.08   0.15  0.696
pre/post(Tx No)    2   2841.98   2841.98  1420.99  15.43  0.000
Error            357  32868.35  32868.35    92.07
Total            383  41655.56
```

GB12^{R}

Tx5 (LI4m^{+1}) and Tx8 (LI4m^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  5, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS    Adj SS   Adj MS      F      P
Subject           23   4351.71   4351.71   189.20   2.25  0.001
Tx No              1    629.99    629.99   629.99   7.50  0.006
pre/post(Tx No)    2   9019.45   9019.45  4509.72  53.70  0.000
Error            357  29978.38  29978.38    83.97
Total            383  43979.54
```

Tx2 (LI4m^{-1}) and Tx6 (LI4m^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   7591.2   7591.2   330.1   2.67  0.000
Tx No              1    247.3    247.3   247.3   2.00  0.158
pre/post(Tx No)    2  10368.6  10368.6  5184.3  41.95  0.000
Error            357  44116.7  44116.7   123.6
Total            383  62323.7
```

Tx4 (NAPm^{+1}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 4
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   4644.9   4644.9   202.0   1.98  0.005
Tx No              1    293.7    293.7   293.7   2.87  0.091
pre/post(Tx No)    2  12549.9  12549.9  6274.9  61.38  0.000
Error            357  36496.0  36496.0   102.2
Total            383  53984.4
```

Tx7 (NAPm^{-1}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6422.6   6422.6   279.2   2.79  0.000
Tx No              1     85.3     85.3    85.3   0.85  0.357
pre/post(Tx No)    2  10771.6  10771.6  5385.8  53.79  0.000
Error            357  35746.9  35746.9   100.1
Total            383  53026.4
```

(c) Does site of needling insertion have an effect?

KI3^{R}

Tx8 (LI4m^{+21}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9588.5   9588.5   416.9   2.91  0.000
Tx No              1     48.4     48.4    48.4   0.34  0.561
pre/post(Tx No)    2   6475.5   6475.5  3237.7  22.62  0.000
Error            357  51098.5  51098.5   143.1
Total            383  67210.8
```

Tx6 (LI4m^{-21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6635.5   6635.5   288.5   2.30  0.001
Tx No              1   1126.1   1126.1  1126.1   8.96  0.003
pre/post(Tx No)    2   4737.9   4737.9  2368.9  18.85  0.000
Error            357  44854.7  44854.7   125.6
Total            383  57354.2
```

Tx5 (LI4m^{+1}) and Tx4 (NAPm^{+1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23  12461.6  12461.6   541.8   3.07  0.000
Tx No              1      0.5      0.5     0.5   0.00  0.956
pre/post(Tx No)    2   4626.7   4626.7  2313.4  13.12  0.000
Error            357  62951.0  62951.0   176.3
Total            383  80039.9
```

Tx2 (LI4m^{-1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5490.9   5490.9   238.7   2.16  0.002
Tx No              1    270.4    270.4   270.4   2.45  0.119
pre/post(Tx No)    2   4692.1   4692.1  2346.0  21.23  0.000
Error            357  39451.3  39451.3   110.5
Total            383  49904.7
```

3^{R}

Tx8 (LI4m^{+21}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6867.0   6867.0   298.6   2.56  0.000
Tx No              1     64.5     64.5    64.5   0.55  0.458
pre/post(Tx No)    2   3623.4   3623.4  1811.7  15.51  0.000
Error            357  41694.5  41694.5   116.8
Total            383  52249.4
```

Tx6 (LI4m^{-21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23  16274.7  16274.7   707.6   4.59  0.000
Tx No              1   2251.5   2251.5  2251.5  14.62  0.000
pre/post(Tx No)    2   5280.0   5280.0  2640.0  17.14  0.000
Error            357  54986.8  54986.8   154.0
Total            383  78793.0
```

Tx5 (LI4m^{+1}) and Tx4 (NAPm^{+1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6180.0   6180.0   268.7   2.07  0.003
Tx No              1    297.6    297.6   297.6   2.29  0.131
pre/post(Tx No)    2   2837.7   2837.7  1418.9  10.94  0.000
Error            357  46305.9  46305.9   129.7
Total            383  55621.2
```

Tx2 (LI4m^{-1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6496.7   6496.7   282.5   2.39  0.000
Tx No              1     27.9     27.9    27.9   0.24  0.628
pre/post(Tx No)    2   2581.8   2581.8  1290.9  10.92  0.000
Error            357  42212.9  42212.9   118.2
Total            383  51319.2
```

ST36^{R}

Tx8 (LI4m^{+21}) and Tx3 (NAPm^{+21})

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	2	3, 8
pre/post(Tx No)	fixed	4	1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	9841.6	9841.6	427.9	3.19	0.000
Tx No	1	8.9	8.9	8.9	0.07	0.797
pre/post(Tx No)	2	2584.9	2584.9	1292.5	9.62	0.000
Error	357	47943.4	47943.4	134.3		
Total	383	60378.9				

Tx6 (LI4m^{-21}) and Tx1 (NAPm^{-21})

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	2	1, 6
pre/post(Tx No)	fixed	4	1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	16274.7	16274.7	707.6	4.59	0.000
Tx No	1	2251.5	2251.5	2251.5	14.62	0.000
pre/post(Tx No)	2	5280.0	5280.0	2640.0	17.14	0.000
Error	357	54986.8	54986.8	154.0		
Total	383	78793.0				

Tx5 (LI4m^{+1}) and Tx4 (NAPm^{+1})

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	2	4, 5
pre/post(Tx No)	fixed	4	1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	6180.0	6180.0	268.7	2.07	0.003
Tx No	1	297.6	297.6	297.6	2.29	0.131
pre/post(Tx No)	2	2837.7	2837.7	1418.9	10.94	0.000
Error	357	46305.9	46305.9	129.7		
Total	383	55621.2				

Tx2 (LI4m^{-1}) and Tx7 (NAPm^{-1})

Factor	Type	Levels	Values
Subject	random	24	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No	fixed	2	2, 7
pre/post(Tx No)	fixed	4	1, 2, 1, 2

Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject	23	6496.7	6496.7	282.5	2.39	0.000
Tx No	1	27.9	27.9	27.9	0.24	0.628
pre/post(Tx No)	2	2581.8	2581.8	1290.9	10.92	0.000
Error	357	42212.9	42212.9	118.2		
Total	383	51319.2				

LI5^{L}

Tx8 (LI4m^{+21}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9083.9   9083.9   395.0   2.47  0.000
Tx No              1    710.4    710.4   710.4   4.44  0.036
pre/post(Tx No)    2   5915.4   5915.4  2957.7  18.48  0.000
Error            357  57149.9  57149.9   160.1
Total            383  72859.6
```

Tx6 (LI4m^{-21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   8679.8   8679.8   377.4  2.02  0.004
Tx No              1     56.8     56.8    56.8  0.30  0.581
pre/post(Tx No)    2   2689.6   2689.6  1344.8  7.21  0.001
Error            357  66620.7  66620.7   186.6
Total            383  78046.9
```

Tx5 (LI4m^{+1}) and Tx4 (NAPm^{+1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23  12527.0  12527.0   544.7   3.23  0.000
Tx No              1     29.4     29.4    29.4   0.17  0.677
pre/post(Tx No)    2   4367.8   4367.8  2183.9  12.94  0.000
Error            357  60248.2  60248.2   168.8
Total            383  77172.3
```

Tx2 (LI4m^{-1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23  11842.0  11842.0   514.9   2.72  0.000
Tx No              1     19.0     19.0    19.0   0.10  0.752
pre/post(Tx No)    2   7498.3   7498.3  3749.1  19.77  0.000
Error            357  67688.6  67688.6   189.6
Total            383  87047.9
```

1[L]

Tx8 (LI4m^{+21}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   7912.3   7912.3   344.0   1.68  0.028
Tx No              1      6.8      6.8     6.8   0.03  0.856
pre/post(Tx No)    2   6580.9   6580.9  3290.4  16.03  0.000
Error            357  73291.2  73291.2   205.3
Total            383  87791.2
```

Tx6 (LI4m^{-21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   5448.4   5448.4   236.9  1.26  0.188
Tx No              1     92.4     92.4    92.4  0.49  0.483
pre/post(Tx No)    2   3372.1   3372.1  1686.1  9.00  0.000
Error            357  66885.0  66885.0   187.4
Total            383  75797.9
```

Tx5 (LI4m^{+1}) and Tx4 (NAPm^{+1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5409.5   5409.5   235.2   1.22  0.223
Tx No              1    166.1    166.1   166.1   0.86  0.354
pre/post(Tx No)    2   6772.2   6772.2  3386.1  17.58  0.000
Error            357  68753.6  68753.6   192.6
Total            383  81101.4
```

