Effect of Laser Acupuncture on Mitigating Anxiety in Acute Stressed Horses: A Randomized, Controlled Study

Wendy Ying DVM, MS, Abhishek Bhattacharjee PhD, Samuel S. Wu PhD

ABSTRACT

Anxiety in sport horses affects their performance, health, and value, as well as the reputation of trainers and competitors. The objective of this study was to determine whether laser acupuncture (LAP), when given before stressful situations, can decrease anxiety and improve performance and health in horses. A 2×2 crossover study was conducted on 24 horses randomly assigned to one of the two groups: Group A - given LAP on Day 1; Group B - given sham LAP with the laser powered off on Day 1. The treatments were switched between the two groups after a 7-day wash-out period. All horses were fitted with a heart rate (HR) monitor before undergoing an acute startle test (AST). Each subject's HR was recorded continuously from 1 minute before to 10 minutes post the AST. The primary outcome data for assessing the treatment effect were HR increase from the baseline (peak HR – baseline HR) following the AST and the time duration needed for HR to return to baseline level (time between peak HR and baseline). The observed outcome data concluded: (1) horses treated with LAP had a smaller HR increase than those treated with sham LAP (p=0.036), and (2) the time it took for the resulting peak HR to return to baseline was shorter in horses treated with LAP than those treated with sham LAP (p=0.045). These findings indicate that laser acupuncture before a known stressful situation could help a horse accept the stressful situation and recover back to homeostasis more rapidly.

Key words: laser acupuncture, crossover study, equine acupuncture, equine anxiety, acute startle test, equine behavior

ABBREVIATIONS

AST	Acute startle test
AP	Acupuncture
BHT	Baseline heart rate
BL	Bladder Meridian
EEG	Electroencephalogram
FDA	Food and Drug Administration
FH	Falcon horn
GaAs	Gallium arsenide
GV	Governing Vessel Meridian
HT	Heart Meridian
HR	Heart rate
LAP	Laser acupuncture
LED	Light emitting diode
LLLT	Low level laser therapy
LoS	Level of stress
PC	Pericardium Meridian
PHR	Peak heart rate
TSR	Time required for stress recovery
TPHR	Time to peak heart rate
TRB	Time to return to baseline
TCVM	Traditional Chinese veterinary medicine

From: Five Elements for Animals, Sarasota, Florida (Ying), School of Mathematical Sciences, University of Northern Colorado, Greeley, Colorado (Bhattacharjee), Department of Biostatistics, University of Florida, Gainesville, Florida (Wu)

The medical definition of anxiety is an abnormal and overwhelming sense of apprehension and fear often marked by physical signs (such as tension, sweating, and increased pulse rate), by doubt concerning the reality and nature of the threat and by self-doubt about one's capacity to cope with it.[1] Levels of anxiety in horses can range from mildly distracted to frantic or manic behavior, but even mild anxiety during competition can be dangerous for horse and rider. Horses are prey animals that rely on their ability to detect predators and react quickly to escape harm. The horse's natural instinct is flight when startled, a strategy that works well in open rangeland.[2] Most horses today, however, are kept in more confined quarters and have to work and live around loud noises, moving vehicles, lights, sounds, large crowds, tents and other animals. Without the natural protection of the other members of the herd, anxiety is common due to the amount of visual and auditory stimuli that must be processed by sport horses living and working in suburban and urban environments.

Anxiety in horses can cause a variety of issues such as gastric and colonic ulcers, head shaking, cribbing, and poor performance.[3] Sport horses face an additional layer of stress when they have to travel to competitions and are stabled in small stalls.[4] Some horses suffer from separation anxiety when leaving the show stabling for competition or when the barn mates leave the stabling area.

There are numerous calming agent supplements available for use by horse owners to reduce horses' anxiety at home and at competitions. Many contain herbs, amino acids, or high levels of minerals that have been shown to be effective for improvement in behavior by anecdotal evidence claimed by the manufacturer themselves or sponsored athletes. To the best of the authors' knowledge, there are currently no published evidence-based studies with statistical evaluation reporting the anxiety reducing effectiveness of a supplement, herbal remedy, or pharmaceutical that is legal for use in equine competition.

