An investigation into the effects of laser acupuncture in isolation and combined with pulsed electromagnetic field therapy on mechanical nociceptive thresholds in the horse's back

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Abstract

The purpose of this study was to identify if laser acupuncture or a combined treatment of laser acupuncture and pulsed electromagnetic field therapy (PEMFT) are effective for treating equine back pain.

Eight healthy horses of mixed breeds and age were used in this randomised single blinded crossover trial. Horses were treated once a week over four weeks with a washout period (one week). Treatments consisted of Laser acupuncture, PEMFT (not reported in this trial), combined (laser acupuncture and PEMFT) and control. Horses were treated by stimulating four acupuncture points bilaterally and the central acupuncture point Bai Hui using commercially available low powered super pulsed (110±20ns) infrared laser (905,860,660nm, 27J/min, 6.75J/cm^2, 450mW, 60sec). For the combined treatment the same protocol was used but were also treated with PEMF therapy (200Hz, 5, 10mins) over the lumbosacral region. Four mechanical nociceptive thresholds (MNTs) were measured bilaterally before and after each treatment (straight after, 60 mins, 120mins and 180mins) by the same blinded assessor. Horses were their own control and treated once a week with either laser acupuncture, PEMFT or combined treatment. They were randomly assigned treatment protocols.

A two way repeated measures ANOVA was performed on SPSS, to identify statistical significance of treatments and time on muscle nociception. Results demonstrated that both laser acupuncture and the combined treatment had a statistically significant effect on increasing MNTs compared to control. There was no statistical significant difference between laser acupuncture or the combined treatment. There was no statistical effect of time on MNTs.

This study suggests that laser acupuncture is an effective treatment for equine back pain. Furthermore, the lack of statistical significance of time suggests carry-over effects of both treatments last up to at least three hours after treatment. It also indicates that there are no beneficial effects of using both treatments, Laser acupuncture is sufficient at increasing MNTs therefore it can be hypothesised that back pain will decrease.

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List of Abbreviations

ROS – Reactive oxygen species

NO – Nitric Oxide

COX – Cytochrome C Oxidase

ATP – Adenosine Triphosphate

PEMFT – Pulsed Electromagnetic Field Therapy

SIJD – Sacroiliac Joint Disease

PBM – Photobiomodulation

MNTs – Mechanical Nociceptive Thresholds

CNS – Central Nervous System

NIR – Near Infra-red

TCVM – Traditional Chinese Veterinary Medicine

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1 Introduction

Back pain is a common cause of poor performance in horses from all levels and disciplines (Riccio *et al*., 2018). If left untreated can cause chronic pain restricting the horse's ability to work, making it a huge concern for veterinarians (Riccio *et al*., 2018). Jeffcott (1979) reported the most common back issues are linked to soft tissue damage of the surrounding musculature of the thoracolumbar spine. Although this research is dated, similar patterns are still seen 40 years later. Landman *et al*., (2004) reported that 74% of horses with back pain also suffered from lameness. Indicating the knock on effects back pain can have, causing worrying economic and welfare concerns. Horses are often referred to physiotherapists to achieve long-term positive results in restoring function (Ridgeway and Harman, 1999).

Physiotherapist's use a variety of techniques to treat and maintain function of the horse's musculoskeletal system. Many electrotherapies have been suggested for the treatment of back pain, however they are costly and invasive. Despite its recent introduction as a complementary therapy, there is a distinct lack of evidence to support its use for other animals compared to other forms of acu-point stimulation (Petermann, 2011). Photobiomodulation therapy (PBM) is becoming one of the most utilized adjunctive tools for veterinary physiotherapists aiding in rehabilitation to either stimulate or inhibit soft tissues.

Acupuncture is a traditional Chinese medicine. Despite being increasingly used in veterinary medicine, it can only be performed by a veterinarian due to the invasive nature of the therapy (Dunkel *et al*., 2017). The traditional techniques have been implemented and developed into modern medicine even though the mechanisms of the therapy are not fully understood (Dunkel *et al*., 2017). More recent research suggests acupuncture triggers a series of actions involving the release of endogenous opioids such as endorphins which modulate pain (Kawakita and Okada, 2014; Dunkel *et al*., 2017). Laser therapy more commonly referred to as PBM therapy has similar mechanisms and is becoming more popular among veterinary physiotherapist's. The combination of the two can be used to provide a less invasive more accessible treatment for physiotherapists. Therefore, greater scientific research is required to support its use and for best practice in physiotherapy.

2 Aims and Objectives

The aim of this study is to demonstrate if laser acupuncture has analgesic effects on equine MNT's.

To establish effective PBM parameters and acu-points to treat equine back pain.

- To identify what laser parameters are best for inducing an analgesic response in equine back muscles
- Identify laser acupuncture increases MNT's
- To identify if laser acupuncture, combined with pulsed electromagnetic field therapy has any more beneficial effects than just laser acupuncture.
- To locate and treat suitable acupuncture points for horses with back pain
- To quantitatively measure MNT's before and after laser acupuncture on equine back muscles

3 Hypotheses

H_1 – Laser acupuncture will increase MNT's

H_0 - Laser acupuncture will have no effect on MNT's

4 Literature Review

4.1 Pain and Mechanical Nociceptive Thresholds

Pain is defined as an unpleasant sensation and emotional experience produced as a process to prevent further damage to tissue (Vanderah, 2007). Pain is a complicated process consisting of highly organised neural and chemical systems (Watkins and Maier, 2000). The neural system consists of nociceptors which are recognised as C-fibres and Alpha δ fibres, which respond selectively to noxious stimuli (Vanderah, 2007). Most nociceptors are small diameter unmyelinated C-fibre axons bundled in fascicles and surrounded by Schwann cells, which speed up conduction velocities (Dubin and Patapoutian, 2010). Sensory information caused by a noxious stimulus is transmitted to the central nervous system (CNS) (spinal cord and thalamus in the brain) via these afferent neurons; a process known as nociception (McGowan *et al*., 2007). Nociceptors are only excited when the noxious stimuli reach the noxious range, suggesting that they possess biophysical and molecular properties, enabling them to detect selectively and only respond to noxious stimuli (Basbaum *et al*., 2009). Vanderah, (2007) notes that the nociceptors are free nerve endings with cell bodies located in the dorsal root ganglion, only able to reach the superficial layers of the dorsal horn (Laminae I and II) of the spinal cord. These nociceptors have both peripheral and central axonal branches that innervate the target organ (Basbaum *et al*., 2009).

When damage or injury occurs, mechanical signals are transduced at the terminal of the primary afferent neuron (Kuner and Flor, 2017). The receptor potential activates a variety of voltage gated ion channels to generate action potentials which are a result of changes in membrane permeability (Pinho-Ribeiro *et al*., 2017). Voltage-gated sodium and potassium ion channels are critical to the generation of these action potentials, conveying signals to the axon terminals. Here signalling molecules are released, transmitting signals from neurons to the target cells across a synapse (Woolf and Salter, 2000; Basbaum *et al*., 2009). At a resting potential the ion channels at the membrane of the neuron are closed with the extracellular space having a positive charge and a higher level of sodium ions (Stucky *et al*., 2001). Consequently, the intracellular space has a negative charge with higher levels of potassium ions (Woolf and Salter, 2000). When a nerve is stimulated, the sodium ion channels open and release sodium into the cell creating a more positive environment inside the neuron, known as depolarisation (Woolf and Salter, 2000; Dubin and Patapoutian, 2010). After depolarisation the channels close and the potassium ion channels open allowing potassium out to recreate the positive environment outside the neuron and negative environment inside, this process is also known as repolarisation (Dubin and Patapoutian, 2010). During this process, the neuron becomes hyperpolarised, where the leaky ion channels allow the neuron to reach its resting potential (Carr *et al.*, 2009). This process (shown in figure 1) causes excitation of the membrane resulting in propagation of the action potentials in both directions away from the stimulus along the nerve fibre (Carr *et al.*, 2009). Once the action potential reaches the end of the axon this causes the presynaptic terminal to open the calcium ion channels increasing the amount of intracellular calcium which causes the release of neurotransmitters into the synapse (Kuner and Flor, 2017).

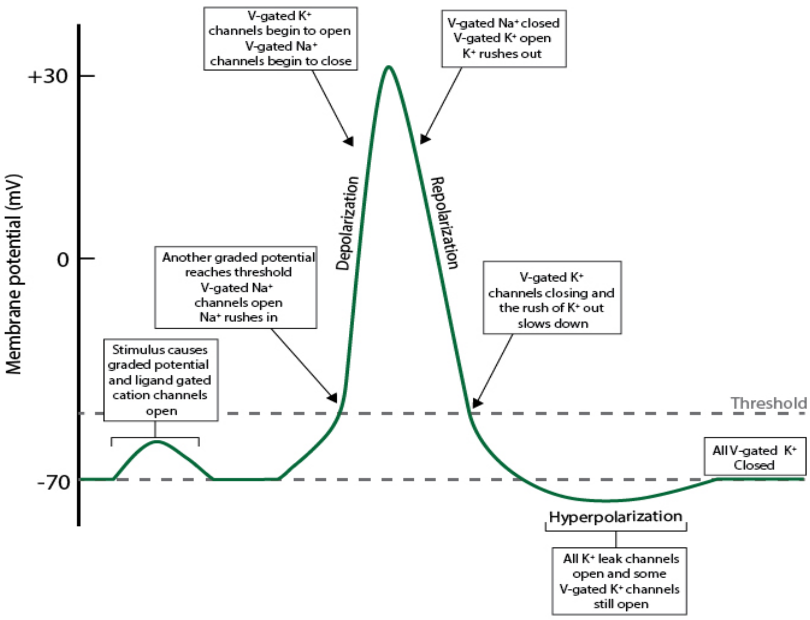

Figure 1. **Demonstrating the process of how action potentials alter membrane potentials (Loyd, 2013).**

On the cell membrane there are receptors that respond to one or more pro-inflammatory or pro-algesic agents, enhancing excitability and depolarisation of that cell. This causes the cells to undergo action potentials, thereby increasing its sensitivity to touch (Stucky *et al.*, 2001). This is why horses may show extreme guarded or aggressive behaviour to even light touch or stroking. There are a wide range of signalling molecules including neurotransmitters, peptides such as substance P and glutamate, cytokines and related lipids (prostaglandins) (Vanderah, 2007; Basbaum *et al*., 2009). These neurotransmitters cause an axon reflex leading to peripheral changes that are common indicators of pain, such as a decrease in nociceptive threshold, tenderness and swelling (Pinho-Ribeiro *et al*., 2017). Lawson *et al* (1997) noted that substance P was expressed in half of all cutaneous nociceptors mainly C-fibres. However, there are neurotransmitters that cause an inhibitory response that cause relaxation and reduce pain such as endorphins and serotonin which aim to bind to the cell receptors to block excitatory neurotransmitters such as substance P (Roeckel *et al*., 2016). The release of excitatory or inhibitory neurotransmitters is dependent on the stimulus and the neurons activated (Brown and Passmore, 2009). This is where physiotherapists can manipulate the nervous system to reduce pain by applying electro-physical agents or electrotherapies.

