Julius S, Shiau D. Comparison of electro-acupuncture frequency and lead placement changes and vascular perfusion of tissue.
Am J Trad Chin Vet Med 2026; 21(1):29-38. https://doi.org/10.59565/001c.154713

Clinical Studies

Comparison of Electro-acupuncture Frequency and Lead Placement Changes and Vascular Perfusion of Tissue

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ABSTRACT
The objective of this study was to assess whether different lead placement patterns and frequencies (measured in hertz, Hz) in electro-acupuncture (EAP) significantly influence tissue vascular perfusion. Thirty-six clinically healthy dogs from a local dog sport community were enrolled in the study. Each subject received 6 EAP treatments, and a 7th treatment which was control (no needle placement). A total of 10 needles were placed at Back-*shu* points (BL-20 through BL-24) bilaterally for 20 minutes. The 6 EAP techniques included lead placement that was contralateral (BL-20 left side to BL-20 right side) or ipsilateral (BL-20 left side to BL-24 left side) and frequency settings at 20, 120, and 80-120 Hz. For each subject, the treatment order was randomized with a 2-week washout period between treatments to avoid carry-over effect. Pre- and post-acupuncture skin temperature changes, measured by infrared thermography, were compared among the 7 treatments. All EAP treatments resulted in significant skin temperature increase ($p<0.05$). No significant temperature change resulted from the no-treatment control ($p=0.983$). The "EAP 20 Hz contralateral" stimulation had significantly greater temperature increase than the other acupuncture treatment methods, with greatest to lowest temperature increase as follows: 20 Hz contralateral, 80-120 Hz ipsilateral, 20 Hz ipsilateral, 80-120 Hz contralateral, 120 Hz contralateral, 120 Hz ipsilateral. Study results indicate that both pattern of lead placement and the frequency used in EAP affect tissue perfusion. These findings are unique and could help shape best practices for selecting EAP frequency and lead placement in future applications.

Keywords: canine, electro-acupuncture frequencies, hertz, thermography, tissue perfusion

ABBREVIATIONS: A-α: A-alpha; A-β: A-beta; AP: acupuncture; A-δ: A-delta; CGRP: calcitonin gene related peptide; EAP: electro-acupuncture; EOP: endogenous opioid peptides; Hz: hertz; IRT: infrared thermography; *κ*: kappa receptors; LP: lead placement; ROI: region of interest

Acupuncture (AP) has been studied for its effects on endogenous substances (e.g. endorphins, serotonin, cortisol, enkephalins), promotion of vasodilation, and tissue temperature increases which are associated with pain reduction through a number of mechanisms of action.[1,2] The vasodilation with subsequent increased tissue perfusion and tissue temperature increase is of particular interest, as nociception (i.e. perception of pain) is closely related to vascular perfusion of tissue.[3] The physiological effect of increasing tissue temperature, similar to local AP effects, has been shown to provide a sedative and analgesic effect, increase vasodilation/circulation, increase relaxed soft tissue extensibility, and provide relaxation of muscle spasms.[4] In addition, sensory and motor nerve conduction velocity can be increased with increases in local tissue temperature, resulting in reduced pain and muscle spasm. Increases in local temperature as low as 1.8 °F (1 °C) have been shown to increase nerve conduction velocity by approximately 2 m/sec.[4]

Acupuncture points are located in areas of low electrical resistance and high electrical conductivity.[5-7] Through histological studies, it has been shown they exist in concentrated areas of free nerve endings, arterioles, lymphatic vessels, and aggregations of mast cells.[1] In dogs, there are approximately 360 acupoints located throughout the body, and in general, their effects can be divided into local, segmental (spinal), and suprasegmental (brain). Local effects start immediately at the site of needle insertion. This includes release of biochemical substances as collagen fibers wrap around the needle stimulating local nerve endings and vasculature. The needle microtrauma

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activates release of Hageman's Factor XII, which in turn activates the local clotting and compliment cascades, leading to the production of plasminogens, protein kinins and prostaglandins; along with mast cell degranulation which creates release of histamine, heparin, proteases and bradykinin. This rapid sequence of events following needle insertion, enhances local tissue immune status, is associated with vasodilation and creates smooth muscle relaxation (reduced muscle spasms).[8-10] Detailed vasoactive effects of acupuncture have been described as an initial short vasoconstriction phase of 15-30 seconds; then a quasi-control state that lasts approximately 10 seconds to 2 minutes; and finally, a vasodilation phase, which lasts 2 minutes to 2 weeks.[8]

Electro-acupuncture (EAP) is a type of acupuncture method that uses the movement of pulse electrical current to stimulate the body tissue by way of pairs of acupuncture needles. The treatment has been used frequently in integrative veterinary clinics (i.e. traditional Chinese veterinary medicine + conventional Western medicine) with various settings, which include needle placement, signal frequency (hertz, Hz), and signal intensity. The electrical stimulation was designed to mimic the movements of manual manipulation of needles but is able to amplify the stimulation and provides more consistency. The enhanced stimulation associated with EAP includes neurotransmitter release, activation of the endorphin system, modulation of neural pathways, stem cell release and an increase in blood circulation.[11]

Differences in frequency and intensity have been studied to determine the appropriate levels for EAP stimulation while maintaining patient comfort. Effects on A-delta (A-δ), A-beta (A-β), A-alpha (A-α), and C fibers have been investigated and established. Based on these findings, suggested frequencies in Hz have been proposed by EAP researchers and practitioners. The A-delta fibers are predominantly stimulated by low-frequency (1-20 Hz) EAP, resulting in release of endogenous opioid peptides (EOP), β-endorphins and met-enkephalins from the CNS.[12] Dynorphin, another EOP, is released from the spinal cord via kappa (κ) receptors, along with predominant C fiber stimulation, with higher-frequency (100 Hz) EAP. Stimulation of serotonergic fibers and release of serotonin and norepinephrine, which are co-localized with EOP, occur at very high-frequencies (200 Hz), and are released from the spinal cord and brain (Table 1).[3,11,13-17]

Despite suggestions on treatment protocols related to A-delta fibers (myelinated, fast sharp pain sensation) and C-nerve fibers (unmyelinated, dull slow pain), practitioners remain divided on treatment protocols for pain.[3,4,11,13] An informal social media survey of 130 integrative veterinary practitioners conducted by the first author (SJ) (unpublished data) found that there was significant debate on what appropriate frequency setting and lead placement (LP) are when applying EAP treatment for lumbar pain. Results of the survey showed the majority of practitioners preferred using 20 Hz (44%), followed by 80-120 Hz (20%), 120 Hz (18%), or 80 Hz (12%). The survey also found that there was a 50/50 split on lead placement (LP) when utilizing 10 needles: contralateral on Back-*shu* points (BL-20 left-to-BL-20 right) with each contralateral pair having leads for a total of 5 leads versus LP ipsilateral (BL-20 left-to-BL-24 left) utilizing only 4 needles for a total of 2 leads.

