Nerve-Specific Local and Systemic Analgesic Effects of Acupuncture in Healthy Adults, Measured by Quantitative Sensory Testing (QST)

Alexandra Dimitrova[1], MD, MA, MCR, Dana Colgan, PhD[1], Barry Oken[1], MD, PhD

[1]Department of Neurology, Oregon Health & Science University

Corresponding Author:
Alexandra Dimitrova, MD, MA, MCR
3181 SW Sam Jackson Park Rd
Mail Code CR 120
Portland, OR 97239
tel. 503-418-2124
fax. (503) 494-9520
Email: dimitroa@ohsu.edu

Dana Colgan, PhD
3181 SW Sam Jackson Park Rd
Mail Code CR 120
Portland, OR 97239
tel. 503-494-7339
fax. (503) 494-9520
Email: colgand@ohsu.edu

Barry Oken, MD, PhD
Oregon Health & Science University
3181 SW Sam Jackson Park Road, Mail Code CR120
Portland, OR 97239
tel. (503) 494-9519
fax. (503) 494-9520
email: oken@ohsu.edu

Funding
This study is supported by the National Center for Complementary and Integrative Health (NCCIH) grant NIH K23 AT008405.

Nerve-Specific Local and Systemic Analgesic Effects of Acupuncture in Healthy Adults, Measured by Quantitative Sensory Testing (QST)

Alexandra Dimitrova[1], MD, Dana Colgan, PhD[1], Barry Oken[1], MD, PhD

[1]Department of Neurology, Oregon Health & Science University

ABSTRACT:
OBJECTIVE: This study aims to assess whether acupuncture analgesia's effects are local or systemic and whether there is a dose response for these effects.

METHODS: 28 healthy volunteers aged 18-45 were randomized to 2 doses of acupuncture, using points closely associated with peripheral nerves in the legs. The lower dose group involved acupoints overlying the deep peroneal nerve(DP) and the higher dose – acupoints overlying the deep peroneal and posterior tibial nerves(DPTN). Baseline and post-acupuncture QST assessments were obtained locally in the calf and great toe and systemically at the hand. Results were analyzed using factorial repeated measures ANOVA for each of the QST variables – cold detection threshold (CDT), vibration detection threshold (VDT), heat pain threshold (HP0.5) and heat pain perception of 5/10(HP5.0). Location (leg/arm) and Time(pre/post-acupuncture) were within-subject factors. Intervention (DP/DPTN) was a between-subject factor.

RESULTS: CDT was increased in the calf($p <0.001$) and in the hand($p <0.001$). VDT was increased in the toe($p <0.001$), but not in the hand. HP0.5 was increased in the calf($p <0.001$) and in the hand ($p <0.001$). HP5.0 was increased in the calf($p = 0.002$) and in the hand($p <0.001$), with the local effect being significantly greater than the systemic ($p=0.004$). In all of the above QST modalities there was no difference between the low-dose (DP) and high-dose (DPTN) acupuncture groups.

CONCLUSIONS: Acupuncture caused comparable local and systemic analgesic effects in cold detection and heat pain perception and only local effects in vibration perception. There was no clear acupuncture dose response to these effects.

INTRODUCTION

In the last few decades there has been a surge in acupuncture research, with multiple evidence-based reviews showing therapeutic benefits of acupuncture for chronic low back pain[1, 2] migraine,[3] tension headache,[4] and other pain conditions.[5] This study is based on the structural theory of acupuncture, which attempts to correlate the location of acupuncture points to peripheral nerves, spinal segments, and spinal plexuses.[6-12] The structural theory dates back to the 1980s and suggests that acupuncture's effect is mediated via afferent input through the peripheral nervous system, eliciting a reflex at the level of the spinal cord via the sympathetic plexuses and via efferents to the visceral organs and skeletal muscle.[13, 14] In the case of local needling in close proximity to a nerve, acupuncture's likely mechanism of action is direct mechanical stimulation, such as pressure on the perineural tissues by the needle in manual acupuncture (MA) or electrical current stimulation of the nerve in electroacupuncture (EA).

The purpose of this study is to assess whether higher dose acupuncture consisting of needles placed in proximity to two peripheral nerves in the leg - the deep peroneal and tibial nerve produces greater analgesic effects, compared to lower dose acupuncture consisting of needles placed over a single leg nerve – the deep peroneal. Our past systematic review or acupuncture for neuropathic pain highlighted the benefits of nerve-oriented acupoint selection.[26] In it, we examined treatment regimens and found that all successful trials of acupuncture for neuropathy involved needle placement in close association with a peripheral nerve,[26] such as in the face for Bell's palsy and in the wrist for carpal tunnel syndrome.

Besides assessing dose response, this study aims to characterize and compare local acupuncture analgesic effects close to the needles, to systemic analgesic effects far from the needles, using quantitative sensory testing (QST). Various acupuncture modalities have been shown to cause analgesia in healthy volunteers, measured by standardized sensory testing,[15, 16] however the local versus systemic effects and the dose response of acupuncture analgesia have not been sufficiently addressed. High-frequency electroacupuncture (HF-EA) is considered inhibitory and has been widely used for treating pain,[17] however its analgesic effects have not been widely studied using standardized, validated sensory testing.

QST assessments have been established as an efficient, precise and reproducible method for sensory testing.[18-20] The Computer Aided Sensory Evaluator IV (CASE IV) is an automated system, which delivers precise, standardized, computer-generated stimuli, based on the study of physiologic perception thresholds and "just noticeable difference (JND)" of heat, cold, vibration and touch-pressure.[21, 22] QST has been used with high-frequency electroacupuncture to measure acupuncture-induced changes in sensation.[15]

METHODS

Study Design

This study is a small sample size randomized controlled comparison between two groups of healthy subjects – DP and DPTN. The intervention was high-frequency electroacupuncture (HF-EA). The QST outcome variables were assessed at baseline and 20 minutes after onset of HF-EA. QST measurements were assessed in the anterolateral calf and foot (local effects) and in the hand (systemic effects). The specific QST outcome measures were: vibration detection threshold (VDT), cold detection threshold (CDT), heat pain detection threshold (HP0.5) and heat stimulation producing an intermediate level, 5, on a 10-point pain scale (HP5.0).