Tx2 (LI4m^{-1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS   Adj SS  Adj MS      F      P
Subject           23   13963.8  13963.8   607.1   2.45  0.000
Tx No              1     370.3    370.3   370.3   1.49  0.223
pre/post(Tx No)    2   13358.7  13358.7  6679.4  26.90  0.000
Error            357   88634.2  88634.2   248.3
Total            383  116327.0
```

PC6^{L}

Tx8 (LI4m^{+21}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5527.9   5527.9   240.3   1.67  0.029
Tx No              1      7.8      7.8     7.8   0.05  0.816
pre/post(Tx No)    2   8925.1   8925.1  4462.6  30.95  0.000
Error            357  51470.6  51470.6   144.2
Total            383  65931.5
```

Tx6 (LI4m^{-21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF    Seq SS   Adj SS  Adj MS      F      P
Subject           23   22686.7  22686.7   986.4   3.82  0.000
Tx No              1     750.9    750.9   750.9   2.91  0.089
pre/post(Tx No)    2    6534.3   6534.3  3267.2  12.66  0.000
Error            357   92147.7  92147.7   258.1
Total            383  122119.6
```

Tx5 (LI4m^{+1}) and Tx4 (NAPm^{+1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   4372.3   4372.3   190.1   1.20  0.242
Tx No              1      0.5      0.5     0.5   0.00  0.957
pre/post(Tx No)    2   5820.5   5820.5  2910.2  18.36  0.000
Error            357  56594.2  56594.2   158.5
```

Tx2 (LI4m^{-1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   8215.1   8215.1   357.2   2.23  0.001
Tx No              1      0.0      0.0     0.0   0.00  0.994
pre/post(Tx No)    2   5560.0   5560.0  2780.0  17.32  0.000
Error            357  57290.6  57290.6   160.5
Total            383  71065.7
```

2[L]

Tx8 (LI4m^{+21}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9159.9   9159.9   398.3   3.15  0.000
Tx No              1    148.0    148.0   148.0   1.17  0.280
pre/post(Tx No)    2   4243.8   4243.8  2121.9  16.76  0.000
Error            357  45201.8  45201.8   126.6
Total            383  58753.5
```

Tx6 (LI4m^{-21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS     F      P
Subject           23   8576.7   8576.7   372.9  2.82  0.000
Tx No              1     78.0     78.0    78.0  0.59  0.443
pre/post(Tx No)    2   2219.6   2219.6  1109.8  8.40  0.000
Error            357  47186.1  47186.1   132.2
Total            383  58060.5
```

Tx5 (LI4m^{+1}) and Tx4 (NAPm^{+1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23  12425.5  12425.5   540.2   2.80  0.000
Tx No              1    185.0    185.0   185.0   0.96  0.328
pre/post(Tx No)    2   6782.1   6782.1  3391.0  17.57  0.000
Error            357  68909.9  68909.9   193.0
Total            383  88302.5
```

Tx2 (LI4m^{-1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5850.0   5850.0   254.3   1.74  0.020
Tx No              1      2.3      2.3     2.3   0.02  0.899
pre/post(Tx No)    2   4289.8   4289.8  2144.9  14.64  0.000
Error            357  52313.5  52313.5   146.5
Total            383  62455.7
```

LI10^{L}

Tx8 (LI4m^{+21}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5976.9   5976.9   259.9   1.32  0.152
Tx No              1   2042.7   2042.7  2042.7  10.35  0.001
pre/post(Tx No)    2  15295.1  15295.1  7647.5  38.74  0.000
Error            357  70471.6  70471.6   197.4
Total            383  93786.3
```

Tx6 (LI4m^{-21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6659.4   6659.4   289.5   1.96  0.006
Tx No              1    876.5    876.5   876.5   5.94  0.015
pre/post(Tx No)    2   4580.9   4580.9  2290.5  15.52  0.000
Error            357  52677.1  52677.1   147.6
Total            383  64793.9
```

Tx5 (LI4m^{+1}) and Tx4 (NAPm^{+1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9353.8   9353.8   406.7   2.51  0.000
Tx No              1    833.6    833.6   833.6   5.14  0.024
pre/post(Tx No)    2   8358.2   8358.2  4179.1  25.75  0.000
Error            357  57932.6  57932.6   162.3
Total            383  76478.2
```

Tx2 (LI4m^{-1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6434.9   6434.9   279.8   1.53  0.059
Tx No              1     11.1     11.1    11.1   0.06  0.806
pre/post(Tx No)    2   6532.6   6532.6  3266.3  17.81  0.000
Error            357  65467.1  65467.1   183.4
Total            383  78445.8
```

LI20^{R}

Tx8 (LI4m^{+21}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5976.9   5976.9   259.9   1.32  0.152
Tx No              1   2042.7   2042.7  2042.7  10.35  0.001
pre/post(Tx No)    2  15295.1  15295.1  7647.5  38.74  0.000
Error            357  70471.6  70471.6   197.4
Total            383  93786.3
```

Tx6 (LI4m^{-21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6659.4   6659.4   289.5   1.96  0.006
Tx No              1    876.5    876.5   876.5   5.94  0.015
pre/post(Tx No)    2   4580.9   4580.9  2290.5  15.52  0.000
Error            357  52677.1  52677.1   147.6
Total            383  64793.9
```

Tx5 (LI4m^{+1}) and Tx4 (NAPm^{+1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9353.8   9353.8   406.7   2.51  0.000
Tx No              1    833.6    833.6   833.6   5.14  0.024
pre/post(Tx No)    2   8358.2   8358.2  4179.1  25.75  0.000
Error            357  57932.6  57932.6   162.3
Total            383  76478.2
```

Tx2 (LI4m^{-1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6434.9   6434.9   279.8   1.53  0.059
Tx No              1     11.1     11.1    11.1   0.06  0.806
pre/post(Tx No)    2   6532.6   6532.6  3266.3  17.81  0.000
Error            357  65467.1  65467.1   183.4
Total            383  78445.8
```

GB12^{R}

Tx8 (LI4m^{+21}) and Tx3 (NAPm^{+21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  3, 8
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   5976.9   5976.9   259.9   1.32  0.152
Tx No              1   2042.7   2042.7  2042.7  10.35  0.001
pre/post(Tx No)    2  15295.1  15295.1  7647.5  38.74  0.000
Error            357  70471.6  70471.6   197.4
Total            383  93786.3
```

Tx6 (LI4m^{-21}) and Tx1 (NAPm^{-21})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  1, 6
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6659.4   6659.4   289.5   1.96  0.006
Tx No              1    876.5    876.5   876.5   5.94  0.015
pre/post(Tx No)    2   4580.9   4580.9  2290.5  15.52  0.000
Error            357  52677.1  52677.1   147.6
Total            383  64793.9
```

Tx5 (LI4m^{+1}) and Tx4 (NAPm^{+1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  4, 5
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   9353.8   9353.8   406.7   2.51  0.000
Tx No              1    833.6    833.6   833.6   5.14  0.024
pre/post(Tx No)    2   8358.2   8358.2  4179.1  25.75  0.000
Error            357  57932.6  57932.6   162.3
Total            383  76478.2
```

Tx2 (LI4m^{-1}) and Tx7 (NAPm^{-1})

```
Factor           Type    Levels  Values
Subject          random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
                                 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
Tx No            fixed        2  2, 7
pre/post(Tx No)  fixed        4  1, 2, 1, 2


Analysis of Variance for %PPT Change, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS      F      P
Subject           23   6434.9   6434.9   279.8   1.53  0.059
Tx No              1     11.1     11.1    11.1   0.06  0.806
pre/post(Tx No)    2   6532.6   6532.6  3266.3  17.81  0.000
Error            357  65467.1  65467.1   183.4
Total            383  78445.8
```

4. Comparison of subjects' perceptions of the acupuncture experience among interventions (Table 4a.5)

PAIN

```
Factor   Type    Levels  Values
subject  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
                         17, 18, 19, 20, 21, 22, 23, 24
Tx No    fixed        8  1, 2, 3, 4, 5, 6, 7, 8


Analysis of Variance for pain, using Adjusted SS for Tests

Source    DF    Seq SS   Adj SS  Adj MS     F      P
subject   23   39009.0  39009.0  1696.0  5.30  0.000
Tx No      7    9749.6   9749.6  1392.8  4.35  0.000
Error    161   51536.0  51536.0   320.1
Total    191  100294.6
```

NEEDLE SENSATION

```
Factor   Type    Levels  Values
subject  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
                         17, 18, 19, 20, 21, 22, 23, 24
Tx No    fixed        8  1, 2, 3, 4, 5, 6, 7, 8


Analysis of Variance for deqi, using Adjusted SS for Tests

Source    DF    Seq SS   Adj SS  Adj MS     F      P
subject   23   52949.2  52949.2  2302.1  6.43  0.000
Tx No      7    6568.2   6568.2   938.3  2.62  0.014
Error    161   57668.5  57668.5   358.2
Total    191  117185.9
```