The considerable variability among practitioners, identified in the survey, indicated there is currently no standardized approach for determining EAP frequency and LP. Instead, decisions are largely shaped by each practitioner's personal experience. Recognizing this inconsistency and lack of literature to support informed evidence-based decisions, the objective of this study was to investigate how various EAP settings, specifically LP and frequency, affect treatment outcomes. These outcomes were measured by monitoring changes in tissue perfusion (reflected as increases in skin temperature) using infrared thermography. The focus on vasodilation, and the resulting increase in tissue perfusion and skin temperature, is especially important because nociception has been linked to vascular perfusion of tissue. Based on the author's (SJ's) experiential evidence, the hypothesis was that EAP with contralateral configuration and 120 Hz frequency would yield the highest skin temperature change, corresponding to increased tissue perfusion.

Table 1: Nerve fiber characteristics[3,11-17]

Fiber Type	Size of Fiber (μm)	Conduction Speed (m/s)	Signal Carried	Substances Released	EAP Stimulation
A-delta nerve fiber (myelinated)	1-5	5-30	Intense fast, sharp pain	EOP, β-endorphins, met-enkephalins	1-20 Hz
C fiber (unmyelinated)	0.2-1.5	0.5-2	Dull, slow pain	Dynorphin	100 Hz
Serotonergic fibers Brain, spinal cord (myelin/unmyelinated)	Small, variable size, depends on myelinated or not: 0.1-0.7	Slow, less than 1 m/s	Very diverse	Serotonin, norepinephrine	200 Hz
A-beta nerve fiber (myelinated)	6-12	35-90	Touch	—	—
A-alpha nerve fiber (myelinated)	12-20	70-120	Proprioception	—	—

EOP=endogenous opioid peptides, μm=micrometers, m/s=meters/second

MATERIALS AND METHODS

Study Subjects

The subject population for this study consisted of clinically healthy canines chosen from a population of local canine athletes that were not receiving acupuncture or spinal manipulation therapy. Study subjects were recruited through the local dog sport community via personally asking and social media. Due to the specific needs of the subjects, an athletic build was ideal. Typically sport dogs are also easy to handle and are acclimated to cooperative care, which is necessary for acupuncture and imaging. The inclusion criteria included 1) short coat; 2) ideal body condition score (4.5-5 of 9); and 3) medium (25-40 lbs) to large (40-70 lbs) size dogs. Subjects that 1) had historical pathology of the lumbar spine (including but not limited to spondylosis, IVDD); 2) initial thermal imaging scan identified increased areas of heat; or 3) had poor temperament (leading to inability to remain calm during treatment) were excluded from study enrollment.

Study Design and Treatment Procedure

This study used a repeated measures design where each study subject received all of the following 7 treatments: Treatment A: EAP 20 Hz and contralateral LP (10 needles inserted, 5 cables used); Treatment B: EAP 120 Hz and contralateral LP (10 needles inserted, 5 cables used); Treatment C: EAP dense and disperse 80-120 Hz with contralateral LP (10 needles inserted, 5 cables used); Treatment D: EAP 20 Hz and ipsilateral LP (10 needles inserted, 2 cables used); Treatment E: EAP 120 Hz and ipsilateral LP (10 needles inserted, 2 cables used); Treatment F: EAP dense and disperse 80-120 Hz with ipsilateral LP (10 needles inserted, 2 cables used); and Treatment G: no-treatment control. Treatments A, B, D, and E utilized a continuous wave form, while treatments C and F utilized a dense and disperse waveform. Dense and disperse relates to administering EAP treatment with alternating high-frequency at 120 Hz (dense) electrical impulses and low-frequency at 80 Hz (disperse) pulses. Treatments (6 different acupuncture treatments+no-treatment control) given to each subject were in a random order generated by utilizing an online random permutation tool[a] (Table 2).

Table 2: Each study subject received all of the following electro-acupuncture treatments (A-F) and a no-treatment session (G). Treatments were separated by 2 weeks, allowing a washout of any potential carryover effects from the previous treatment.

Treatment Method	Frequency	Lead Placement
A	20 Hz	Contralateral
B	120 Hz	Contralateral
C	80-120 Hz	Contralateral
D	20 Hz	Ipsilateral
E	120 Hz	Ipsilateral
F	80-120 Hz	Ipsilateral
G	No treatment	No treatment

Based on the randomly assigned treatment order, each of the 7 treatments was performed on every study dog. Treatments were separated by 2 weeks, allowing a washout of any potential carryover effects from the previous treatment. For all acupuncture treatments, 10 needles[b] (34-guage×1.0-inch copper) were placed to full depth of 1-inch in Back-*shu* points BL-20, BL-21, BL-22, BL-23, and BL-24 bilaterally (Table 3, Figure 1). Lead placement was at the junction of the copper wire and stainless-steel needle shaft, which affected needle depth by approximately 2 mm on needles with leads attached. For contralateral LP, leads were placed on Back-*shu* points BL-20 connected bilateral, BL-21 connected bilateral, BL-22 connected bilateral, BL-23 connected bilateral, and BL-24 connected bilateral. For ipsilateral LP, leads were placed on Back-*shu* points BL-20 left connected to BL-24 left and BL-20 right connected to BL-24 right. This placement was chosen based on the previously conducted informal unpublished survey regarding LP. It is important to note that despite only using 2 leads for ipsilateral cable placement, all study subjects had 10 needles placed for all treatments, except Treatment G, the no-treatment control. All study subjects were amendable to this size needle and depth. The needle size remained consistent for the same subject throughout the study. It has been the author's (SJ's) experience that the 34-guage×1.0-inch copper needle is well tolerated by this demographic.

