Original Scientific Reports

The Effectiveness of Electro-Acupuncture on Experimental Lameness in Horses

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ABSTRACT

Six normal experimental Thoroughbred horses, 4-8 years old, were selected for this study. Each subject was fitted with special shoes on the front feet designed to produce a focal area of controlled sole pressure to induce lameness. A standardized lameness scale from 0-3, based on specific clinical behaviors, was established prior to the study. A focal area of compression was induced by tightening one screw on the left side of the left front shoe until a lameness score of 2-3 was attained in the left front limb. Each horse received three different treatments, with a one-week washout period between each treatment, in a randomly assigned order using a double 3 x 3 Latin Square study design. The three treatments each horse received were: electro-acupuncture connecting acupoints *Bai-hui* to *Duan-xue*, the left SI-9 to *San-yang-luo*, and the left *Qian-chan-wan* to *Qian-jiu* for 45 minutes with a frequency of 80-120 Hz, a 0.5% bupivacaine HCL nerve block subcutaneously injected into the area of the lateral palmar nerve where it passes lateral to the lateral sesamoid bone (as a positive control to block the left lateral area of the sole the screw was compressing) and a sham nerve block using saline at the same site (as a negative control). Lameness was evaluated by 2 separate investigators using the standardized lameness scale at the beginning of the study prior to lameness induction, after lameness was induced and after each treatment. One of the investigators evaluated only videos of the lameness examination and was blinded as to which treatment group each horse was in at the time. Radioimmunological assays (RIA) were used to measure plasma concentration of β-endorphin, cortisol and ACTH before and after the 3 treatments. Electro-acupuncture (EA) decreased the lameness score and reduced the degree of lameness significantly ($P<0.001$). EA also significantly increased plasma β-endorphin concentrations and reduced cortisol levels compared to the other groups, but had no effect on ACTH levels. These results suggest that the release of β-endorphin may be one of the mechanisms by which acupuncture relieves pain. The cause for the reduced cortisol levels was unknown. The results of this study further support the use of EA to treat natural occurring lameness from hoof disease in horses.

Key Words: Equine, lameness, electro-acupuncture, hoof pain

The ultimate goal of equine medicine is to relieve animal pain and suffering. Equine practitioners may accomplish this goal without recognizing pain; however, it is not possible for them to assess the effectiveness of treatments without defining the expression of pain. Thus, pain models have been developed for the evaluation of pain-relieving and analgesic effects of interventions in horses.[1,2] Many reported pain models assess the effectiveness of analgesics and sedatives in horses. Based on the well-known effect of a stone in the shoe, Merkens and Schamhardt developed a reversible lameness model.[3] A special horseshoe was constructed so that a screw could be turned to apply focal pressure against the sole, resulting in lameness of adjustable severity. After removal of the screw, the pain and lameness in the horses vanished immediately. [3]

The most common and rewarding aspect of equine acupuncture is the diagnosis and treatment of lameness due to musculoskeletal pathology. [4,5] Electro-acupuncture (EA) has been found to effectively restore normal activity and function in the event of musculoskeletal abnormalities and treat

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lameness in horses.[6,7] However, few controlled studies have been conducted on how acupuncture relieves lameness. In this study, an experimental reversible lameness model was used in horses. The objectives of the study were to determine if EA would reduce the degree of lameness in this experimental model and provide some insight into the mechanisms by which this may occur. This study was approved by the University of Florida Institutional Animal Use and Care Committee (IACUC).

MATERIALS AND METHODS

Six experimental Thoroughbred horses, aged 4-8 years of age, were used in this study. There were 3 mares, 2 geldings and 1 stallion. They were all housed at the College of Veterinary Medicine at the University of Florida. Two days before the start of the experiment, both front feet of each horse were fitted with special shoes designed to allow controlled pressure to be applied to the sole of the foot by tightening a screw on one side (Figure 1). The subjects were randomly assigned to 3 treatment groups and each horse received three treatments as outlined in Table 1. The three treatments were acupuncture, a nerve block to serve as a positive control and a sham nerve block with saline to serve as a negative control. There was a washout period of one week between each treatment.

Table 1: Individual horse assignment by treatment group

Horse #	Random Assignment of Treatment (Double 3 x 3 Latin Square Design)		
	1st	2nd	3rd
1	AT	PC	SC
2	PC	SC	AT
3	SC	AT	PC
4	AT	SC	PC
5	SC	PC	AT
6	PC	AT	SC

AT=acupuncture treatment; PC= bupivacaine HCL positive control; SC= saline injection negative control

In the acupuncture treatment (AT) group, electro-acupuncture (EA) stimulation was conducted on each horse for 45 minutes with a frequency of 80-120 Hz using a standard EA instrument [a]. In the positive control (PC) group, 2 ml of 0.5 % bupivacaine HCL [b] were injected subcutaneously into the area of the lateral palmar nerve where it passes lateral (abaxial) to the lateral sesamoid bone in order to block the region of sole compression induced by the tightened screw. In the sham control (SC) group, 2 ml of saline were injected into the pastern in the same area of the lateral palmar nerve.

At 0800 hours, the test horse was taken to a quiet barn or stocks and a catheter was placed in the left jugular vein, filled with heparinized saline and taped to the neck. At 0900 hours, the horse was placed on a treadmill to evaluate for pre-study lameness. At 0930 hours, the left lateral screw on the left shoe was tightened until the lameness score was 2-3 while standing, walking and trotting on the treadmill. At 1015 hours, EA stimulation was given to the AT group, pastern injections of bupivacaine HCL in the PC group and pastern injections of saline in the SC group. At 1145 hours, the screw on the left shoe was loosened. At 1330 hours, the experiment was terminated and the horse returned to the field for a week before the process was repeated and the next type of treatment administered.