In the racing and show world, the improvement of equine performance without the use of drugs continues to promote research in acupuncture for reducing anxiety in horses. Villas-Boas et al. reported in a 6-horse study, that dry needle acupuncture at GV-1, HT-7, BL-52, and GV-20 for 20 minutes reduced autonomic and endocrine response to startle, however, no behavioral changes were noted.[5] Ferguson and colleagues used acoustic startle reflex (AST) and change in heart rate (HR) to demonstrate a positive effect of lavender oil aromatherapy as a means to control stress in horses.[6] Kim et al. have investigated changes in the electroencephalogram (EEG) to assess and document the sedative effect of acupuncture in dogs.[7]

In traditional Chinese veterinary medicine (TCVM), anxiety is defined as a *Shen* disturbance. The *Shen* is the mind and spirit and is associated with the Heart (HT) and Pericardium (PC) Meridians. The current gold standard for treating *Shen* disturbance in horses is using aqua-acupuncture with injection of 6-10 cc vitamin B-12 into the *An-Shen* acupoint bilaterally.[8] Aqua-acupuncture stimulates acupoints for a longer period than laser or dry needle stimulation because the fluid creates special configuration changes to the acupoint space and causes mild irritation which continues to stimulate the acupoint.[9]

Compared to dry needle or aqua-acupuncture, laser acupuncture is less invasive and may be performed by veterinarians, technicians, or horse owners legally and safely during competition. Laser acupuncture has been used in equine practice in the United States since the mid-1980s, but there has been little scientific research on its effectiveness. Klide and Martin demonstrated that dry needle, laser, and aqua-acupuncture were equally effective for treating back pain in horses.[10] More recently, Peterman showed that a significant reduction of pain in severely laminitic horses could be achieved using low level laser therapy (LLLT).[11] There have been no clinical studies evaluating the effectiveness of LLLT on behavior modification in horses to date. The objective of this study was to determine whether laser acupuncture, when given before stressful situations, can decrease anxiety that could lead to improvement of performance and health in horses. Specifically, through a crossover study, the hypothesis was that horses treated with laser acupuncture have lower anxiety level from an acute startle test (AST) than those receiving sham laser acupuncture.

MATERIALS AND METHODS

Horses for the study were recruited from the Sarasota, Florida, USA area. To participate in the study, owners were asked to enter a verbal agreement which allowed use of their animals and all data collected from them as defined in the study protocol. All animals were housed and fed prior to the study according to the husbandry practices of each individual owner. Study protocol defined humane treatment of animals enrolled in the study which included allowing horses to be loose in their own stalls during the AST and hay withheld only during the time of treatment and data collection period.

Inclusion criteria for the study was defined as a healthy horse of any age, sex and breed. Horses that had been on herbal or pharmaceutical calming agents within the previous 6 months were excluded. The study utilized a 2-period, 2-treatment (2x2) crossover design, in which study horses were randomly assigned to either Group A or Group B using a commercial software[a]. Horses in Group A received the test treatment (real laser acupuncture) in the first period (Day 1) and, after a 7-day wash-out period, received the control treatment (sham laser acupuncture) for the second period (Day 8). With the same procedure and schedule, horses assigned to Group B received the control treatment on Day 1 and test treatment on Day 8.

Laser acupuncture was performed with a commercial class I laser[b] (133mW average power output) which delivers a synergistic synchronization of super pulsed (25W) laser light at 905 nm from a gallium arsenide (GaAs) cluster probe plus infrared and red light at 875 nm and 640 nm from light emitting diodes (LEDs).[12] Acupuncture points were stimulated at each site for 1 minute with the utility probe using mode 3 (1000 Hz) to deliver 2 J per cm^2.[13] When receiving the test treatment of laser acupuncture, the horse was placed in cross ties and laser acupuncture was performed at points commonly used to control anxiety, which included *An-shen*, GV-24, HT-7, PC-6, BL-14, and BL-15 (Table 1). For the control treatment, the same procedure was followed; however, the laser was not powered on. Caretakers were not aware if the horse was in the treatment group or control group, but the principal investigator (PI) was not blinded.

