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Basic Science Studies

Decoding Bioelectric Signals at Acupuncture Points in Horses and Humans Using the Electroacugram Device

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ABSTRACT

The objective of this study was to investigate the bioelectrical properties of acupuncture points in horses and humans. A modular platform (electroacugram) was designed for high-fidelity capture and analysis of bioelectric signals over the frequency range of 220 Hz to 310 Hz generated at acupoints. The electroacugram (EAG) system was tested on Heart (HT) Meridian equine and human acupoints. In the horse (n=8), effects of external stimulation of bioelectric activity were analyzed for HT-7+HT-9 and HT-7+HT-4. In humans (n=8), bioelectric activity was recorded between HT-9 and each of the following acupoints: HT-8, HT-7, HT-6, HT-5, HT-4, HT-3, HT-2. Study findings demonstrated that these acupoints generate distinct bioelectric signals when compared to sham points, with observable differences in amplitude and unique waveforms. It was also observed that external stimuli, such as gentle forehead touches on horses and cold stimuli for humans, significantly influenced these signals. By demonstrating consistent and measurable bioelectric activity at specific acupoints, and the ability to capture the bioelectric changes consistent with physiological stimuli (touch or cold) in the study population; the EAG system was able to non-invasively explore some of the physiological responses underlying acupuncture. Additionally, the EAG system successfully recorded bioelectric activity using both needle electrodes and non-invasive pad electrodes in humans, underscoring suitability for clinical and research applications. These findings provide new insights into bioelectrical characteristics pertinent to acupoints and suggest that bioelectric signal profiling may play an important role in validating acupuncture's therapeutic claims and open avenues for future research.

Keywords: acupuncture, bioelectric signals, electroacugram, equine, human, stimulation

ABBREVIATIONS: DNP: do not populate; EAG: electroacugram; Hz: hertz; ISI: inter-spike interval; ms: milliseconds; VCC: voltage at the common collector

As acupuncture continues to gain traction in the realm of "Complementary and Integrative Medicine" globally, the urgency for a scientifically rigorous understanding of its underlying mechanisms and evidence-based effectiveness has escalated. Existing literature points to the potential of acupuncture-based treatments as promising solutions for managing anxiety and pain in humans.[1-8] For instance, research indicates that stimulating acupuncture points (acupoints) can effectively alleviate depression and anxiety in human cancer patients.[9-11] Additionally, acupuncture has been shown to produce faster and more effective relief for general anxiety disorder compared to clonazepam in humans.[12] Furthermore, integrative acupoint stimulation has been proven to reduce postoperative pain and associated morphine side effects in humans.[13]

Acupoints exhibit distinct bioelectrical properties when stimulated compared to non-acupoints, including altered impedance characteristics, increased conductance, and variable electrical potentials.[13-15] Notably, the frequency-dependent nature of impedance responses may reflect complex physiological behaviors beyond simple changes in conductivity. Previous methods that relied on skin impedance measurements to identify acupoints have been questioned for their validity and susceptibility to interference from factors like skin texture and sweat glands.[14,16] Studies focusing on non-bio-resistance have revealed that bioelectric signals at acupoints are significantly

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stronger than those at adjacent non-acupoint areas.[17] Bioelectricity serves as a crucial link between cognitive processes (brain neural networks) and physiological functions (muscle systems), playing an essential role in the well-being induced by acupuncture.[2,18-22] This bioelectric activity is instrumental in both diagnosing and treating conditions like anxiety and pain.[18,19,23,24]

At rest, acupuncture points exhibit predominantly slow oscillatory potentials in the ultra-low frequency range. Human acupoints have been shown to carry 0-10 Hz baseline fluctuations with significantly higher power (~15% greater) than adjacent non-acupoint skin which peak around 0-2 Hz.[25] Under stimulation, however, much faster electrical activity is observed. For example, acupuncture needle manipulation can induce bursts of afferent nerve firing at tens of hertz (≈35-50 Hz) in animal models.[26] In equine studies, needle electrodes at myofascial trigger-points (analogous to reactive acupoints) record high-frequency spike discharges (brief energy extending into the hundreds or thousands of hertz), whereas idle acupoint signals remain confined to lower frequencies.[27] Therefore, filtering the electroacugram (EAG) to ~220-3100 Hz is appropriate to isolate these fast bioelectrical components (neural spikes, muscle fiber potentials) from slow drift and noise.

While acupoints have been extensively studied and hold significance, our scientific grasp of their bioelectric properties remains incomplete. The relationship between bioelectric signals induced by acupuncture and established biological systems, such as neural and muscular networks, is complex and not yet fully understood. Acupuncture's potential to evoke nerve activity in the somatosensory system through needle stimulation at a human acupoint such as Heart-7 (HT-7) further highlights its significance.[28]

To address key questions on the bioelectric properties of acupoints, a modular platform (electroacugram) was designed for high-fidelity capture and analysis of bioelectric signals generated at specific acupoints. The primary objective of the study was to investigate the reliability of the electroacugram to detect distinct bioelectric signals at acupoints, both at baseline and linked to physiological responses. It was hypothesized that EAG recordings would demonstrate unique consistent and measurable bioelectric activity at acupoints in both horses and humans which were linked to physiological responses.

MATERIALS AND METHODS

Electroacugram Device Development

The EAG system was engineered to serve as a comprehensive platform for monitoring bioelectrical signals, particularly those emanating from acupoints. The system has a modular design, comprised of three primary components: the electrode assembly, a bioelectrical amplifier with specialized filtering capabilities, and a data recorder and analyzer (Figure 1).