Research volunteers were recruited from Oregon Health and Science University (OHSU)'s Study Participation Opportunities website. The study's recruitment advertisement, research protocol, consent form and all other related materials were approved by OHSU's Institutional Review Board. Healthy male and female volunteers, ages 18-45 were screened via a telephone interview. The exclusion criteria aimed to exclude those unable to tolerate QST; those with contraindications to acupuncture and with chronic pain conditions (**Table 1**). Volunteers who were determined eligible were provided with additional information and scheduled for the study, which consisted of a single 2.5-3 hour session. Upon arrival to the lab, the principal investigator answered all questions and obtained informed consent. Following enrollment, the subjects underwent baseline QST testing in the leg and arm as follows:

Baseline Quantitative Sensory Testing (QST) Measurements

QST measurements were performed using the Computer-assisted Sensory Evaluation (CASE) IV system (WR Electronics, Minneapolis, MN, USA), as follows:

Vibratory Detection Threshold (VDT)

Vibration stimuli were delivered as 25 discrete levels ranging from 0.0 to 350 micrometers (μm) of displacement, based on previously established "Just Noticeable Difference" (JND) values.[21, 22] Each stimulus was presented with an exponential onset, and turned off with an exponential decay, in order to eliminate the touch-pressure artifact, which is caused by an instantaneous on/off. Stimulation in the leg was delivered at the tip of the right great toe and in the hand - at the fingernail bed of the right index finger **(Figure 1).**

Cold Detection Threshold (CDT)

The CASE IV thermal stimulator uses a four-degree-per-second ramp up and down and is operated in a range from 8-50°C, with an accuracy of 0.25 to 1.25°C, depending on temperature. It is used both for heating and cooling stimuli. For high-magnitude thermal (cooling) stimuli, the absolute temperature is limited to 8°C. Thermal stimulation in the leg was delivered at the proximal anterior calf **(Figure 1)** and in the arm, at the mid-dorsum of the hand.[21 22]

The 4-2-1 stimulus presentation algorithm for VDT and CDT assessment

Subjects were tested using the 4-2-1 stimulus presentation algorithm as developed by Dyck et al.[23] Testing began at an intermediate level (level 13 of 25 JND units). The stimulus was increased (if not felt) or decreased (if felt) by four steps to the point of <u>turnaround</u> (felt at the higher level when not felt at lower levels, or not felt at the lower level when it had been felt at the higher level). After the first turnaround, stepping was in steps of two JND. After the second turnaround, stepping was by steps of one JND. A total of 20 stimulus events were used, with five of them being randomly distributed null stimuli. A positive response (indicating perception) to more than one null stimulus aborted the program. The subject was re-instructed, and the test was re-run. Three consecutive failures (due to spurious answers to null stimuli) indicated that the algorithm could not be used for this subject and the subject was excluded from further participation in the study.

Heat Pain Assessment (HP)

Heat pain was tested using a non-repeating ascending algorithm with interspersed null stimuli. Subjects were instructed to determine when a stimulus begins to feel painful, not merely hot, in order to develop a "pain perception profile."[24] Subjects were asked to rate each presented stimulus on a scale from 0-10, with zero corresponding to any stimulus the subject cannot feel, feels as warm, or merely hot. Subjects rated painful feelings associated with a stimulus on a scale from 1-10, with 1 being the least painful, and 10 being the most. Testing terminated as soon as subjects reported 5/10 pain intensity. Two pain threshold values were calculated: HP0.5 – the onset of pain sensation and HP5.0 - a standardized intermediate level of pain. These values were calculated from a quadratic equation, which was a least-squares-fit of the final zero and all the nonzero responses given by the subject. The difference between the HP5.0 and the HP0.5 (HP 5.0-0.5) was also calculated and was an indicator of how tolerant a subject is to increasingly painful stimuli.[24]

The final baseline values for VDT, CDT, HP0.5 and HP5.0 were obtained by averaging 2 trial measurements. If the 2 trials were more than 20% different, a third trial was run and the average of the 3 trials was used.

Randomization

Following collection of QST baseline values, subjects were randomized to 2 intervention groups: group 1 – bilateral deep peroneal intervention **(DP)** and group 2 – bilateral deep peroneal plus tibial nerve intervention **(DPTN).** Randomization was conducted using a specially designed computer program, which allowed for stratified randomization based on 2x2 categories with one category being sex (M/F) and the other - projected median age split of 31, as normative data shows that sensory threshold vary with sex and age. Specifically, sensory thresholds, measured by QST, tend to rise with age.[21, 25] The order of baseline QST testing – leg vs hand was also randomly assigned, with the computer program assigning 50% of subjects within an intervention group to leg QST first and the other 50% - to hand QST first, in order to minimize QST learning effects.

The outcome assessors were blinded to the randomization. This was accomplished by placing a screen so that the subject's legs were hidden from the outcome assessor.

Acupuncture Intervention:

Following randomization, single-use MAC acupuncture needles (0.22 x 25 mm, TianJin Haing Lim Sou Won Medical Equipment Co, Ltd, South Korea) were placed in the following acupuncture points bilaterally: DP group (over the Deep peroneal nerve) – GB34, ST36, ST41, LR3 (**Figure 1**); DPTN group - over the Deep peroneal and Posterior tibial nerves) - GB34, ST36, ST41, LR3, KD9, SP6, KD3, KD1 (**Figure 1**). Needling was performed by a licensed acupuncturist, in accordance with standard clinical practice. Once the needles were placed, electroacupuncture was delivered to all acupupoints at 100 Hz continuous stimulation, using the Electrostimulator 6c.Pro (Pantheon Research, Venice, CA), for 20 minutes. The stimulus intensity was titrated to a level the subject perceived as moderate stimulation and titrated every 5 minutes as needed to maintain a level of moderate stimulation.

Post-acupuncture QST

Follow-up QST measurements were performed in the same way as baseline, with the acupuncture going, but without further changes in the electrostimulation level of intensity.