Before each treatment, the horse was placed in a box stall for 20 minutes, and then was taken out of the stall and hosed at the girth and withers. Conducting gel was applied to the electrodes and the polar equine heart monitor belt was placed on the horse with monitor and electrodes on the left side of the barrel. A Bluetooth receiver[c] was worn on the investigator's (Ying) wrist and linked to the heart rate (HR) monitor. The horse was then allowed back into the stall. After an approximate 3-minute stall acclimation period, the HR recording was started and the start time was noted. The recording lasted for approximately 11 minutes, which started at 1 min before the AST and ended at 10 min post AST (Figure 1). The AST was performed

Table 1: Acupuncture points used to treat anxiety (*Shen* Disturbance) in horses with the location and needle depth for each acupoint[8]

Acupoint	*Pin Yin* Name	Location	Approximate Needle Depth*
	An-shen Calm *Shen*	Just behind the ear, halfway between the rostral and caudal ear bases bordered by the cranial wing of the atlas	1 cun
GV-24	*Da-feng-men* Great Wind Gate	Dorsal midline at the base of the forelock; at the external sagittal crest	1 cun
HT-7	*Shen-men* Spirit Gate	Caudolateral radius in the depression caudal to the lateral ulnar muscle just proximal to the accessory carpal bone	1 cun
PC-6	*Nei-guan* Inner Pass	Cranial to the lower border of the chestnut, in the groove between the flexor carpi radialis and flexor carpi ulnaris muscles	1 cun
BL-14	*Jue-yin-shu* Pericardium Association point	At the 9th intercostal space approx. 9 cm lateral to the dorsal midline in the iliocostal muscle groove	1 cun
BL-15	*Xin-shu* Heart Association point	At the 10th intercostal space approx. 9 cm lateral to the dorsal midline in the iliocostal muscle groove	1 cun

*Laser light penetrates to approximate 3 cm depth[17]