TENSION

```
Factor   Type    Levels  Values
subject  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
                         17, 18, 19, 20, 21, 22, 23, 24
Tx No    fixed        8  1, 2, 3, 4, 5, 6, 7, 8


Analysis of Variance for tension, using Adjusted SS for Tests

Source    DF   Seq SS   Adj SS  Adj MS     F      P
subject   23  18809.5  18809.5   817.8  5.68  0.000
Tx No      7   2083.1   2083.1   297.6  2.07  0.050
Error    161  23174.4  23174.4   143.9
Total    191  44067.0
```

ANXIETY

```
Factor   Type    Levels  Values
subject  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
                         17, 18, 19, 20, 21, 22, 23, 24
Tx No    fixed        8  1, 2, 3, 4, 5, 6, 7, 8


Analysis of Variance for anxiety, using Adjusted SS for Tests

Source    DF    Seq SS    Adj SS  Adj MS     F      P
subject   23   6316.25   6316.25  274.62  3.21  0.000
Tx No      7    799.67    799.67  114.24  1.34  0.236
Error    161  13761.08  13761.08   85.47
Total    191  20877.00
```

ACUPUNCTURIST'S BEHAVIOUR

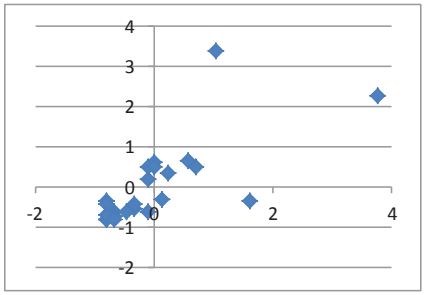
```
Factor   Type    Levels  Values
subject  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
                          17, 18, 19, 20, 21, 22, 23, 24
Tx No    fixed        8  1, 2, 3, 4, 5, 6, 7, 8


Analysis of Variance for Acup Behaviour, using Adjusted SS for Tests

Source    DF   Seq SS   Adj SS  Adj MS     F      P
subject   23  1520.76  1518.68   66.03  1.36  0.144
Tx No      7   341.48   341.48   48.78  1.00  0.433
Error    137  6673.66  6673.66   48.71
Total    167  8535.90
```

5. Scatterplots and Pearson product moment correlation coefficient r and r^2 for the VAS scores for pain, needle sensation, tension, anxiety, recorded for the 21 minutes intervention period for each intervention (Section 4.9)