Due to horses previously being prey animals they have adapted to suppress pain allowing for a flight response. The pain gate control theory suggests that noxious stimuli are modulated pathways in the CNS (Melzack and Wall, 1965). Descending pathways are able to modulate the number of neurotransmitters released causing analgesia and allow the flight response (Kuner and Flor, 2017). Analgesia also occurs due to previous experiences, causing descending signals to inhibit pain (Vanderah, 2007). Furthermore, physiotherapeutic techniques such as laser acupuncture and pulsed electromagnetic field therapy can help to cause analgesia increasing pain thresholds and break chronic pain cycles (Wilson *et al*., 2018). There are two main clinical definitions of pain, acute and chronic. Acute pain can be defined as a protective mechanism that alerts the individual to a harmful stimulus immediately to protect the body (Kuner and Flor, 2017). Chronic differs not only in the time of onset and duration but the mechanisms (Masoumipoor *et al*., 2014). Horses suffering from chronic pain may not demonstrate obvious clinical signs however will be compensating elsewhere, causing further musculoskeletal injury making diagnosis and treatment difficult (Allen *et* al., 2010; Spadavecchia *et al*., 2016). Chronic pain unlike acute, can be present without inflammation or noticeable tissue damage (Allen *et al*., 2010). It is often associated with guarded behaviour and usually manifests as micro-trauma from chronic overuse, often due to poor saddle fit and riding techniques (Walstock, 2009; Allen *et al*., 2010). Pain can originate from various structures along the back such as muscles or various other types of connective tissue such as tendons, ligaments and afferent nerve endings (Walstock, 2009). The presence of back pain in some horses can lead to activation imbalances and neuromuscular fatigue in the spinal muscles (Oddsson and De Luca, 2003). Zaneb *et al*., (2009) noted that there were preferential use of back muscles and different maximum muscle activity values on one side over the other. It was suggested that this could be due to handedness of the horses, possibly as a result of human domestication in which it is an unwritten rule everything is done on the left side (Groesel *et al*., 2010).

4.1.1 Measurement of Pain

Measurement of pain is very subjective, therefore objectively measuring the presence of pain can be very difficult (Haussler and Erb, 2006). Many clinicians often use passive manual palpation to detect local tenderness, which are a manifestation of musculoskeletal pain (Haussler and Erb, 2006). Tools such as pressure algometry are used to quantify and monitor musculoskeletal nociception however, there is still some subjectivity with this technique. Fishcher (1987) defined MNT's as the minimum amount of pressure that induces a pain response. Nociception can vary greatly dependant on age, sex, weight and exercise activity. Janczak *et al*., (2012) noted that the sex of piglets had a significant effect of Mechanical nociceptive thresholds (MNT's) furthermore, as the piglets gained weight over time (age) repeatability reduced and MNT's increased. Suggesting these factors all need to be taken into account when using pressure algometry in scientific research. Haussler and Erb, (2006) noted that that there was significant increase in MNT's in ridden horses compared to non-ridden horses. Reasons for this has been suggested to be because of the endogenous opioid system producing analgesia following exercise as well as reduced repetitive trauma on the back (Mehl *et al*., 1999). Haussler and Erb, (2006) demonstrated that there were trends of higher MNT's in young heavy, non-thoroughbred geldings, however not significantly. While Varcoe-Cocks *et al*., (2006) noted that horses with suspected sacroiliac joint dysfunction (SIJD) and muscle pain had significantly lower MNT's (Varcoe-Cocks *et al*., 2006). These horses also showed greater differences in mean algometry readings between left and right sides compared to control horses. Suggesting that more pain is present on the side of sacroiliac injury. However, Balaguier *et al*., (2016) reports difference in pain sensitivity is only usually on adjacent sites and not bilateral homologous locations. Pain sensitivity on adjacent sites is thought to be because of change in skin thickness and density of nociceptors (Pongratz and Licka, 2017). However, this study is based on human subjects unlike the Varcoe-Cocks study, therefore differences in the anatomy as well as injuries and pathologies are likely to be the cause of left-right side differences. This supports the role of pressure algometry in providing objective means of quantifying musculoskeletal pain and its response to physiotherapy treatments.

4.2 Photobiomodulation

Photobiomodulation involves the use of low levels of visible or near infrared light (NIR) this can reduce pain, inflammation, oedema, and promote healing (Hamblin, 2010). Laser is a coherent monochromatic light source, resulting in all waves being the same wavelength (Watson, 2017). The cellular mechanisms of PBM involves light energy in the form of photons supplied to chromophores in mammalian tissue (de Freitas and Hamblin, 2016). The primary chromophore in the mammalian tissue is thought to be Cytochrome C Oxidase (Cox) which has a similar absorption spectrum to the wavelength of red to NIR light (600-1000nm) (Chung *et al.*, 2011; de Freitas and Hamblin, 2016). Cytochrome C oxidase is an enzyme located in the last phase of the electron transport chain in the mitochondrial membrane (Hamblin, 2010; de Freitas and Hamblin, 2016). Albuquerque-Pontes *et al.,* (2015) studied irradiated skeletal muscle with three different doses of PBM therapy and demonstrated that Cox activity increased with three different doses (660nm 1J, 830nm 3J and 905nm 1J) at 5 minutes and 24 hours after treatment. Which suggests that at these doses therapeutic benefits can occur.

The first law of photobiology states that for PBM to have effects on living tissue the photons have to be absorbed by electronic absorption bands of the chromophores which leads to an electronically excited state (Sutherland, 2002). This produces an increase in electron transport reactions leading to molecular and cellular alterations via cellular signalling reactions as shown in plate 1 (Riegel and Godbold, 2016). Increases in electron transport improves adenosine triphosphate (ATP) production (Hamblin and Demidova-Rice, 2007).

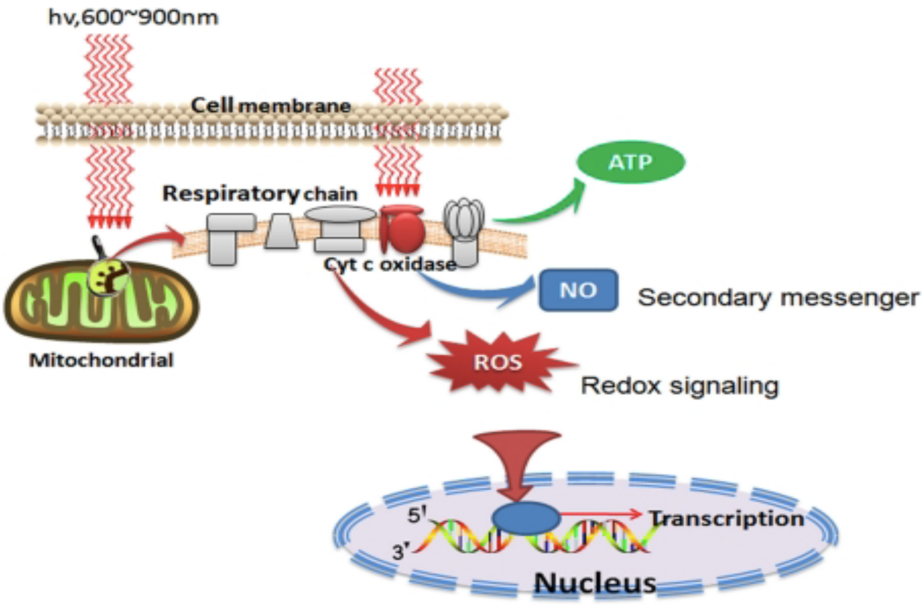

Plate 1. **Diagram showing the cellular signalling pathways caused by PBM therapy. After photons are absorbed by chromophores respiration and ATP production is increased and signalling molecules such as ROS and NO are produced and released (Huang *et al.*, 2009).**

Karu and Kolyakov (2005) were the first to show evidence that nitric oxide (NO) is involved in PBM. It is thought that the process of increased ATP production is due to photodissociation of inhibitory binding of NO from COX to allow for increased oxygen binding as a result, the rate of cellular respiration will increase upregulating ATP production and improving cellular function (Hamblin and Demidova-Rice, 2007). It was thought that inhibitory NO binds to COX to reduce oxygen binding, this occurs to divert oxygen to other cellular tissue that requires it (Vamanan, 2010). However, Cotler *et al.*, (2015) suggests it is more likely to occur as a result of injured or hypoxic cells. Consequently, Brown (2001) suggests that COX consumes around 90% of oxygen in mammals which is important for nearly all energy production in the body. When NO is released it causes vasodilation increasing blood and lymph flow (Pryor and Millis, 2015). Increases in blood flow will provide more nutrients and oxygen to the cell causing a further increase in ATP production via oxidative phosphorylation (Cotler *et al.*, 2015). This causes secondary mechanisms which aid in reducing muscle spasm, pain and inflammation, as well as increasing cell proliferation (Pryor and Millis, 2015).

The metabolism of oxygen produces chemically reactive oxygen molecules known as reactive oxygen species (ROS), as a result of an overall shift in redox potential to greater oxidation (Cotler *et al.*, 2015).

Release of ROS leads to a decrease in reactive nitrogen species and the activation of the transcription factor nuclear kappa B, which acts as a redox-sensor. This in turn initiates signal transduction pathways leading to protein modification and gene expression (Huang *et al*., 2011). If ROS are produced at high levels they can cause cell damage, however at low concentrations ROS act as messengers within cells and have beneficial effects which induce neural differentiation and activation of transcription factors, which leads to reduced cell death (de Freitas and Hamblin, 2016).

4.2.1 PBM for pain

Possible mechanisms of PBM for treating pain are becoming more widely understood. Studies such as, de Oliveira and de Freitas, (2016) have demonstrated that PBM can provide instant protective effects which lead to enhancement of functional activity of damaged nerves. Additionally, it influences tissue healing and reduces degeneration of neural tissues (de Oliveira and de Freitas, 2016).

Low-intensity PBM has been found to stimulate mitochondria and raise the mitochondrial membrane potential, increasing cell metabolism and the firing rate of action potentials in nerves (Cotler *et al*., 2015). However, at high doses PBM acting on a nerve can inhibit mitochondrial metabolism in C and Alpha δ nerve fibres and therefore reducing mitochondrial membrane potential, which induces a nerve blockade of the peripheral and sympathetic nerves (Cotler *et al*., 2015). This causes fast acting pain relief and can occur within minutes and leading to the release of neuromuscular contraction and to a reduction in muscle spasm and hypertonicity (Olavi *et al*., 1989; Carrasco *et al*., 2009; Cotler *et al*., 2015). However, Karu (2013) suggests PBM works by decreasing inflammation and consequentially reduces pain sensitivity. Initially the effect of PBM is at the epidermal neural network, although the effects travel to nerves in the subcutaneous tissues, sympathetic ganglia and finally the neuromuscular junction within muscles and nerves (Cotler *et al*., 2015). When PBM is applied with the correct dose it can cause inhibition of action potentials where there has been reported a 30% neural blockade within 10-20 minutes, however this is reversed within 24 hours (Bashiri, 2013).

It has been reported that ATP production plays an important role in pain relief. In neurons ATP is synthesized by mitochondria located in the dorsal root ganglion (Cotler *et al*., 2015). When PBM is applied it decreases the mitochondrial membrane potential in the dorsal root ganglion and then ATP production is reduced. Therefore, it has been suggested that the reduction of ATP could be the cause of neural blockades (Chow *et al*., 2007). However, Karu (2010) reports that PBM stimulates the sodium/potassium pump at the cell membrane. This causes the hyperpolarisation of the neural membrane leading to inhibition of action potentials therefore pain thresholds increase (Karu, 2010). This inhibitory response of peripheral nociceptors increases the MNTs, but also decreases the release of pro-inflammatory mediators and neuropeptides such as substance P. PBM also regulates neurotransmitters which provide further pain relief such as endogenous opioids for example β-endorphin and serotonin into the cerebrospinal fluid (Hagiwara *et al*., 2007; Cotler *et al*., 2015). This suggests that PBM not only has short fast acting pain relief mechanism but also medium and long term effects (Cotler *et al*., 2015). Furthermore, increases in blood and lymph flow removes inflammatory mediators reducing swelling and oedema within in hours or days. By reducing swelling and oedema osmotic pressure will be reduced, releasing pressure off nerve endings and therefore further reducing pain (Karu, 2013; Cotler *et al*., 2015).