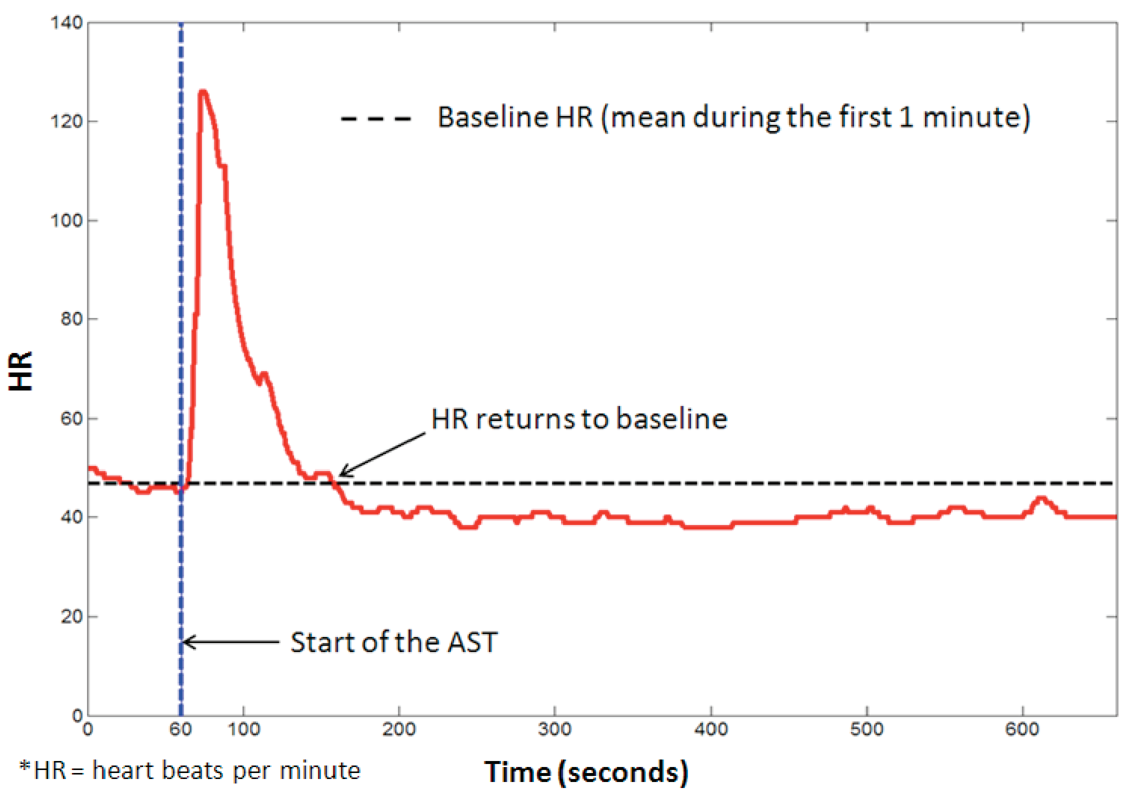

Figure 1: An example of a typical 11-minute heart rate (HR) reading demonstrating a rapid increase to peak heart rate (PHR) in response to the acute startle test (AST) and then a return to baseline HR (TRB). The black horizontal line denotes the baseline HR (BHR) before the AST, and the blue vertical line stands for the time when the AST occurred.

using a portable signal horn[d] that produced 112 decibels at a distance of 10 feet for 10 seconds. Data collected from the HR recording included: (1) baseline average HR (BHR; i.e. mean HR during the first minute of recording), (2) peak HR after AST (PHR; i.e. maximal HR after the baseline period), (3) time when PHR observed (TPHR; i.e. time after AST when peak heart rate is recorded), and (4) time when HR returned to baseline (TRB; i.e. time when HR returned to baseline after AST). Based on these data, the study further derived two outcome measurements: (1) LoS (level of stress) = PHR – BHR and **(2)** TSR (time required for stress recovery) = TRB – TPHR.

Two statistical hypotheses were tested: (1) H_0: The mean LoS in horses receiving the test treatment is the same as those receiving the control treatment; vs. H_A: The mean LoS in horses receiving test treatment is lower than those receiving the control treatment; and (2) H_0: The mean TSR in horses receiving the test treatment is the same as those receiving the control treatment; vs. H_A: The mean TSR in horses receiving the test treatment is shorter than those receiving the control treatment.

With the 2×2 crossover study design, the statistical inference on these hypotheses was based on the group comparison (Group A vs. Group B) with respect to the within-subject difference between the two periods (i.e. outcome from Day 1 – outcome from Day 8). For each subject, the study calculated dLoS = LoS_{d1} – LoS_{d8} and dTSR = TSR_{d1} – TSR_{d8} and applied either two-sample t-test or Wilcoxon Rank Sum test (depending on the result of normality test) on dLoS and dTSR to test the first and second hypotheses, respectively. All tests were 2-sided and a null hypothesis was rejected when the resulting (two-sided) *p*-value was less than 0.05. Based on the power calculation adjustment on effective size for a crossover trial, with a sample size of 12 subjects per group, the applied two-sample t-test on this 2×2 crossover study should have a 91% power to reject the null hypothesis with a 0.05 significance level when the difference between group means was at least equal to the overall outcome deviation.[14] R statistical software[e] was used for all descriptive and quantitative statistical analyses.

RESULTS

A total of 24 healthy horses (19 geldings and 5 mares) ranging in age from 6 to 29 years old were enrolled in this study. All horses resided in Sarasota, Florida and were kept on pasture most of the day. Six of the horses were stalled at night, while the other 18 were stalled for a few hours in preparation for work. Twelve of the horses were fed hay only, while the other 12 had hay and concentrate, which varied based on dietary needs and exercise level. Breeds included Arabians, Quarter Horses, Warmbloods, Tennessee Walkers and mixed-breeds. Performance disciplines study horses were ridden in included jumpers, hunters, dressage, driving, fox hunting, and trail.