$NAPm^{-21}$

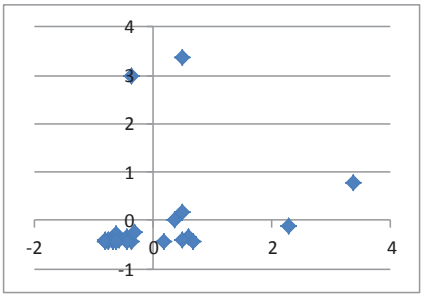
Pain and Deqi

r = 0.72 r^2 = 0.51



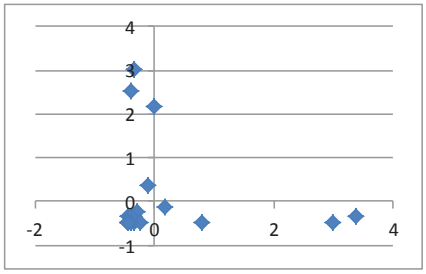
Pain and Tension

r = 0.44 r^2 = 0.19



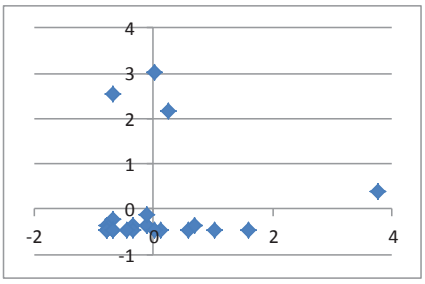
Tension and Anxiety

r = -0.10 r^2 = 0.01



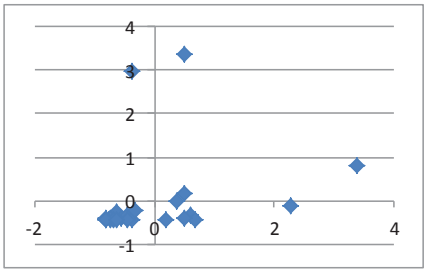
Pain and Anxiety

r = 0.06 r^2 = 0.00



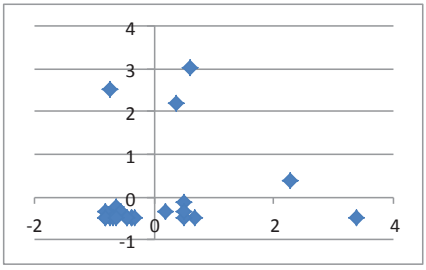
Deqi and Tension

r = 0.24 r^2 = 0.06



Deqi and Anxiety

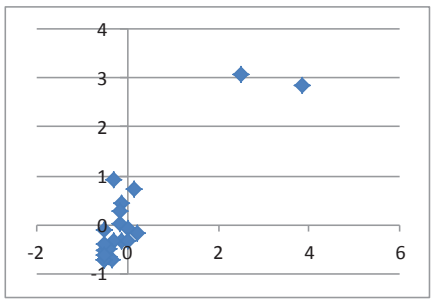
r = 0.11 r^2 = 0.01



LIm^{-1}

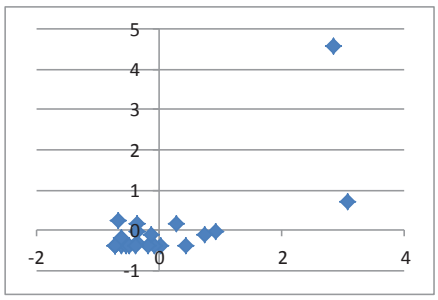
Pain and Deqi

r = 0.90 r^2 = 0.82



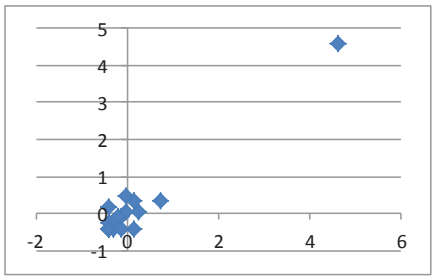
Pain and Tension

r = 0.88 r^2 = 0.78



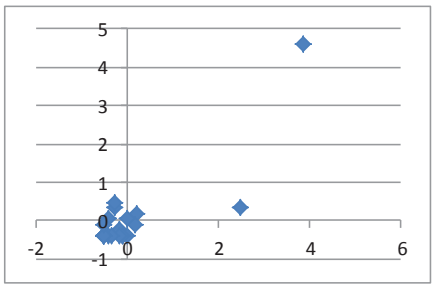
Tension and Anxiety

r = 0.97 r^2 = 0.95



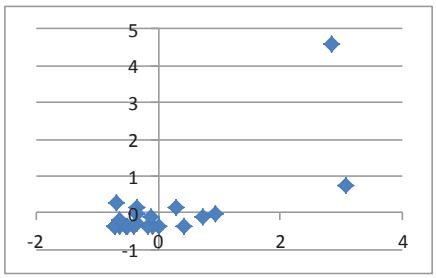
Pain and Anxiety

r = 0.85 r^2 = 0.73



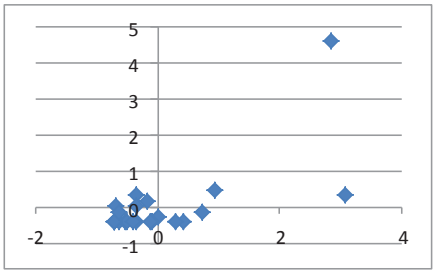
Deqi and Tension

r = 0.72 r^2 = 0.52



Deqi and Anxiety

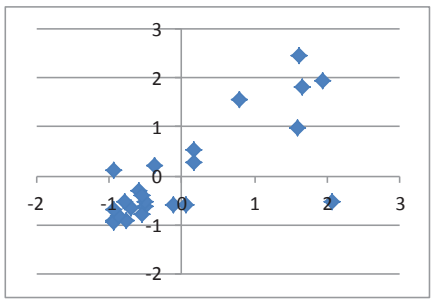
r = 0.69 r^2 = 0.47



NAPm^{+21}

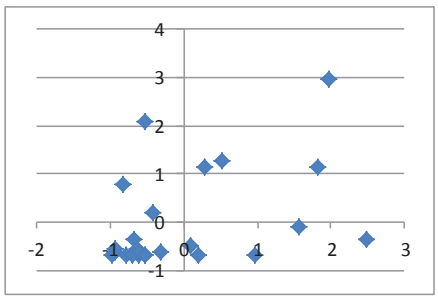
Pain and Deqi

r = 0.77 r^2 = 0.60



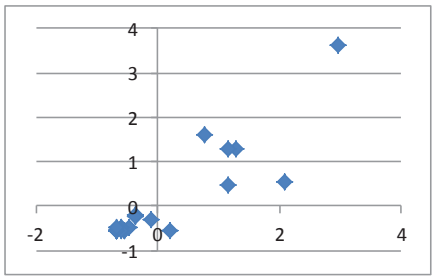
Pain and Tension

r = 0.62 r^2 = 0.39



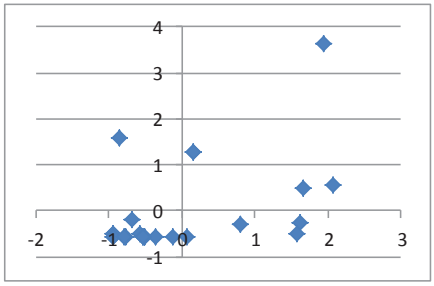
Tension and Anxiety

r = 0.90 r^2 = 0.81



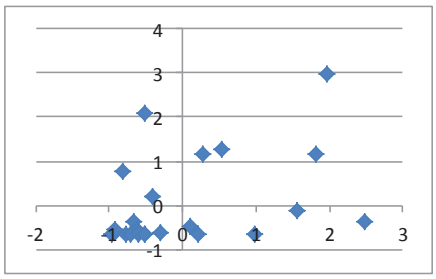
Pain and Anxiety

r = 0.47 r^2 = 0.22



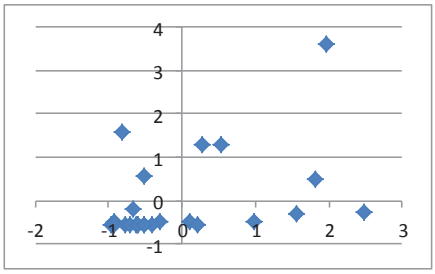
Deqi and Tension

r = 0.42 r^2 = 0.18



Deqi and Anxiety

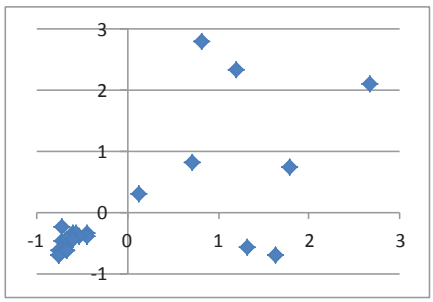
r = 0.42 r^2 = 0.18



NAPm^{+1}

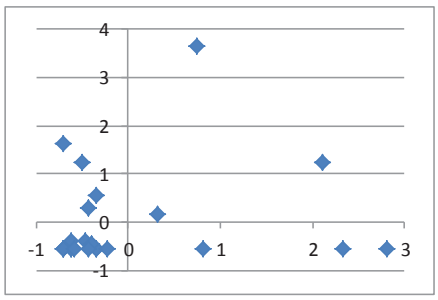
Pain and Deqi

r = 0.66 r^2 = 0.44



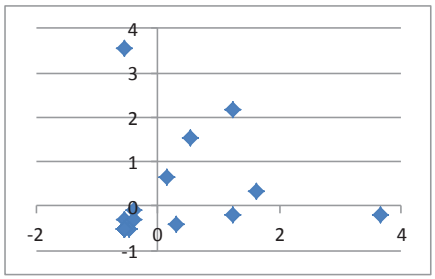
Pain and Tension

r = 0.56 r^2 = 0.31



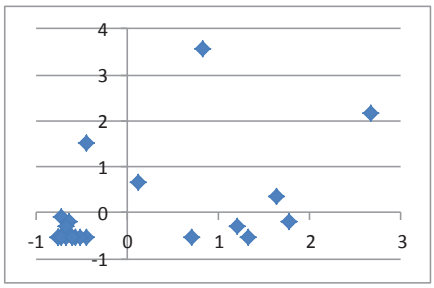
Tension and Anxiety

r = 0.21 r^2 = 0.04



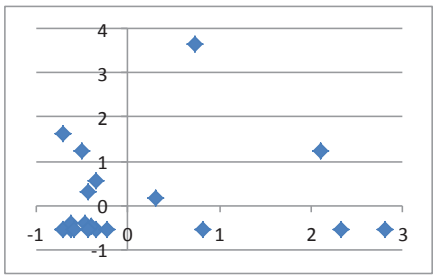
Pain and Anxiety

r = 0.47 r^2 = 0.22



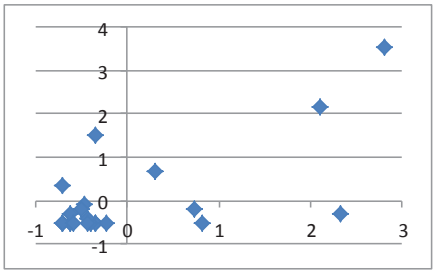
Deqi and Tension

r = 0.15 r^2 = 0.02



Deqi and Anxiety

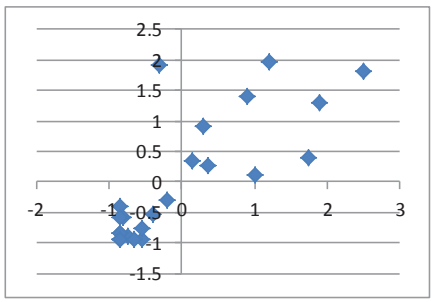
r = 0.68 r^2 = 0.46



LI4m^{+1}

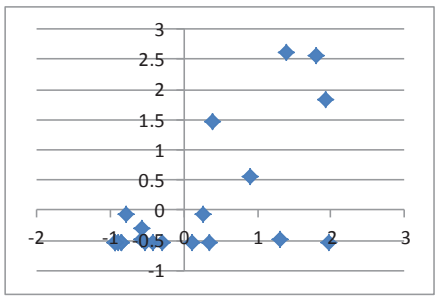
Pain and Deqi

r = 0.79 r^2 = 0.62



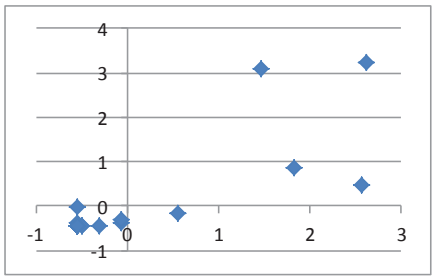
Pain and Tension

r = 0.57 r^2 = 0.32



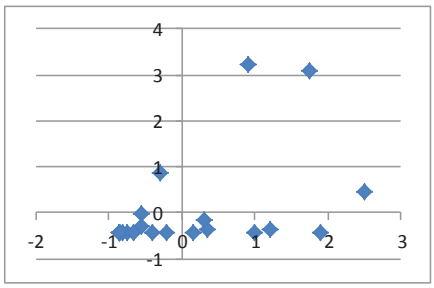
Tension and Anxiety

r = 0.80 r^2 = 0.65



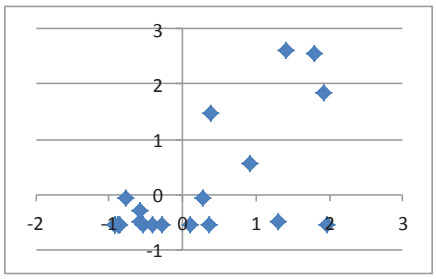
Pain and Anxiety

r = 0.47 r^2 = 0.22



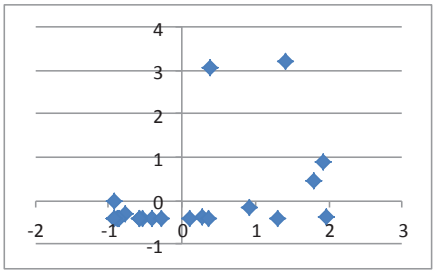
Deqi and Tension

r = 0.67 r^2 = 0.45