Sample Size Estimate

Acupuncture's effect was estimated from the single available related study from the literature, which applied local acupuncture to the leg and measured QST effects in the leg.[15] Lang et al[15] used manual and electro-acupuncture to assess analgesia in the legs, using 24 healthy subjects, 12 men and 12 women, in a cross-over design (6 groups of 4 healthy subjects each). The authors detected a 0.2°C difference in CDT, which was statistically significant. Sample size estimates were generated by a mixed model simulation using Power Analysis and Sample Size (PASS) 14.0 software (NCSS, Kaysville, Utah, USA). 100 simulations were run using a 0.05 Alpha level, a linear up effects pattern, and an estimated minimal detectable difference of 0.2°C in CDT. Simulation results revealed that 12 subjects provided a study power of 0.88 (0.79-0.93). As a conservative over-estimate 14 subjects per group were enrolled.

Statistical Analysis Plan

All analyses were conducted using SPSS version 25.0 (IBM Corp, Armonk, NY). The means and standard deviations (SD) were calculated for five variables on both the hand (or finger) and calf (or toe): VDT, CDT, HP05, HP5.0 and HP5.0-0.5. Baseline group characteristics were compared using independent sample t-tests. Factorial repeated measures analysis of variance (ANOVAs) were computed for each of the five variables independently. For each ANOVA, there were two within-subjects factors: location (two levels: leg, arm) and time (two levels: pre- and post-acupuncture). A between-subjects factor was treatment group (2 levels: DP, DPTN). When a significant interaction was detected, simple effects analyses were conducted to determine the direction of the interaction. A Greenhouse–Geisser correction was used if Mauchly's test showed that sphericity could not be assumed. The Wilcoxon signed-rank test was used for pairwise comparisons. For this exploratory study, results were interpreted according to the level of statistical significance $p \leq 0.05$ and effect size reported as partial eta squared. Since this was a pilot study there was no adjustment for multiple comparisons but p values less than 0.005 would be statistically significant even with conservative adjustments.

RESULTS

At baseline, there were no significant differences in age and sex and in all QST parameters between the 2 treatment groups (**Table 2**). Factorial repeated measures analysis of variance (ANOVA) for cold detection threshold revealed highly significant effect of acupuncture on CDT in the leg (calf) - $p = 0.000007$ and in the arm (hand), $p = 0.000074$ (**Table 3**). There were no significant differences between the low dose (DP) and the high dose (DPTN) treatment groups. Also, there was no significant difference between the CDT effect in the leg compared to its effect in the arm (p=0.437), at both acupuncture doses.

In contrast to cold detection sensation, factorial repeated measures ANOVA for vibration detection threshold revealed only local significant effect, in the toe, $p = 0.00032$ (**Table 3**). There was no significant change in the finger VDT. These findings did not differ by treatment group.

In heat pain detection threshold (HP0.5), similarly to CDT, acupuncture had significant analgesic effect both in the leg (calf) - $p = 0.000031$ and in the arm (hand), $p = 0.000205$ (**Table 3**). Again, there were no significant differences between the low dose (DP) and the high dose (DPTN) groups. There was no significant difference between the HP0.5 analgesic effect in the leg, compared to the arm (p=0.883), at either acupuncture dose.

Standardized intermediate pain perception (HP5.0) represented subject-reported pain level of 5/10 on the Visual Analog Scale (VAS). In HP5.0, acupuncture again showed significant analgesic effect, with significant increase in the HP5.0 in the leg (calf) - $p = 0.002$ and in the arm (hand), $p = 0.000290$ (**Table 3**). In addition, the analgesic effect on HP5.0 was significantly greater locally in the leg, compared to systemically in the hand (p=0.004). There was no difference between the low dose (DP) and the high dose (DPTN) treatment groups.

HP5.0-0.5 represented the subject's tolerance to increasingly painful stimuli, by measuring the sensory span of pain perception, from pain onset defined as 1/10 on the VAS to standardized intermediate pain defined as 5/10 on the VAS. This was a simple subtraction of HP5.0-HP0.5 for each subject. In terms of pain perception spans, there was a rightward shift towards higher heat pain sensory threshold levels (HP0.5) and higher intermediate pain (HP5.0) levels in both treatment groups in the arm and in the leg (**Figure 2**). The sensory spans in the arm shifted to the right from the already reported higher HP0.5 and HP5.0 values but the spans were not significantly changed with acupuncture. In the leg, locally, acupuncture caused an extended pain perception span, $p = 0.002$ (**Table 3**). There was a trend towards significance in the comparison of the leg vs the arm pain perception span (p=0.06). There was no difference between the low dose (DP) and the high dose (DPTN) treatment groups.

DISCUSSION

Early acupuncture research suggested that its analgesic effects are mediated by the endogenous opioid system.[27-29] It is well established that acupuncture increases cerebrospinal fluid levels of endorphins, enkephalins, and adrenocorticotropic hormone and its effect can be blocked by the endorphin antagonist naloxone.[27, 29] Unfortunately these early studies did compare local to systemic analgesic effects, or build a strong case for point specificity, or explore dose response. This study aims to address some of these gaps by characterizing both local and systemic analgesic effects and dose response.

Local vs Systemic Analgesia in Acupuncture Analgesia

This study found that acupuncture's effects on VDT are local. In contrast, the analgesic effects on CDT, heat pain detection threshold (HP0.5) and intermediate level pain perception (HP5.0) were both local and systemic. Additionally, there was a significant difference in intermediate level pain perception (HP5.0) and in the tolerance to increasingly painful stimuli (HP5.0-0.5) locally compared to systemically. The findings of similar local and systemic responses to heat pain and cold perception are not surprising, as pain and temperature information is processed somewhat similarly by the nervous system. Pain and temperature sensation involves thermoreceptors and nociceptors and travels in the spinothalamic tracts.[30] Acupuncture's likely mechanism of action, in addition to release of endogenous opioids, is activation of heat- and cold-sensitive C-fibers and cold-sensitive A-delta fibers. [31,32,33] Needles placed in close proximity to a nerve stimulate the nerve fibers directly, via an electric current or a physical stimulus from manual acupuncture, with or without additional needle manipulation.