Table 3: Summary table of anatomic location of acupuncture points used in the study (BL-20 through BL-24) and acupoints that marked masking tape boundaries for imaging area

Acupuncture Point	Anatomical Location Description
BL-18	On the dorsolateral aspect of the spine, 1.5 *cun* lateral to the caudal border of the dorsal spinous process of T10 bilaterally
BL-20	On the dorsolateral aspect of the spine, 1.5 *cun* lateral to the caudal border of the dorsal spinous process of T12 bilaterally
BL-21	On the dorsolateral aspect of the spine, 1.5 *cun* lateral to the caudal border of the dorsal spinous process of T13 bilaterally
BL-22	On the dorsolateral aspect of the spine, 1.5 *cun* lateral to the caudal border of the dorsal spinous process of L1 bilaterally
BL-23	On the dorsolateral aspect of the spine, 1.5 *cun* lateral to the caudal border of the dorsal spinous process of L2 bilaterally
BL-24	On the dorsolateral aspect of the spine, 1.5 *cun* lateral to the caudal border of the dorsal spinous process of L4 bilaterally
BL-26	On the dorsolateral aspect of the spine, 1.5 *cun* lateral to the caudal border of the dorsal spinous process of L6 bilaterally

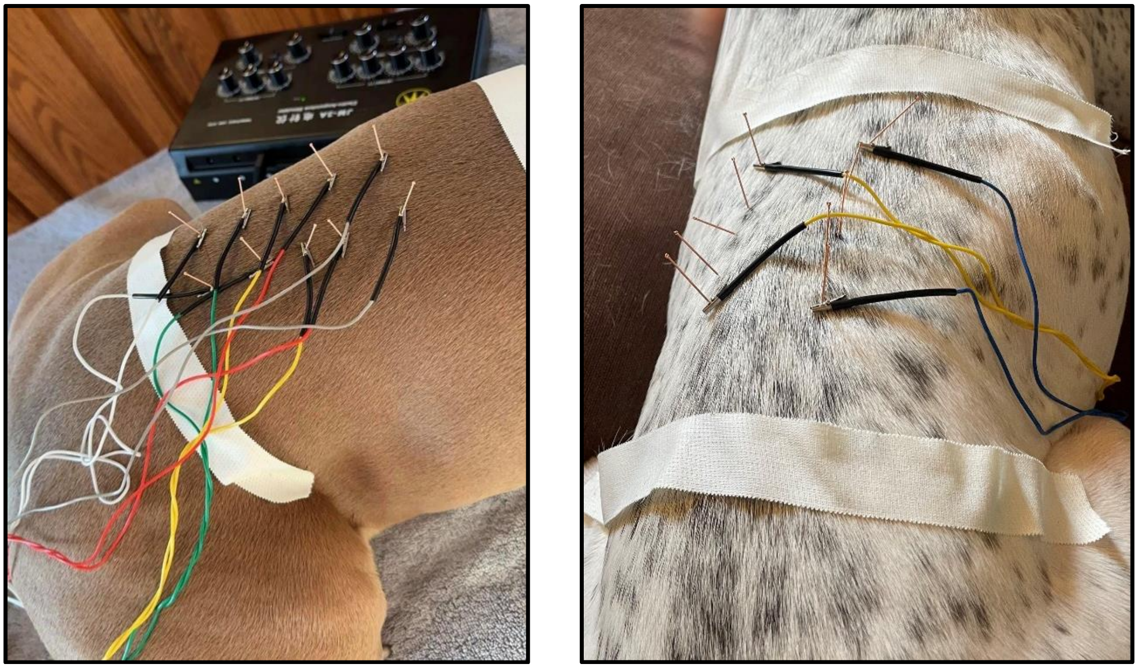

Figure 1: These pictures demonstrate the 2 types of lead placement associated with stimulating 10 acupuncture needles, placed from BL-20 through BL-24 for electro-acupuncture treatment: left picture-contralateral lead placement (i.e. BL-20 right to BL-20 left); right picture-ipsilateral lead placement (BL-20 left to BL-24 left).

Prior to receiving each treatment, the study subject was allowed to acclimate to the room for 20 minutes where the treatment was performed. This would allow body temperature to regulate and the subject to mentally acclimate to their surroundings. During this time the thermal imaging camera was also turned on to acclimate to the environment. Prior to needle placement (or no needle placement in reference to no-treatment control), a strip of 1-inch porous tape[c] was placed at T10 (BL-18), extending from left rib cage to right rib cage, and another placed at L6 (BL-26), extending from left flank to right flank (Table 3). Tape application facilitated consistent thermal imaging over the area of interest that would be measured (Figure 2).

Once the tape was applied, infrared thermal imaging was performed with the region of interest (ROI) being the caudal dorsal aspect of the spine, specifically BL-20 to BL-24. This region was selected due to its typically flat nature, which aids in a consistent imaging pattern. The distance between the camera and the ROI varied based on the size dog but was consistent with guidelines for veterinary infrared thermography (IRT), which states the subject of the image should fill at least 75% of the image.[18] Every precaution was taken to reduce outside interference (e.g. closed windows, set temperature of room, shades closed to prevent airflow, no human or animal proximity to ROI). To capture images, the IRT imaging lens was held perpendicular to the ROI of the taped off rectangular configuration of the thoracolumbar spine. The images were first captured with grey scale to ensure accurate focusing and were later changed to medical palette for analysis.

All acupuncture treatments had the needles placed for a total of 20 minutes while the subject rested comfortably in the owners' presence. During EAP treatments, leads were placed on the acupuncture needle at the junction of the copper wire and stainless-steel needle shaft prior to insertion, allowing time to be equal for all needle treatments. During the no-treatment control, the dog rested comfortably in the owner's presence, but no needles were placed. The intensity setting of the electro-acupuncture unit[d] was determined based on a patient's comfort level, with the machine knob setting placed halfway between the upper one-third of 0 and 1. Immediately following the removal of acupuncture needles and/or after the 20-minute no-treatment control concluded, a post-treatment infrared thermal imaging on the same area was performed (Figure 3).