4.2.2 PBM dose response

The biphasic dose response was developed back in the late 18th century to develop the Arndt-Schulz law (Chung *et al.,* 2011). In 1887 Hugo Schulz demonstrated the activity of various poisons such as iodine at low doses had stimulatory effects on the metabolism of yeast (Huang *et al*., 2011). Later Schulz shared this research with Rudolph Arndt and together developed the theory that weak stimuli cause slight biological activity, whilst moderate stimuli will cause an optimal response causing a biostimulatory response and finally strong stimuli will create a biohibitory response (Chung *et al*., 2011). The Arndt-Schulz curve was produce to depict these effects as shown in figure 2. The biphasic dose response has been demonstrated with the use PBM therapy. Huang *et al*., (2011) irradiated neurons with different doses (0.3,3,10 or 30J/cm^2 at 25mW/cm^2, 810nm) and measured levels of ROS, mitochondrial membrane potential and ATP. At lower doses it increased ATP and membrane potentials and at higher doses these decreased. ROS were measured at their highest at 30J/cm^2 while a reduction in membrane potential was noted (Huang *et al.*, 2011). While hyperpolarisation of the mitochondrial membrane potentials is wanted to reduce pain thresholds, high amount of ROS can cause cytotoxic effects (Huang *et al.*, 2011). Results from this study show how important acquiring the correct doses are for the desired effects. However, it is important to bare in mind that when reducing pain with PBM therapy that highest doses may increase pain and have damaging effects on cells.

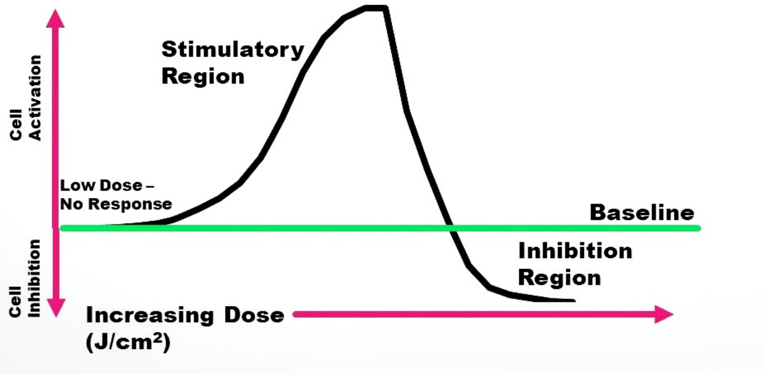

Figure 2. **The biphasic dose response curve demonstrating the effect of photobiomodulation on cell activation, adapted from (Carroll, 2008).**

4.2.3 Penetration Depths

There still remains a lot of uncertainty around penetration depths of PBM therapy, however wavelength appears to be a determining factor in affecting the penetration depths (Ryan and Smith, 2007). For successful penetration depths of target tissues, the optimal dose must be determined accurately (Dolugan *et al.*, 2017). Many companies selling high powered lasers believe increased power improves penetrative quality of light however research suggests otherwise (Kasten *et al.*, 2017). Hudson *et al.*, (2013) studied penetration depths of two wavelengths (808nm and 980nm, 1W/cm^2) on bovine tissue samples and determined that 808nm light penetrated 54% deeper than 980nm. This is likely due to the absorption of photons by water leading to greater heat generation rather than the photons reaching the cells. Additionally, Joesen *et al.*, (2012) looked at penetration depths of two commercially available lasers (810nm, 200mW continuous wave or 904nm, 60mW super pulsed laser). Although laser light penetration (810nm) was stable at 20% of the initial optical output, the super pulsed laser revealed a linear increase in penetrating energy from 38%-58% when irradiated for 150 seconds. This suggests although both wavelengths are suitable at penetrating tissue the super pulsed characteristic makes it more efficient. Furthermore, findings correspond well with PBM dose analysis in systematic reviews on PBM for musculoskeletal problems (Joesen *et al*., 2012).

Penetration of PBM is not only dependant on wavelength, but the scatter and absorption properties (Laakso *et al*., 1993). In addition, penetration of PBM is dependent on competing photochemical pathways within the skin as well as how the light behaves with the air and skin will have large effects on PBM penetration (Laakso *et al*., 1993; Duesterdieck-Zellmer *et al*., 2016).

In veterinary medicine, depth of penetration proves more difficult due to hair and pigment therefore, recommendations have been made to clean and clip the area that is going to be treated (Ryan and Smith, 2007). However, this is impractical, time consuming and many owners will not allow this. Ryan and Smith, (2007) note that when tendons where clean and clipped it caused an increase in penetration ($p = 0.0032$) (810nm, 500mW continuous beam). Furthermore, the study concluded that although hair colour did not affect penetration, grey horses seemed to have higher penetration depths. However, there was only three greys included in the study and the rest were bays (n=9). Therefore, the reliability of the study can be questioned, as it is not wholly representative of a range of different coloured horses. This does suggest varying penetration depths is dependent on skin pigment, which is further supported by Husby *et al.,* (2016) who noted chestnut skin samples allowed greater penetration of laser compared to bay horses skin. This study concluded that having varying wavelengths (660nm, 800nm and 970nm) catered for all skin colours. A more recent study by Monici *et al*., (2018) also looked at penetration depths of PBM (905nm, 25W or 808nm or 1.1W both pulsed) on the superficial digital flexor tendon and suspensory ligament and found penetration depths were 0.16mm and 0.5mm respectively. Despite providing a good insight into penetration depth into various biological tissues the study was *in vitro* and thus blood was not circulating the limb making the results less reliable for practice as blood is known to absorb photons at certain wavelengths (Hamblin and Demidova-Rice, 2007). Although the study does give good insight into penetration depth of various biological tissues. This is also supported by the study of Kleinkort and Foley (1984) whom found long wavelengths can penetrate up to 15mm deep into tissues. Controversially, other researchers believe that although the direct effects of laser penetration is superficial, this indirectly causes penetration depths up to 50mm due to photochemical pathways spreading through tissue (Kolari, 1985; Esnouf *et al*., 2007).

4.3 Current research on acu-point stimulation

Acupuncture has a long treatment history for domestic animals, however stimulating acu-points with PBM is of relatively recent origin (Whittaker, 2004). Laser acupuncture is performed transcutaneously to a single anatomically defined acu-point which overlies a number of peripheral nerves (de Oliveira and de Freitas, 2016). An issue surrounding PBM studies, is the lack of consistency in reporting laser parameters and choice of acu-points. Thus proving difficult to assess and compare research as well as repeatability of clinical findings. The location of acu-points in animals is thought to be derived empirically or transpostionally from human points on to animal anatomy (Chan *et al.,* 2001; Whittaker, 2004). However, this approach is clearly open to subjective interpretation given the obvious anatomical differences. Melzack *et al*., (1977) was the first to study the anatomic and clinical pain correspondences of trigger and acupuncture points, finding a 71% correspondence however, the study did not attempt to look for similarities in referred-pain patterns of trigger points and the meridians of their anatomically corresponding acupuncture points. A more recent study by Dorsher and Fleckenstein (2009) found trigger points regions had a 93.3% anatomical correspondence with classic acu-points. Furthermore, they found a strong consistency of the distributions of trigger point region referred pain patterns to acupuncture meridians. There is a lot of research on the use of PBM therapy for trigger point, however very little on acu-points. These studies suggest PBM therapy on trigger points has a close relation to laser acupuncture, therefore viewing this literature may be useful in understanding the benefits of laser acupuncture.

Stimulating acu-points using dry needles has shown to have positive effects on pain (Wang *et al*., 2008). It has been a part of Traditional Chinese Veterinary Medicine (TCVM) for centuries. The continued development has resulted in playing an important role of veterinary medicine. However, despite its importance it is not accessible to physiotherapists due to its invasive nature for animals. Thus, limiting a physiotherapists rehabilitation strategy for treating equine back pain. The mechanisms of acupuncture are still unknown; however new advances are being made to explain its effect on the animal body. It is believed that acupuncture can trigger a cascade of events causing a release of endogenous opioids such as enkephalin and β-endorphin which as previously mentioned modulate pain (Kawakita and Okada, 2014).

The channels or meridians in TCVM play an important role in Chinese medicine and are responsible for transport of *Qi* (Shmalberg and Xie, 2011; Pelligrini *et al*., 2018). In conventional medicine *QI* is perceived as a form of energy in the form of adenosine triphosphate (ATP) (Petermann, 2017). Produced by the Krebs cycle responsible for aerobic respiration within the mitochondria in all living cells (Petermann, 2017). When energy (*Qi*) is produced, this causes upregulation of cells for life processes ranging from cell proliferation to muscle relaxation (VanderPloeg and YI, 2009). It is noted that when disease or injury occurs, there is a disturbance in the flow of *Qi* along the bodies meridians resulting in a loss of balance between *yin* and *yang* (Kaptchuk, 2002). Injury and disease can also affect the nervous system by irritating afferent nerves causing pain, restriction of blood flow and lymphatic drainage, restricting the flow of *Qi* (Petermann, 2017). Overtime this leads to a vicious cycle of worsening pain and other compensatory issues (Ezzo *et al*., 2000). Therefore, when laser is applied with the correct parameters to acu-points light, energy is converted into ATP via oxidative phosphorylation to produce energy needed, to upregulate cells and increase cell metabolism, therefore reducing injury, disease and breaking the pain cycle (Petermann, 2017; Pelligrini *et al*., 2018).

There are a few ways in which acu-points can be stimulated to reduce pain, these have been explored through research. A recent pilot study by Dunkel *et al*., (2017) looked into the effects of traditional acupuncture treatment on objective and subjective gait parameters in horses, this reported that treatment decreased hip hike while increasing pelvic limb push-off, and pelvic movement symmetry (Dunkel *et al*., 2017). This suggests a lesser degree of discomfort, although the mechanisms of how the treatment achieved this was not identified. This method of treatment provides evidence that acupuncture can help with musculoskeletal discomfort. The gall bladder meridian seems to be of particular interest when treating horses with back pain (Still, 2013). There are various forms of musculoskeletal pain that have come across in clinical practice in sport horses (Still, 2013) such as, myofascial trigger points, strained muscles and ligaments or irritated nerves. While these conditions can directly affect performance and welfare of horses, they can be easily identified and diagnosed, but also treated using acupuncture (Still, 2013).

A study treating pain along the gall bladder meridian (n=15) on horses using dry-needling acupuncture (Still, 2015). Found that there was a marked reduction of tenderness along the previously sore sections of the gall bladder meridian within 30-120 seconds after the needles were inserted. Immediately after treatment, pain relief was seen in all horses. However, five days' post treatment only 12 horses were rated cured, whilst only three horses had improved, with pain still not completely resolved (Still, 2015). Although this study suggest acupuncture has positive effects and lasting carry over effects on musculoskeletal pain, similar to previous studies it is not known how this occurs. Furthermore, the treatment and assessment was completed by the author, demonstrating potential bias. The assessment was also completed subjectively, thus it is likely there is some inaccuracy in the results. There was a distinct lack of control and small sample size indicating that it is unrepresentative of the equine population.