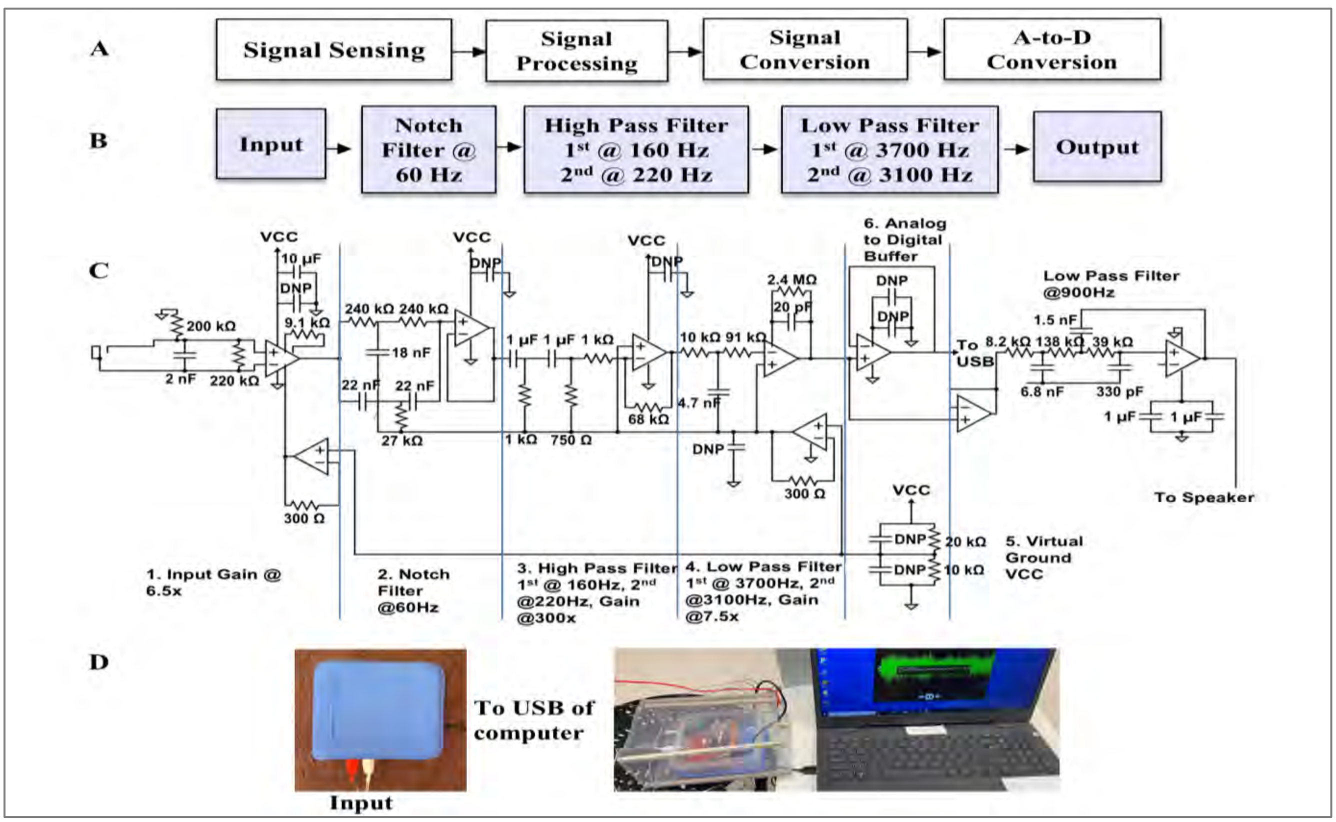

Figure 1: Architectural overview of the acupoint EAG device. **A.** Flowchart illustrating the overall signal pathway, from initial sensing by the electrode sensor through sequential signal processing stages. **B.** Schematic representation of the filter module, with annotations describing each step in the signal conditioning process. **C.** Detailed block diagram of the signal processing architecture, highlighting key components: (1) initial input amplification (Gain=6.5×); (2) 60 Hz notch filter for noise suppression; (3) dual-stage high-pass filtering at 160 Hz and 220 Hz with a combined gain of 300×; (4) dual-stage low-pass filtering at 3700 Hz and 3100 Hz with a combined gain of 7.5×; (5) virtual ground reference system; and (6) analog-to-digital conversion for digital signal acquisition. **D.** Photographic image of the EAG device prototype showing its physical configuration, which consists of an electrode pad (left), connected to a coverless view of the bioelectrical amplifier and noise suppression unit (middle), connected to a laptop (right). DNP=do not populate, VCC=voltage at the common collector.

Electrode assembly: Two types of electrodes were developed to meet distinct experimental requirements: needle electrodes for both animal and human studies, and pad electrodes specifically for human studies. The needle[a]-electrodes (28-gauge, 2 inch/0.35×50 mm) were designed for direct insertion into acupoints, providing high-fidelity signal capture. The pad-electrodes offered a non-invasive alternative for surface-level measurements on human subjects.

Bioelectrical amplifier and noise suppression: Central to the EAG system is a bioelectrical amplifier[b] equipped with a series of filters designed to minimize noise and enhance signal clarity. The amplifier incorporated a "Notch Filter" at 60 Hz to eliminate electrical interference commonly found in laboratory settings. Additionally, it featured a dual-stage "High Pass Filter" with the first stage set at 160 Hz and the second at 220 Hz, as well as a dual-stage "Low Pass Filter" with the first stage at 3700 Hz and the second at 3100 Hz. These filters work in tandem to isolate the bioelectric signals of interest, thereby improving the signal-to-noise ratio.

EAG signal recording with and without stimulation: Bioelectric signals between two specific acupoints are continuously recorded under two conditions: baseline (no stimulation) and during stimulation (gentle forehead touch for equine subjects or hand immersion in ice water for human subjects), as illustrated in the block diagram (Figure 2). A laptop computer[c] served as the final component of the EAG system, handling digital conversion, data storage, and analysis of the recorded signals (Figure 1D).

Data analysis: The recorded bioelectrical signals were saved as audio files (.wav extension) and analyzed using Spike Recorder software[b]. Various parameters like average waveforms, inter-spike intervals (ISI), and autocorrelogram were calculated. Signal-to-noise ratios were calculated, and any anomalies or outliers were carefully examined and accounted for in the final analysis.

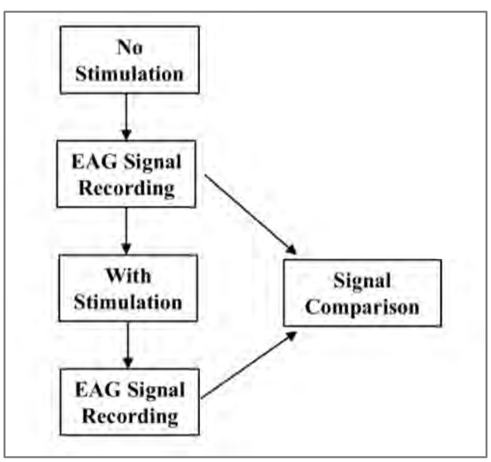

Figure 2: Continuous EAG recording of bioelectric activity between acupoints under baseline and stimulated conditions. This diagram illustrates a continuous operational flow in which bioelectric signals are recorded first under baseline conditions (without stimulation) and then under stimulated conditions (either via gentle forehead touching in horses or ice-water immersion in humans), without interrupting the recording process. Continuous recordings were captured to compare signal patterns between these two states.

Equine Acupoint Bioelectrical Signal Measurement

Animals

Eight clinically healthy horses from the Chi University herd and client-owned horses, all free from any known neurological or musculoskeletal disorders, were selected for the study to test the reliability of the bioelectrical signal recordings. They ranged in age from 6 to 21 years, included 5 different breeds, both intact males/females and geldings (Table 1). The study protocols for equine EAG recording were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Chi University (#CH-IACUC101).

Equine EAG Recording Overview

The equine phase of this study recorded bioelectric activity potential between HT-7 and HT-9 or between HT-7 and HT-4. Sterile, single-use acupuncture needles[a] were carefully inserted into specific acupoints, namely HT-9 and HT-7 or HT-4 and HT-7 on the left foreleg by a licensed veterinarian certified in veterinary acupuncture (Table 2).[29] Sham acupuncture (needle insertion at non-acupuncture points) was performed on points positioned 10 mm away from true acupoints on the left front limb, and served as control. The area was wiped down with alcohol prior to needle[a] insertion (0.35 mm × 50 mm, copper handle, guide tube used). The insertion depth and angle were maintained consistently across all subjects to ensure uniformity in the recordings.

Table 1: Baseline demographic characteristics of horses in the electroacugram (EAG) assessments.

	Horse #	Age	Breed	Sex
1	#05	21	Thoroughbred	Gelding
2	#06	18	Arabian	Gelding
3	#11	11	Warmblood	Gelding
4	#20	16	Machador	Gelding
5	#32	10	Warmblood	Mare
6	#33	18	Quarter Horse	Mare
7	#37	10	Thoroughbred	Gelding
8	#39	6	Quarter Horse	Gelding

Table 2: Description of each test acupoint stimulated along the equine Heart Meridian with their anatomic location and primary actions.[29]

Equine Acupoint	Anatomic Location (Horse)	Primary Actions	Needle Insertion Depth (approx.)
HT-4	5 *cun* proximal to carpus, medial to flexor tendons	Regulates Heart *Qi*, calms *Shen*	0.5–1.0 *cun*
HT-7	On carpal joint crease, medial to flexor tendon	Tonifies Heart, calms *Shen*, sedative effect	0.3–0.5 *cun*
HT-9	Medial coronary band of the hoof	Revives consciousness, clears Heat	0.2–0.5 *cun*