Outcome Measurements

The thermal imaging system[e] produced before and after images of each treatment to determine skin temperature changes associated with each study treatment. The software had a guided recheck option, which retained a silhouette of the previously imaged area and allowed for more accuracy of the targeted imaging area when repeating the trials for the same subject.

Infrared software[f] analysis of the areas was performed by creating a square or rectangle (determined by dog breed) around the treated area, utilizing the inside aspect of the tape edges cranially and caudally and the left and right body wall edges as parameters (Figure 3). The software then performed calculations yielding the minimum, maximum, and average temperature within the imaging area. To avoid using an extreme reading within an area (i.e. due to artifact), only the average temperature was used for analysis. The temperature referred to hereafter is the average temperature within the imaging area. The difference between pre- and post-temperatures was calculated for comparisons among the acupuncture treatments.

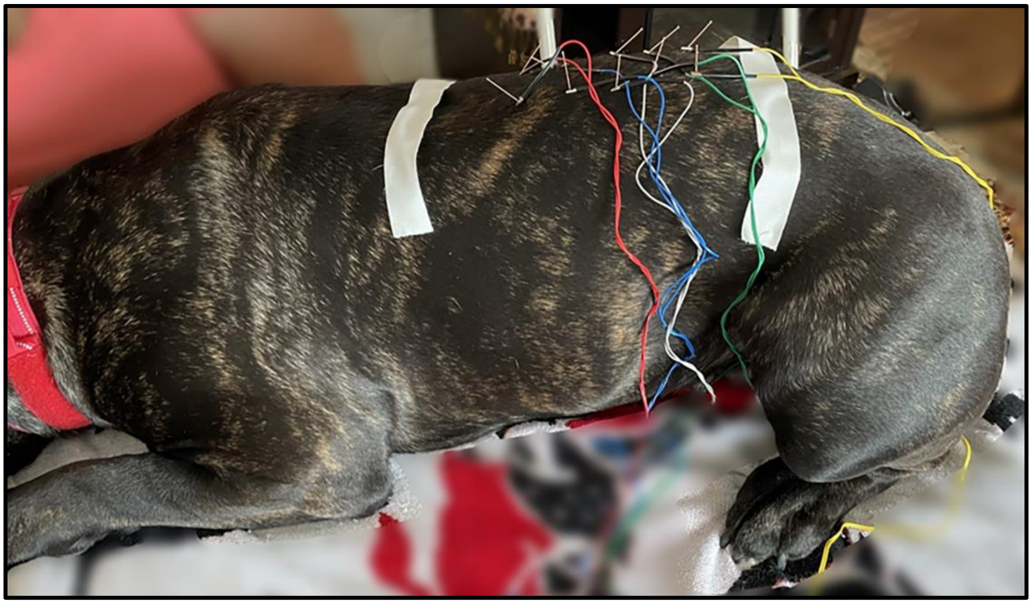

Figure 2: As illustrated in the picture**,** porous tape was placed at T10 (BL-18), extending from the left to right side of the rib cage, and another strip of tape was placed at L6 (BL-26) extending from the left to right flank. Placement of the tape facilitated consistent thermal imaging over the area of interest investigated by the study.

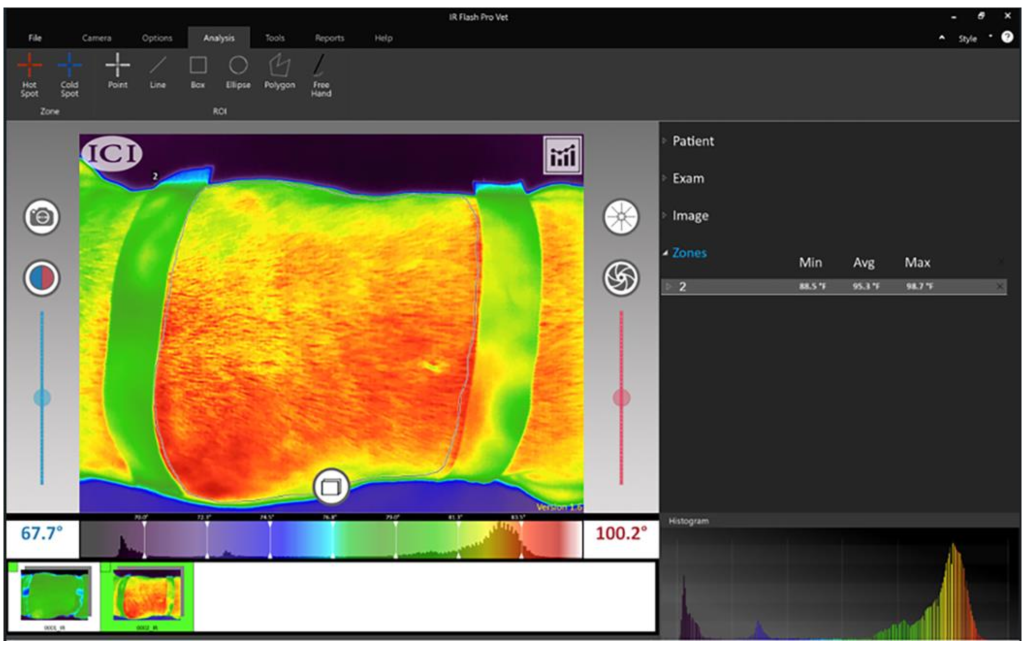

Figure 3: Immediately following removal of acupuncture needles, post-treatment infrared thermal imaging of the treatment area, as shown in the picture, was performed. Using IRT imaging software, calculation of minimum, maximum and average temperature present between tape edge borders was calculated. The average temperature of the imaging area was used for analysis to avoid extreme readings within an area due to artifact.

Statistical Analysis

Open-source public statistical software[g] was used for all statistical analyses and graphical presentation of the data. The study hypothesized that different treatments would result in temperature change differences. Based on the outcome measurement described, the study tested the following statistical hypotheses: H_0–The mean values of temperature change are the same among all treatments; versus H_A–There exists a difference among the 7 treatments in mean value of temperature change. In addition, effects of frequency and LP factors, as well as their interaction effect on the temperature changes were also tested.