Deqi and Anxiety

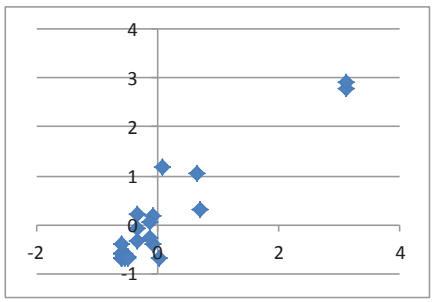
r = 0.44 r^2 = 0.19



LI4m^{-21}

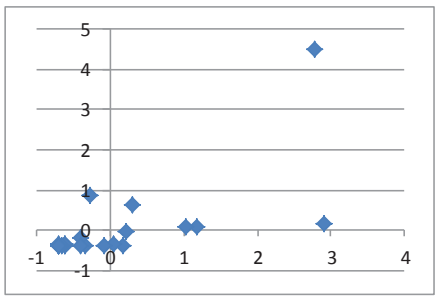
Pain and Deqi

r = 0.94 r^2 = 0.88



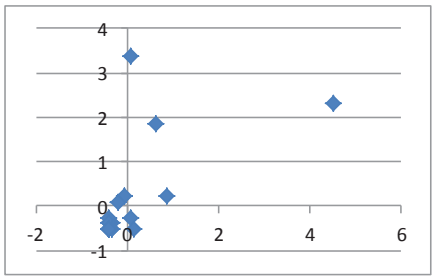
Pain and Tension

r = 0.74 r^2 = 0.54



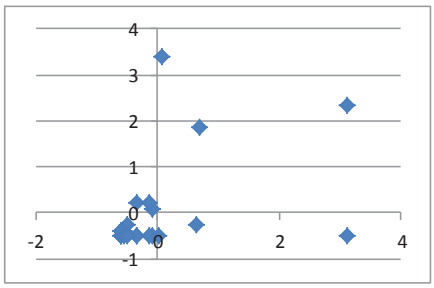
Tension and Anxiety

r = 0.62 r^2 = 0.38



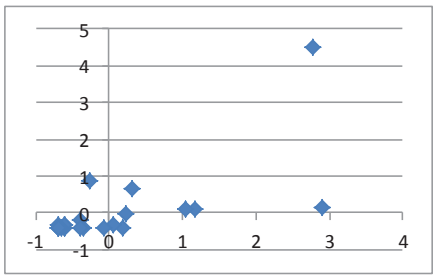
Pain and Anxiety

r = 0.42 r^2 = 0.17



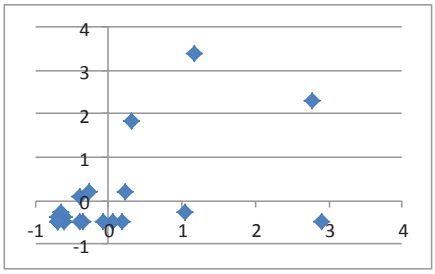
Deqi and Tension

r = 0.68 r^2 = 0.46



Deqi and Anxiety

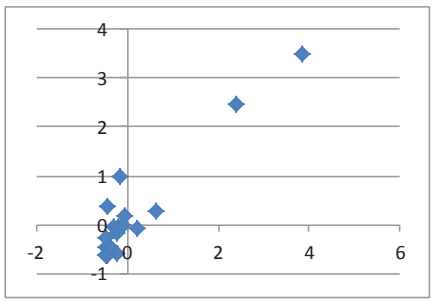
r = 0.53 r^2 = 0.28



$NAPm^{-1}$

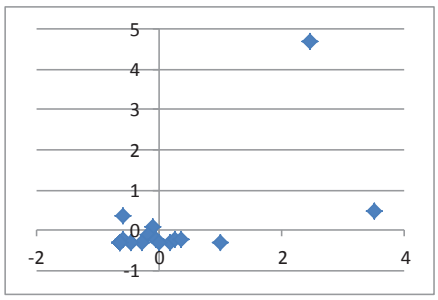
Pain and Deqi

r = 0.94 r^2 = 0.88



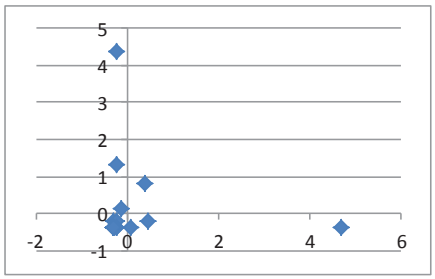
Pain and Tension

r = 0.62 r^2 = 0.38



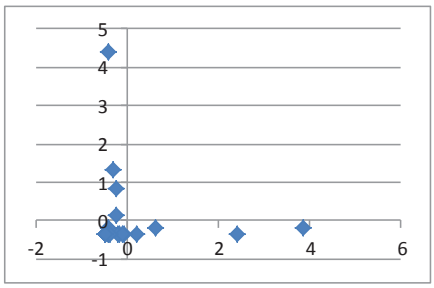
Tension and Anxiety

r = -0.04 r^2 = 0.00



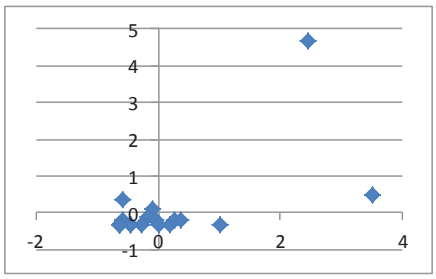
Pain and Anxiety

r = -0.09 r^2 = 0.01



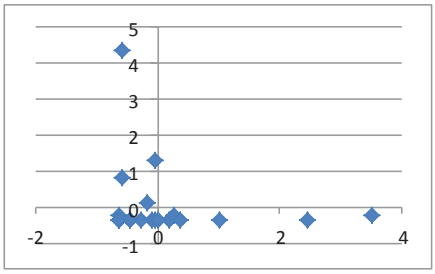
Deqi and Tension

r = 0.61 r^2 = 0.37



Deqi and Anxiety

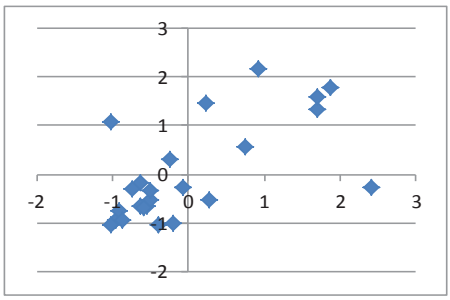
r = -0.13 r^2 = 0.02



LI4m^{+21}

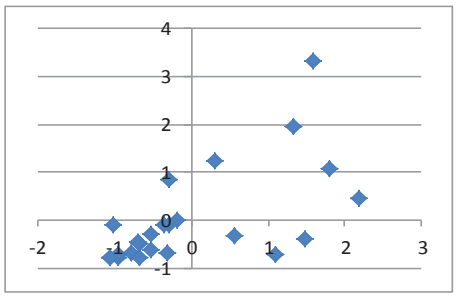
Pain and Deqi

r = 0.63 r^2 = 0.40



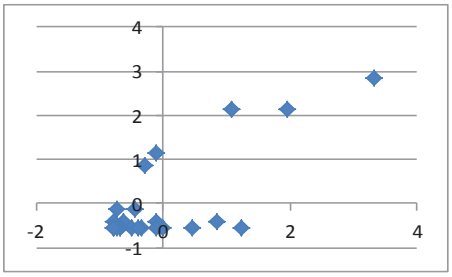
Pain and Tension

r = 0.77 r^2 = 0.59



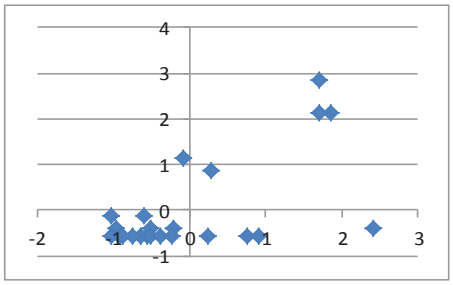
Tension and Anxiety

r = 0.76 r^2 = 0.58



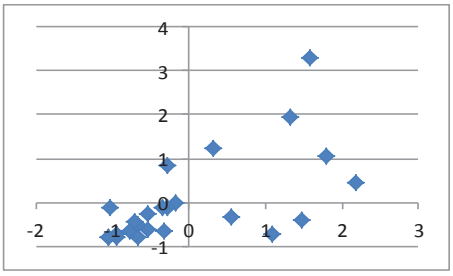
Pain and Anxiety

r = 0.63 r^2 = 0.39



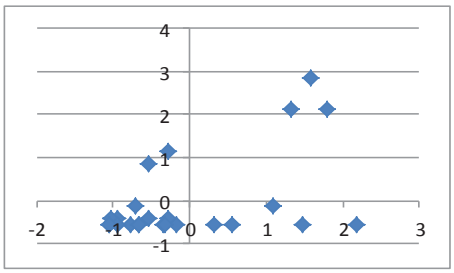
Deqi and Tension

r = 0.62 r^2 = 0.39



Deqi and Anxiety

r = 0.51 r^2 = 0.26



Part II: Needling pain, sensation and regional PPT profiles during the intervention phase

5. Needle sensation intensity profiles – comparing all eight interventions (Section 4.12)

Results for: t=1

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for De Qi, using Adjusted SS for Tests

Source       DF   Seq SS   Adj SS  Adj MS     F      P
Tx No         7    829.7    829.7   118.5  0.48  0.850
Subject No   23  42689.9  42689.9  1856.1  7.47  0.000
Error       161  39984.3  39984.3   248.3
Total       191  83503.9
```

Results for: t=4

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for De Qi, using Adjusted SS for Tests

Source       DF   Seq SS   Adj SS  Adj MS     F      P
Tx No         7   3283.6   3283.6   469.1  4.07  0.000
Subject No   23  11166.4  11166.4   485.5  4.21  0.000
Error       161  18554.0  18554.0   115.2
Total       191  33004.0
```