While an increasing number of studies have assessed various aspects of acupuncture analgesia using QST or other standardized measures of sensation, Lang et al[15] remains the single known study to-date to employ continuous HF-EA. Similar to our findings, the authors reported that HF-EA affects cold detection threshold locally and systemically, although they tested the contralateral leg as the systemic QST location. We chose the hand as control, because it makes a better case for systemic effect, as hand sensory changes cannot be attributed to spinal segmental level changes. In contrast to our findings, Lang et al found only local effect on heat pain threshold (HP0.5 equivalent). Other findings included local effect on mechanical pain threshold and both local and systemic effects on pressure pain threshold, measured by algometer. One possibility to explain the greater systemic effects observed in this study is the nerve-specific point selection. Another is the greater number of needles used and the bilateral needle placement, which are addressed later.

In contrast to HF-EA, manual acupuncture (MA) and low-frequency electroacupuncture have been used more extensively with sensory testing. Studies vary widely in needle placement, methods of sensory testing (QST, pressure algometer, electric pain, etc) and testing location. While these varied methodologies make for difficult comparisons, other studies have also found local and systemic analgesic effects of acupuncture. In one study, 5-minunte administration of either MA or mixed high/low-frequency electroacupuncture in the arm were found to increase the pressure pain threshold in the foot, during and immediately post-acupuncture.[34] Electroacupuncture (EA) had significantly greater analgesic effect during acupuncture, but not immediately after, when the effects of EA and MA were comparable.

In a different study, MA or a combination of 2Hz/100Hz mixed frequency EA were applied unilaterally in the left leg for 30 min and 13 different QST modalities were tested post-acupuncture in both legs and arms. The authors found only changes in the pressure pain threshold locally in the left leg and only with EA. They attributed this change to segmental inhibition at the level of the spinal cord and saw a trend toward pressure pain threshold changes in the contralateral leg. They also suggested a different mechanism of action for EA compared to MA. Four acupoints were chosen on the basis of being frequently used for pain. In our study, we also used 4 acupoints in the lower dose group (DP), but our point selection was based on proximity to a large peripheral nerve. We also applied acupuncture bilaterally and kept acupuncture going during the second QST assessment. This may explain why we observed a greater, multi-modality QST change both locally and systemically. There is some evidence that longer periods of EA produce greater heat-pain analgesia and the effect extends to the contralateral leg and thigh.[35]

A recent comprehensive review/meta-analysis on acupuncture's effect on sensory thresholds concluded that acupuncture affects various sensory thresholds, with the strongest evidence for effect on pressure pain threshold and moderate evidence for heat pain and cold pain detection.[16] There was some evidence that acupuncture affects pin-prick-like pain and lastly, the authors found no evidence that acupuncture affects vibration detection and mechanical detection thresholds. Interestingly, some studies suggested that sham acupuncture may cause changes in sensory thresholds as well.[5, 36, 37] Even though few of the included studies addressed local vs systemic analgesic effects, the authors observed that the analgesic effects of acupuncture were stronger locally and ipsilaterally, compared to contralaterally or at more remote body sites, invoking spinal segmental inhibition as a likely mechanism. We applied acupuncture bilaterally to achieve a more powerful acupuncture effect and assumed that the sensory threshold changes in both legs were identical and tested only one leg in the interest of time. As our systemic testing occurred in the hand, we cannot attribute our findings to spinal segmental effects and feel that a higher central mechanism must be invoked to explain this finding. Further imaging and electrophysiologic studies could help characterize acupuncture's analgesic effect on pain processing areas in the rostral spinal cord or brain.

Acupuncture and Dose Response

To-date, there is no clear concept of an appropriate dose of acupuncture and how much treatment is needed for a given medical condition.[38] The relationship between number of needles and acupuncture effect is also unclear and is probably not linear. Common standardization problems with acupuncture research and practice include varied point selection, number of needles used, needle retention time, needling depth, amount of needle manipulation, and adjunct use of moxibustion and EA. Acupoint selection for this study was based on our systematic review of acupoint selection in successful trials of peripheral neuropathy[26] and on the standardized nerve-specific protocol for the treatment of peripheral neuropathy, which was based on this review and on clinical observations.[39] The selected acupoints, even though they were based on anatomical correlations with the underlying nerves, are frequently used for pain and neurologic conditions such as numbness and paralysis.

Surprisingly, we found no differences between the DP and DPTN treatment groups. Both caused powerful analgesic effects and this may be partly due to the fact that continuous HF-EA was administered to all points bilaterally. Even at the lower dose subjects underwent continuous stimulation at 8 different acupoints in the lower legs (4 per leg). It is possible that even the DP group received a relatively high dose of acupuncture. However, there is no way to make meaningful comparison to other studies using QST as outcomes, since they do not use the same acupoints. Lang et al used 4 points in one leg[15] and other studies, which showed systemic analgesic effect used only 2 needles in the one arm[33] or leg.[34, 35]

Adverse Events

No adverse events were observed in the course of this study and there were no drop-outs. Even though the study consisted of a single session, subjects were provided contact information to report any problems, concerns or other issues, of which there were none. During the study session, all subjects tolerated acupuncture well, without significant discomfort, serious bruising or bleeding.

Study Limitations

While QST has been shown to be reproducible, reliable[18] and has been widely used in research and clinical practice, the validity of QST results is highly dependent on the subject's ability to cooperate and sustain attention on stimulus presentation. Therefore, QST cannot be administered when the patient is inattentive or uncooperative. We adjusted our exclusion criteria to select for subjects who will not have any issues complying with QST assessment and followed standard QST administration procedures. This study was focused on local and systemic QST outcomes and did not pursue any central mechanisms of pain mediation, including possible placebo and expectancy effects. This approach is somewhat reductionistic as it is well established that positive expectancy increases acupuncture's therapeutic potential.[40, 41] Subjects were not tested on their attitudes towards and expectations of acupuncture, as this study was exploratory and aimed to assess whether acupuncture's analgesic effect is dose-specific and local vs systemic. Future studies will need to factor in various psychological aspects of pain perception, including tolerance and habituation.