Historically veterinary practitioners have always used conventional needles, however modern technology is being used to stimulate acu-points (Xie *et al*., 2001). Electricity, lasers and injectable agents have been more recently used, resulting in improved accessibity to physiotherapists, due to the less invasive nature. The influence of electro-acupuncture on pain thresholds in horses has been studied by Xie *et al*., (2001) who noted electrical stimulation (80-120Hz) had a greater analgesic effect compared to lower frequencies (20Hz) on a limb withdrawal reflex. However, it was also found that the analgesic effect of electro-acupuncture depends upon the acu-points chosen. Furthermore, plasma concentration of cortisol levels decreased while β-endorphin significantly increased in the treated group, and continued to increase over a 60-minute period and returned back to baseline at 90 minutes. This suggests a possible mechanism of analgesia after acupuncture treatment. In a later study Xie *et al*., (2005) compared electro-acupuncture to phenylbutazone or saline solution injections to treat chronic thoracolumbar pain in horses and found that MNT's increased in the acupuncture group after three treatments and lasted up to two weeks. Suggesting acupuncture is an effective treatment of chronic thoracolumbar pain in horses with beneficial carry over effects.

Currently, there is very limited research in laser acupuncture for treating back pain in horses. To the authors knowledge, there is only one study supporting the use of laser acupuncture, however it is over 30 years old thus, lacking scientific relevance (Martin and Klide, 1987). Researchers are still focussing on the underlying mechanism of laser acupuncture, in order to build scientific basis for clinical practice. Varying opinions and concerns in its mechanisms largely surround the lack of mechanical stimulation. It is therefore assumed that laser acupuncture does not share the same pain modulation pathways as traditional needling (Law *et al.*, 2015). In comparison primary advantages of laser acupuncture concern its lack of pain while treating, thus reducing the risk of injury to the physiotherapists in cases where horse react negatively (Martin and Klide, 1987).

A study by Martin and Klide (1987) was completed on (n=14) horses with back pain. Horses were treated with laser (904nm, 300μw, 360pulses/sec, 2mins/point) on five acu-points bilaterally, with one treatment each week for an 8-16 week period. Treatment was stopped once the horse performed to a suitable level. Making the study subjective as therapists may define a suitable level differently, therefore results cannot be compared accurately. Results demonstrated that 10/14 horses had alleviated clinical signs. Although the study lacked control and had a small sample size it does provide evidence that laser acupuncture is a clinically useful tool for treating chronic back pain in sporting horses. A later study completed by Martin and Klide, (1989) compared dry needling, injection and laser for stimulating acupuncture points to treat chronic back pain. The study found that laser was the weaker treatment (possibly due to how superficial the treatment is), although results between groups revealed not significantly (P=0.8). This suggests it has a beneficial effect on reducing back pain, while being less invasive, thus more therapeutic and accessible for paraprofessionals. Furthermore, the study was poorly designed as improvements after treatment were measured subjectively and the study was very open to bias. Additionally, sample groups were small therefore more recent research is needed to gather more representative data.

A systematic review completed by Law *et al*., (2015) evaluated the effects of laser acupuncture on musculoskeletal pain and functional outcomes in humans. Two-thirds (n=31/49) of the studies reported positive effects. It was also noted that there was a distinct correlation between studies reporting positive effects and high methodological quality and reported laser parameters. Negative studies have previously failed to demonstrate this. Studies looking at long-term follow up effects were more likely to report positive outcomes on pain. The review suggests that there is moderate quality of evidence to support the use of laser acupuncture for managing and improving functional recovery.

Simunovic, (1996) used laser (632.8nm, 830nm continuous wave and 904nm pulsed) over trigger points on a range of painful conditions. Results demonstrated that acute pain decreased by 70% and chronic by 60%. It was noted that no negative effects of these laser parameters were observed and if the subjects were on analgesic drugs before, the amount taken was considerably reduced or excluded. This suggests that PBM can be used as a monotherapy or complementary therapy to reduce pain providing the correct dosage is given. In comparison Venancio *et al.,* (2005) demonstrated that six sessions of laser (780nm, 30mW, 6.3J/cm^2) on trigger points did not significantly affect pain thresholds 15, 30 and 60 days after the last treatment. This suggests these parameters would be not effective on acu-points to reduce back pain. In contrast to this Lonrenzi *et al*., (2009) used a lower dosage of PBM (670nm, 5.25mJ/mm^2, pulsed) on acu-points and found it significantly reduced pain thus improving muscular performance. This study was completed on rats whereas the subjects in the Venancio *et al*., (2005) study where humans. This indicates the importance of utilising the correct dosage parameters to induce the desired response based on a range of factors such as skin pigmentation and thickness. A review by Uemoto *et al*., (2013) note that studies have found the use of laser is more efficient than dry needling. It was suggested that improvements in microcirculation from the application of PBM therapy, may cause increases in oxygen supply to damaged cells, thereby upregulating cell metabolism and breaking the chronic pain cycle reducing spasms and pain (Uemoto *et al*., 2013).

4.4 Pulsed Electromagnetic Field Therapy

Therapeutic modalities such as Pulsed Electromagnetic Field Therapy (PEMFT), are becoming increasingly important in veterinary physiotherapy. Due to a better understanding of their value and mechanisms (Wilson *et al*., 2018. The cellular mechanisms through which PEMFT influence the body's natural processes, are complex and are still not fully understood (Gaynor *et al*., 2018). Many steps have been made to try and understand the processes by which it works, to address healing and reducing pain and give physiotherapists a more rounded holistic approach to treating horses (Gaynor *et al*., 2018). This study aims to identify the mechanisms of PEMFT and provide clinical evidence on the effects of PEMFT combined with laser acupuncture on equine back pain. Studies have reported that PEMFT modulates the release of transforming growth factors and cytokines, thus improving cell health and enhancing healing processes (Aaron *et al*., 2004; Tepper *et al*., 2004). There are also reports that the release of nitric oxide aids in vasodilation thus, reducing inflammation and associated pain (Pilla *et al*., 2011). Pilla *et al*., (2011) notes that when exposing tissues to PEMFT it targets the cell membrane first, by modulating ion binding and transport across the membrane by manipulating the voltage-ion-gates to open and close. PEMFT causes the release of intracellular calcium, leading to increased binding to calmodulin which is the primary transduction pathway (Pilla *et al*., 2011). This causes a signalling cascade upregulating cell metabolism to enhance healing and cell health after injury or disease (Gaynor *et al*., 2018). Despite the on-going research into the mechanisms of PEMFT there is still a lot of controversy on this topic (Biermann *et al*., 2014). However, evidence-based medicine can provide data for its efficiency, even though the mechanisms are not fully understood (Akai and Hayashi, 2002).

A study by Biermann *et al*., (2014) tested PEMFT (40min/day, 50 microtesla, variable frequency 1 to 30Hz) applied over the back of polo ponies and assessed MNT's and spinal flexion tests before and after two, 10-day treatment periods. Results demonstrated that the ponies had reduced MNTs within and between treatments in both the placebo and PEMFT treatments. Flexion tests revealed stiffness and avoidance in 19/20 horses and over the treatment periods reactions increased. It was suggested that back pain may have not been severe enough to allow a significant affect. However, the settings used could be another factor that may cause a lack of effectiveness. Laycock, (1995) suggests that all cells have a voltage of about -70mv and damage to the cells alters the membrane potentials increasing the amount of interstitial fluid leading to swelling and oedema. PEMFT works by hyperpolarising the cells membrane to -90mv therefore pain in blocked. Laycock, (1995) further suggests the most effective frequencies are at 200Hz with a pulse rate of 5Hz. This would suggest why the previous study had no effect on equine back pain. Therefore, this study will identify if the setting Laycock, (1995) suggests are effective at reducing equine back pain.

In summary this study will evaluate the effects of a commercially available laser and the recommended doses on mechanical nociceptive thresholds. Furthermore, it will explore whether laser acupuncture is effective on its own or whether the use of PEMFT with it, will acquire larger increases in mechanical nociceptive thresholds. Additionally, it will assess a set of acu-points and if these are effective at treating equine back pain. The results from this study will provide clinical evidence that may influence veterinary physiotherapeutic treatments as well as providing evidence on an underutilized therapy.

5 Materials and Methods

5.1 Ethical Considerations

The study was approved by the Research ethics committee of Writtle University College of Essex. The trial was supervised by a qualified physiotherapist. All owners signed an owner consent form before the trial (see appendix ii). Lame or unhealthy horses were not included in the study and a welfare monitoring protocol was followed closely. Horses were monitored throughout the whole process to ensure they were well enough to be used. They were checked before and after each treatment and if they were considered to be in too much pain they were removed and arrangements made, to be seen by a vet.

5.2 Horses (*Equus Caballus*)

Eight healthy sound horses (E. *Caballus*) were sourced from an external yard and used in the study to measure the effects of laser acupuncture and pulsed electromagnetic field therapy on mechanical nociceptive thresholds. The number of horses was based on similar studies and on the sample size resource calculation. There were four mares, three geldings and one stallion, ages ranged from 5-21 years old with a mean age of 14.625 ± 5.829. Horses were of varying breeds (Irish Draught n=1, Irish Sport pony n=1, Thoroughbred n=1, Shetland n=1, Warmblood n=2, New Forrest n=1 and Welsh D n=1) and heights ranged from 11.2hh-16.2hh. Majority of the horses had black skin apart from one which had pink skin. Coat colour varied with two chestnuts, five bays and a skewbald. Coat length also varied three horses were clipped, three had long coats and finally two had very long coats. Horses were stabled in their normal stables with access to hay and water on trial days. However, went about their normal routine during the wash-out period.

5.3 Study design and randomisation

The study was a randomised, crossover, single blinded control trial. Horses were randomly allocated a treatment sequence by a computerised randomisation calculator. There were four treatment groups which were laser acupuncture, PEMFT, Combined (laser acupuncture and PEMFT) and finally a control group. The study was a twin trial whereby two people were measuring mechanical nociceptive thresholds before and after two different treatments (laser acupuncture and PEMFT) Therefore, a combined group of both laser acupuncture and PEMFT was used to seek the benefits of both treatments together. The trial was completed once a week over four weeks so every horse had each treatment. A one-week wash-out period was used in between treatment days to reduce the risk of carry over effects of PBM, which has been suggested as a suitable length for a washout period (Leal-Junior *et al*., 2009; Miranda *et al.,* 2016; Aver Vanin *et al*., 2017).

5.4 Algometry

Pressure algometry overcomes the subjectivity of deep palpation usually adopted by veterinarians and physiotherapists (Varcoe-Cocks *et al*., 2006). Although palpation plays an important role in assessing and diagnosing of equine back pain its subjectivity limits the strength as a clinical research tool (Dyce *et al*., 2002; Varcoe-Cocks *et al*., 2006). Therefore, algometry is able to quantify muscular tenderness along the back making it a more reliable research tool (Varcoe-Cocks *et al*., 2006).

Before any readings were taken four algometry points were marked up bilaterally using chalk and a round marker by the authors (one measuring and one marking) every week to reduce human error as shown in plate 2. Horses were restrained in there stable with a head collar and lead rope for every reading. Baseline readings were taken before each treatment every week using a digital Wager FPX 25 Algometer (Newtons) with a blunt 1cm^2 probe area. After treatment, MNT readings were taken straight after, and every hour after for three hours by the same trained blinded assessor (Nussbaum and Downes, 1998; Pelfort et al., 2015; Pongratz and Licka, 2017). Three readings were taken at each point so a mean could be taken.