To test the hypothesis that compares mean temperature changes among the 7 treatments with the randomized complete block design, after finding that the temperature change data for some treatments were not normally distributed (by Shapiro-Wilk normality test), the nonparametric Friedman test (based on ranks rather than means) was used to determine the rejection of the null hypothesis. When the null hypothesis that all treatment temperature changes are equal was rejected, post hoc multiple comparisons test (Nemenyi's nonparametric test) was carried out to determine which pairs of acupuncture treatments were different. To test the hypotheses concerning the effects of frequency setting and LP, respectively, and their interaction effect, the aligned rank transform ANOVA for two-factor factorial design with the block variable was applied.

With 7 treatments compared using Friedman test, a sample size of 40 subjects would offer an approximately 90% power to reject the null hypothesis with a 0.05 significance level, assuming a detectable treatment mean difference equal to the overall within-treatment standard deviation. Such a sample size would offer over 90% power to detect the factor effect (either frequency setting or LP) with 0.05 significance level, assuming a detectable mean difference with the factor equal to the overall standard deviation.

RESULTS

There were 36 dogs that met inclusion criteria and were enrolled in the study. All enrolled dogs completed 7 rounds (in a randomized order) of the tested AP treatments (including the no-treatment control). Among the subjects, 61.1% were females and 38.9% were males. The mean±SD age was 4.7±3.0 years old and the weight was 58.9±17.3 lbs. The group consisted of 9 breeds, where the top three were American Staffordshire Terrier (Amstaff), German Shorthair Pointer (GSP), and Doberman Pinscher (Table 4).

The mean temperature among subjects before and after each treatment were analyzed (Figure 4). Before treatment, the temperatures were not significantly different among the 7 treatment methods (6 AP + no-treatment control); p=0.198. After experimental treatment, all 6 AP treatment methods resulted in significant skin temperature increase (A-D, F p-value<0.0001 and E p-value<0.05), with the method "EAP 20 Hz contralateral" (treatment A) resulting in the greatest temperature increase (p=5.8×10^{-11}) and "EAP 120 Hz ipsilateral" (treatment E) with the lowest temperature (p=0.027) increase (Table 5, Figure 5). As expected, a subject's skin temperature remained almost unchanged from before to after the "no-treatment control" session (p=0.983). Statistically, there existed a significant difference (p=2.8×10^{-7}) in temperature changes among the 7 treatment methods (Figure 6). Based on the post hoc multiple comparisons test, except for AP method "EAP 120 Hz ipsilateral" (treatment E), the other 5 AP treatment methods had significantly greater temperature increase than the no-AP treatment (Table 6).

With respect to the effects of the EAP settings, each of the six EAP methods (A to F) was a combination of two factors: frequency setting (20, 120, or 80-120 Hz) and LP method ("contralateral" or "ipsilateral"). Statistical inference was carried out to determine how these 2 factors affected the outcome (temperature increase) and if there existed an interaction effect between the two factors (i.e. did frequency's effect depend on LP or vice versa). The findings revealed that there existed significant interaction between frequency setting and LP (p=0.013), which indicated that the effect from one factor (i.e. LP) was different between different levels of the other factor (i.e. frequency setting). For example, the outcome differences among different frequency settings of the "contralateral" LP could be different from those of the "ipsilateral" LP. Since the between-factor interaction exists, it is not meaningful to look at individual factor effects; it is more meaningful to look into the differences within one factor for each level of the other factor, such as comparing a frequency of 20 Hz contralateral to 120 Hz contralateral LP.

Table 4: Summary statistics of subject signalment data (n=36)

Demographic	Results
Sex	Female: 22/36=61.1%; male: 14/36=38.9%
Age (yrs)	1.5-13; mean±SD=4.7±3.0; median=3.8
Weight (lbs)	26-120; mean±SD=58.9±17.3; median=55
Breed	Amstaff (13), GSP (11), Doberman (6), Aust. Cattle Dog (1), Beagle (1), Boerboel (1), Great Dane (1), Rott/APBT (1), and Whippet/BC (1)

Amstaff=American Staffordshire Terrier, GSP=German Shorthair Pointer, Aust=Australian, Rott/APBT=Rottweiler/American Pitbull dog, Whippet/BC=Whippet/Border Collie

Table 5: Greatest to smallest temperature change (increase) between pre- and post-acupuncture treatment detected by infrared thermography

AP Treatment Method	Percentage Change from Pre-AP Temperature Mean	*p*-value
EAP 20 Hz Contralateral (A)	(+2.38%)	5.8×10^{-11}
EAP 80-120 Hz Ipsilateral (F)	(+1.44%)	1.4×10^{-7}
EAP 20 Hz Ipsilateral (D)	(+1.22%)	1.9×10^{-5}
EAP 80-120 Hz Contralateral (C)	(+1.07%)	1.4×10^{-5}
EAP 120 Hz Contralateral (B)	(+1.05%)	3.2×10^{-4}
EAP 120 Hz Ipsilateral (E)	(+0.43%)	0.027

Table 6: The *p*-values of post hoc multiple comparisons test (Dunnett's test), where each acupuncture treatment was compared with the no-treatment control in order of greatest to least statistical significance

Compared to No Acupuncture Treatment Control	*p*-value
EAP 20 Hz Contralateral (A)	1.3×10^{-9}
EAP 80-120 Hz Ipsilateral (F)	0.0005
EAP 120 Hz Contralateral (B)	0.010
EAP 80-120 Hz Contralateral (C)	0.012
EAP 20 Hz Ipsilateral (D)	0.016
EAP 120 Hz Ipsilateral (E)	0.062