Results for: t=7

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for De Qi, using Adjusted SS for Tests

Source       DF    Seq SS    Adj SS  Adj MS     F      P
Tx No         7   3355.31   3355.31  479.33  5.08  0.000
Subject No   23   8111.56   8111.56  352.68  3.74  0.000
Error       161  15184.44  15184.44   94.31
Total       191  26651.31
```

Results for: t=10

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for De Qi, using Adjusted SS for Tests

Source       DF    Seq SS    Adj SS  Adj MS     F      P
Tx No         7   4138.92   4138.92  591.27  6.35  0.000
Subject No   23   6874.42   6874.42  298.89  3.21  0.000
Error       161  14992.58  14992.58   93.12
Total       191  26005.92
```

Results for: t=13

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for De Qi, using Adjusted SS for Tests

Source       DF    Seq SS    Adj SS  Adj MS     F      P
Tx No         7   4309.08   4309.08  615.58  7.68  0.000
Subject No   23   4952.49   4952.49  215.33  2.69  0.000
Error       161  12909.05  12909.05   80.18
Total       191  22170.62
```

Results for: t=16

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for De Qi, using Adjusted SS for Tests

Source       DF    Seq SS    Adj SS  Adj MS     F      P
Tx No         7   5970.67   5970.67  852.95  9.19  0.000
Subject No   23   6384.25   6384.25  277.58  2.99  0.000
Error       161  14936.33  14936.33   92.77
Total       191  27291.25
```

Results for: t=19

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for De Qi, using Adjusted SS for Tests

Source       DF    Seq SS    Adj SS  Adj MS     F      P
Tx No         7   5480.48   5480.48  782.93  8.71  0.000
Subject No   23   7300.48   7300.48  317.41  3.53  0.000
Error       161  14469.02  14469.02   89.87
Total       191  27249.98
```

Results for: t=22

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for De Qi, using Adjusted SS for Tests

Source       DF   Seq SS   Adj SS  Adj MS     F      P
Tx No         7   4038.9   4038.9   577.0  5.67  0.000
Subject No   23   7665.8   7665.8   333.3  3.28  0.000
Error       161  16384.7  16384.7   101.8
Total       191  28089.5
```

6. Pain intensity profiles (Section 4.12)

Results for: t=1

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for Pain Score, using Adjusted SS for Tests

Source       DF   Seq SS   Adj SS  Adj MS     F      P
Tx No         7   2561.9   2561.9   366.0  1.74  0.103
Subject No   23  25382.7  25382.7  1103.6  5.25  0.000
Error       161  33817.1  33817.1   210.0
Total       191  61761.7
```

Results for: t=4

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for Pain Score, using Adjusted SS for Tests

Source       DF    Seq SS    Adj SS  Adj MS     F      P
Tx No         7   3536.66   3536.66  505.24  5.33  0.000
Subject No   23   3667.99   3667.99  159.48  1.68  0.033
Error       161  15248.46  15248.46   94.71
Total       191  22453.12
```

Results for: t=7

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for Pain Score, using Adjusted SS for Tests

Source       DF    Seq SS    Adj SS  Adj MS      F      P
Tx No         7   6472.58   6472.58  924.65  10.70  0.000
Subject No   23   2910.17   2910.17  126.53   1.46  0.090
Error       161  13913.17  13913.17   86.42
Total       191  23295.92
```

Results for: t=10

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for Pain Score, using Adjusted SS for Tests

Source       DF    Seq SS    Adj SS  Adj MS     F      P
Tx No         7   4594.73   4594.73  656.39  6.85  0.000
Subject No   23   3363.73   3363.73  146.25  1.53  0.069
Error       161  15427.02  15427.02   95.82
Total       191  23385.48
```

Results for: t=13

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for Pain Score, using Adjusted SS for Tests

Source       DF    Seq SS    Adj SS  Adj MS      F      P
Tx No         7   5139.91   5139.91  734.27  11.24  0.000
Subject No   23   4286.99   4286.99  186.39   2.85  0.000
Error       161  10513.96  10513.96   65.30
Total       191  19940.87
```

Results for: t=16

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for Pain Score, using Adjusted SS for Tests

Source       DF   Seq SS   Adj SS  Adj MS      F      P
Tx No         7   9205.2   9205.2  1315.0  12.54  0.000
Subject No   23   5206.7   5206.7   226.4   2.16  0.003
Error       161  16888.0  16888.0   104.9
Total       191  31299.9
```

Results for: t=19

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for Pain Score, using Adjusted SS for Tests

Source       DF   Seq SS   Adj SS  Adj MS     F      P
Tx No         7   9191.3   9191.3  1313.0  8.68  0.000
Subject No   23   5734.0   5734.0   249.3  1.65  0.039
Error       161  24341.7  24341.7   151.2
Total       191  39267.0
```

Results for: t=22

```
Factor      Type    Levels  Values
Tx No       fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject No  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
                            16, 17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for Pain Score, using Adjusted SS for Tests