Plate 2. **Demonstrating points were marked for algometry on each horse and technique used**

The algometer was placed perpendicular on each of the eight points with no pressure for three seconds to reduce startle effects (Pelfort et al., 2015; Pongratz and Licka, 2017). After this, pressure was gradually applied over two, three second intervals to create a smooth transition by pushing the algometer on to the skin. As soon as a pressure-pain response was elicited the pressure was removed and the measurement of the force applied was taken from the digital display on the algometer. Pain response was measured based on subtle behavioural changes such as change in facial expression, ear movement and chewing/yawning (Gleerup et al., 2014; Mullard et al., 2017). If these subtle signs are not shown then reactions such as skin fasciculation, holding of breath, swishing tail and movement away from assessor will be seen as a pain sign (Wagner, 2010). The assessor did not view the algometry readings during the application of pressure and set it to zero after every reading to reduce any bias.

The four points tested (bilaterally) were located using anatomical landmarks and measuring distance between points using a 30cm ruler. Point three was the first to be located, found at the mid-point between the *tuber sacrale* and the *tuber coxae* on the belly of the middle gluteal, point two was measured 20cm cranial to point three on the thoracolumbar longissimus, point one was located 15cm cranial to point two over the caudal thoracic longissimus muscle (Dyce *et al*., 2002; Varcoe-Cocks *et al*., 2006). Finally point four was measured 20cm dorsal to the greater trochanter on the vertebral head of the *biceps femoris* (Dyce *et al*., 2002; Varcoe-Cocks *et al*., 2006). Previous studies have tested these points for MNT's and have found easily repeatable results (Dyce *et al*., 2002; Varcoe-Cocks *et al*., 2006).

5.5 Treatments

5.5.1 Acu-point selection and photobiomodulation stimulation

A class one Multi Radiance MR4 ACTIVet™ PRO Super Pulsed Laser (Solon, OH, USA) was used throughout the study and applied by the same individual throughout the trial, with the treatment parameters as recommended by the manufacturer. The Multi Radiance laser runs through three wavelengths (905nm, 860nm, 660nm), with a super pulse of 110±20ns. The parameters are shown in table 1. For horses with longer coats the hair was split to increase the amount of laser delivered to the skin.

Table 1. **Laser parameters and dose**

Treatment	Wavelength (nm)	Static magnetic induction (mT)	Power (mW)	Pulse rate (ns)	Radiation aperture (cm^2)	Time (s)	Dose (J)
Multi-Radiance Laser	905, 860, 660	35 ± 10	450	110 ± 20	4 ± 0.4	60	27J/min 6.75J/cm^2

***Plate 3*. Demonstrating the application of laser on the acu-point BL28**

The acu-points used in this study were adapted from Rosin *et al.,* (2006) Laser acupuncture guide for treating equine back pain as well as points previously used in similar studies (Martin and Klide, 1987; Xie *et al*., 1996; Fleming, 2001; Dunkel *et al*., 2017). Points used were GV 03 (Bai Hui), BL 28, SI 03, BL 67, BL 60. All points were used bilaterally apart from Bai-Hui point as it is a central point. All acu-points were located using palpation and were radiated with direct contact of the laser to the horse's skin. See table 2 for point description and figure 3 for location.

***Table 2*. Points radiated with laser (Rosin *et al*., 2006)**

Point	Location	Description
GV 03 (Bai Hui)	Located between L5 and the sacral bone on the dorsal midline of the back.	Central trigger point for inhibiting inflammation, and for relaxation
BL 28	10cm from the dorsal midline of the back between sacral vertebrae 2 and 3.	Relaxes gluteal musculature and the iliosacral joint
BL 60	To the side, on the hollow on the cranial side of the calcaneal tuberosity.	Master point for pain
BL 67	Proximally to the coronet, on the rearward-sideways aspect of the rear leg	Conducts excess blockage from the bladder meridian
SI 03	To the side, between the cannon bone and the head of the splint bone	Conducts excess/blockage from the governor vessel on the midline of the back.

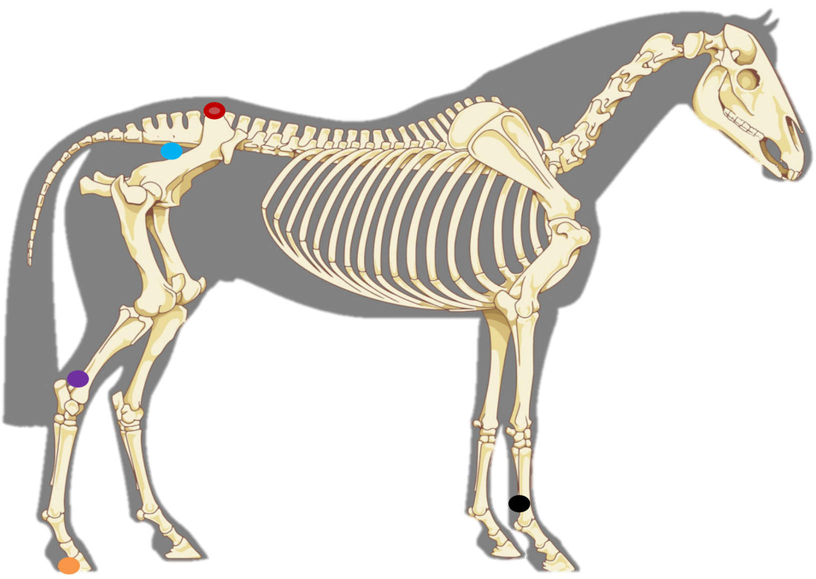

***Figure 3*. Estimated anatomical location of acu-points treated bilaterally. Red: Bai Hui, Blue: BL 28, Purple: BL 60, Orange: BL67 Black: SI03**

5.5.2 PEMF stimulation

Application of PEMFT was completed by the same individual throughout the trial using a Westville biomag 2. The settings that were used are 200Hz, 5μs for 10 minutes. An applicator was used over the lumbosacral area as shown in plate 4.

***Plate 4.* Demonstrating the PEMF machine and the application throughout the trial**

5.5.3 Combined

In the combined treatment Laser was applied first and then PEMFT. The reason for this is because PEMFT will increase blood flow. However, increasing blood flow into an area were laser is being applied will affect the absorption of photons. Blood contains haemoglobin which is a chromophore therefore photons will be absorbed before it has reached the target tissues. The same parameters and acu-points were used throughout for laser. The same setting for PEMFT were also used throughout.

5.6 Data collection

Pressure algometry was used to collect quantitative data of MNTs in horses. Horses were prepared in the morning by marking the individual algometry points (8) with chalk which were left on all day (see chapter 3.4). Horses were given their treatment for that given week and MNTs were measured straight after treatment, 60 minutes after treatment, 120 minutes after treatment and finally 180 minutes after treatment. A data collection sheet was made (see appendix i) to collect raw data and then data was placed into Microsoft Excel 2016 (V.15.27). Reactions to treatment, colour of skin, coat length and temperature were also noted.

A crossover repeated measures design was used so each horse acted as its own control which provided a more efficient comparison between interventions. This was beneficial so a fewer number of horses could be used as resources were limited, but statistical results were still provided. A week wash-out period was used to prevent any carry-over effects of PBM, therefore data collection took four weeks however data was collected once a week over the four weeks.

6 Results

Firstly, percentage differences were worked out between the baseline readings and readings after treatment. Left and right data was assessed for normality by Shapiro-Wilk and was not normally distributed ($p < 0.05$). Therefore, a Wilcoxon's rank paired test was run and null hypothesis was rejected ($p = 0.06$); Thus, left and right sides cannot be grouped.

Muscle nociception percentage differences were subjected to a two-way repeated measures ANOVA analysis of variance having three levels of treatment (laser acupuncture, combined and control) and four levels of time (immediately after treatment, 1, 2 and 3 hours). Not all effects were statistically significant at 0.05 significance level. The sections below show the results of all points 1-4 with left and right shown separately with variations between points.

Analysis of the studentized residuals showed that there was normality, as assessed by the Shapiro-Wilk test of normality and no outliers, as assessed by no studentized residuals greater than ±3 standard deviations. There was sphericity for the interaction term, as assessed by Mauchly's test of sphericity ($p > 0.05$). Data are mean (M) or standard deviation (SD), unless otherwise stated.

6.1 Point 1 left

The data was normally distributed, as assessed by Shapiro-Wilk test ($p > 0.05$). The assumption of sphericity was met for the main effects of treatments, as assessed by Mauchly's test of sphericity $X^2(2) = 3.691$, $p = 0.158$. The main effect of treatment showed a statistically significant increase on mechanical nociceptive thresholds, $F(2,14) = 8.6$, $p = 0.004$ as shown in figure 4. Therefore, a pairwise comparisons table was consulted. There was no difference in MNTs between laser and combined groups (0.042(95% CI -0.369 to 0.453) N, $p = 1.000$. Closer analysis showed MNTs to be higher after laser acupuncture, compared to combined. However, Laser acupuncture significantly increased MNTs compared to control (0.386(95% CI 0.152 to 0.619) N, $p = 0.004$. Indicating laser acupuncture was more effective than the control at increasing MNTs. When comparing combined treatment to the control there was a significant difference (0.344(95% CI 0.56 to 0.632) N, p = 0.022. Indicating that MNTs were significantly higher after the combined treatment compared to the control.

For main effects of time, the assumption of sphericity was met, as assessed by Mauchly's test of sphericity $X^2(5)$ = 3.04 p = 0.657. Main effects of time yielded an F ratio of $F(3, 21)$ = 0.160, p = 0.922, indicating there is not a significant effect of time on MNTs. There was not a statistically significant two-way interaction between treatment and time on muscle nociception suggesting MNTs did not increase or decrease over time, $F(6,42)$ = 1.719 p = 0.140.

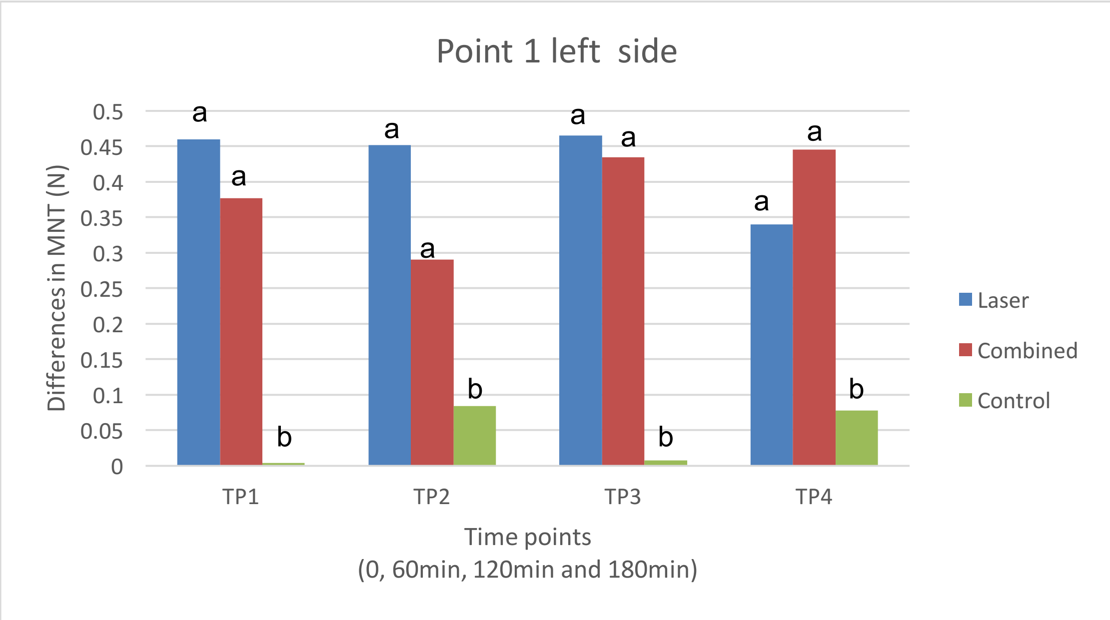

Figure 4. **The average values comparing the difference between the laser, combined and control treatment the four different time points at point 1 left. Different letters on bars indicate significant difference (p < 0.05).**

6.2 Point 1 right

The data was normally distributed at each time point, as assessed by boxplot and Shapiro-Wilk test (p > 0.05). For the main effects of treatment, the assumption of sphericity was met, as assessed by Mauchly's test of sphericity $X^2(2)$ = 0.233 p = 0.890. MNT's did not significantly increase after treatment $F(2,14)$ = 1.848 p = 0.194 (see figure 5). MNTs did increase after treatments but not significantly.