All 24 horses enrolled completed the study. There were no adverse events associated with either the treatment modality or testing procedures. The group random assignment resulted in 12 horses in Group A and the remaining 12 in Group B. Age and gender variables were statistically compared between test groups (Table 2). Age in Group A ranged from 6 to 29 years old, with a mean ± standard deviation (M±SD) of 15.0 ± 7.75 years old and Group B's age ranged from 6 to 28 years old, with a M±SD of 13.33 ± 6.05 years old. The difference of mean age between the two groups was not significant (*p* = 0.5633, by two-sample t-test). With respect to a horse's gender, both groups were dominated by geldings; Group A: 10 geldings/2 mares and Group B: 9 geldings/3 mares. Proportions of gender were not significantly different between the two groups (*p* = 1.00, two-sample test of proportions).

Typical results of the 11-minute reading included a rapid increase to peak heart rate in response to the AST and then a return to baseline HR over time (Figure 1). The LoS outcomes of each horse were calculated (PHR-BHR) for each of the two treatment periods (Day 1 and Day 8) and compared (Figure 2). The mean LoS in Group A from Day 1 trial (test treatment) was 35.6 ± 22.7 and 59.3 ± 33.6 from Day 8 trial (control treatment). In Group B, the mean LoS from Day 1 trial (control treatment) was 52.2 ± 29.7 and 46.2 ± 41.0 from Day 8 trial (test treatment). Statistical evaluation (Wellek and Blettner method) did not find carryover effect to be significant (*p* = 0.931).[14] The mean LoS difference from Day 1 trial to Day 8 trial in Group A was -23.8 ± 31.3 and 6.3 ± 34.6 in Group B. The difference between Group A and Group B with respect to the trial difference was statistically significant (*p* = 0.036), and therefore the data supported the hypothesis (rejected H_0): the mean LoS in horses receiving test treatment is lower than those receiving the control treatment (Table 3).

Similar analyses were conducted on the time required for stress recovery (TSR) data and compared (Figure 3). In Group A, the mean TSR in Day 1 trial (test treatment) was

Table 2: Summary statistics of study horse age and gender

	Group A	Group B	*p*-value
Age (years) (Mean ± SD)	15.0 ± 7.75	13.33 ± 6.05	0.5633
Gender	Gelding: 83.3% Mare: 16.7%	Gelding: 75.0% Mare: 25%	1.00

Table 3: Summary statistics of the outcome measurements level of stress (LoS) and time required for stress recovery (TSR)

	Group A (Test → Control)	Group B (Control → Test)	*p*-value
LoS (Mean±SD)	Day 1 = 35.6±22.7	Day 1 = 52.2±29.7	
	Day 8 = 59.3±33.6	Day 8 = 46.2±41.0	
	Day 1 – Day 8 = -23.8±31.3	**Day 1 – Day 8 = 6.3±34.6**	**0.036**
TSR (Mean±SD)	Day 1 = 107.3±151.8	Day 1 = 176.6±164.8	
	Day 8 = 220.2±225.3	Day 8 = 65.0±37.9	
	Day 1 – Day 8 = -112.8 ±296.4	**Day 1 – Day 8 = 111.6 ±172.0**	**0.045**

LoS = level of stress (unit = beats per minutes); TSR= time required for stress recovery (unit = seconds)