Source       DF   Seq SS   Adj SS  Adj MS     F      P
Tx No         7   9559.9   9559.9  1365.7  9.11  0.000
Subject No   23   5693.5   5693.5   247.5  1.65  0.039
Error       161  24147.6  24147.6   150.0
Total       191  39401.0
```

7. Needle sensation and pain intensity profiles – comparing $NAPm^{+21}$ and $LI4m^{+21}$ (Section 4.12)

	Needle sensation				Pain	
Time	p value	$F_{1,23}$ =		**Time**	p value	$F_{1,23}$ =
t=1	0.783	0.08		t=1	0.243	1.44
t=4	0.763	0.09		t=4	0.656	0.20
t=7	0.322	1.02		t=7	0.669	0.19
t=10	0.312	1.07		t=10	0.385	0.79
t=13	0.607	0.27		t=13	0.949	0.00
t=16	0.758	0.10		t=16	0.543	0.38
t=19	0.784	0.08		t=19	0.821	0.05
t=22	0.237	1.48		t=22	0.856	0.03

8. Needle sensation and pain intensity profiles – comparing $NAPm^{-21}$, $LI4m^{-1}$, $NAPm^{+1}$, $LI4m^{+1}$, $LI4m^{-21}$ and $NAPm^{-1}$ (Section 4.12)

	Needle sensation				**Pain**	
Time	p value	$F_{5,115}$ =		**Time**	p value	$F_{5,115}$ =
t=1	0.696	0.61		t=1	0.107	1.86
t=4	0.093	1.94		t=4	0.621	0.71
t=7	0.851	0.4		t=7	0.738	0.55
t=10	0.924	0.28		t=10	0.516	0.85
t=13	0.833	0.42		t=13	0.386	1.06
t=16	0.909	0.31		t=16	0.560	0.79
t=19	0.931	0.27		t=19	0.475	0.91
t=22	0.376	1.08		t=22	0.611	0.72

9. Relationship between pain and needle sensation perceptions (Section 4.12)

$NAPm^{+21}$	t=1		t=4		t=7		t=10		t=13		t=16		t=19		t=22	
	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res
None	2	-1.63	7	0.41	4	-0.82	4	-0.82	4	-0.82	3	-1.22	4	-0.82	4	-0.82
Pain only	0	-2.45	2	-1.63	1	-2.04	3	-1.22	1	-2.04	1	-2.04	1	-2.04	3	-1.22
NS only	4	-0.82	4	-0.82	3	-1.22	4	-0.82	3	-1.22	2	-1.63	1	-2.04	2	-1.63
Pain and NS	18	4.90	11	2.04	16	4.08	13	2.86	16	4.08	18	4.90	18	4.90	15	3.67
p-value	0.00		0.05		0.00		0.01		0.00		0.00		0.00		0.00	
chi-square value	33.3		7.7		23.0		11.0		23.0		32.3		33.0		18.3	

$LI4m^{+21}$	t=1		t=4		t=7		t=10		t=13		t=16		t=19		t=22	
	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res
None	4	-0.82	2	-1.63	3	-1.22	5	-0.41	4	-0.82	4	-0.82	3	-1.22	3	-1.22
Pain only	0	-2.45	1	-2.04	1	-2.04	1	-2.04	1	-2.04	2	-1.63	1	-2.04	2	-1.63
NS only	5	-0.41	2	-1.63	2	-1.63	4	-0.82	2	-1.63	4	-0.82	6	0.00	5	-0.41
Pain and NS	15	3.67	19	5.31	18	4.90	14	3.27	17	4.49	14	3.27	14	3.27	14	3.27
p-value	0.00		0.00		0.00		0.00		0.00		0.00		0.00		0.00	
chi-square value	20.3		37.7		32.3		15.7		27.7		14.7		16.3		15.0	

$NAPm^{-21}$	t=1		t=4		t=7		t=10		t=13		t=16		t=19		t=22	
	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res
None	6	0.00	11	2.04	15	3.67	16	4.08	20	5.72	19	5.31	19	5.31	18	4.90
Pain only	0	-2.45	0	-2.45	0	-2.45	0	2.45	0	-2.45	0	-2.45	0	-2.45	0	-2.45
NS only	7	0.41	6	0.00	6	0.00	5	0.41	2	-1.63	3	-1.22	3	-1.22	4	-0.82
Pain and NS	11	2.04	7	0.41	3	-1.22	3	1.22	2	-1.63	2	-1.63	2	-1.63	2	-1.63
p-value	0.02		0.02		0.00		0.00		0.00		0.00		0.00		0.00	
chi-square value	10.3		10.3		21		24.3		44		38.3		38.3		33.3	

$LI4m^{-21}$	t=1		t=4		t=7		t=10		t=13		t=16		t=19		t=22	
	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res
None	2	-1.63	11	2.04	14	3.27	15	3.67	18	4.90	16	4.08	15	3.67	12	2.45
Pain only	1	-2.04	0	-2.45	0	-2.45	0	-2.45	0	-2.45	0	-2.45	0	-2.45	0	-2.45
NS only	5	-0.41	8	0.82	7	0.41	7	0.41	4	-0.82	6	0.00	7	0.41	7	0.41
Pain and NS	16	4.08	5	-0.41	3	-1.22	2	-1.63	2	-1.63	2	-1.63	2	-1.63	5	-0.41
p-value	0.00		0.01		0.00		0.00		0.00		0.00		0.00		0.01	
chi-square value	23.7		11.0		18.3		22.3		33.3		25.3		22.3		12.3	

$NAPm^{+1}$	t=1		t=4		t=7		t=10		t=13		t=16		t=19		t=22	
	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res
None	3	-1.22	17	4.49	18	4.70	16	4.08	18	4.90	17	4.49	19	5.31	17	4.49
Pain only	0	-2.45	1	-2.04	1	-2.10	0	-2.45	0	-2.45	0	-2.45	0	-2.45	0	-2.45
NS only	5	-0.41	2	-1.63	3	-1.30	6	0.00	3	-1.22	5	-0.41	3	-1.22	3	-1.22
Pain and NS	16	4.08	4	-0.82	3	-1.30	2	-1.63	3	-1.22	2	-1.63	2	-1.63	4	-0.82
p-value	0.00		0.00		0.00		0.00		0.00		0.00		0.00		0.00	
chi-square value	24.3		27.7		29.9		25.3		33.0		29.0		38.3		28.3	

$LI4m^{+1}$	t=1		t=4		t=7		t=10		t=13		t=16		t=19		t=22	
	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res
None	1	-2.04	13	2.86	15	3.67	15	3.67	17	4.49	18	4.90	17	4.49	17	5.13
Pain only	0	-2.45	1	-2.04	1	-2.04	1	-2.04	0	-2.45	0	-2.45	0	-2.45	1	-1.85
NS only	7	0.41	7	0.41	7	0.04	7	0.04	5	-0.41	4	-0.82	5	-0.41	2	-1.42
Pain and NS	16	4.08	3	-1.22	1	-2.04	1	-2.04	2	-1.63	2	-1.63	2	-1.63	1	-1.85
p-value	0.00		0.00		0.00		0.00		0.00		0.00		0.00		0.00	
chi-square value	27.0		14.0		22.0		22.0		29.0		33.3		29.0		35.2	

$NAPm^{-1}$	t=1		t=4		t=7		t=10		t=13		t=16		t=19		t=22	
	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res
None	5	-0.41	13	2.86	13	2.86	16	4.08	16	4.08	16	4.08	16	4.08	16	4.08
Pain only	1	-2.04	0	-2.45	0	-2.45	0	-2.45	0	-2.45	0	-2.45	0	-2.45	1	-2.04
NS only	6	0.00	7	0.41	8	0.41	6	0.00	5	-0.41	5	-0.41	6	0.00	5	-0.41
Pain and NS	12	2.45	4	-0.82	3	-1.22	2	-1.63	3	-1.22	3	-1.22	2	-1.63	2	-1.63
p-value	0.02		0.00		0.00		0.00		0.00		0.00		0.00		0.00	
chi-square value	10.3		15.0		16.3		25.3		24.3		24.3		25.3		23.7	

$LI4m^{-1}$	t=1		t=4		t=7		t=10		t=13		t=16		t=19		t=22	
	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res	f	Std Res
None	5	-0.41	14	3.27	15	3.67	16	4.08	15	3.67	17	4.49	18	4.90	17	4.49
Pain only	0	-2.45	1	-2.04	1	-2.04	0	-2.45	1	-2.04	0	-2.45	0	-2.45	1	-2.04
NS only	5	-0.41	3	-1.22	6	0.00	7	0.41	6	0.00	4	-0.82	3	-1.22	5	-0.41
Pain and NS	14	3.27	6	0.00	2	-1.63	1	-2.04	2	-1.63	3	-1.22	3	-1.22	1	-2.41
p-value	0.00		0.00		0.00		0.00		0.00		0.00		0.00		0.00	
chi-square value	17.0		16.3		20.3		27.0		20.3		28.30		33.0		28.7	

10. Mean % PPT change for the three regional sites ($KI3^R$, $LI10^L$ and $GB12^R$) measured during (t=4, t=10 and t=16) and post-intervention (t=22-27), shown separately for the eight interventions. Also shown for each intervention are the p-values (based on ANOVA) for the between site comparison at each time interval. Statistical significant increases in mean % PPT from baseline are shown next to the mean values (*). (Section 4.15)

Tx1 - $NAPm^{-21}$

Time	Mean % ppt change			p-value
	$KI3^R$	$LI10^L$	$GB12^R$	
4	4.2	10.4	4.8	0.327
10	6.7	16.7*	9.9*	0.221
16	10.4*	11.4	12.6*	0.925
22-27	9.6*	10.6	11.5*	0.914

Tx2 - $LI4m^{-1}$

Time	Mean % ppt change			p-value
	$KI3^R$	$LI10^L$	$GB12^R$	
4	11.3*	19.9*	8.6*	0.057
10	12.3*	17.6*	13*	0.404
16	12.0*	15.2*	13.4*	0.670
22-27	5.1	7.6	11.9*	0.051

Tx3 - $NAPm^{+21}$

Time	Mean % ppt change			p-value
	$KI3^R$	$LI10^L$	$GB12^R$	
4	9.9*	15.2*	8.5*	0.133
10	11.8*	14.9*	11.8*	0.594
16	17.5*	13.0*	14.0*	0.389
22-27	8.9*	9.4*	9.6*	0.955

Tx4 - $NAPm^{+1}$

Time	Mean % ppt change			p-value
	$KI3^R$	$LI10^L$	$GB12^R$	
4	8.3	9.9	11.6*	0.650
10	15.4*	14.2*	10.5*	0.490
16	12.3*	17.8*	13.5*	0.318
22-27	7.0	7.9	13.0*	0.053

Tx5 - $LI4m^{+1}$

Time	Mean % ppt change			p-value
	$KI3^R$	$LI10^L$	$GB12^R$	
4	8.3*	8.9*	7.0*	0.818
10	7.2	8.3	7.0*	0.932
16	10.2*	13.3*	12.7*	0.624
22-27	6.9	7.9	6.8*	0.906

Tx6 - $LI4m^{-21}$

Time	Mean % ppt change			p-value
	$KI3^R$	$LI10^L$	$GB12^R$	
4	7.8	9.8*	7.5	0.841
10	6.7	13.7*	13.7*	0.108
16	7.7	11.6*	12.0*	0.531
22-27	2.7	5.0	8.7*	0.164

Tx7 - $NAPm^{-1}$

Time	Mean % ppt change			p-value
	$KI3^R$	$LI10^L$	$GB12^R$	
4	8.4*	8.3	7.4*	0.949
10	14.1*	8.2	9.6*	0.199
16	12.1*	9.6	9.8*	0.755
22-27	8.5*	7.6	9.6*	0.745

Tx8 - $LI4m^{+21}$

Time	Mean % ppt change			p-value
	$KI3^R$	$LI10^L$	$GB12^R$	
4	14.3*	16.7*	11.1*	0.457
10	11.2*	16.2*	15.3*	0.477
16	12.6*	20.9*	16.3*	0.176
22-27	7.5	9.9	12.1*	0.198

11. Between intervention comparisons for the combined mean % PPT change of the three regional sites ($KI3^R$, $LI10^L$ and $GB12^R$) for each time interval (Section 4.15).

ANOVA
Results for: t=4