The shoeing procedure was based on the Merkens's method. [3] A M8-nut (φ 8mm) was welded to the inner rim of each branch of a shoe between the quarters and the bars and a round headed screw (2.5 cm length) was screwed into the nut (Figure 1). Pressure on the intact sole could be developed by further tightening the screw against the sole. The degree of lameness could be varied by turning the screw tighter or looser, thereby altering the pressure on the sole. The lateral screw on the left shoe was tightened until the horse's lameness score was 2-3. Most of the horses showed lameness when the screw was turned 3 rounds after it touched the sole. Lameness score, stride lengths and treadmill exercise were evaluated on each subject at pre-shoeing and post-shoeing to determine the baseline of these measurements.

Lameness evaluation was based on a lameness grading score and the stride length. [8,9]

A lameness grading score (0 to 3) was

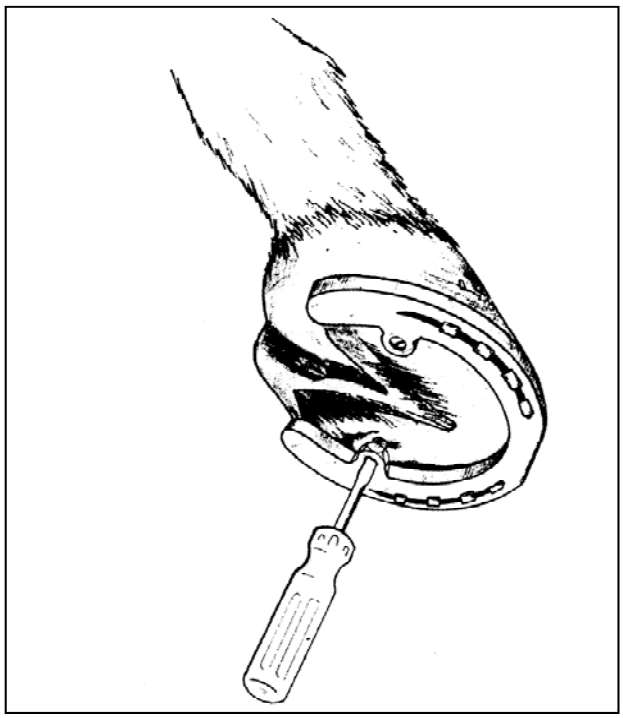

Figure 1: Pressure against hoof sole induced by turning the adjustable screw (The lateral screw of the left front limb was tightened to induce lameness)

designed for evaluating the severity of lameness based on the clinical signs and the degree of lameness present while standing, walking and trotting on the treadmill (Table 2). All the lameness evaluations were recorded on video-tape. Even with a defined lameness scale, the evaluation of lameness in horses can be subjective so to reduce the subjective bias in this study, one investigator performed all live lameness evaluations and these were videotaped and the other investigator based their evaluation on the video-tape and was blinded to the treatment group assignment of each horse. The final lameness score was the average score of the two different investigators' scores.

Stride lengths were measured from the hoof imprints after the horse walked over a sand pit. The stride length was the average of five continual stride measurements. There are three types of stride length measurements: total stride length (TSL), front half stride length (FHSL) and back half stride length (BHSL) (Figure 2). The TSL is the distance between the cranial edge of each hoof imprint for successive imprints of that hoof. The TSL of one limb is divided into two parts by the contralateral hoof imprint. The cranial part is the FHSL and the caudal part is the BHSL (Figure 2). The difference between front and hind stride length (DFB) was DFB (cm) =FHSL (cm) - BHSL (cm).

Table 2: Lameness scoring scale

Score	Characteristics
0	The horse stands, walks and trots easily and willingly without an abnormal gait Will readily bear weight on the affected limb when the other limb is lifted
1	The horse lifts the affected limb incessantly and alternately Not reluctant to move No lameness is evident at a walk, but at a trot the horse moves with a short and stilted gait Will readily bear weight on the affected limb when the other limb is lifted
2	The horse lifts the affected leg incessantly and alternately Reluctant to move but will Lameness is evident at a walk or trot and the gait is short and stilted Will bear weight on the affected limb when the other limb is lifted only when forced
3	The horse will not bear body weight on the affected limb when standing Will not move unless forced Lameness is severe at a walk or trot and the gait is short and stilted Refuses to bear weight on the limb when the other limb is lifted

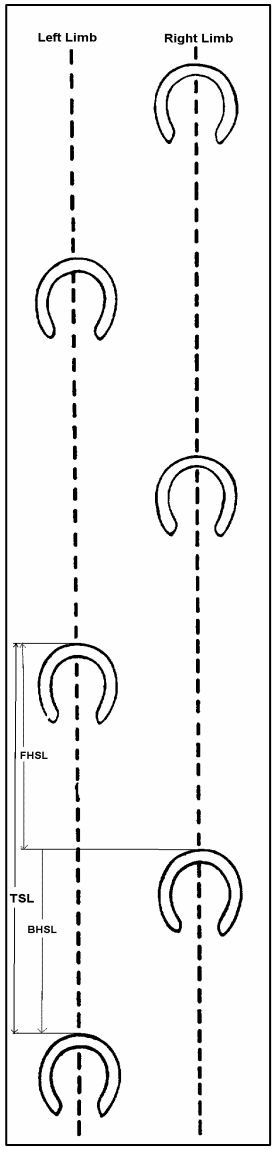

Figure 2: The measurement of stride length in horses
TSL= total stride length (cm); FHSL= front half stride length (cm);
BHSL= back half stride length (cm)