Main effects of time where analysed, the assumption of sphericity was not met, as assessed by Mauchly's test of sphericity $X^2(5)$ = 12.064 p = 0.036. Epsilon (ε) was 0.480 using Greenhouse-

Geisser, and was used. Muscle nociception did not significantly change over time, $F(1.441,10.088) = 2.010$ $p = 0.187$. Treatment and time did not have a significant two-way interaction on muscle nociception $F(6,42) = 1.240$ $p = 0.306$ therefore MNT's did not decrease over time.

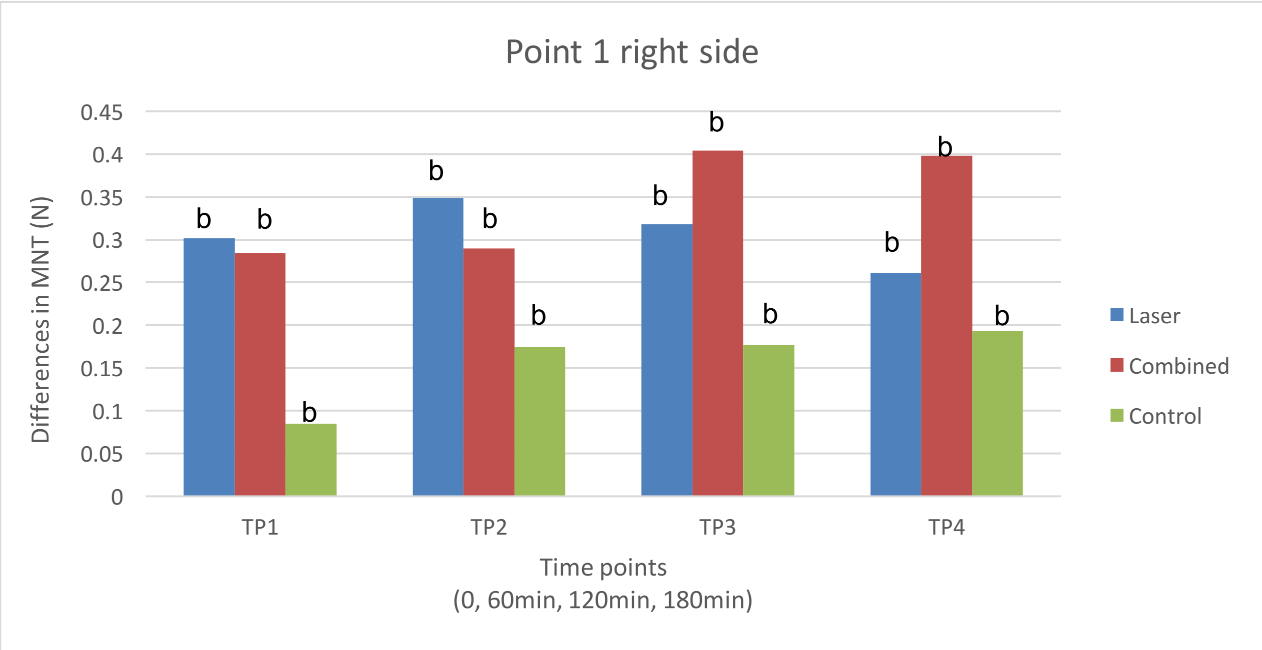

Figure 5. **The average values comparing the difference between the laser, combined and control treatment the four differenr time points at point 1 right. Different letters on bars indicate significant difference (p < 0.05).**

6.3 Point 2 left

All data were normal distributed at each time point, as assessed by boxplot and Shapiro-Wilk test ($p > 0.05$). Mauchly's test of sphericity indicated that the assumption of sphericity had been violated for the two-way interaction, $X^2(20) = 39.158$ $p = 0.015$. Therefore, Epsilon (ε) was 0.408 using Greenhouse-Geisser correction. There was a significant interaction between time and treatment on muscle nociception $F(2.446, 17.124) = 3.935$ $p = 0.033$. Muscle nociception decreased over time after laser acupuncture.

The main effects of time were analysed the assumption of sphericity had not been violated, $X^2(5) = 5.960$ $p = 0.316$. There was not a significant effect of time on muscle nociception $F(3, 21) = 0.740$ $p = 0.540$. The main effects of treatment were analysed; the assumption of sphericity had not been violated $X^2(2) = 0.394$ $p = 0.821$. MNTs significantly increased after treatments, $F(2,14) = 13.520$ $p = 0.001$.

Pairwise Comparisons show that there were no significant differences between the laser and combined groups (0.054(95% CI -0.198 to 0.306) N, p = 1.000. There was a significant difference between the laser group and the control group (0.355(95% CI 0.153 to 0.556) N, p = 0.003. There was a significant difference between the combined group and control group (0.301(95% CI 0.068 to 0.534) N, p = 0.015 (see figure 6).

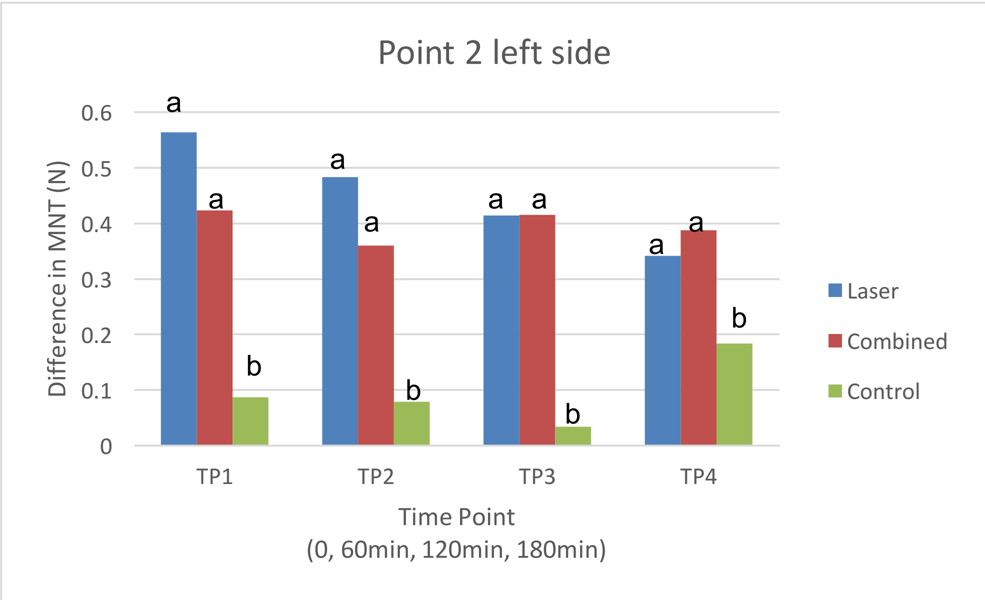

Figure 6. **The average values comparing the difference between the laser, combined and control treatment the four different time points at point 2 left. Different letters on bars indicate significant difference (p < 0.05).**

6.4 Point 2 right

All data was normally distributed at each time point, as assessed by boxplot and Shapiro-Wilk test (p > 0.05). For the within subject's effect of time, assumption of sphericity as assessed by Mauchly's test of sphericity was met $X^2(5) = 10.018$ p = 0.079. There was a statistically significant effect of time on muscle nociception $F(3,21) = 5.384$ p = 0.007. Pairwise comparisons between time point one and two show no significant difference (-0.091(95% CI -0.199 to 0.017) N, p = 0.111. There was a statistical significance between time point one and three (-0.105(95% CI -0.163 to -0.047) N, p = 0.002. There was not a statistical significant effect between time point one and four (-0.077(95% CI -0.177 to 0.022) N, p = 0.154. There was no statistical significant effect between time point two and three (-0.015(95%

CI -0.081 to 0.052) N, *p* = 1.000. The effects between time points two and four were not statistically significant (0.013(95% CI -0.135 to 0.162) N, *p* = 1.000. Finally, there was not a statistical significant effect of time point three and four (0.028(95% CI -0.088 to 0.144) N, *p* = 1.000.

For the effect of treatments assumptions were assessed and not violated, $X^2(2) = 0.238$ $p = 0.888$. There was no significant effect of treatment on muscle nociception, $F(2,14) = 2.122$ $p = 0.157$ as shown in figure 7. Mauchly's test of sphericity assessed the sphericity of the time and treatment interaction and assumptions were met $X^2(20) = 17.166$ $p = 0.732$. There was a two way interaction between time and treatment $F(6,42) = 4.747$ $p = 0.001$, muscle nociception in the laser group decreased over time.

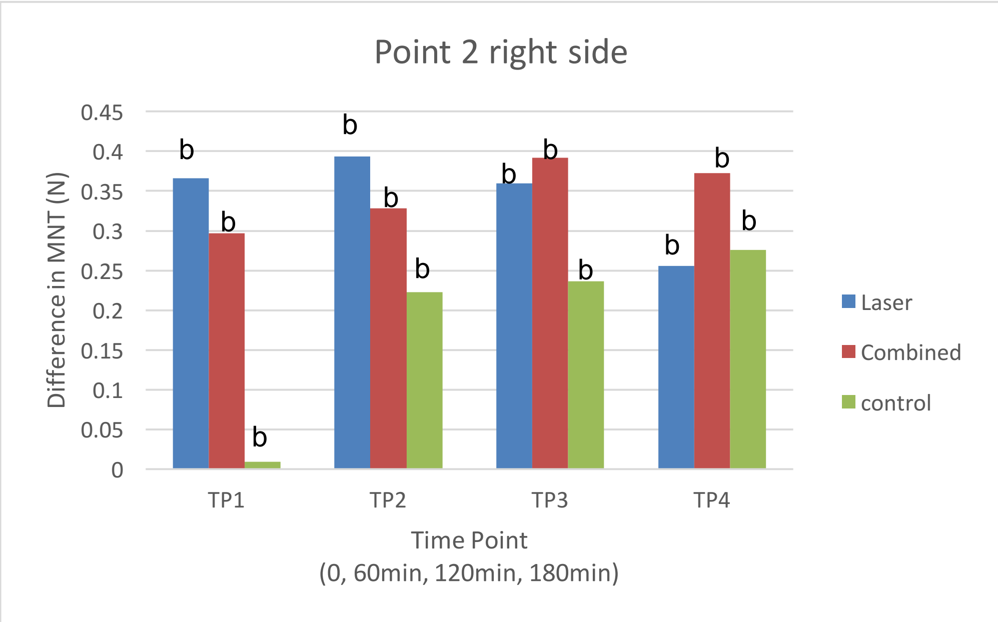

Figure 7. **The average values comparing the difference between the laser, combined and control treatment the four different time points at point 2 right. Different letters on bars indicate significant difference (p < 0.05).**

6.5 Point 3 left

Normality was assessed by Shaprio-Wilk test and was found to be parametric ($p > 0.05$). The assumption of sphericity was met for time as assessed by Mauchly's test of sphericity, X^2 (5) = 8.177 $p = 0.152$. There was no effect of time on muscle nociception $F(3, 21)$ 0.267 $p = 0.849$.