```
Factor   Type    Levels  Values
Tx Type  fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
                         17, 18, 19, 20, 21, 22, 23, 24

Analysis of Variance for %ppt change, using Adjusted SS for Tests

Source    DF    Seq SS    Adj SS  Adj MS     F      P
Tx Type    7    3659.2    3659.2   522.7  2.41  0.019
Subject   23   11585.0   11585.0   503.7  2.33  0.000
Error    545  117997.2  117997.2   216.5
Total    575  133241.4
```

Results for: t=10

```
Factor   Type    Levels  Values
Tx Type  fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
                         17, 18, 19, 20, 21, 22, 23, 24


Analysis of Variance for %ppt change, using Adjusted SS for Tests

Source    DF    Seq SS    Adj SS  Adj MS     F      P
Tx Type    7    2623.6    2623.6   374.8  1.50  0.163
Subject   23   17548.7   17548.7   763.0  3.06  0.000
Error    545  135872.2  135872.2   249.3
Total    575  156044.6


S = 15.7895   R-Sq = 12.93%   R-Sq(adj) = 8.13%
```

Results for: t=16

```
Factor   Type    Levels  Values
Tx Type  fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
                         17, 18, 19, 20, 21, 22, 23, 24


Analysis of Variance for %ppt change, using Adjusted SS for Tests

Source    DF    Seq SS    Adj SS  Adj MS     F      P
Tx Type    7    2506.7    2506.7   358.1  1.50  0.163
Subject   23   24389.0   24389.0  1060.4  4.45  0.000
Error    545  129769.8  129769.8   238.1
Total    575  156665.4
```

Results for: t=22

```
Factor   Type    Levels  Values
Tx Type  fixed        8  1, 2, 3, 4, 5, 6, 7, 8
Subject  random      24  1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
                         17, 18, 19, 20, 21, 22, 23, 24


Analysis of Variance for %ppt change, using Adjusted SS for Tests

Source    DF   Seq SS   Adj SS  Adj MS     F      P
Tx Type    7   1317.6   1317.6   188.2  1.45  0.183
Subject   23  15726.8  15726.8   683.8  5.27  0.000
Error    545  70711.8  70711.8   129.7
Total    575  87756.2
```

Appendix VII: An inter-text comparison of the operational definitions of acupoint LI4

Source	Site	Description
Intestine 4 (LI4) Source	Site	Description
Baker and Deadman (2000)	1	"On the dorsum of the hand, between the first and second metacarpal bones, at the midpoint of the second metacarpal bone and close to its radial border." (p 103)
Ellis et al (1989)	2	"In the depression where the index finger and the thumb bones part." (p 39)
Lade (1989)	2	"…between the first and second metacarpals which form a depression…when the thumb is abducted." (p 40)
Mi (1994)	2	"…at the articulation of the forking (metacarpal) bones of the thumb and index fingers."(p 178)
O'Connor and Bensky (1981)	3	"…slightly to the index finger side of the area between the 1st and 2nd metacarpal bones." (p 132)
Rogers and Rogers (1989)	1	"On the dorsum of the hand in the middle of the 2nd metacarpal on the lateral side (thumb side)." (p 12)
Shandong Medical College (1988)	1	"On the middle point of the os metacarpale II, on the prominence of the 1st musculus inter ossei dorsales slightly towards the side of the index [finger]." (p 11)

Comment: While the local anatomy is more than likely to prevent nearby acupoints, namely Large Intestine 3 (LI3) and 5 (LI5) in this case, from being mistakenly needled, there is no guarantee that the structures stimulated at site #1 will be the same as those at site #2 or #3.

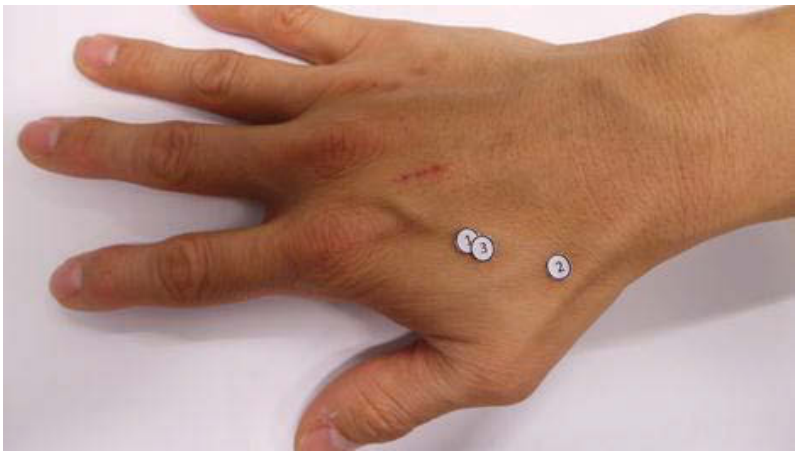


Photo showing the location of LI4 according to the different sources summarised in the above table.

Appendix VIII: Summary of the needling parameters used in the studies that were reviewed in section 2.1

Authors	Condition	Verum Acupuncture	Control/Sham	Manipulation	PPT measured at	Retention Time
Karsts et al 2000	Tension headache	**LI4**, LV3 and other points (Gb14, GB21, Gb41, Bl2 BL10, BL60, LU7, TE5 ST8, ST36, ST44, GV20, EX1) depending on symptoms and local tender muscular points; twice/week for 5 weeks	Blunt needle at GB20	No	On the left and right temporal region	30 mins
Choi et al 2013	Healthy subjects	Cross over design: SP6, ST36, GB39 and SP9 on left leg	Cross over desigh with 3 needling conditions: 1. sperficial, 2. deep, 3. manipulation; 48h washout between each	Manual for intervention 3	In the middle of the 4 acupuncture points on the right leg	5 mins
Mavrommatis et al 2012	Osteoarthritis of the knee	ST36, SP9, SP10, GB34, Ex-LE2, Ex-LE5, **LI4**, KI3, ST40 and SP6 twice/week for 8 weeks	Retractable needles at same points	Electro at the following pairs ST36-SP9 and GB34-SP10 as from week 3	At a specific trigger point on the panful knee	Failed to disclose
He at al 2004	Neck and shoulder pain	Body (LI11, GB31); ear (shenmen, cervical spine, shoulder, shoulder joint, shoulder back) and electro (ExHN, Gb12, BL12, Gv14, SI15, SI14, **LI4**); 3 txs/wk for 3-4 weeks, total of 10 txs	Distal points from actual acupuncture points	Electro	On 5 shoulder muscles namely trapezius, levator scapula, teres minor, sub occipital and supraspinatus	45 mins
Nabeta and Kawakita 2002	Neck and shoulder pain	Tender points, only one tx	Blunt needle on tender points	Manual	Trigger points on shoulder and neck	5 mins
Itoh et al 2011	exercise induced pain	Most tender point on the *extensor digital* muscle (skin and muscle groups and on the *anterior tibial* muscle (non-segmental group); only 1 tx	Lying down with no intervention on a plinth table	None	20 mm distal to the maximum tender point	30 mins
Lang et al 2010	Healthy subjects	Cross over design: SP6, ST36, GB39 and SP9	Cross over desigh with 3 needling conditions: 1. manual acup, 2. low freq electro, 3. high freq electro; 41 week washout between each	Manual for intervention 1 and electro for interventions 2 and 3	Failed to disclose	30 mins
Zhang et al 2011	Plantar fasciitis	PC7; ten daily treatment sessions over 2 week	Acupuncture at LI4	Manual	At the most painful site on the painful foot	30 mins
Hübscher et al 2008	Exercised-induced DOMS in healthy subjects	GB34, LU3, LU5, LI11, SP10 and *ashi* (tender) points; 24 and 48 hours after DOMS were induced	sham acupuncture (superficial needling at nonacupoints), and control (no needling).	Manual	On 7 equidistant points along a line joining the insertion of the biceps brachii on the radius and the acromion	15 mins
Targino et al 2008	Fibromyalgia female patients	Ex-HN-3, bilateral LR3, LI4, PC6, GB34 and SP6; twice per week for 10 weeks	Standard care consisted of 12.5–75 mg of tricyclic antidepressants per day, walking for 30 min twice a week at their own pace, breathe deeply and mental relaxation exercises for another 30 min.	No	At 18 fibromyalgia points across the body	20 mins
Shen and Goddard 2007	Myofascial pain subjects	LI4, single tx	Blunt needle at LI4	Manual	On the right masseter muscle	15 mins
Barlas et al (2000)	Exercised-induced DOMS in healthy subjects	Tx Gp1: PC2, LI11, LU5 LI4; Tx Gp2: 'tender' points on the *biceps brachii*	Control (20 min rest), placebo (minimal needling at four the non-acupuncture points between the biceps brachii and the humerus	Manual	On eight equidistant points along a line joining the insertion of the biceps brachii on the radius and the acromion	20 mins