45 minutes using a standard electro-acupuncture instrument.[a]

Blood samples were collected 14 times respectively at 0800, 0900, 0915, 9030, 0945, 1000, 1015, 1030, 1045, 1100, 1115, 1145, 1200 and 1330 hours for each subject in all 3 groups. Blood samples (20 ml) were collected from the jugular vein after clearing the catheter of heparin. Blood was collected into a venipuncture tube containing EDTA as the anticoagulant. The sample tube was immediately inserted into ice, then blood was centrifuged within 30 minutes of collection and plasma was stored at -20 ^{0} C until assayed. Radioimmunological assays (RIA) were used to measure plasma concentrations of β-endorphin , cortisol and ACTH. [10-12] Standard β-endorphin kits [e], coat-a-count cortisol kits[d] and ACTH kits[d] were used.

The study design was a double 3 x 3 Latin Square design with 2 factors. The data was presented as the mean ± standard error (S.E.) and analyzed using the general linear model (GLM). The significance level was p=0.05 when the Duncan's Multiple Range Test was used to compare data for the different time periods in the same group. The significance level was p=0.01 when Least Squares Means were used to compare data for the different treatment groups at each measuring time. All statistical analyses were performed using SAS for Windows (version 6.12).

RESULTS

The lameness score results are summarized in Table 4. The lameness score increased significantly (P<0.001) in all groups after the screw was tightened. After the saline injection, the lameness score did not change significantly (P>0.05). After the bupivacaine HCL injection, the lameness score decreased significantly (P<0.001) and returned to the baseline level. After the acupuncture treatment, the lameness score decreased significantly (P<0.001), but did not return to the baseline level.

The total stride length (TSL) of the front right or left limb did not change significantly (P>0.05) among different time periods in all groups (Table 5). The front half stride length (FHSL) did not significantly (P>0.05) change among different time periods in all groups (Table 6). The back half stride length (BHSL) did not significantly (P>0.01) change among different time periods in AT and PC groups, however, it decreased (P<0.05) after saline injection in SC group (Table 6).

Prior to tightening the screw, no lameness occurred and the difference between the front and back half stride length (DFB) was -1.2±0.9 cm in the AT group, -0.6±1.6 cm in the PC group and 0.3±1.3 cm in the SC group (Table 7). When the screw was tightened and lameness occurred, DFB

Table 3: Location and insertion technique of the acupuncture points used in this study

Name of Acupoint	Location	Insertion Technique
Bai-hui	Between the spinous processes at the lumbosacral junction	Perpendicular insertion 6 cm deep
Duan-xue (GV-6)	Between the spinous processes of T18 and L1	Perpendicular insertion 5 cm deep
Qiang-feng (SI-9)	In the depression formed by the intramuscular groove that separates the long and the lateral heads of triceps brachii muscle just caudal to the deltoideus muscle	Perpendicular insertion 7.5 cm deep
San-yang-luo	In the intramuscular groove between lateral digital extensor and ulnaris lateralis muscle, about 3 cm distal to the proximal head of the radius	Angular insertion 6 cm deep
Qian-chan-wan (SI-3)	On the caudolateral side of the fetlock, just proximal to the proximal sesamoid bones	Perpendicular insertion 2 cm deep
Qian-jiu (PC-9)	On the palmer aspect of the front hoof, on the palmer median line, just proximal to the heel bulbs	Angular insertion 2 cm deep

Table 4: Lameness scores in horses before and after tightening of the hoof screw, after treatment in each group and after loosening the screw

Group	Horses per group	**Lameness Score** (Mean ± S.E.)				
		Before tightening	**After tightening**	**After treatment**	**After loosening**	**95 minutes later**
AT	6	$0.2 \pm 0.2^{a\,1}$	$2.7 \pm 0.2^{c\,1}$	$1.3 \pm 0.2^{b\,2}$	$0 \pm 0^{a\,1}$	$0 \pm 0^{a\,1}$
PC	6	$0.1 \pm 0.1^{a\,1}$	$2.3 \pm 0.2^{b\,1}$	$0.3 \pm 0.2^{a\,1}$	$0 \pm 0^{a\,1}$	$0.1 \pm 0.1^{a\,1}$
SC	6	$0.2 \pm 0.2^{a\,1}$	$2.5 \pm 0.2^{b\,1}$	$2.0 \pm 0.4^{b\,2}$	$0.3 \pm 0.2^{a\,1}$	$0.2 \pm 0.2^{a\,1}$

S.E.= standard error; AT= acupuncture treatment; PC= bupivacaine HCL treatment; SC=saline treatment
Alphabetic superscripts ^{a,b,c} : Mean values within the group without a common alphabetic superscript are statistically different ($P<0.05$) by the Duncan's Multiple Range Test; Numerical superscripts ^{1,2}: Mean values between groups without a common numerical superscript are significantly different ($P<0.01$) by the Least Squares Means comparison.

increased ($P<0.01$) to 10.8±2.4 cm in the AT group and 8.6±3.3 cm in the SC group. After acupuncture treatment, DFB decreased ($P<0.01$) to 3.8 ± 1.3 cm in the AT group, while DFB remained increased at 8.9 ±3.2 cm in the SC group after saline injection. This result indicates that the DFB may be an objective parameter to assess lameness in horses. When lameness was present, the DFB was greater than 3.8 cm and when the DFB was less than 0.5 cm or -2.4 cm, the horse was not lame (Table 7).