This study compared two acupuncture regimens, based on anatomical associations with large peripheral nerves. The structural theory of acupuncture postulates that acupuncture is mediated via large peripheral nerves eliciting a reflex at the level of the spinal cord and engages the autonomic nervous system. While there is consensus that the nervous system is vital for processing the effects of acupuncture, the neurophysiologic testing to support these theories is lacking so far. The stronger local effects observed in vibration detection and in intermediate heat pain perception (HP5.0) suggest that the observed analgesic effects cannot be simply attributed to the endogenous opioid system. A direct measurement such as by microneurography in conjunction with acupuncture may shed light on the acupuncture needle effects on the underlying nerve.

All measurements were conducted in a single study session, which does not address important clinical questions about the duration of acupuncture analgesia and acupuncture washout effects. Further studies should include additional QST measurements, spaced over time. Furthermore, as this was a mechanistic study focused on dose response, if did not include a placebo acupuncture group or another form of control. Future studies should incorporate sham acupuncture, including sham-electroacupuncture in acupuncture-naïve subjects.

CONCLUSIONS

This study revealed that high-frequency electroacupuncture administered to the legs bilaterally causes local changes in vibration detection threshold and both local and systemic changes in cold detection threshold, heat pain detection threshold and intermediate level pain perception. In heat pain, there is significantly stronger local effect of acupuncture on intermediate level pain perception (HP5.0) and on the tolerance to increasingly painful stimuli (HP5.0-0.5). This study's findings are more robust compared to prior similar studies, in terms of multiple QST modalities affected by acupuncture and the findings of numerous systemic analgesic effects. The strong analgesic effects observed here could be attributed to the selection of acupoints with close anatomic associations to the peripheral nerves in the leg. Acupuncture analgesia is likely mediated by direct needle effects on the underlying nerves, although further studies are needed to elucidate this mechanism and the presence of systemic effects necessitates further studies of acupuncture's effect on the central nervous system.

REFERENCES:

1. Manheimer E, White A, Berman B, Forys K, Ernst E. Meta-analysis: acupuncture for low back pain. *Ann Intern Med.* Apr 19 2005;142(8):651-663.
2. Furlan AD, van Tulder M, Cherkin D, et al. Acupuncture and dry-needling for low back pain: an updated systematic review within the framework of the cochrane collaboration. *Spine (Phila Pa 1976).* Apr 15 2005;30(8):944-963.
3. Linde K, Allais G, Brinkhaus B, Manheimer E, Vickers A, White AR. Acupuncture for migraine prophylaxis. *Cochrane Database Syst Rev.* 2009(1):CD001218.
4. Linde K, Allais G, Brinkhaus B, Manheimer E, Vickers A, White AR. Acupuncture for tension-type headache. *Cochrane Database Syst Rev.* 2009(1):CD007587.
5. Vickers AJ, Cronin AM, Maschino AC, et al. Acupuncture for chronic pain: individual patient data meta-analysis. *Arch Intern Med.* Oct 22 2012;172(19):1444-1453.
6. Dung HC. Acupuncture points of the cranial nerves. *Am J Chin Med.* Summer 1984;12(1-4):80-92.
7. Dung HC. Acupuncture points of the cervical plexus. *Am J Chin Med.* Summer 1984;12(1-4):94-105.
8. Dung HC. Acupuncture points of the brachial plexus. *Am J Chin Med.* 1985;13(1-4):49-64.
9. Dung HC. Acupuncture points of the lumbar plexus. *Am J Chin Med.* 1985;13(1-4):133-143.
10. Dung HC. Acupuncture points of the sacral plexus. *Am J Chin Med.* 1985;13(1-4):145-156.
11. Dung HC. Acupuncture points of the typical spinal nerves. *Am J Chin Med.* 1985;13(1-4):39-47.
12. Bossy J. Morphological data concerning the acupuncture points and channel network. *Acupunct Electrother Res.* 1984;9(2):79-106.
13. Cheng K. Neuroanatomical basis of acupuncture treatment for some common illnesses. *Acupunct Med.* 2009;27(2):61-64.
14. Cheng K. Neuroanatomical characteristics of acupuncture points: relationship between their anatomical locations and traditional clinical indications. *Acupunct Med.* 2011;29(4):289-294.
15. Lang PM, Stoer J, Schober GM, Audette JF, Irnich D. Bilateral acupuncture analgesia observed by quantitative sensory testing in healthy volunteers. *Anesth Analg.* May 1 2010;110(5):1448-1456.
16. Baeumler PI, Fleckenstein J, Takayama S, Simang M, Seki T, Irnich D. Effects of acupuncture on sensory perception: a systematic review and meta-analysis. *PloS one.* 2014;9(12):e113731.
17. Aung SKH, Chen WPD. *Clinical Introduction to Medical Acupuncture*: Thieme; 2007.
18. Geber C, Klein T, Azad S, et al. Test-retest and interobserver reliability of quantitative sensory testing according to the protocol of the German Research Network on Neuropathic Pain (DFNS): a multi-centre study. *Pain.* Mar 2011;152(3):548-556.
19. Shy ME, Frohman EM, So YT, et al. Quantitative sensory testing: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology.* Mar 25 2003;60(6):898-904.
20. Dyck PJ, Kratz KM, Lehman KA, et al. The Rochester Diabetic Neuropathy Study: design, criteria for types of neuropathy, selection bias, and reproducibility of neuropathic tests. *Neurology.* Jun 1991;41(6):799-807.
21. Dyck PJ, Zimmerman IR, O'Brien PC, et al. Introduction of automated systems to evaluate touch-pressure, vibration, and thermal cutaneous sensation in man. *Ann Neurol.* Dec 1978;4(6):502-510.
22. O'Brien PC, Dyck PJ, Kosanke JL. A computer evaluation of quantitative algorithms for measuring detection thresholds of cutaneous sensation. In: Munsat, ed. *Quantification of Neurologic Deficit.* Boston: Butterworth-Heinemann; 1989:197-206.
23. Dyck PJ, O'Brien PC, Kosanke JL, Gillen DA, Karnes JL. A 4, 2, and 1 stepping algorithm for quick and accurate estimation of cutaneous sensation threshold. *Neurology.* Aug 1993;43(8):1508-1512.
24. Dyck PJ, Zimmerman IR, Johnson DM, et al. A standard test of heat-pain responses using CASE IV. *Journal of the neurological sciences.* Mar 1996;136(1-2):54-63.
25. Magerl W, Krumova EK, Baron R, Tolle T, Treede RD, Maier C. Reference data for quantitative sensory testing (QST): refined stratification for age and a novel method for statistical comparison of group data. *Pain.* Dec 2010;151(3):598-605.
26. Dimitrova A, Murchison C, Oken B. The Case for Local Needling in Successful Randomized Controlled Trials of Peripheral Neuropathy: A Follow-Up Systematic Review. *Medical acupuncture.* Aug 1 2018;30(4):179-191.
27. Pomeranz B, Cheng R. Suppression of noxious responses in single neurons of cat spinal cord by electroacupuncture and its reversal by the opiate antagonist naloxone. *Exp Neurol.* May 1979;64(2):327-341.
28. Cheng RS, Pomeranz B. Electroacupuncture analgesia could be mediated by at least two pain-relieving mechanisms; endorphin and non-endorphin systems. *Life Sci.* 1979 25(23):1957-1962.