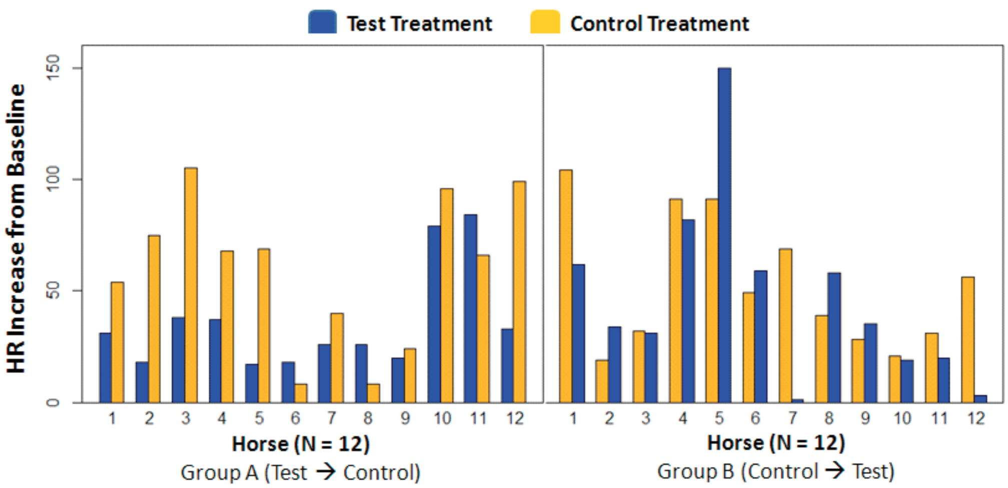

Figure 2: Level of Stress (LoS) of each individual horse under treatment, quantified as the increase of HR (baseline HR → peak HR) after the AST during each of the two treatment periods (Day 1 and Day 8; Test or Control). The mean LoS in Group A test treatment was 35.6 ± 22.7 (Day 1) compared to control treatment at 59.3 ± 33.6 (Day 8). In Group B, the mean LoS test treatment was 46.2 ± 41.0 (Day 8) while control treatment was 52.2 ± 29.7 (Day 1). The mean LoS in horses receiving test treatment was lower than those receiving the control treatment (p=0.036).

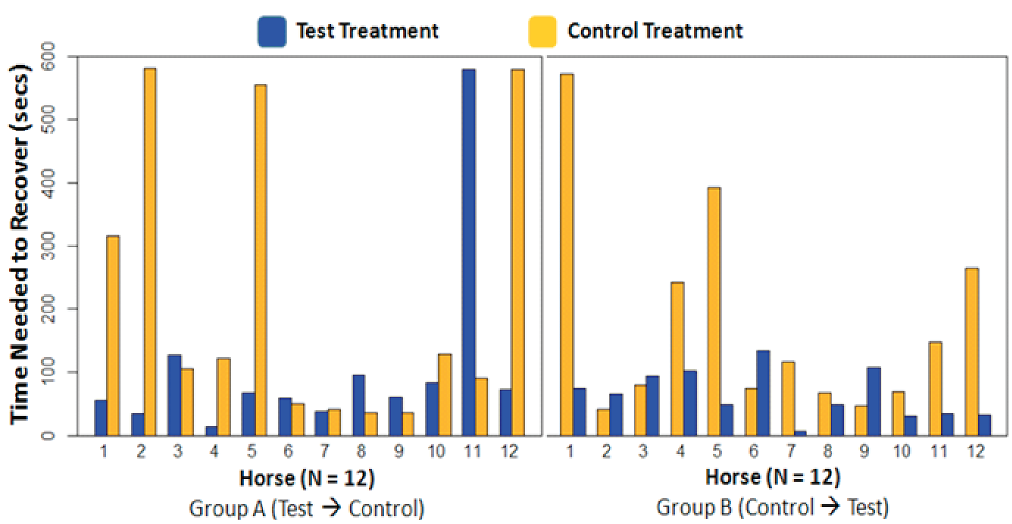

Figure 3: Time needed for stress recovery (TSR), quantified as time length to recover from the peak HR to baseline HR during each of the two treatment periods (Day 1 and Day 8; Test or Control). The mean TSR in Group A test treatment was 107.3 seconds ± 151.8 (Day 1) compared to control treatment at 220.2 seconds ± 225.3 (Day 8). In Group B, the mean TSR test treatment was 65.0 seconds ± 37.9 (Day 8) while control treatment was 176.6 seconds ± 164.8 (Day 1). The mean TSR in horses receiving test treatment was shorter than those receiving the control treatment (p=0.045).