In the acupuncture treatment group, the plasma concentration of beta-endorphin started to decrease significantly at 0945 hours, but increased significantly ($P<0.001$) during acupuncture treatment, returned to the baseline after termination of acupuncture treatment and declined through the end of the test (Table 8). For the bupivacaine HCL treatment group, the plasma concentration of beta

Table 5: Total stride length (TSL) in centimeters of the right and left front limb in horses before and after tightening of the hoof screw, after treatment in each group and after loosening the screw

Group	Limb	**Total Stride Length (cm)** (Mean ± S.E.)				
		Before tightening	**After tightening**	**After treatment**	**After loosening**	**95 minutes later**
AT	right	253.5±9.9$^{a\ 1}$	234.4 ±8.9$^{a\ 1}$	230.1±10.8$^{a\ 1}$	241.8±13.0$^{a\ 1}$	247.3±11.1$^{a\ 1}$
	left	253.2±9.9$^{a\ 1}$	234.0±9.2$^{a\ 1}$	231.1±11.1$^{a\ 1}$	242.3±12.9$^{a\ 1}$	247.8±11.1$^{a\ 1}$
PC	right	254.0±11.9$^{a\ 1}$	232.7 ±6.0$^{a\ 1}$	245.5 ±9.7$^{a\ 1}$	253.4 ±9.1$^{a\ 1}$	243.8 ±7.0$^{a\ 1}$
	left	254.3±11.8$^{a\ 1}$	232.7±5.6$^{a\ 1}$	244.7±9.6$^{a\ 1}$	253.2±9.0$^{a\ 1}$	244.1±6.8$^{a\ 1}$
SC	right	243.7 ±9.5$^{a\ 1}$	238.4 ±4.0$^{a\ 1}$	227.2 ±6.1$^{a\ 1}$	249.5 ±6.9$^{a\ 1}$	237.3 ±7.8$^{a\ 1}$
	left	245.9±9.5$^{a\ 1}$	237.9±4.0$^{a\ 1}$	227.9±6.1$^{a\ 1}$	250.7±7.0$^{a\ 1}$	238.0±8.1$^{a\ 1}$

S.E.= standard error; TSL = the distance between the front edges of the imprint of each front leg between successive strides; AT= acupuncture treatment; PC= bupivacaine HCL treatment; SC=saline treatment
Alphabetic superscripts ^{a,b}: Mean values within the group without a common alphabetic superscript are statistically different (P<0.05) by the Duncan's Multiple Range Test; Numerical superscripts ^{1,2}: Mean values between groups without a common numerical superscript are significantly different (P<0.01) by the Least Squares Means comparison.

Table 6: The front half stride length (FHSL) and back half stride length (BHSL) of the left front limb in horses before and after tightening of the hoof screw, after treatment in each group and after loosening the screw

Group	Half	**Stride Length** (cm) (Mean ± S.E.)				
		Before tightening	**After tightening**	**After treatment**	**After loosening**	**95 minutes later**
AT	FHSL	126.1±5.1$^{a\ 1}$	122.4±4.5$^{a\ 1}$	117.5±6.1$^{a\ 1}$	120.0±6.5 $^{a\ 1}$	123.0 ±5.4$^{a\ 1}$
	BHSL	127.3±4.9$^{a\ 1}$	111.6±5.0$^{a\ 1}$	113.7±5.0$^{a\ 1}$	122.3±6.5$^{a\ 1}$	124.8±5.7$^{a\ 1}$
PC	FHSL	126.9 ±6.0$^{a\ 1}$	119.7 ±3.7$^{a\ 1}$	123.4 ±6.0$^{a\ 1}$	126.8 ±4.4$^{a\ 1}$	121.1 ±2.7$^{a\ 1}$
	BHSL	127.4±6.0$^{a\ 1}$	112.9±2.6$^{a\ 1}$	122.1±3.9$^{a\ 1}$	126.3±4.8$^{a\ 1}$	122.9±4.4$^{a\ 1}$
SC	FHSL	123.1 ±4.9$^{a\ 1}$	123.2 ±2.8$^{a\ 1}$	118.4 ±3.0$^{a\ 1}$	124.5 ±4.0$^{a\ 1}$	118.7 ±3.7$^{a\ 1}$
	BHSL	122.8±4.7$^{a\ 1}$	114.7±2.3$^{a,b\ 1}$	109.5±3.8$^{b\ 2}$	126.2±3.0$^{a\ 1}$	119.3±4.5$^{a,b\ 1}$

S.E.= standard error; AT= acupuncture treatment; PC= bupivacaine HCL treatment; SC=saline treatment
Alphabetic superscripts ^{a,b}: Mean values within the group without a common alphabetic superscript are statistically different (P<0.05) by the Duncan's Multiple Range Test; Numerical superscripts ^{1,2}: Mean values between groups without a common numerical superscript are significantly different (P<0.01) by the Least Squares Means comparison.

endorphin started to decrease at 0945 hours and continued to decline through the end of the test (Table 8). In the saline treatment group, the plasma concentration of beta-endorphin started to decrease at 1000 hours and continued to decline until the termination of experiment.