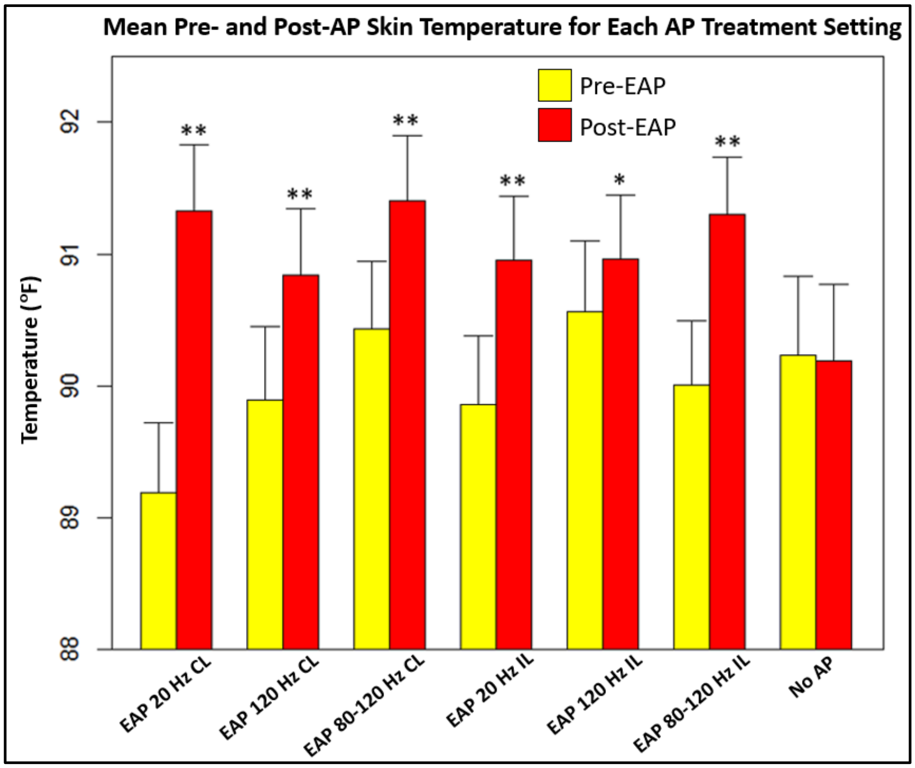

Figure 4: Mean skin temperature over all subjects before and after receiving the assigned treatment. CL=contralateral; IL=ipsilateral; ** $p < 0.001$; * $p < 0.05$ compared to the pre-AP temperature

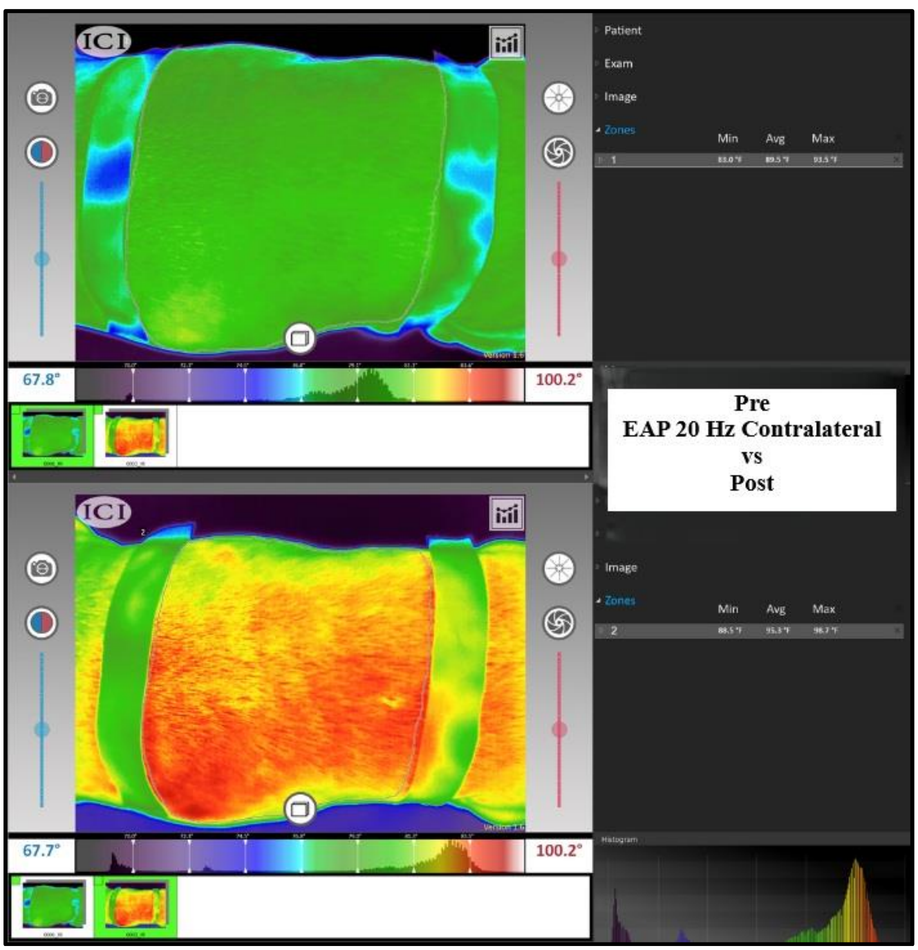

Figure 5: Comparison of pre-treatment (top picture) and post-treatment (bottom picture) with EAP 20 Hz, contralateral LP in a study dog.