29. Cheng RS, Pomeranz B. A combined treatment with D-amino acids and electroacupuncture produces a greater analgesia than either treatment alone; naloxone reverses these effects. *Pain.* 1980;8(2):231-236.
30. Waldman SD. *Pain Review*: Saunders/Elsevier; 2009.
31. LaMotte RH, Campbell JN. Comparison of responses of warm and nociceptive C-fiber afferents in monkey with human judgments of thermal pain. *Journal of neurophysiology.* Mar 1978;41(2):509-528.
32. Yarnitsky D, Ochoa JL. Warm and cold specific somatosensory systems. Psychophysical thresholds, reaction times and peripheral conduction velocities. *Brain : a journal of neurology.* Aug 1991;114 (Pt 4):1819-1826.
33. Verdugo R, Ochoa JL. Quantitative somatosensory thermotest. A key method for functional evaluation of small calibre afferent channels. *Brain : a journal of neurology.* Jun 1992;115 (Pt 3):893-913.
34. Schliessbach J, van der Klift E, Arendt-Nielsen L, Curatolo M, Streitberger K. The effect of brief electrical and manual acupuncture stimulation on mechanical experimental pain. *Pain medicine (Malden, Mass.).* Feb 2011;12(2):268-275.
35. Leung AY, Kim SJ, Schulteis G, Yaksh T. The effect of acupuncture duration on analgesia and peripheral sensory thresholds. *BMC complementary and alternative medicine.* May 1 2008;8:18.
36. Irnich D, Salih N, Offenbacher M, Fleckenstein J. Is sham laser a valid control for acupuncture trials? *Evidence-based complementary and alternative medicine : eCAM.* 2011;2011:485945.
37. Linde K, Niemann K, Schneider A, Meissner K. How large are the nonspecific effects of acupuncture? A meta-analysis of randomized controlled trials. *BMC medicine.* Nov 23 2010;8:75.
38. White A, Cummings M, Barlas P, et al. Defining an adequate dose of acupuncture using a neurophysiological approach--a narrative review of the literature. *Acupunct Med.* Jun 2008;26(2):111-120.
39. Dimitrova A. Introducing a Standardized Acupuncture Protocol for Peripheral Neuropathy: A Case Series. *Medical acupuncture.* Dec 1 2017;29(6):352-365.
40. Prady SL, Burch J, Vanderbloemen L, Crouch S, MacPherson H. Measuring expectations of benefit from treatment in acupuncture trials: a systematic review. *Complementary therapies in medicine.* Apr 2015;23(2):185-199.
41. Kong J, Kaptchuk TJ, Polich G, et al. An fMRI study on the interaction and dissociation between expectation of pain relief and acupuncture treatment. *NeuroImage.* Sep 2009;47(3):1066-1076.

Table 1. Study Exclusion Criteria

Contraindications for Electroacupuncture • Pregnancy • Coagulopathy/ current anti-coagulation treatment • Presence of an implantable electronic device such as a cardiac pacemaker/defibrillator, deep brain stimulator or vagus nerve stimulator
Inability to Participate in QST • Significant cognitive impairment interfering with alertness and/or attention such as attention deficit/hyperactivity disorder or uncontrolled anxiety • Hospitalization for anxiety or depression in the past 3 months • Presence of a psychiatric diagnosis other than anxiety or depression • Change of psychoactive medication such as anti-depressants in the last 3 months • Use of any investigational drugs within the previous six months • Marijuana use in the past week • Other Illicit drug use in the past month • Current alcohol abuse (> 2 drinks/day)
Impaired Pain Perception • Presence of chronic pain condition such as neck pain, low back pain, etc. • Presence of neuropathy, other sensory impairment or other neurologic conditions • Use of opioids, benzodiazepines or other sedating medications

Table 2. Group Baseline Characteristics

Group Makeup and Baseline QST values	DP Group	DPTN Group	Significance
Average Age	26.79	26.57	0.909*
Sex			
Women (%)	11 (78.6%)	10 (71.4%)	
Men (%)	3 (21.4%)	4 (28.6%)	0.190**
Leg QST	Mean (SD)	Mean (SD)	
Baseline Calf CDT	9.53 (1.96)	9.50 (2.76)	0.973*
Baseline Toe VDT	9.75 (2.04)	9.83 (2.00)	0.916*
Baseline Calf HP 0.5	19.72 (1.76)	19.98 (1.72)	0.688*
Baseline Calf HP 5.0	23.66 (1.88)	24.45 (2.05)	0.292*
Baseline Calf HP 5.0-0.5	4.09 (1.05)	4.40 (1.19)	0.466*
Arm QST			
Baseline Hand CDT	5.13 (0.97)	5.46 (1.62)	0.516*
Baseline Finger VDT	8.83 (1.81)	8.20 (1.74)	0.358*
Baseline Hand HP 0.5	18.38 (3.13)	18.32 (1.71)	0.945*
Baseline Hand HP 5.0	23.74 (1.88)	24.04 (1.99)	0.687*
Baseline Hand HP 5.0-0.5	5.32 (2.34)	5.73 (2.11)	0.636*