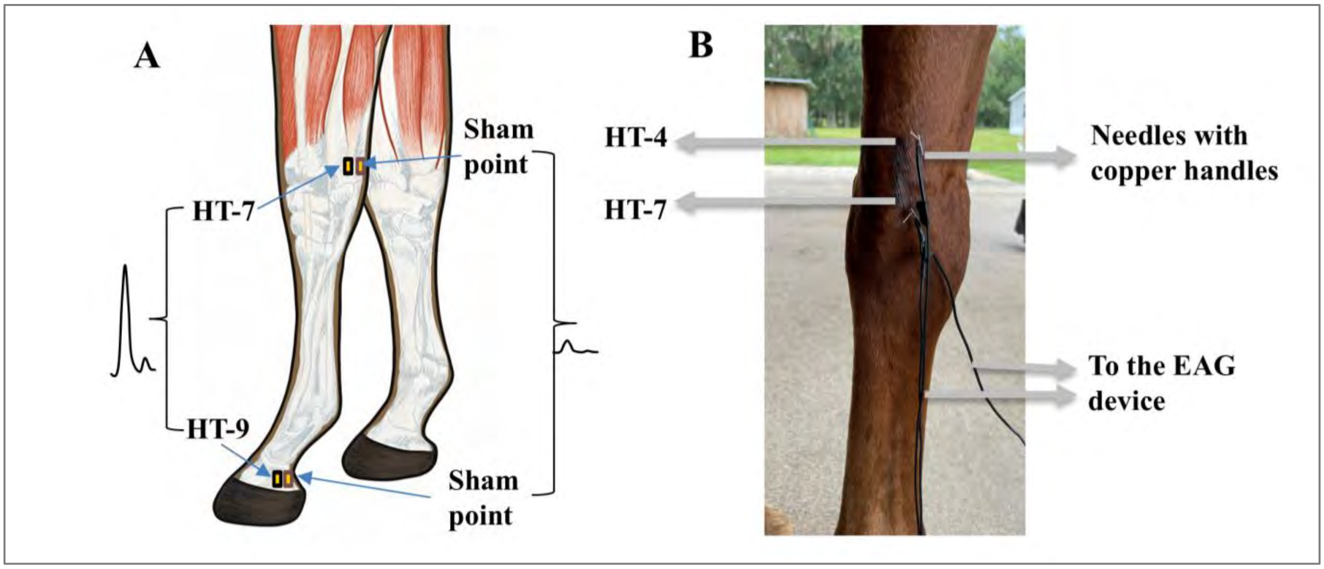

Figure 3: Illustration of acupuncture needle placement at acupoints. **A.** Measurement of bioelectric potential between HT-7 and HT-9 or between sham points. **B.** Photograph showing needles inserted at HT-4 and HT-7, connected to the EAG device.

The EAG device was calibrated using the physical simulants of biological tissues (5% gelatin in saline) and then connected to the inserted acupuncture needles with clamps that were wire-connected to the EAG device (Figure 3). The resulting bioelectric potentials between these points were captured using the EAG device. After the signals were stable, the signals were recorded and stored digitally for durations ranging from 1 to 5 minutes.

Bioelectric activity was recorded under 2 different physiological conditions: baseline (no stimulation) and external stimulation. The HT-7 acupoint was selected for use in this phase of the study as it is commonly used to treat equine anxiety, which can heighten responses to stimuli. To assess the effects of external stimulation on bioelectric activity between HT-7 and HT-9 or between HT-4 and HT-7, gentle forehead touches were applied to the horses, a technique known to evoke pleasurable sensations. Initially, the bioelectric potential between HT-9 and HT-7 or between HT-4 and HT-7 was recorded under baseline conditions. Then, forehead touching was introduced while continuously monitoring the bioelectric potential. Three recordings were taken for each condition (i.e. baseline or touching head) to ensure data reliability.

Human Acupoint Bioelectrical Signal Measurement

Study Population

The study protocols for human EAG recording were reviewed and approved by the Institutional Review Board (IRB) at Chi University (CH-IRB#09). Written informed consent was obtained from all human subjects participating in the study, ensuring that they were fully aware of the study's objectives and procedures. The study included 8 adult human subjects, both sexes, 23 to 63 years old and representing three different races, with no known history of neurological or musculoskeletal disorders (Table 3). Participants with potential confounding factors such as asthma, atopy, inserted prosthesis, burns on the arms or legs, excessive sweating, skin diseases, metal allergy, surgery history within the previous year, or medication use in the previous month were excluded. The recruitment process adhered to stringent standards, ensuring that participants were fully informed.

Human EAG Recording Overview

To establish the translational relevance of the equine model in reflecting human acupoint bioelectric characteristics, bioelectric activity assessments were conducted on acupoints (HT-2 to HT-9) both with and without stimulation (i.e. cold) using the EAG device (Table 4, Figure 4).[30,31] Seeking to transition from minimal to non-invasive assessments, comparative analysis of EAG features was conducted with acupuncture needle and pad electrodes. Certified acupuncturists identified and marked the eight acupoints HT-2 to HT-9 on the right hand of each participant. Sterile stainless steel acupuncture needles (0.30 mm×40 mm) with copper handles[d] were used, and the area was wiped down with alcohol. The needles were then carefully inserted into these acupoints, adhering to standardized insertion techniques to ensure consistency across all subjects. Alternatively, pad-electrodes were placed over the identified acupoints, after the areas were wiped down with alcohol (Figure 4).

Table 3: Baseline demographic characteristics of human subjects in the EAG assessments.

Human Subject	Age	Race	Sex
P001	62	Asian	Male
P002	27	White	Male
P003	59	Asian	Female
P004	23	Asian	Female
P005	24	Asian	Female
P006	60	Asian	Female
P007	61	Black	Male
P008	63	Asian	Male

Table 4: Description of each test acupoint stimulated along the human Heart Meridian with their anatomic location and primary actions.[30,31]

Human Acupoint	Anatomic Description	Primary Actions	Needle Insertion Depth (approx.)
HT-2 (*Qing-ling*)	3 *cun* above the medial end of the transverse cubital crease, on the line connecting HT-1 and HT-3.	Activates the Channel and relieves pain, especially along the Heart Meridian.	0.5–1.0 *cun*
HT-3 (*Shao-hai*)	At the medial end of the transverse cubital crease, in the depression anterior to the medial epicondyle of the humerus.	Calms the spirit, transforms Phlegm, clears Heat, benefits the arm.	0.5–1.0 *cun*
HT-4 (*Ling-dao*)	1.5 *cun* above the transverse crease of the wrist, on the radial side of the tendon of flexor carpi ulnaris.	Calms the spirit, benefits the voice and speech, relaxes muscles and sinews.	0.3–0.5 *cun*
HT-5 (*Tong-li*)	1 *cun* above the transverse crease of the wrist, on the radial side of the tendon of flexor carpi ulnaris.	Calms the spirit, regulates heart rhythm, treats palpitations and aphasia.	0.3–0.5 *cun*
HT-6 (*Yin-xi*)	0.5 *cun* above the transverse crease of the wrist, on the radial side of the tendon of flexor carpi ulnaris.	Nourishes Heart *Yin*, clears Deficiency Fire, stops sweating and treats night sweats.	0.3–0.5 *cun*
HT-7 (*Shen-men*)	At the ulnar end of the transverse crease of the wrist, in the depression on the radial side of the tendon of flexor carpi ulnaris.	Calms the spirit, regulates and tonifies the Heart, treats insomnia and anxiety.	0.3–0.5 *cun*
HT-8 (*Shao-fu*)	On the palm, between the 4th and 5th metacarpal bones, where the tip of the little finger rests when a fist is made.	Clears Heat from the Heart and Small Intestine, calms the spirit, regulates urination.	0.2–0.3 *cun*
HT-9 (*Shao-chong*)	On the radial side of the little finger, about 0.1 *cun* from the corner of the nail.	Revives consciousness, clears Heat, and benefits the tongue and eyes.	0.1–0.2 *cun*