The plasma concentration of ACTH did not change significantly (P>0.05) for the different time periods (Table 9). The plasma cortisol concentration increased numerically in the acupuncture group after treatment, but decreased significantly (P<0.001) at 1200 and 1330 hours. For both the bupivacaine HCL and saline treatment groups, the plasma cortisol concentration did not change significantly (P>0.05) during the different time periods (Table 10). No significant difference

Table 7: The difference between front and back half stride length (DFB) of the left front limb in horses before and after tightening of the hoof screw, after treatment in each group and after loosening the screw

Group	Horses per group	**Difference Between the Front and Back Half Stride Length** (cm) (Mean±S.E.)				
		Before tightening	**After tightening**	**After treatment**	**After loosening**	**95 minutes later**
AT	6	-1.2±0.9 $^{a\,1}$	10.8±2.4 $^{c\,1}$	3.8 ±1.3 $^{b\,1}$	-2.4±0.4 $^{a\,1}$	-1.8±0.8 $^{a\,1}$
PC	6	-0.6±1.6 $^{a\,1}$	6.8 ±2.9 $^{a\,1}$	0.5 ±4.2 $^{a\,1}$	0.5±2.0 $^{a\,1}$	-1.8±2.8 $^{a\,1}$
SC	6	0.3± 1.3 $^{a\,1}$	8.6 ±3.3 $^{b\,1}$	8.9 ±3.2 $^{b\,2}$	-1.7±1.1 $^{a\,1}$	-0.6±1.5 $^{a\,1}$

S.E.= standard error; AT= acupuncture treatment; PC= bupivacaine HCL treatment; SC=saline treatment
Alphabetic superscripts ^{a,b,c}: Mean values within the group without a common alphabetic superscript are statistically different (P<0.05) by the Duncan's Multiple Range Test; Numerical superscripts ^{1,2}: Mean values between groups without a common numerical superscript are significantly different (P<0.01) by the Least Squares Means comparison.

Table 8: The mean plasma concentration of beta‑endorphin (pg/ml) in horses by group over time

Time (hours)	**Action**	**Plasma Concentration of Beta‑endorphin** (Mean±S.E.)		
		AT (n=6)	**PC (n=6)**	**SC (n=6)**
0800		50.02 ±2.8 $^{c,d\,1}$	49.62±4.7 $^{a\,1}$	52.91± 17.8 $^{a\,1}$
0900		51.73 ± 2.6 $^{c,d\,1}$	49.32 ± 6.1 $^{a\,1}$	53.52±24.2 $^{a\,1}$
0915		50.21 ±2.3 $^{c,d\,1}$	46.69± 4.4 $^{a,b\,1}$	45.93±19.4 $^{a,b\,1}$
0930	Tighten screw	44.28±2.5 $^{d,e\,1}$	44.2± 8.8 $^{a,b,c\,1}$	46.63± 22.0 $^{a.b\,1}$
0945		38.76 ± 4.1 $^{e\,1}$	40.24 ±9.1 $^{b.c.d\,1}$	43.36± 17.3 $^{a,b\,1}$
1000		39.19 ±2.8 $^{e\,1}$	40.54 ±8.9 $^{b,c,d\,1}$	31.51±18.3 $^{b,c\,1}$
1015	Treatment given	45.68± 2.8 $^{d,e\,1}$	38.58 ±5.0 $^{b,c,d\,1,2}$	31.24±14.3 $^{b,c\,2,3}$
1030		63.44 ± 4.1 $^{b\,1}$	36.75 ±3.6 $^{c,d\,2}$	24.13±11.5 $^{c,d\,2}$
1045		88.36 ±2.1 $^{a\,1}$	32.44 ±6.7 $^{d,e\,2}$	23.42± 11.0 $^{c,d\,2}$
1100	AT ended	92.38 ±3.6 $^{a\,1}$	27.14 ±10.5 $^{e\,2}$	20.16±6.7 $^{c,d\,2}$
1115		59.39±3.9 $^{b,c\,1}$	25.05 ±7.3 $^{e,f\,2}$	20.98± 12.2 $^{c,d\,2}$
1145	Loosen screw	49.49±4.9 $^{c,d\,1}$	17.75 ± 6.1 $^{f,g\,2}$	11.92±5.3 $^{c,d\,2}$
1200		37.27± 1.7 $^{e\,1}$	11.39±2.6 $^{g\,2}$	8.68±2.0 $^{d\,2}$
1330		24.40±2.2 ^{f}	10.29±2.3 ^{g}	7.31± 2.9 ^{d}

S.E.= standard error; n= number of horses in the group; AT= acupuncture treatment; PC= bupivacaine HCL treatment; SC=saline treatment; Alphabetic superscripts ^{a,b,c,d,e,f,g}: Mean values within the group without a common alphabetic superscript are statistically different (P<0.05) by the Duncan's Multiple Range Test; Numerical superscripts ^{1,2,3}: Mean values between groups without a common numerical superscript are significantly different (P<0.01) by the Least Squares Means comparison.

Table 9: The mean plasma concentration of ACTH (pg/ml) in horses by group over time

Time (hours)	Action	Plasma Concentration of ACTH (pg/ml) (Mean ± S.E.)		
		AT (n=6)	PC (n=6)	SC (n=6)
0800		84.88 ± 20.3 [a 1]	96.11± 5.4 [a 1]	97.13±15.2 [a 1]
0900		77.19 ± 15.3 [a 1]	87.24 ±6.6 [a 1]	86.05±9.7 [a 1]
0915		69.28 ± 16.1 [a 1]	92.33± 4.9 [a 1]	86.34 ±12.5 [a 1]
0930	Tighten screw	70.51± 15.4 [a 1]	86.37± 7.9 [a 1]	85.73±11.6 [a 1]
0945		74.75 ±18.8 [a 1]	84.24 ± 6.3 [a 1]	81.98±11.0 [a 1]
1000		74.25 ± 14.7 [a 1]	87.56 ± 7.8 [a 1]	86.93±14.4 [a 1]
1015	Treatment given	69.03± 14.7 [a 1]	92.91 ±9.8 [a 1]	82.50± 13.8 [a 1]
1030		83.79 ± 20.6 [a 1]	91.17 ± 10.2 [a 1]	84.42±15.6 [a 1]
1045		69.87 ±13.6 [a 1]	89.67±10.5 [a 1]	96.04± 14.9 [a 1]
1100	AT ended	69.71 ±14.4 [a 1]	91.79 ± 10.6 [a 1]	101.27 ±17.5 [a 1]
1115		71.79 ± 14.9 [a 1]	93.05±11.1 [a 1]	101.59±14.1 [a 1]
1145	Loosen screw	64.24± 14.0 [a 1]	86.36 ± 5.8 [a 1]	92.51±11.5 [a 1]
1200		67.31±12.8 [a 1]	98.87±9.0 [a 1]	86.14±11.7 [a 1]
1330		71.88±15.0 [a 1]	104.5±13.5 [a 1]	82.94±8.8 [a 1]