DP = deep peroneal nerve, DPTN = deep peroneal and tibial nerve
*independent samples t-test; ** chi-square test

Figure 1. Acupuncture Points, QST probes placement

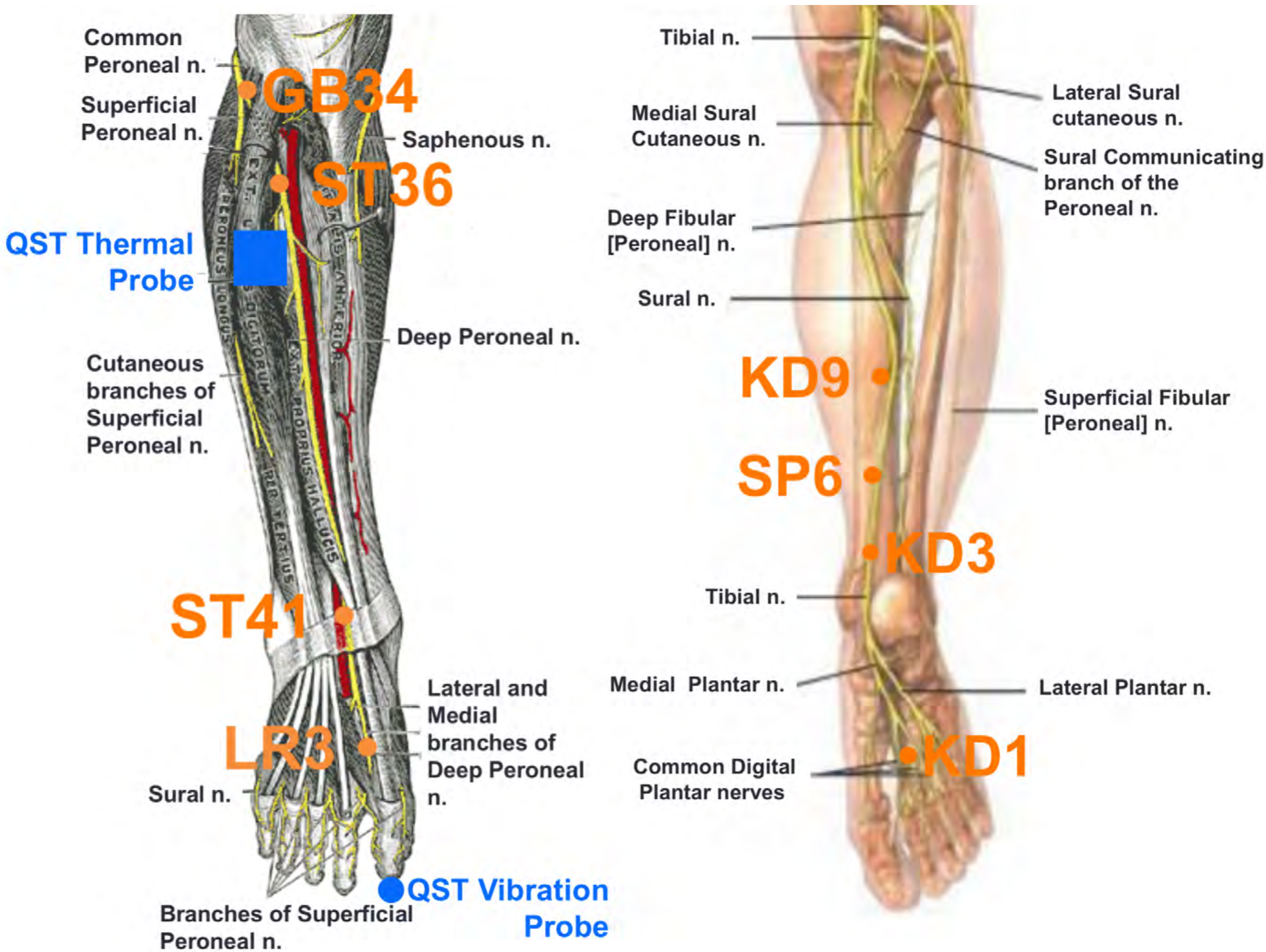
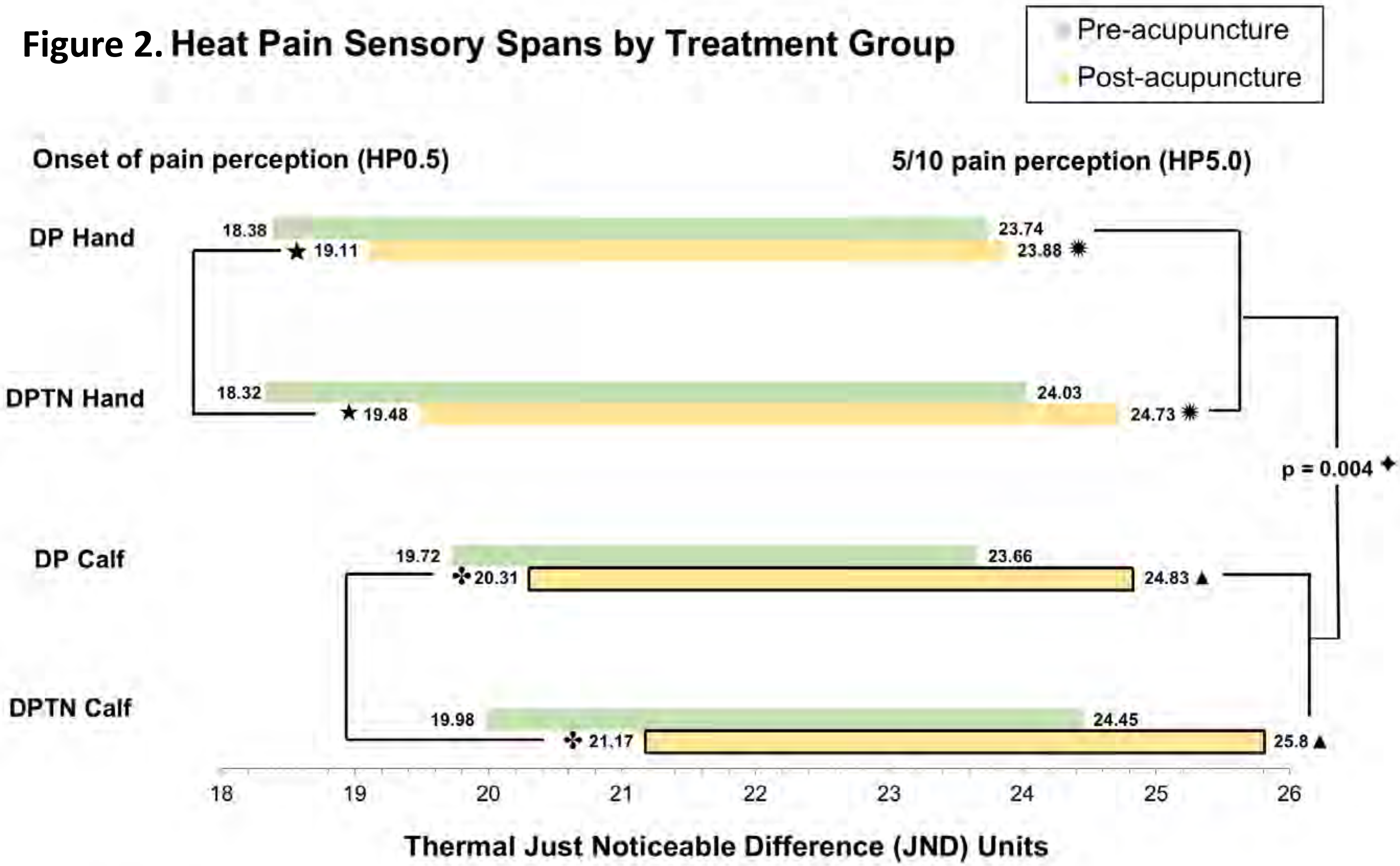