The assumption as assessed by Mauchly's test of sphericity was met X^2 (2) = 2.986 $p = 0.225$. There was a significant effect of treatment on muscle nociception $F(2,14) = 14.349$ $p = 0.000407$. Pairwise comparisons showed that there was no significance between the laser group and combined group

(0.114(95% CI -0.205 to 0.433) N, p = 0.902. There was a significant difference between the laser and control groups (0.417(95% CI 0.227 to 0.607) N, p = 0.001. There was a significant difference between the combined and control groups (0.303(95% CI 0.075 to 0.531) N, p = 0.013 (see figure 8). The sphericity of time and treatments was not violated as assessed by Mauchly's test of Sphericity $X^2(20)$ = 27.426 p = 0.197. There was a significant two-way interaction between time and treatment on muscle nociception $F(6,42)$ = 3.276 p = 0.010.

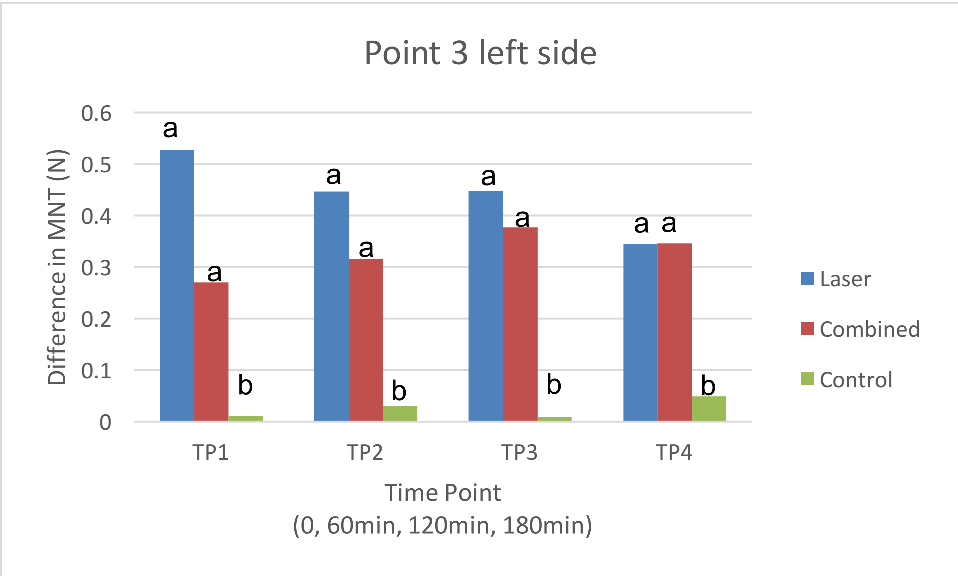

Figure 8. **The average values comparing the difference between the laser, combined and control treatment the four different time points at point 3 left. Different letters on bars indicate significant difference (p < 0.05).**

6.6 Point 3 right

Normality was assessed by Shaprio-Wilk test and was found to be parametric (p > 0.05). The assumption of sphericity for time was not met, as assessed by Mauchly's test of sphericity, $X^2(5)$ = 15.126 p = 0.011. Epsilon was 0.480, using Greenhouse- Geisser, and was used. Muscle nociception did not significantly change over time, $F(1.439, 10.075)$ = 0.231, p = 0.726. The assumption of sphericity for treatment was violated, as assessed by Mauchly's test of sphericity, $X^2(2)$ = 9.797 p = 0.007. Epsilon was 0.554, using Greenhouse- Geisser, and was used. There was not a significant effect of treatment on muscle nociception, $F(1.108,7.758)$ = 0.528 p = 0.507. There was no significant two-way interaction between time and treatments $F(1.991,13.940)$ = 0.765 p = 0.483.

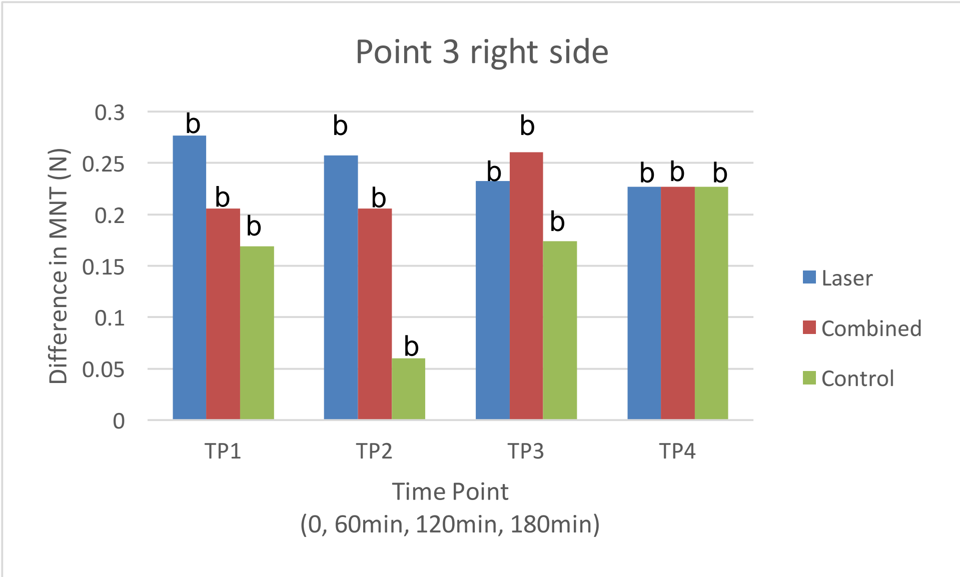

Figure 9. **The average values comparing the difference between the laser, combined and control treatment the four different time points at point 3 right. Different letters on bars indicate significant difference (p < 0.05).**

6.7 Point 4 left

Normality was assessed by Shaprio-Wilk test and was found to be parametric (p > 0.05). Mauchly's test of sphericity was used to test the sphericity of time and the assumption was violated $X^2(5) = 18.231$ = 0.003. Epsilon was 0.507, using Greenhouse- Geisser, and was used. There was not effect of time on muscle nociception $F(1.522,10.656) = 0.340$ $p = 0.663$. The assumption of sphericity for treatment was met $X^2(2) = 2.313$ $p = 0.315$. There was a significant effect of treatment on muscle nociception $F(2,14) = 6.074$ $p = 0.013$. Pairwise comparisons showed that there was no significant difference between laser and the combined group (0.033(95% CI -0.239 to 0.305) N, $p = 1.000$. There was a significant difference between laser and the control group (0.247(95% CI 0.088 to 0.406) N, $p = 0.005$. There was no significant difference between the combined and control group (0.214(95% CI -0.059 to 0.487) N, $p = 0.132$. There was not a significant two-way interaction between treatment and time $F(2.258,15.805) = 1.309$, $p = 0.301$.

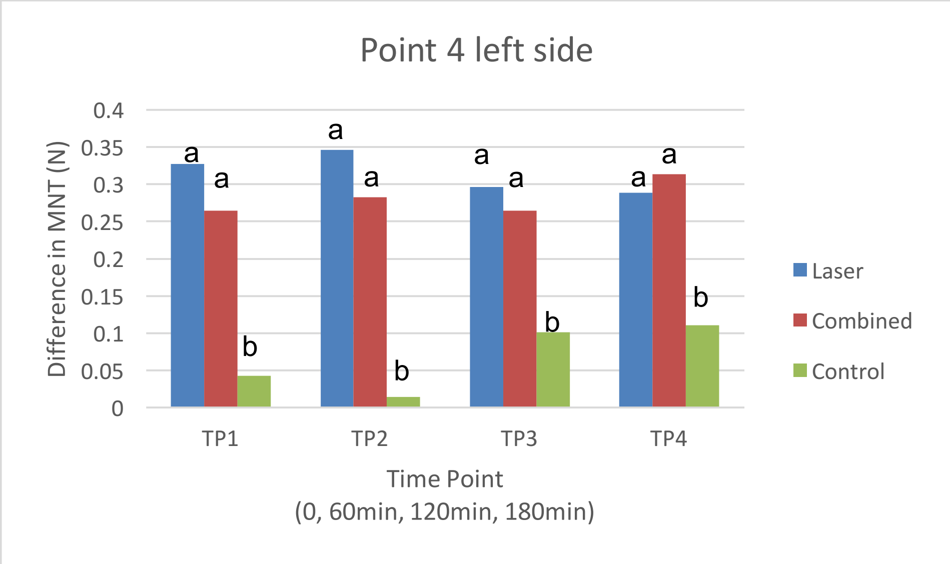

Figure 10. **The average values comparing the difference between the laser, combined and control treatment the four different time points at point 4 left. Different letters on bars indicate significant difference (p < 0.05).**

6.8 Point 4 right

Normality was assessed by Shaprio-Wilk test and was found to be parametric (p > 0.05). The assumption of sphericity for time was not violated, as assessed by Mauchly's test of sphericity, $X^2(5) = 0.626$, $p = 0.987$. Time did not have a significant effect on muscle nociception $F(3,21) = 0.494$, $p = 0.690$. The assumption of sphericity for time was met, as assessed by Mauchly's test of sphericity, $X^2(2) = 0.344$, $p = 0.842$. Treatment had a significant effect on muscle nociception $F(2,14) = 4.425$, $p = 0.032$. Pairwise comparisons demonstrated that there was no significant difference between laser and combined treatments (0.072(95% CI -0.165 to 0.309) N, $p = 1.000$. There was significant difference between laser and the control treatments (0.204(95% CI 0.011 to 0.396) N, $p = 0.039$. There was no significant difference between the combined and control groups (0.132(95% CI -0.089 to 0.352) N, $P = 0.312$. There was no significant two-way interaction between time and treatments $F(6,42) = 1.989$, $p = 0.089$.

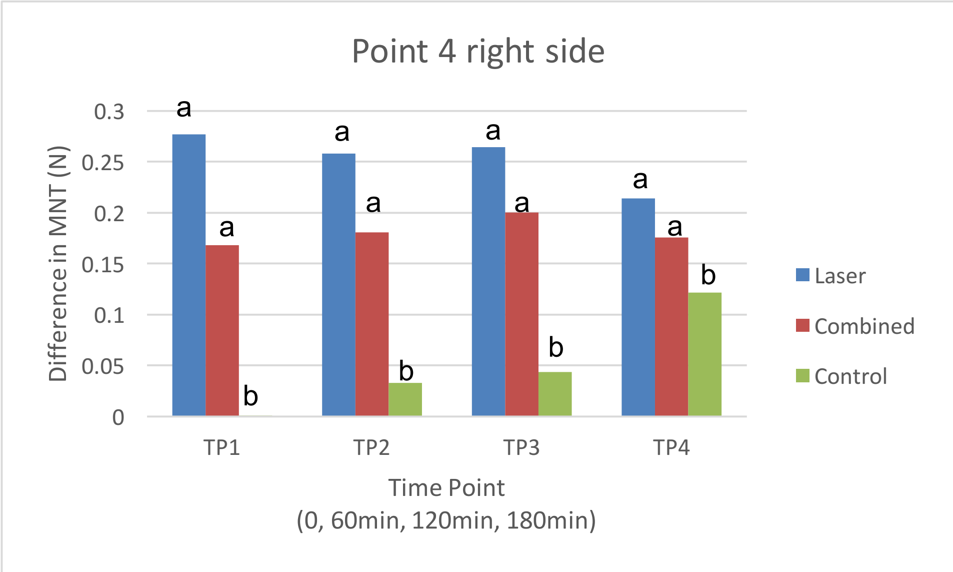

Figure 11. **The average values comparing the difference between the laser, combined and control treatment the four different time points at point 4 right. Different letters on bars indicate significant difference ($p < 0.05$).**

7 Discussion

The purpose of this study was to investigate the effects of laser acupuncture and PEMFT on equine back pain. The study was a development from a systematic review which recognised a lack of publications on the use of laser acupuncture for back pain.