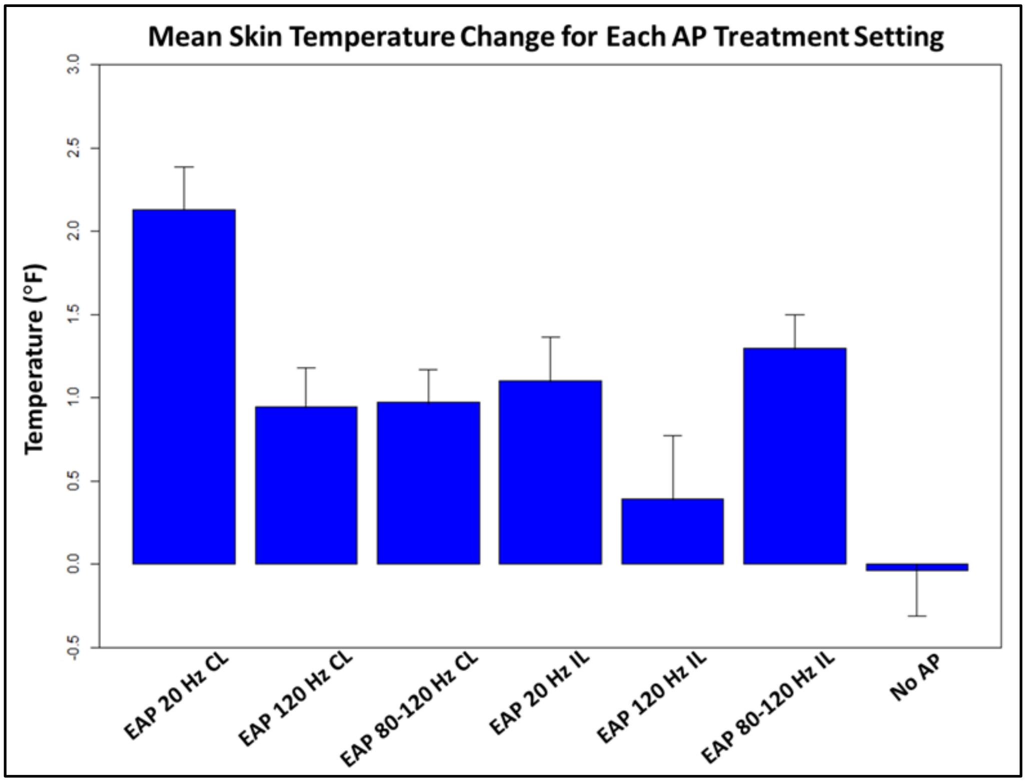

Figure 6: Mean skin temperature changes after receiving the assigned acupuncture treatment. EAP= electro-acupuncture; CL=contralateral; IL=ipsilateral

The comparison among the three frequency settings for each LP showed that when using "contralateral LP", 20 Hz frequency setting resulted in significantly greater temperature increase than the other two frequency settings; compared to 120 Hz (p=0.014), or 80-120 Hz (p=0.0002). The difference was not significant between the 120 Hz and the 80-120 Hz frequency settings (p=1.000). When using "ipsilateral LP", there were no significant differences among the three frequency settings (all p>0.05).

The comparison between the two LPs (contralateral versus ipsilateral) for each frequency setting (20 Hz, 120 Hz, vs 80-120 Hz) revealed that 1) when applying 20 Hz frequency, the "contralateral LP" resulted in significantly greater temperature increase than the "ipsilateral LP" at 20 Hz (p=0.006); 2) when applying 120 Hz frequency, the temperature increases between pre- and post-EAP were not significantly different between the two LP methods (contralateral versus ipsilateral; p=0.506); and 3) when applying 80-120 Hz frequency, the temperature increases between pre- and post-EAP were not significantly different between the two LP methods (contralateral vs ipsilateral; p=0.247).

DISCUSSION

This randomized controlled trial with repeated measures sought to determine whether changes in electro-acupuncture lead placement and stimulation frequency would variably increase vascular perfusion of tissue, as detected by thermal imaging temperature increases. The data from 36 clinically healthy canines treated with 3 different EAP frequencies (20, 80-120, 120 Hz) and 2 different patterns of lead placement (contralateral, ipsilateral) produced the following statistically significant findings: 1) all 6 EAP treatments resulted in thermography detected skin temperature increase (A-D, F p-value<0.0001 and E p-value < 0.05); 2) there existed a significant difference in temperature changes ($p=2.8\times10^{-7}$) among the 7 treatment methods; 3) the 20 Hz contralateral lead placement had the greatest temperature increase ($p=5.8\times10^{-11}$); 4) 5 of the 6 EAP settings (A-D, F) had significantly greater temperature increase than no-AP treatment (p<0.05); and 5) there existed significant interaction between frequency setting and LP (p=0.013). In summary, the results of the data collected in the present study, demonstrated that all EAP treatments resulted in significant mean temperature increase between pre- and post-EAP. Comparison of the different EAP techniques identified the greatest to lowest temperature increases as follows: 20 Hz contralateral, 80-120 Hz ipsilateral, 20 Hz ipsilateral, 80-120 Hz contralateral, 120 Hz contralateral, 120 Hz ipsilateral.

The use of IRT has been investigated with standardized protocols established for providing quantitative measurements of body surface temperature that can be extrapolated into determining tissue perfusion and its clinical usefulness.[18-22] Collins demonstrated that, in 24 dogs with pre-existing pathology of the back, there was a significantly greater temperature change in the group receiving dry needle acupuncture than dogs receiving no treatment.[23] Dai et al., during convenience sampling of horses presented for TCVM exam of various medical issues, discovered that in 23 of the 41 animals examined, there was increased acupoint sensitivity at key AP diagnostic points (LI-18 and/or PC-1) for front hoof pain.

The horses with increased sensitivity had corresponding increases in thermal readings of the front hooves; whereas those with no sensitivity at these points revealed no significant difference in thermal readings of bilateral front hooves.[19]

Acupuncture stimulation activates the mechanoreceptors in superficial cutaneous tissues and deep muscular tissues.[24] Acupoints contain abundant free nerve endings, encapsulated cutaneous receptors (Merkel, Meissner, Ruffini, and Pacinian corpuscles), sarcous sensory receptors (muscle spindles and tendon organs), and ergo-receptors, as well as their associated afferent fibers. How an acupuncture needle is inserted and stimulated, as well as needle length in comparison to patient size, can determine what type of receptors are activated.[24] That association may explain the finding that EAP lead placement and frequency setting have a significant interaction that affects local tissue temperature, as assessed with IRT.

Axons that transmit information about pain fall into two main categories: A-δ (delta) group of myelinated axons, with a conduction rate of 5-30 m/s, and the C fiber group of smaller unmyelinated axons, with a much slower conduction rate of 2 m/s (Table 1). Both A-δ (delta) and C fibers are active when painful stimuli occur. The fast-conducting A-delta nociceptors respond to dangerously intense mechanical stimuli or both intense mechanical and thermal stimuli. The slower conducting unmyelinated C fiber nociceptors, acting as polymodal, respond to mechanical and chemical stimuli. Putting these together, it is recognized that there are three major classifications of nociceptive afferents that supply the skin: A-delta mechanosensitive nociceptors, A-delta mechanothermal nociceptors, and polymodal nociceptors.[3] Based on histological studies, the connective tissue layer contains A-β (beta) afferent fibers and the skeletal muscle layer contains A-delta and C afferent fibers.[24] Based on this, it can be inferred that the 34-gauge×1.0-inch acupuncture needle used in this study activated all 3 fiber types simply due to needle placement and depth.