Table 3. Quantitative Sensory Testing (QST) data

QST Modality	Descriptive Statistics: Leg (JND units) DP Group Mean (SD)	Leg (JND units) DPTN Group Mean (SD)	Arm (JND units) DP Group Mean (SD)	Arm (JND units) DPTN Group Mean (SD)	Pre/Post Acupuncture Effects: ANOVA Factor Leg	ANOVA Interaction Leg x Group	ANOVA Factor Arm	ANOVA Interaction Arm x Group	ANOVA Interaction Arm x Leg	ANOVA Interaction Arm x Leg x Group
CDT Pre Post	9.53 (1.96) 10.44 (2.59)	9.50 (2.76) 10.39 (2.47)	5.13 (0.97) 6.47 (2.10)	5.46 (1.62) 6.61 (2.74)	F 162.33 **p <0.00001***	F 0.20 p 0.67	F 22.08 **p 0.00007**	F 0.05 p 0.82	F 0.62 p 0.44	F 0.03 p 0.86
VDT Pre Post	9.75 (2.04) 9.71 (2.25)	9.83 (1.99) 10.04 (1.98)	8.83 (1.81) 8.97 (1.97)	8.20 (1.73) 8.24 (1.74)	F 17.14 **p 0.0003**	F 2.05 p 0.16	F 0.43 p 0.52	F 0.08 p 0.79	F < 0.001 p 0.99	F 0.61 p 0.44
HP 0.5 Pre Post	19.72 (1.76) 20.31 (1.41)	19.98 (1.72) 21.17 (1.52)	18.38 (3.12) 19.11 (2.08)	18.32 (1.71) 19.48 (1.59)	F 25.29 **p 0.00003**	F 0.50 p 0.49	F 18.62 **p 0.0002**	F 1.46 p 0.24	F 0.02 p 0.88	F 0.04 p 0.84
HP 5.0 Pre Post	23.66 (1.88) 24.83 (1.38)	24.45 (2.05) 25.80 (2.27)	23.74 (1.88) 23.88 (1.63)	24.04 (1.99) 24.73 (1.90)	F 12.54 **p 0.002**	F 0.90 p 0.35	F 17.49 **p 0.0003**	F 0.82 p 0.37	F 9.68 **p 0.004**	F 0.48 p 0.50
HP 5.0-0.5 Pre Post	4.09 (1.05) 4.51 (1.33)	4.40 (1.18) 4.56 (1.42)	5.32 (2.34) 4.80 (1.69)	5.73 (2.11) 5.22 (1.51)	F 12.00 **p 0.002**	F 0.21 p 0.65	F 0.35 p 0.56	F 0.11 p 0.74	F 3.88 p 0.06	F 0.16 p 0.70

JND = just noticeable difference; CDT = cold detection threshold; VDT = vibration detection threshold; HP 0.5 = onset of pain sensation; HP 5.0 = pain 5/10 intensity; HP 5.0-0.5 = range of pain sensation from 1/10 to 5/10 on visual analog scale. *p = 0.000007

Figure 2. **Heat Pain Sensory Spans by Treatment Group**



★ $p < 0.001$ = effect of acupuncture on HP0.5 in the hand, no difference between DP and DPTN;

✣ $p < 0.0001$ = effect of acupuncture on HP0.5 in the leg, no difference between DP and DPTN;

✷ $p < 0.001$ = effect of acupuncture on HP5.0 in the hand, no difference between DP and DPTN;

▲ $p = 0.02$ = effect of acupuncture on HP5.0 in the leg, no difference between DP and DPTN;

✦ $p = 0.004$ = greater effect of acupuncture on HP5.0 in the leg compared to its effect on HP5.0 in the hand, no difference between DP and DPTN.

☐ expanded heat pain sensory span in the leg compared to before acupuncture, no difference between DP and DPTN, $p = 0.002$