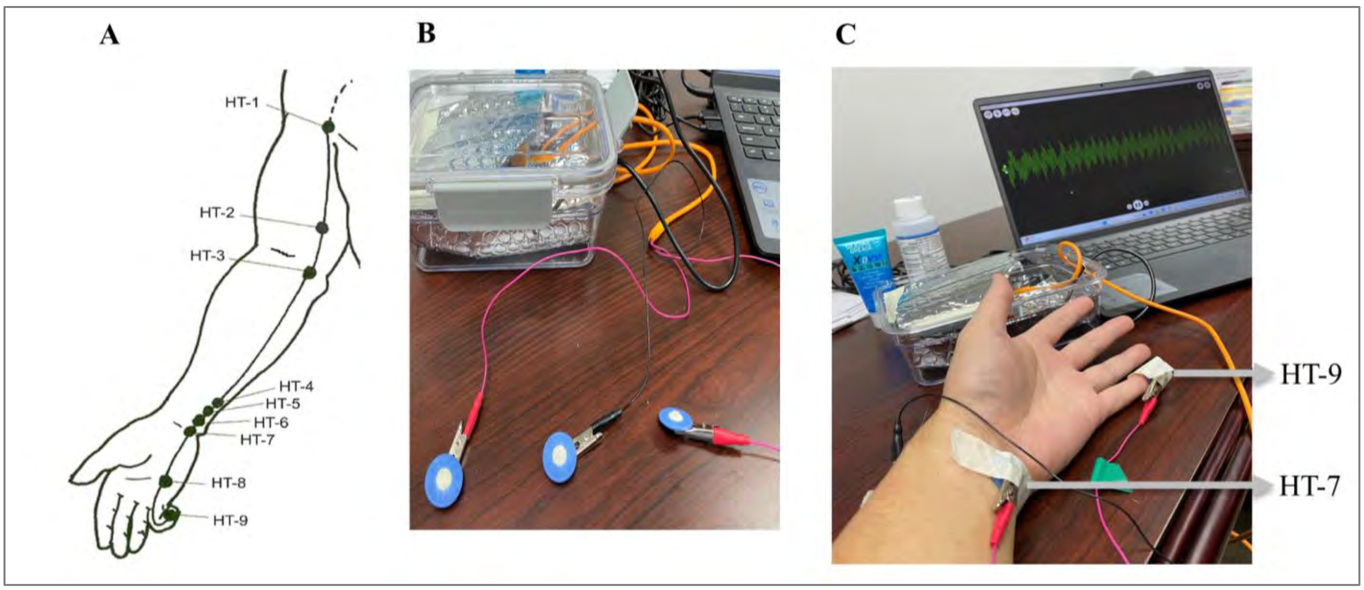

Figure 4: A. Illustration of Heart Meridian acupoints along the right arm of a human. EAG assessments were conducted at HT-2 through HT-9. **B.** Photograph displaying pad electrodes connected to the EAG device. **C.** Photograph showing pad electrodes positioned at HT-7 and HT-9, with a reference pad electrode attached to the back of the hand.

The EAG device was calibrated using physical simulants (5% gelatin in saline) prior to each recording session. The device was connected to the inserted acupuncture needles or pad-electrodes over the acupoints (Figure 4). Baseline bioelectric activity was recorded at acupoints along the Heart Meridian, specifically between HT-9 and each of the following points: HT-8, HT-7, HT-6, HT-5, HT-4, HT-3, and HT-2. This included measurements of bioelectric potential between HT-9 and HT-8, HT-9 and HT-7, HT-9 and HT-6, HT-9 and HT-5, HT-9 and HT-4, HT-9 and HT-3, as well as HT-9 and HT-2, with particular focus on the activity between HT-7 and HT-9 on the right hand. The EAG measurements were additionally captured while the subject's left hand was submerged in ice-cold water for 1 to 2 minutes, aiming to provoke a cold or pain response. Bioelectric potentials were recorded for a predetermined duration, typically ranging from 1 to 5 minutes. Three recordings were taken for each condition/acupoint pair and each subject to ensure data reliability. Saved files (.wav extension) were analyzed using Spike Recorder software[b].

Quality Control

To ensure the integrity of the study, all procedures were conducted by trained and certified veterinary and human acupuncturists. Comprehensive protocols were followed for data collection, with equipment weekly calibrated, to provide a robust and reliable dataset for the bioelectrical activities at equine and human acupoints under various conditions.

RESULTS

Bioelectric Potentials Generated from Equine Acupoints HT-7 and HT-9

The EAG device was utilized to record the bioelectric potential between HT-7 and HT-9, as well as between sham points (Figure 3). The amplitudes recorded between HT-7 and HT-9 (Figures 5A and 5B) were significantly higher than those observed at the sham points (Figure 5C). Additionally, the electric signals from the acupoints (Figure 5B) exhibited distinct wave patterns that were notably different from those recorded at the sham points (Figure 5D). To further assess the functional connectivity along the Heart Meridian in equine subjects, bioelectric potentials were recorded between acupoints HT-4 and HT-7 using the EAG system in eight horses (Figure 6). The recordings revealed consistent bioelectric activity across all subjects, with distinct signal profiles and spike patterns observed at this acupoint pair. These dominant waveform characteristics, visualized across multiple horses, suggest a reproducible electrophysiological signature between HT-4 and HT-7, reinforcing their physiological relevance within the Heart Meridian pathway.

The bioelectric potential between HT-7 and HT-9 with and without gentle forehead touching (stimulation) was compared (Figure 7). Forehead stimulation induced notable changes in the bioelectric potential between HT-7 and HT-9. Specifically, signal amplitude and waveform complexity increased during stimulation in several horses, suggesting heightened electrophysiological responsiveness of these acupoints. Similarly, EAG recordings between HT-4 and HT-7 in four horses comparing no-stimulation and stimulation conditions, demonstrated stimulation elicited enhanced signal activity including increased spike frequency and greater signal variability (Figure 8). These results indicate that gentle tactile stimulation may modulate bioelectric signaling along the Heart Meridian, reinforcing the functional sensitivity of these acupoints to external input.

The spike waveforms recorded between HT-7 and HT-9 or HT-4 and HT-7 revealed three recurring patterns: (1) an upward sharp wave pattern, typically observed at acupoints under baseline conditions without stimulation; (2) a spike-and-wave pattern, stronger at acupoints and weaker at sham points during stimulation; and (3) a downward sharp wave pattern, predominantly recorded at sham points (Figure 9).

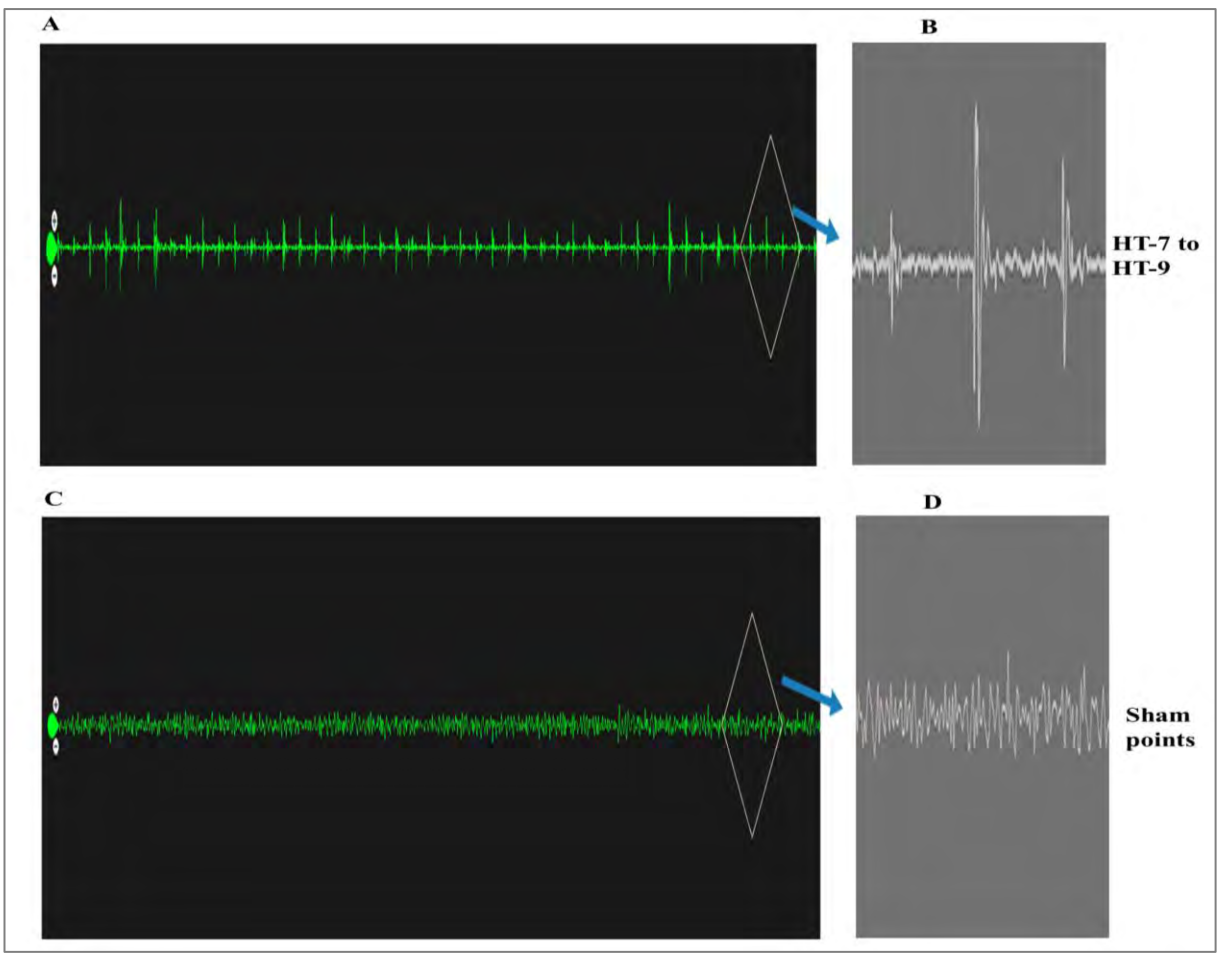

Figure 5: Capturing bioelectrical activity (baseline, without stimulation) at equine acupoints using the EAG device. **A.** Bioelectric potentials recorded between acupoints HT-7 and HT-9. **B.** Representative examples of dominant spike patterns commonly observed between HT-7 and HT-9. **C.** Bioelectric potential variability recorded at sham (non-acupoint) locations. **D.** Representative examples of dominant spike patterns commonly observed at sham acupoints.