107.3 seconds ± 151.8 and was 220.2 seconds ± 225.3 in Day 8 trial (control treatment). For Group B subjects, the mean TSR from Day 1 trial (control treatment) was 176.6 seconds ± 164.8 and only 65.0 seconds ± 37.9 from Day 8 trial (test treatment). Statistical evaluation (Wellek and Blettner method) did not find carryover effect in TSR to be significant (p = 0.590). The mean TSR difference from Day 1 and Day 8 trials in Groups A and B was -112.8± 296.4 and 111.6 ±172.0 respectively, which demonstrated a significant difference between Group A and Group B (p = 0.045). The statistical inference concluded that the data supported the hypothesis (rejected H_0): the mean TSR in horses receiving test treatment is shorter than those receiving the control treatment (Table 3).

DISCUSSION

Non-pharmaceutical management of anxiety in performance horses is a crowded field with a variety of products claiming efficacy through antidotal evidence or support by sponsored athletes but lack evidence-based studies looking at true efficacy. This is the first randomized, 2 period cross-over design study with statistical evaluation investigating a non-pharmaceutical treatment for equine stress through the application of laser acupuncture. The results of the study demonstrated a statistically significant lower heart rate increase (p=0.036) and a quicker return to baseline heart rate (p=0.045) after a stress producing event in horses treated with LAP at anxiolytic acupoints. These findings satisfied the study objective and hypothesis that LAP could be used for equine behavior modification and treated horses would more readily accept a stressful event with more rapid return to homeostasis than horses treated with sham laser acupuncture.

Acupuncture has been used to reduce anxiety in both humans and animals since ancient times. Li et al. showed that acupuncture stimulates production of enkephalins in the brain.[15] Enkephalins are neurotransmitters that regulate sympathetic outflow. Limiting sympathetic outflow before a stressful situation should help maintain a calm *Shen* and help the animal rebalance to a neutral state of *Yin* and *Yang*. In other words, after stimulation of the sympathetic system, a return to homeostasis and balance between sympathetic and parasympathetic systems should occur more rapidly with laser acupuncture than without. Quah-Smith et al. mapped stimulation of different parts of the brain with MRI during laser acupuncture treatment in humans and found that neural activity varies by acupoints, suggesting that neurological effects vary with site stimulation.[16]

The results of this study show that laser acupuncture influences some heart parameters related to stress and controlled by the parasympathetic nervous system. Return to baseline was achieved more rapidly in study horses treated with LAP similar to results described by Ferguson et al. in response to treatment with lavender essential oils given by nebulizer and exposed to AST.[6]

Limitations to this study included an experimental design which did not allow blinding of the primary investigator (PI) during data collection as the laser had a handset blinking light and red aiming light when delivering treatment. This did not affect outcome of study results as data collection was performed with the PI outside the stall. In addition, parameters measured were HR and time which are independent non-subjective data and unaffected by PI blinding. Other limitations of this experimental design included control of the external environment for horses on the two data collection days. Variations could include ambient temperature, amount of work horses performed between test periods, external noises and in the case of mares, hormonal balance. Finally, there were 3 horses in each study group that were non-responders for both peak heart rate and time to recover; Group A: #6, #8, #11 and Group B: #2, #6, #9. Signalment of these horses (breed, age, sex) were evaluated (Table 4). No consistent characteristics of these individuals were identified, however, more consistent age, sex, breed or temperament (constitution) might allow better assessment of which animals respond optimally to LAP treatment for stress episodes.