S.E.=standard error; n= number of horses in the group; AT= acupuncture treatment; PC= bupivacaine HCL treatment; SC=saline treatment; Alphabetic superscript [a]: Mean values within the group are not significant (P>0.05) by the Duncan's Multiple Range Test; Numerical superscript [1] : Mean values between groups are not significantly different (P>0.05) by the Least Squares Means comparison.

of the plasma concentration of ACTH and cortisol was found among acupuncture, bupivacaine HCL and saline treatment groups (P>0.05).

DISCUSSION

The results of this experimental model showed that EA significantly reduced the degree of lameness and the lameness score in this group of horses. These findings agree with the numerous clinical studies in which acupuncture treatment effectively resolved lameness associated with joint contusion, muscular atrophy, chronic back pain and muscle pain. [13-16] While the local anesthetic bupivacaine HCL completely resolved the lameness and the lameness score returned to the baseline, EA only reduced the lameness and decreased the lameness score by 52% from 2.7 to 1.3. This suggests that the mechanism of the EA analgesic effect is possibly related to a separate effect on the nervous system unrelated to the mechanism of a local anesthetic.

Acupuncture stimulation of ST-36 in the hind limb of rats caused a decrease in renal sympathetic nerve activity (RNA) and mean arterial blood pressure (MAP), but transection of the sciatic and femoral nerves completely abolished these RNA and MAP responses following acupuncture. [17] In a pain study of guinea pigs, sciatic nerve transection abolished the acupuncture analgesic effects.[18] These results suggest that the effect of acupuncture may be via afferent nerve fibers.

Peripheral nerve fibers can be divided into 2 categories: myelinated and unmyelinated. There are 5 myelinated fibers: Aα, Aβ, Aγ, Aδ and B and one unmyelinated fiber referred to as C fibers (Table 11).[19] The primary sensory fibers utilized for pain perception are called the nociceptive afferents. Nociceptive afferents generally fall into the myelinated Aδ fiber and the unmyelinated C

Table 10: The mean plasma concentration of cortisol (μg/dL) in horses by group over time

Time (hours)	Action	Plasma Concentration of Cortisol (μg/dL) (Mean±S.E.)		
		AT (n=6)	PC (n=6)	SC (n=6)
0800		21.48 ±1.3 [a,b,c,d 1]	20.90± 4.3 [a 1]	19.41±3.6 [a 1]
0900		22.44 ± 2.7 [a,b,c 1]	14.0±3.1 [a 1]	19.89±3.8 [a 1]
0915		26.40± 2.3 [a,b 1]	17.86 ±3.4 [a 1]	20.44 ±3.7 [a 1]
0930	Tighten screw	21.76 ± 1.4 [a,b,c,d 1]	16.77 ± 3.5 [a 1]	16.87±3.2 [a 1]
0945		20.33 ± 1.2 [c,b,d 1]	16.70± 2.9 [a 1]	14.33±3.2 [a 1]
1000		22.87±1.9 [a,b,c 1]	17.32 ±1.8 [a 1]	18.09±4.2 [a 1]
1015	Treatment given	26.14± 2.1 [a,b 1]	21.97 ± 2.1 [a 1]	22.64±5.1 [a 1]
1030		25.97 ± 2.4 [a,b 1]	22.62 ±2.0 [a 1]	22.1 ±4.0 [a 1]
1045		29.11±3.2 [a 1]	20.03±2.4 [a 1]	23.91±3.6 [a 1]
1100	AT ended	26.71±2.0 [a,b 1]	18.57±2.5 [a 1]	25.49±3.7 [a 1]
1115		25.70±4.3 [a,b,c 1]	19.35±3.0 [a 1]	26.76±3.2 [a 1]
1145	Loosen screw	17.83 ±2.4 [c,d,e 1]	13.6±3.2 [a 1]	19.94±2.3 [a 1]
1200		12.72±2.6 [e 1]	15.93 ±3.1 [a 1]	17.73 ±2.6 [a 1]
1330		14.05±2.8 [e 1]	17.49±2.9 [a 1]	12.81±3.8 [a 1]

S.E.= standard error; n= number of horses in the group; AT= acupuncture treatment; PC= bupivacaine HCL treatment; SC=saline treatment; Alphabetic superscripts [a,b,c,d,e]: Mean values within the group without a common alphabetic superscript are statistically different (P<0.05) by the Duncan's Multiple Range Test; Numerical superscripts [1] : Mean values between groups are not significantly different (P>0.05) by the Least Squares Means comparison.

fiber categories. The Aα, Aβ, Aγ and B fibers are called non-nociceptive afferents. [19] In general, nociceptive afferents have a higher threshold to stimulation than non-nociceptive afferents. Aδ fibers respond to thermal and mechanical stimuli (e.g. pressure), while C fibers respond to noxious, mechanical, thermal and chemical stimuli and thus are called C-polymodal nociceptors (C-PMNs).[20]

Local anesthetic agents block intensive prolonged pain and secondary prolonged dull sensation by preferentially and directly blocking the C-PMNs. Low-intensity electrical stimulation preferentially activates the largest fibers such as Aα and Aβ. [21] As EA is a type of low-intensity electrical stimulation and most of acupoints are motor points, it seems likely that Aα and Aβ fibers are involved in transmission of EA signals. [22-23] Since 6 out of 6 horses in this study were restless and expressed discomfort when the needle was initially inserted into an acupoint, it seems likely that EA also stimulates the myelinated Aδ and unmyelinated C fibers. Other authors have also suggested that acupuncture was mediated by afferents Aδ and C fibers. [19] So, EA most likely stimulates afferent Aα, Aβ, Aδ and C nerve fibers.