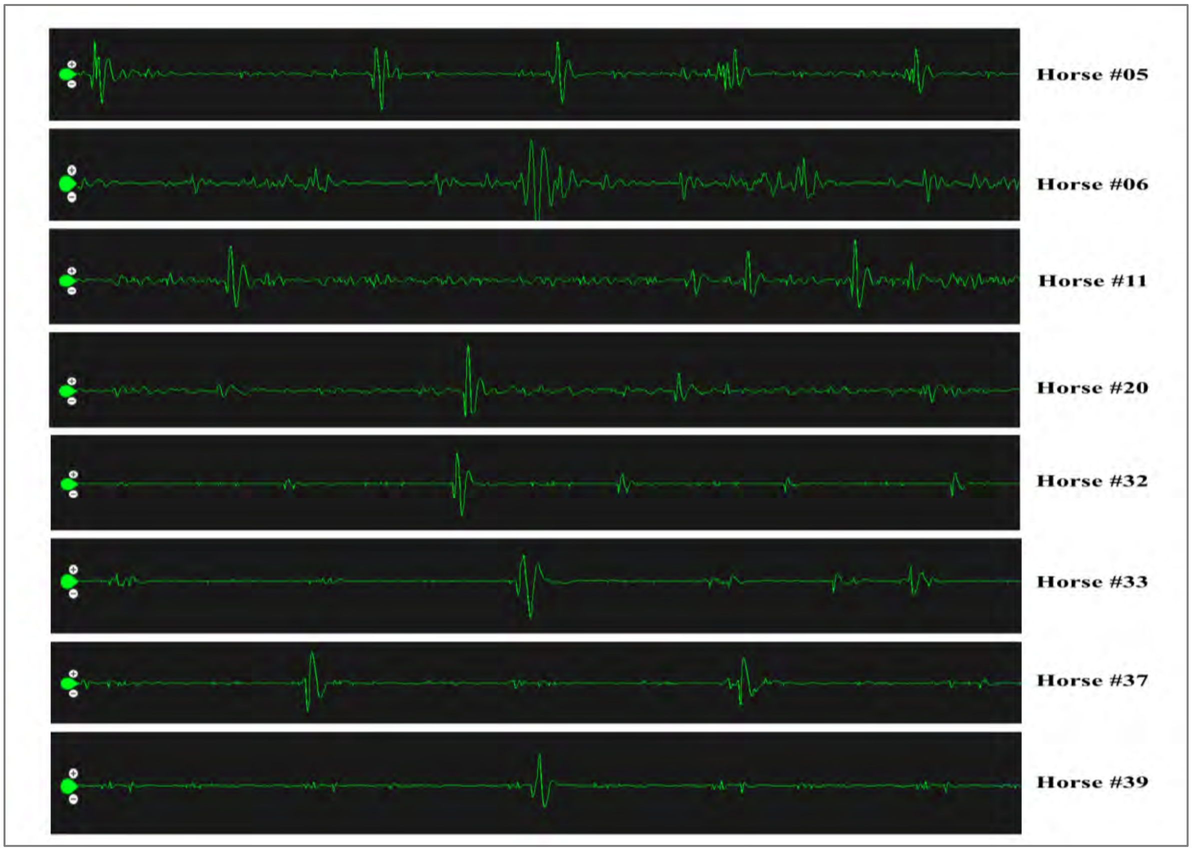

Figure 6: Bioelectric potential measurement (baseline) between equine HT-4 and HT-7 acupoints using the EAG system. This figure depicts the range of bioelectric potentials recorded between HT-4 and HT-7 in a sample of 8 horses. Additionally, it highlights representative dominant spike patterns commonly observed in the data.

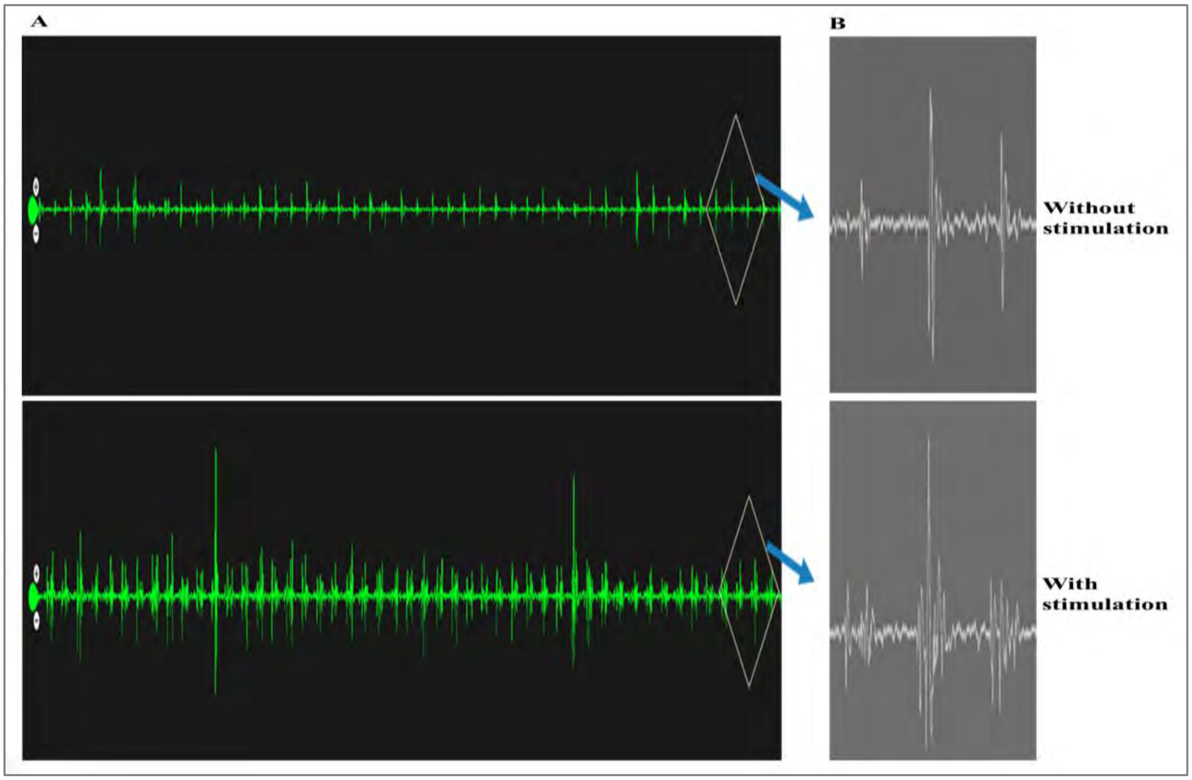

Figure 7: Comparison of EAG responses at equine HT-7 and HT-9 acupoints before and after stimulation. **A.** Variation in bioelectric potentials between acupoints HT-7 and HT-9 under different conditions: without (upper panel) and with (lower panel) gentle external stimulation (forehead touch). **B.** Representative display of dominant spike patterns frequently observed.