With a similar study protocol, future investigations might include evaluating LAP's ability to reduce stress for both mare and foal during the weaning process. The HR monitor transmits data by Bluetooth wirelessly, therefore researchers would need only a minimal amount of handling to reduce exogenous stress to the foal during data collection. The protocol could also be used for the conduct of canine studies evaluating treatment of thunderstorm phobia, gunshot and firework anxiety. Further studies might evaluate the length of effect of LAP treatment to reduce an AST response and determine optimum time between treatments.

Broader use of the AST protocol developed for this study, could evaluate the efficacy of other anxiety treatments such as herbal therapies, dry needle, electro-acupuncture, aqua-acupuncture, mineral supplements, and pharmaceuticals. In addition, ASTs could be developed that involve motion or a novel object that startles horses who are not affected by loud noises. Choice of treatment could then be selected by evidence-based rather than anecdotal evidence.

In summary, findings from this study concluded that horses treated with laser acupuncture before a stressful event had a statistically significant smaller HR increase than those treated with sham LAP (p=0.036) and the time it took for the resulting peak HR to return to baseline was statistically significantly shorter in horses treated with LAP than those treated with sham LAP (p=0.045). These findings indicate that laser acupuncture before a known stressful situation could help a horse more readily accept the stressful event with shorter time to homeostasis recovery.

Table 4: Signalment of study horses. The 3 horses that did not respond to laser acupuncture treatment (lower peak heart rate, more rapid recovery) in each group are highlighted. There are no consistent characteristics noted among these individuals.

Group and Number	Subject #	Age	Gender	Breeding	Period 1 Assignment	Period 2 Assignment
A 1	**#1**	16	Gelding	Half Arab	Treatment	Control
A 2	**#5**	6	Gelding	Arab	Treatment	Control
A 3	**#6**	18	Gelding	Arab	Treatment	Control
A 4	**#7**	10	Mare	WB (KWPN)	Treatment	Control
A 5	**#8**	29	Gelding	WB (Han)	Treatment	Control
A 6	**#11**	6	Gelding	WB (KWPN)	Treatment	Control
A 7	**#12**	14	Gelding	WB (KWPN)	Treatment	Control
A 8	**#15**	29	Gelding	WB (KWPN)	Treatment	Control
A 9	**#16**	16	Gelding	QH	Treatment	Control
A 10	**#19**	8	Mare	Draft-cross	Treatment	Control
A 11	**#20**	11	Gelding	Tenn Walker	Treatment	Control
A 12	**#21**	17	Gelding	KY Mountain Horse	Treatment	Control
B 1	**#2**	16	Gelding	Half Arab	Control	Treatment
B 2	**#3**	8	Gelding	Half Arab	Control	Treatment
B 3	**#4**	6	Gelding	Arab	Control	Treatment
B 4	**#9**	8	Mare	TB	Control	Treatment
B 5	**#10**	12	Gelding	WB (Han/TB)	Control	Treatment
B 6	**#13**	15	Mare	Freisian	Control	Treatment
B 7	**#14**	9	Mare	QH	Control	Treatment
B 8	**#17**	28	Gelding	TK	Control	Treatment
B 9	**#18**	19	Gelding	Draft-cross	Control	Treatment
B 10	**#22**	11	Gelding	Paint	Control	Treatment
B 11	**#23**	16	Gelding	Welsh	Control	Treatment
B 12	**#24**	12	Gelding	WB (Han/TB)	Control	Treatment

WB=warmblood; KWPN=Royal Dutch Sport Horse; Han=Hanoverian; QH=Quarter Horse; Tenn=Tennessee; Ky=Kentucky; TB=Thoroughbred; TK=Trakehner

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FOOTNOTES

[a] GraphPad Quickcalc, GraphPad Software, La Jolla, CA, USA

[b] MR4 Activet Multiradiance laser, Multiradiance Medical Solon, OH, USA

[c] Polar RS800CX heart rate monitor, Polar Electro Inc., Lake Success, NY, USA

[d] Falcon portable signal horn, Falcon Safety Products, Branchburg, NJ, USA

[e] R Statistical Software, R Foundation for Statistical Computing, Vienna, Austria

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