The large Aα and Aβ fibers may be dominant during the transmission of EA stimulation because the non-nociceptive afferents Aα and Aβ have a lower threshold to stimulation than the nociceptive afferents Aδ and C and EA is a type of low intensity stimulation. Also after their initial response, the horses did not show any evidence of pain during the EA stimulation in this and other studies indicating that Aδ and C fibers are probably not being stimulated. [24]

Pain signals could be influenced and

Table 11: Overview of the classification of peripheral nerve fibers [19]

Fiber group	Innervation	Mean diameter (μm)	Mean conduction velocity (m/sec)
Myelinated			
Aα	Primary muscle spindle motor to skeletal muscles	15	100
Aβ	Cutaneous touch and pressure afferents	8	50
Aγ	Motor to muscle spindle	6	20
Aδ	Mechanoreceptors, nociceptors	<3	15
B	Sympathetic preganglionic	3	7
Unmyelinated C	Mechanoreceptors, nociceptors, sympathetic postganglionic	1	1

μm=micron; m/sec=meter/second

partially or completely blocked by EA stimulation before they reach the dorsal horn of the spinal cord. [25] In an example of carpal injury due to acute contusion, pain signals from the carpal joints reached the dorsal horn of the spinal cord via the nociceptive afferent Aδ and C fibers (Figure 3). EA signals from acupoints are transmitted to the dorsal horn of the spinal cord predominantly by Aα and Aβ fibers, but also by some Aδ and C fibers. When pain signals and EA signals arrive at the spinal cord simultaneously, there are 3 possible outcomes. First they may not influence each other and the pain does

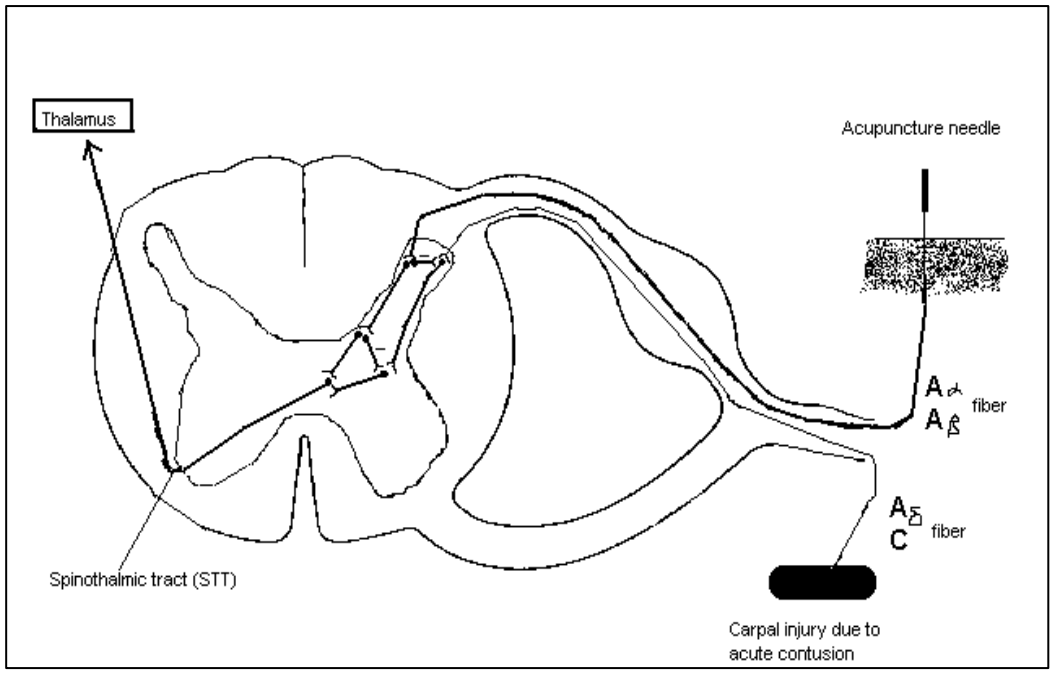

Figure 3: Transduction and transmission of EA and painful signals. Pain signals were transmitted from carpal joints to the dorsal horn of spinal cord via the small afferent Aδ and C fibers. During an EA treatment, EA signals reach the dorsal horn via large Aα and Aβ. EA signals may inhibit painful signals.

not change. Second, EA signals might enhance pain signals and result in increased pain. Lastly, EA signals may inhibit pain signals and result in less pain or no pain. As Aα and Aβ have a larger diameter and faster conduction velocity, EA signals will reach the spinal cord before pain signals. When EA signals engage neuronal sites on dorsal horn neurons, arriving pain signals have fewer sites with which to interact and pain signals are blocked resulting in a reduction of perceived pain (Figure 3). In this way, EA may induce a segmental spinal inhibition of nociceptive inputs and result in an immediate, short-term, segmental, non-opioid analgesia. [26]