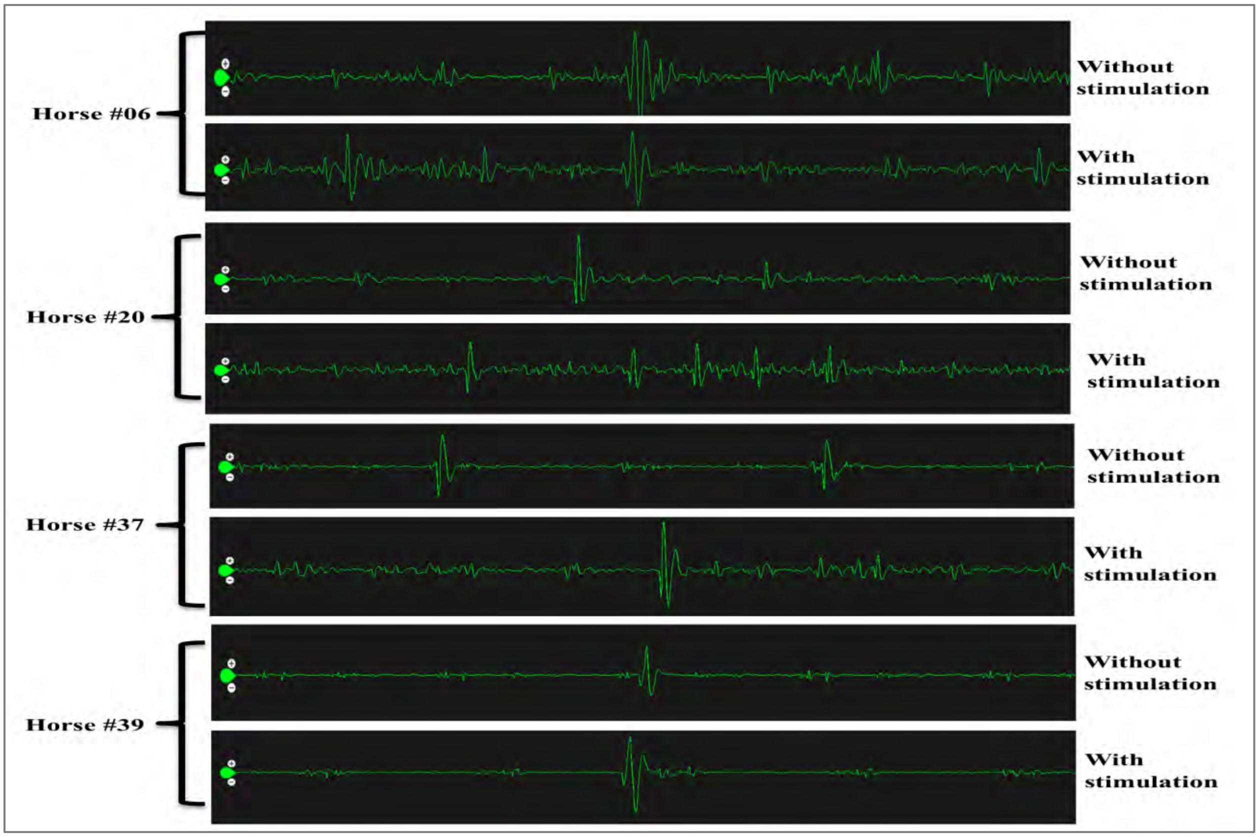

Figure 8: Effects of external stimuli on EAG signals between equine acupoints HT-4 and HT-7. This figure illustrates the difference in bioelectric activity between acupoints HT-4 and HT-7 under two conditions: with and without gentle external stimulation (forehead touching). Representative examples of common spike patterns are also presented.

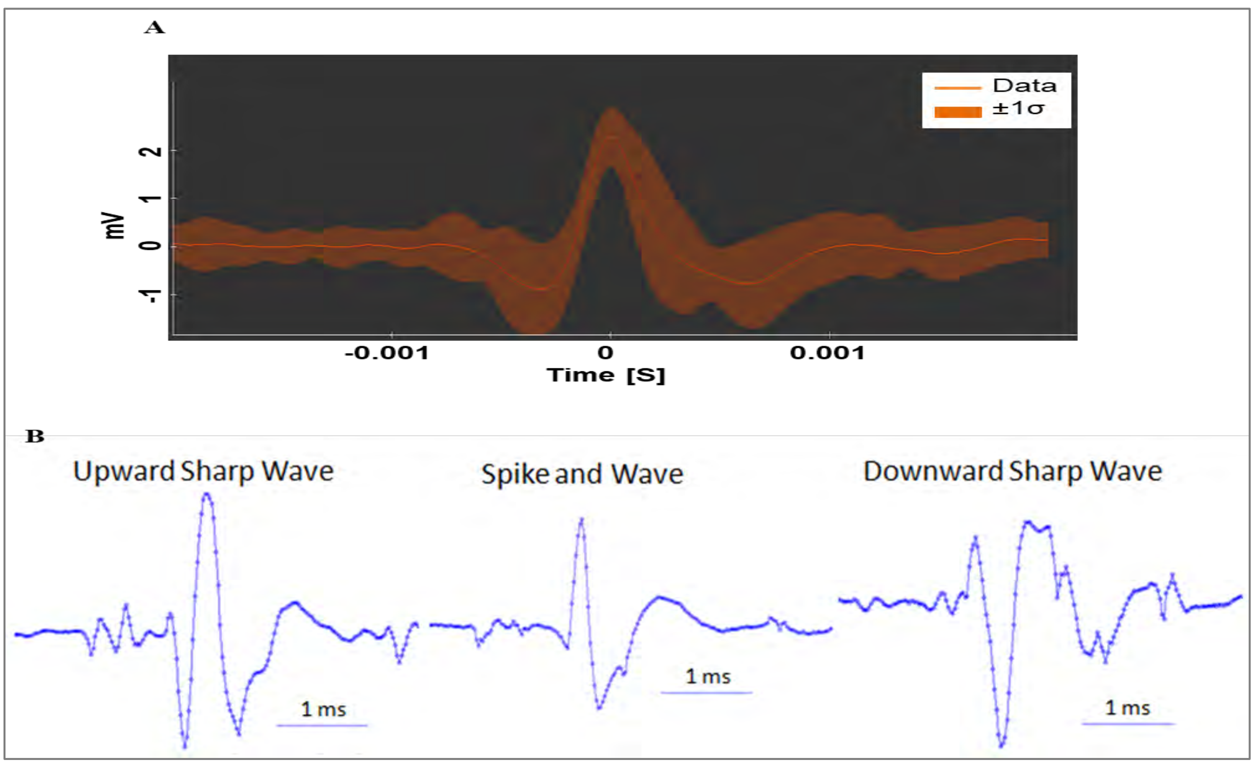

Figure 9: EAG spike waveform characteristics at equine HT-7 and HT-9 acupoints. **A.** Spike waveforms recorded between acupoints HT-7 and HT-9. The horizontal axis represents time in milliseconds (ms), centered around the positive peak, while the vertical axis indicates the average amplitude of the spike waveforms in millivolts (mV). The central bright line depicts the mean waveform, with the shaded area representing the standard deviation. **B.** Representative EAG waveforms showcasing three common wave patterns observed in the recordings: upward sharp wave (baseline acupoint), spike & wave (stimulation acupoint), downward sharp wave (sham points).

Bioelectric Potentials Generated from Human Heart Meridian Acupoints

Bioelectric potentials at human acupoints HT-7 and HT-9 were recorded and analyzed, revealing consistent spike patterns similar to those observed in horses (Figure 10A). Cold stimulation resulted in increased peak amplitudes, indicating heightened bioelectric activity (Figure 10B). Comparison of both electrode types (pad versus needle) demonstrated successful captured bioelectric activity with comparable waveform patterns (Figure 11). The recorded signals exhibited similar spike morphologies, including waveform shape, amplitude range, and spike frequency, indicating high fidelity in detecting bioelectric signals regardless of electrode type.

The bioelectric potential between the terminal acupoint of the Heart Meridian (HT-9) and other acupoints along the Meridian (HT-8, HT-7, HT-6, HT-5, HT-4, HT-3, and HT-2) was assessed using the EAG device (Figure 4). EAG patterns recorded from different acupoints exhibited distinct variability (Figures 12A, 12B, and 12C). For example, the waveforms recorded between HT-9 and HT-8 as well as between HT-9 and HT-7 displayed lower frequencies (Figure 12A), while higher frequencies were observed in recordings between HT-9 and HT-6, HT-5, HT-4, HT-3, and HT-2 (Figures 12B and 12C).