Based on previous research of various frequencies used for EAP treatment, it has been demonstrated that low frequency EAP stimulates A-delta and high frequency predominately stimulates C fibers (100 Hz) and serotonergic fibers of brain/spinal cord (200 Hz).[11,13-15] The A-delta fiber when stimulated from acupuncture or muscle activity releases a neuropeptide (calcitonin gene related peptide/CGRP), that acts as a potent capillary bed vasodilator; restoring muscle blood flow and acting as a growth factor contributing to angiogenesis by producing trophic effects on the endothelial cells. The C fibers in muscle also contribute to the release of CGRP during the acute stages of tissue damage through neurogenic inflammation, providing a protective effect of vasodilation, extravasation and edema, which facilitates physiological tissue healing.[24] It can therefore be hypothesized that all EAP settings in this investigation activated the A-delta (δ) and C fibers with subsequent release of CGRP; with "EAP 20 Hz contralateral LP" treatment providing the greatest vasodilation. This led to a statistically significant increase in temperature change, directly correlating to an increase in tissue perfusion, which could be considered to provide benefits to pain management and healing.

When evaluating frequency as an individual analysis and comparing the same frequency but paired with different lead placement patterns, it should be acknowledged as a study limitation that the contralateral pattern utilized 5 leads and 10 needles, while ipsilateral LP utilized 2 leads and 10 needles. Although the electrical current is flowing through the Bladder Meridian from BL-20 through BL-24 in both the contralateral and ipsilateral lead placement patterns, the variable that 5 leads (10 needles) connected to one body area may provide more energy than 2 leads connected to 4 needles across the same size body area cannot be discounted under the experimental conditions of this study. It is noted that the only statistically significant finding for frequency comparisons was 20 Hz contralateral compared to 20 Hz ipsilateral, where the 20 Hz contralateral yielded significantly greater temperature increase. With this, one can infer that perhaps the number of needles and number of leads used are not independent factors on influencing tissue temperature increases. Instead, frequency and LP configuration work together to yield specific results, as found in the statistically significant change of 20 Hz contralateral LP when compared to all other frequencies and LP.

At study start it was hypothesized that higher frequency (120 Hz) with contralateral lead placement would yield higher thermography detected skin temperature change. When looking at the results of the data collected, all EAP treatments resulted in significant temperature changes, however, the 20 Hz frequency with contralateral LP technique reflected the largest change in skin temperature between pre- and post-EAP. Although the LP technique (contralateral) was consistent with the study hypothesis, the low frequency (20 Hz) was an unexpected finding and inconsistent with the study hypothesis, which predicted the higher frequency (120 Hz) would produce greater skin temperature change. It is hypothesized the unexpected finding reflects greater vasodilation at low frequency when compared to higher frequencies. This may be related to greater production of CGRP associated with low frequency (1-20 Hz) A-delta fiber stimulation than higher frequency stimulation of C fibers, although both fibers release CGRP.[25] Neuropeptides were not measured in this study, or comparisons made to clinical efficacy, therefore, the unexpected finding can only be analyzed in regard to local effects.

Limitations to the study included a slightly smaller than planned sample size. This may have limited statistical power to detect smaller differences among the 6 acupuncture treatments. The study was comprised of 36 subjects, only 4 fewer than the planned sample size and therefore, still offered a statistical power of 85% for detecting a treatment difference. For example, a larger sample size might have achieved statistical significance for Treatment E skin temperature increase when compared with no treatment control (Table 6).

Nevertheless, the study still offered over 95% for the power to determine the significant effect of an EAP factor (frequency or LP). That stated, ideally the study would include more subjects to increase the statistical power for more meaningful findings. Additionally, to add robustness to this study, comparison with dry needle only technique would have added information on the effects of needle only insertion.

In conclusion, it was discovered that an EAP frequency of 20 Hz with leads placed contralaterally produced the largest temperature change between pre- and post-AP treatments when comparing 6 EAP treatments with different frequencies and lead placements. It is important to note that all EAP treatments studied produced statistically significant increases in pre- and post-treatment temperature of the region studied when compared to no acupuncture treatment of the area. It can be inferred that any of the EAP techniques evaluated are more effective at increasing tissue perfusion in this area than doing nothing at all. Electro-acupuncture is an easy-to-use modality for the trained professional. The supplies are accessible and affordable with the procedure easily performed in a clinic setting. Future clinical research in the field of EAP frequency settings and their interactions with patterns of lead placement, along with investigation of effect on disease syndromes, are needed to optimize EAP treatment. These studies would be an important step towards shaping best practices for veterinary patients based on evidence-based research rather than practitioner preference.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Xie for his continued teaching and contributions to advance TCVM, members of the Chi University Master Thesis Committee, Dr. Jianliang Zhang and Dr. Joyce Carnevale, who aided in countless communications for guidance of this project as well as its countless edits, the AJTCVM editorial team, and finally to the dog owners who allowed their pets to contribute to science and the advancement of TCVM research.

Declaration of Interest and Funding

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this paper and the author did not receive any specific grant of funding from any organization in the public, commercial or non-profit sectors.

FOOTNOTES

[a] Random permutation, http://www.jerrydallal.com/random/random_permutation.htm

[b] Acupuncture needles (Jing Mei 34-guage×1.0-inch copper needles), Wuxi Jiajian Medical Instruments Co., Ltd, Wuxi, Jiangsu, China

[c] Standard porous tape (1-inch), Cardinal Health 200, LLC, Waukegan, Illinois, USA

[d] Electro-acupuncture unit (JM-03A), Wuxi Jiajian Medical Instruments Co., Ltd, Wuxi, Jiangsu, China

[e] Digatherm IR Tablet 640 digital thermal imaging system, WellVu Veterinary Thermal Imaging (Formerly Digatherm) LLC, Ocala, Florida, USA

[f] IR Flash Pro infrared software, WellVu Veterinary Thermal Imaging (Formerly Digatherm) LLC, Ocala, Florida, USA

[g] R version 4.4.1. The R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org

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