In this study, the stride length was defined as the distance between the front cranial edge of each hoof imprint for successive imprints of that hoof. One stride length consists of the front and back half parts. The difference between the front and back half was called the DFB in this study. The front half stride length is similar to Adam's anterior phase of stride and the back half stride length to Adam's posterior phase of the stride. [9] The results show that the total stride length of the front right or the left limb did not change significantly among different time periods for all treatment (including saline control) groups. This is because the length of the stride must be the same as the opposite limb to keep the balance even when the horse is lame. [5]

The results showed clearly that the DFB increased significantly when the horse was lame. The degree of lameness was directly proportional to the DFB. When the screw was tightened against the sole, the horse showed supporting leg lameness associated with shortening of the posterior phase or back half stride length (BHSL). This finding agrees with the general principle that lameness from hoof pain should cause a shorter posterior phase of the stride. [9] A decreased BHSL is associated with a compensatory lengthening of the front half stride length (FHSL) or anterior phase, resulting in an increased DFB. When the screw was tightened sufficiently against the sole to induce obvious lameness (lameness scores $\geq$ 2.0), the DFB increased up to 10.8 ± 2.4 cm in the acupuncture treatment group, 6.8 ± 2.9 cm in the positive control (bupivacaine HCL treatment) group and 8.6±3.3 cm in the negative control group (saline treatment). When the DFB was greater than 3.8 cm, the horse was lame and when the DFB was less than 0.5 cm or -2.4 cm, lameness was not observed. While this indicates that the DFB could be used as an objective parameter to measure lameness in horses, the precise role of the DFB in lameness evaluation requires further clarification.

Electro-acupuncture reduced the DFB by 65% from 10.8 ± 2.4 cm to 3.8 ± 1.3 cm and partially reduced the lameness. In contrast, the local anesthetic bupivacaine HCL completely resolved lameness and the DFB returned to the baseline.

The plasma concentration of beta‑endorphin increased significantly ($P<0.001$) after acupuncture treatment. EA simultaneously decreased the lameness score in the experimental hoof pain model and reduced the degree of lameness significantly ($P<0.001$). These results suggest that the release of β-endorphin may be one of the mechanisms by which acupuncture relieves pain.

In this study, β-endorphin tended to decrease in the afternoon. In the negative control group (saline treatment), plasma concentration of β-endorphin was 52.91±17.8 pg/ml at 0800 hours, decreased significantly to 31.51 ±18.3 pg/ml at 1000 hours and then fell to 7.31 ± 2.9 pg/ml at 1330 hours. In the EA treatment group, plasma concentration of β-endorphin decreased significantly from 50.02 ± 2.8 pg/ml at 0800 hours to 38.76 ± 4.1 pg/ml at 0945 hours prior to treatment. When EA treatment started at 1015 hours, a significant increase in plasma β-endorphin concentration began. By 1030 hours the levels had increased to 63.44 ± 4.1 pg/ml, at 1045 hours they were 88.36 ± 2.1 pg/ml and by 1100 hours, when EA treatment was complete, they had increased to 92.38 ± 3.6 pg/ml. A diurnal variation in plasma β-endorphin has been shown. [27] Immunoreactive β-endorphin plasma concentrations were lower at 0600 hours, elevated at 0900 hours, dropped at 1200 hours and slightly elevated at 1500 hours, and then dropped again at 1800 hours and 2400 hours. The lowest level of β-endorphin was at 0600 hours and 1200.[27] The EA treatment group showed a clear increase in β-endorphins which was not likely associated with simple diurnal variations.

The plasma levels of ACTH did not change during any time periods in all groups in this study. This suggests that EA may not cause a significant ACTH response. This result correlates with another study conducted in healthy human volunteers. [28] It is interesting that ACTH did not change while β-endorphin increased after EA stimulation, since β-

endorphin is released in a one-to-one molar ratio with ACTH from the pituitary. [29] The change of ACTH should parallel that of β-endorphin, if both have the same pituitary origin. The lack of significant change in plasma ACTH concentration after acupuncture stimulation in the present study suggests that β-endorphin may be released into peripheral blood from sources other than the pituitary. Some studies have demonstrated that EA stimulation induced the release of β-endorphin from the reticularis paragigantocellularis lateralis (RPGL) and from the preoptic area (PA) within the brain. [30-31] High concentrations of β-endorphin are also found in the hypothalamus, periaquaductal grey matter (PAG) and locus coeruleus.[29] Since EA induced a significant change in β-endorphin but not ACTH in this study, this suggests that acupuncture induced-biochemical changes, including the release of β-endorphin, can not be simply due to an effect of "stress".

In the acupuncture group, plasma levels of cortisol increased numerically after treatment, but decreased significantly ($P<0.001$) at 1200 and 1330 hours. For both the bupivacaine HCL and saline groups, plasma concentration of cortisol did not change significantly ($P>0.05$) among different time periods. The cause for decrease in cortisol associated with EA treatment is unknown at this time.

In summary, EA decreased the lameness score and reduced the degree of lameness significantly ($P<0.001$) and increased the plasma β-endorphin concentrations in horses in this experimental hoof pain model. The results further suggest that the release of β-endorphin may be one of the mechanisms by which acupuncture relieves pain. The findings of this study further support the use of EA to treat natural occurring lameness associated with hoof disease in horses.

a. WQ6F, Donghua Electronic Instrument Factory, Beijing, China.
b. 0.5 % bupivacaine HCL, Abbott Lab, North Chicago, IL
c. Suzhou Medical Instrument Factory, Jiangsu, China
d. Nichols Institute Diagnostics, San Juan Capistrano, CA.
e. Diagnostic Systems Laboratories, Inc. Webster, TX.

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