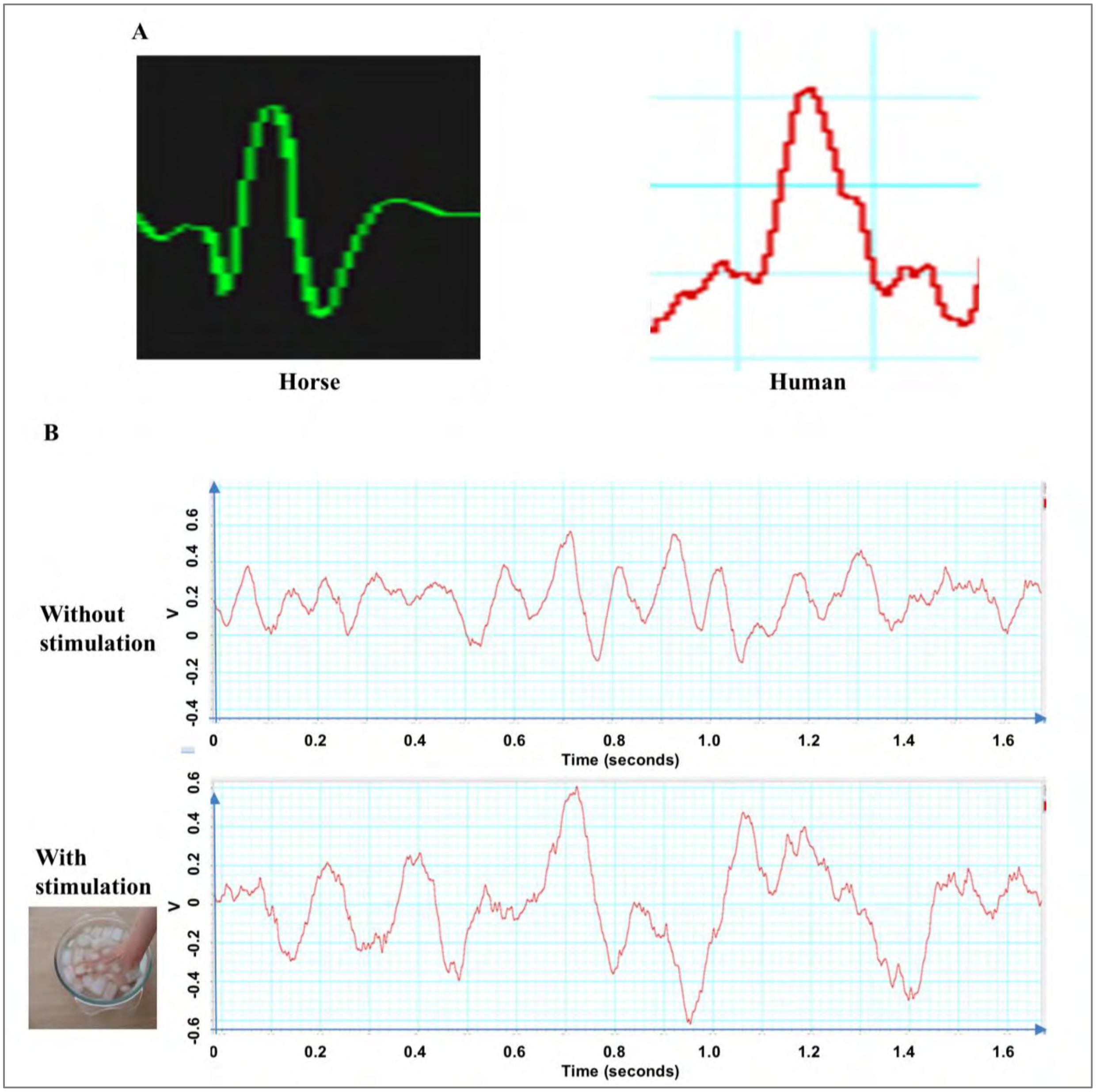

Figure 10: Human acupoint EAG analysis. **A.** The EAG device is connected to needles at HT-7 and HT-9 acupoints in human subjects to measure bioelectric potential variations. Representative potential patterns are shown for horses (left panel) and humans (right panel). **B.** Waveform amplitude is affected by cold stimulation. EAG recordings were taken from HT-7 and HT-9 on the right hand, with and without the left hand immersed in ice-cold water. The horizontal axis represents time, and the vertical axis shows the amplitude of the average spike waveforms in millivolts (mV).

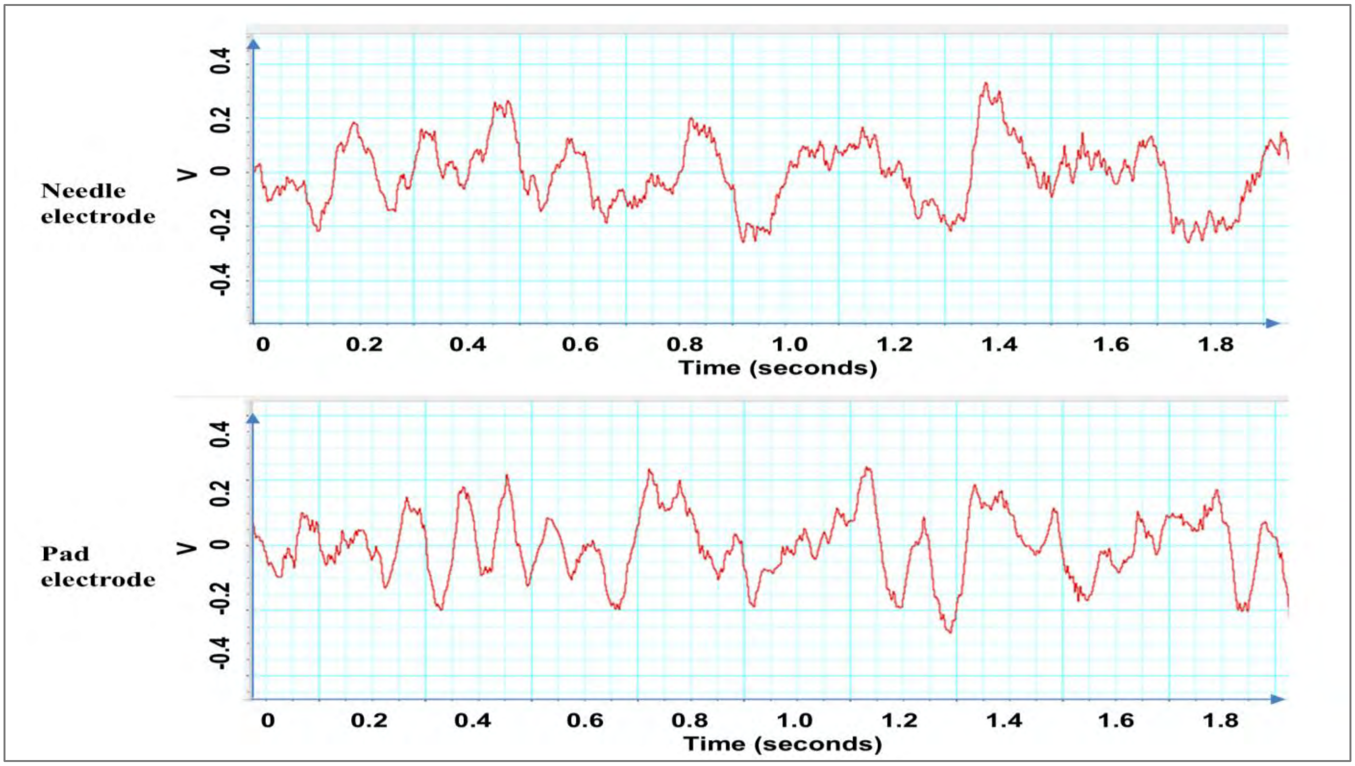

Figure 11: Comparison of electrode types used for human acupoint recording. The EAG device is connected either to needles inserted at HT-7 and HT-9 acupoints or to pad electrodes placed over these points.

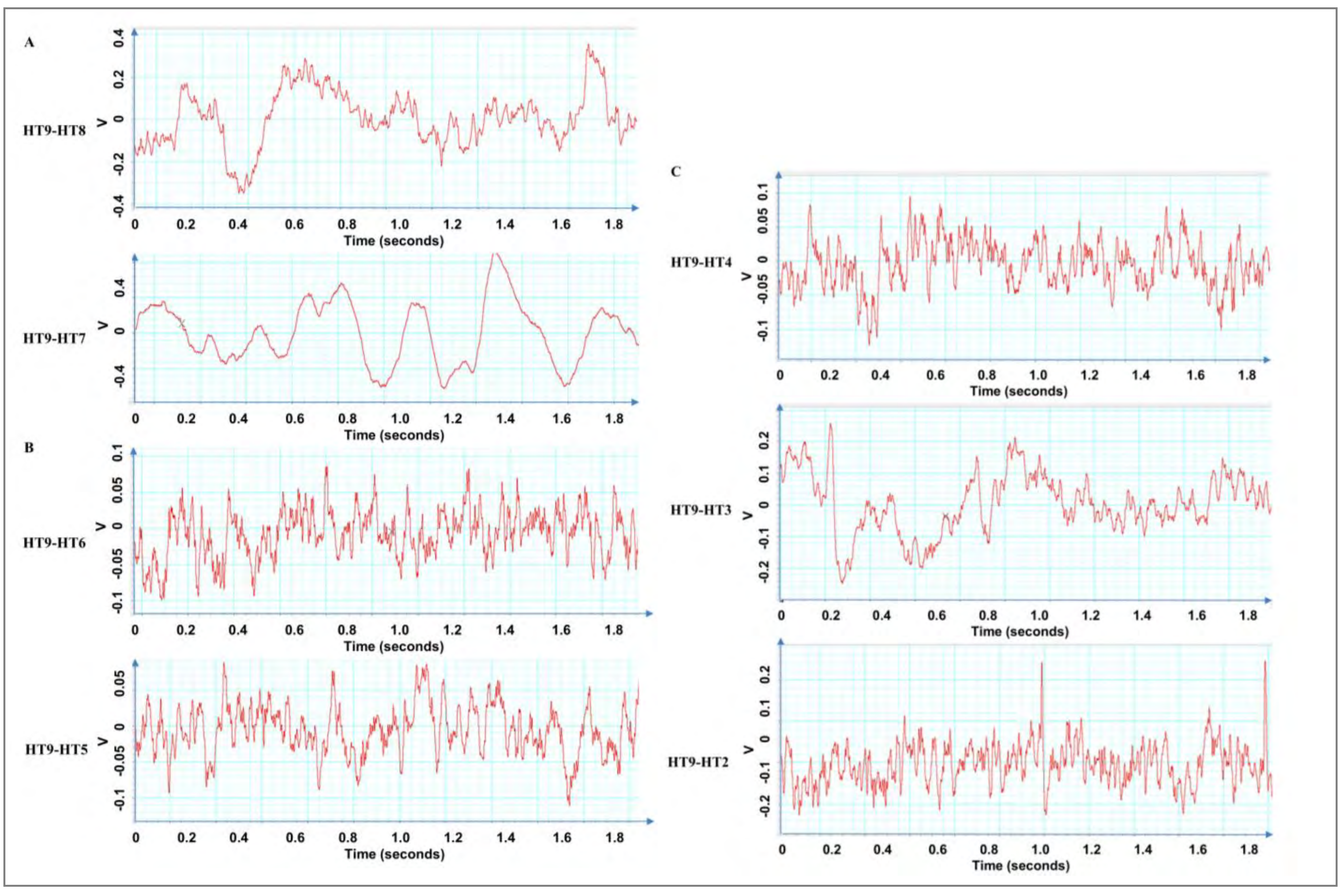

Figure 12: EAG bioelectric potential measurement across human acupoints along the Heart Meridian. The EAG device is connected to a pad electrode at acupoint HT-9 and another electrode at HT-8 or HT-7 (**panel A**), HT-6 or HT-5 (**panel B**), and HT-4, HT-3, or HT-2 (**panel C**). Bioelectric potential changes between these acupoint pairs are recorded and analyzed to identify distinct patterns.

DISCUSSION

This study explored the bioelectric properties of equine and human acupuncture points. A versatile platform (electroacugram/EAG) was designed to detect and monitor bioelectrical signals at acupoints. The system's modular architecture included specialized electrodes and advanced filtering capabilities, designed to capture high-fidelity bioelectric signals. Research was then conducted: 1) to investigate the new system's efficacy in differentiating genuine acupoints from sham points, 2) compare bioelectric change at acupoints during physiologic stimulation (forehead stroking, hand in ice) to baseline measurement (no stimulation), and 3) measure bioelectric pattern similarity between the use of electrode pads to acupuncture needles.

During phase 1 research, the EAG system was used to measure bioelectric potential between HT-7 and HT-9 and between sham points in horses. The recorded amplitudes between HT-7 and HT-9 were notably higher compared to those from the sham points. Furthermore, the electric signals from the acupoints displayed distinct wave patterns that differed significantly from those observed at the sham points. The authors suggest the signals from sham points likely originated from background noise or a mixture of noise and weak bioelectric signals generated by cells at sham point locations. These observations indicate that acupoints HT-7 and HT-9, but not sham acupoints, have the ability to generate unique measurable bioelectric activity in horses.

During the second phase, to establish the translational relevance of the equine model in reflecting human acupoint bioelectric characteristics, bioelectric potentials at human acupoints, HT-7 and HT-9, were recorded and analyzed. A consistent spike pattern was observed, akin to that in horses, demonstrating translational relevance across equine and human models. Additionally, the research delved into the impact of external physiological stimulation on bioelectric activity between HT-7 and HT-9. Gentle forehead touching/stroking was performed on the horses, while the humans were requested to immerse a hand in ice water. The EAG demonstrated a resultant change in pattern associated with physiological stimulation. The horses showed a strong spike and weave pattern at acupoints versus baseline (upward sharp wave); while cold or pain stimulation in humans led to an increase in peak amplitudes, indicating heightened bioelectric activity.

Research conducted on a comparative analysis between needle-based stimulation and non-invasive pad electrodes revealed similar EAG signal characteristics, supporting the effectiveness of both methods for capturing bioelectric activity. The consistency in EAG signals obtained through both needle and pad electrodes underscores the potential utility of the EAG device for non-invasive profiling of bioelectric signals at acupoints in humans. This is particularly relevant for clinical settings where minimal invasiveness is desired.

When compared to existing literature, our observations align with earlier research demonstrating the physiological impact of acupuncture stimulation.[32,33] The consistent EAG patterns identified in both human and equine models echo findings by Choi et al., who reported that the physiological effects of acupuncture are significantly influenced by both needle manipulation techniques and the patient's perceived needling sensation. Specifically, Choi and colleagues found that enhanced clinical outcomes, including pain reduction and improved autonomic response, were closely associated with stronger "*De-qi*" sensations—a composite of aching, tingling, or heaviness—elicited through manual needle rotation or depth variation. Their randomized trial demonstrated that manipulating acupuncture needles to enhance needling sensation produced greater therapeutic efficacy compared to shallow or static needling. This supports the hypothesis that sensory input from acupuncture is not merely a passive event, but an active modulator of neuromodulatory pathways. The observed amplification of EAG signals during external stimulation in our study—such as increased spike amplitude and complexity following forehead touches in horses and cold-induced stress in humans—suggests that acupoints are highly responsive to sensory inputs, consistent with the mechanistic model proposed by Choi et al. Therefore, the variability in bioelectric signal patterns across different stimulation conditions may reflect physiologically relevant mechanisms linked to the efficacy of acupuncture therapy, and underscores the importance of needling technique and patient sensation in clinical outcomes.[34]

Despite the strengths of our findings, several limitations should be considered. The study's focus on equine and human models limits the generalizability of our results to other species. Additionally, the long-term effects of modulating bioelectric activity remain unexplored. Addressing these gaps in future research will be critical to fully understanding the therapeutic potential of bioelectric modulation.

In summary, findings from this study satisfy the hypothesis that EAG recordings at acupoints would demonstrate unique consistent and measurable bioelectric activity in horses and humans and fills a critical gap by introducing a specialized EAG device for acupoint bioelectric signal analysis. This system is a significant advancement over previous methods that relied on skin impedance measurements, fraught with validity concerns.[35] Furthermore, this study explored the impact of external physiologic stimuli on bioelectric activity, identifying distinct EAG patterns that may have therapeutic implications. Overall, our findings demonstrate the EAG system's utility in bioelectric research and also open new avenues for understanding the role of acupoint bioelectrical signals and potential therapeutic applications.

AUTHOR CONTRIBUTION

Jianliang Zhang: Conceptualization, Investigation, Methodology, Writing – review & editing
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FOOTNOTES

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