Overall, treated horses had a larger increase in MNTs compared to the control, which suggests back pain reduced. This study demonstrated that laser acupuncture and the combined treatment significantly increases MNTs, therefore is able to reduce equine back pain. The results in this study similar to the results obtained by Martin and Klide (1987). Encouragingly, there was a significant effect of treatment on increasing muscle nociception on five points (62.5%) (**chapters 6.1, 6.3, 6.5, 6.7 and 6.8**), however on three points MNTs did not significantly increase. Interestingly they were all on the right side (**chapters 6.2, 6.4 and 6.6**). A possible explanation for this is, would be because baseline MNT's on the right side were already higher than the left therefore, they did not significantly increase after treatment. The fact a small number of horses were used could have also contributed to this. Although both laser acupuncture and the combined treatment had significant effects on increasing MNTs, there was no statistical difference between laser acupuncture and the combined treatments. Suggesting there is no advantage of using both laser acupuncture and PEMFT to treat pain. This provides a reasonable level of evidence to suggest laser acupuncture on its own is effective at reducing back pain, therefore the null hypothesis can be rejected and the alternative accepted. The data does suggest that laser acupuncture had a slightly larger increase on MNTs over the combined group but not a significantly. Thus, results show both are equally as good as each other. To the authors knowledge there is no other study to have used these treatments together to treat back pain, therefore results cannot be compared. However, it does provide physiotherapists with evidence to suggest one electrotherapy is more beneficial than two. For all points (87.5%) apart from point two right ($p = 0.007$) there was no significant effect of time on muscle nociception (**chapters 6.1, 6.2, 6.3, 6.5, 6.6, 6.7 and 6.8**). This would suggest that long term benefits need to be established to understand the carry-over effects of the two treatments, for example at what point MNT's start to reduce such 24, 48 and 72hrs after treatment.

This study found that there was only a significant two-way interaction between treatment and time at point two left ($p = 0.033$) (**chapter 6.3**) and not at any other point suggesting this may be an anomaly. The results show MNTs reduced over time at point two left, however at the other points MNTs did not reduce over time therefore, it can be presumed that the carry over effects of laser and PEMFT last up to three hours. Many studies have reported longer carry over effects for up to two weeks (Tseng *et al*., 2016; White and Hunt, 2019). The findings in this study are supported by Lonrenzi *et al*., (2009) who found that pulsed laser (670nm, 5.25mJ/mm^2) significantly reduced pain associated to inflammation and muscle damage therefore increasing muscular performance ($p < 0.05$). However, it was noted that there was no two-way interaction between time and treatment here even up to 72hrs after treatment. It was suggested that the possible mechanisms for analgesic effects of the laser acupuncture are related to the conduction of electromyographic signals at the acu-points and the transmission down the meridians (Lonrenzi *et al*., 2009). Furthermore, it was noted that the opioid systems are activated therefore changing brain chemistry and sensation. Although the dosage of laser is low in the Lonrenzi *et al*., (2009) study the subjects were rats therefore dosages used would be relative to body size and skin thickness.

The laser parameters used in this study were effective at increasing MNTs. Venancio *et al*., (2002) suggest that dosage ranges are dependent on the type of pain (acute or chronic), target tissue and skin pigment. The results a broadly consistent with other animal and human based research looking at laser acupuncture. Venancio *et al*., (2002) notes that for chronic pain and trigger points dosages at 4J/cm^2 with more sessions are best. Whereas doses for more acute pain should be around 24J/cm^2 just five times for a week. Simunovic, (1996) further supports this by suggesting decreases in doses but increasing frequency of application for chronic pain is very important, probably more important than wavelength due to potential harmful effects of over dosing causing negative effects (Uemoto *et al*., 2013). However, Hudson *et al*., (2013) notes that wavelengths at 808nm penetrate 54% deeper than 980nm on bovine tissue. Suggesting that wavelength is still a very important factor when applying laser especially on different skin pigmentation.

Traditionally the application of PBM is through the animal's coat however research suggests that this is an inefficient method of delivering photons to the target tissue (Ryan and Smith, 2007). Ryan and Smith, (2007) found that laser applied to a superficial digital flexor tendon had best penetration when the limb was clipped and cleaned. However, when treating back pain this process is inefficient and many owners will not allow patches of hair to be clipped out of their horses. But to access the deep structures of the back this might have to be implicated in horse's with deeper musculoskeletal pain. Conversely, Martin and Klide, (1987) did not clip the horses before applying laser acupuncture and still demonstrated good results (10/14 had alleviation of back pain). However, this study used a pulsed laser rather than a continuous beam that Ryan and Smith, (2007) used. Like the Lonrenzi *et al*., (2009) study, Joesen *et al*., (2012) had good results with a pulsed laser compared to continuous lasers. Finding that pulsed lasers can penetrate up to 58% deeper than a continuous wave. This could suggest why there was such a high statistical significance in muscle nociception after treatment and follows the same trend as the Martin and Klide (1987) study. However, more research is needed to compare continuous and pulsed lasers for equine back pain. Petermann, (2017) suggest that that pulsed or super pulsed lasers provide energy at a much greater penetration depth without emitting so much energy that the target tissue heats up. This suggests why the horses in this study had a greater improvement in MNTs after just one treatment. Furthermore, the horses in the Martin and Klide, (1987) study had a minimum of eight treatments rather than just one treatment like the horses in this study.

Ryan and Smith, (2007) indicate that a greater amount of light was transmitted through tendons of grey subjects compared to bays however this was not significant, therefore skin pigmentation did not affect penetration depths. This however would not significantly affect the results in this study as majority of the horses had black skin and were bay or chestnut with only one horse having pink skin, but this horse's results were not significantly different to the others. This could possibly be due to the three wavelengths that the laser used in this trial emits, therefore different skin colours are supplied with the optimum wavelength for effective penetration. More research is need to understand the exact penetration depths through a variation of skin colours with the three wavelengths used. However, it was noted that the two chestnuts included in this study had lower baseline MNTs but and larger increases after treatment. There is an age old belief that chestnut horses are more sensitive than any other coloured horse. It is thought that chestnut horses have one less dermal layer than others, which may suggest why these two horses had such a large improvement in MNTs. Finn *et al*., (2016) suggest mutations in the genes influence melanocytes which not only affects colour but also impacts on physiological and behavioural functions therefore may be more sensitive to pain. Making their response to a stimulus more dramatic (Finn *et al*., 2016). Husby *et al*., (2016) noted that chestnut skin samples allowed 15.4% laser light penetration (660nm, 800nm and 970nm) compared to bay horses skin at 2.5%, which further suggests chestnuts may have thinner skin or are able to absorb laser light more efficiently. This is another important variable to think about when applying PBM in practice as it is important not to over treat and cause damaging effects to cells.

7.1 Algometry

There is a distinct lack of scientific evidence for objective evaluation of back sensitivity in horses. However, pressure algometry is described as an objective tool to quantify pressure-pain responses, by giving mechanical nociceptive threshold values. Within this study pressure algometry proved to be a consistent and useful tool to objectively measure MNTs before and after treatment. Therefore, it should be able to detect the effects of analgesics and a dose-response relationship (De Heus *et al*., 2010; Haussler and Erb, 2010). This will help to determine optimal doses of laser and PEMFT for subsequent clinical evaluation and to apply these doses in practice (Love and Whay, 2011). A common problem when testing on horses is that they may learn to respond to the stimulus as soon as it is perceived as an aversion technique, therefore they may seem more painful than they actually are (Chambers *et al*., 1994). The main issue with pressure algometry is individual variability. Variation in skin thickness, hair length, epidermal pigmentation and nociceptor distribution are thought to influence peripheral pain perception (Love and Whay, 2011). Within this study the two horses with very long coat lengths had higher MNTs throughout. This could have been for a number of reasons for example if the coat is longer, the assessor is less likely to see the pain response such a skin fasciculation therefore the results for these two horses are likely to be less reliable than the horses with shorter coats. Furthermore, one of the long coated subjects was a Shetland pony which are known for being more resilient animals due to their ancestry.

One of the most common trends with the horses in this study was that the majority of baseline MNTs were always lower on left compared to right. This corresponds to the Varco-Cocks *et al*., (2006) study which demonstrated that there were differences between left and right MNTs. However, Balaguier *et al*., (2016) findings contradict this, although this studies subjects were human rather than horses, this could explain the differences in distribution of pain. Left and right differences in the Varco-Cocks *et al*., (2006) study was thought to correspond to the side injury of the sacroiliac joint. Although the current study did not look in to SIJD, there is a direct link between the SIJ capsule and the *multifidus*. Damage to the left or right side of the SIJ could trigger a reflex lowering the thresholds of nerve endings of the surrounding musculature such as the parspinals and gluteal musculature (Indahal *et al*., 1999; Varcoe-Cocks *et al*., 2006).

There are a number of other reasons why there were such apparent differences in left and right, such as poor saddle fit (De Cocq *et al*., 2006), rider asymmetries and weight (Fruehwirth *et al*., 2004), or due to horsemanship tradition. Riders handle and mount horses from the left side which could have knock on effects on the horses back (Geutjens *et al*., 2008). These findings correspond to Zaneb *et al*., (2009) results where preferential use of back muscles may cause handedness and is often due to human handling techniques. Geutjens *et al.,* (2008) notes that when a rider mounts from the floor or a step, peak pressures increase however, mounting from the floor had significantly higher pressure peaks than a raised platform ($p = 0.05$). This suggests that when mounting, the horse will stabilise itself by muscular contraction. Thus when repeatedly mounting from the same side the horse will continually brace leading too muscular asymmetries as well as discomfort due to the torque of the saddle on the horses back (Fruehwirth *et al*., 2004; Geutjens *et al*., 2008; Groesel *et al*., 2010).

The points used within this study demonstrated moderate to good repeatability. Points 1-3 showed lower MNT's than point 4 which was also described in in the Varco-Cocks *et al*., (2006) study. If this study was repeated, it would be suggested that all points were moved 10cm cranially from the original location. Covering a greater area of the paraspinals and gluteals. It was also noted that from MNTs decreased from point 1 going caudally, it has been suggested is could be down to altering skin thickness which supports Pongratz and Licka, (2017) findings that the thoracic region has significantly lower MNTs compared to the lumbar region which corresponded to skin thickness on *in vitro* specimens. Furthermore, in ridden work a saddle would cover points 1 and 2 so it is also important to bare in mind micro-trauma from the rider and saddle may have caused reduced MNTs.

7.2 Limitations

One of the main limitations in this study is the small sample size, therefore the study is not as representative of the equine population compared to larger sample sizes. This makes it more difficult to generalize from the sample to the general population. Another limitation of the study was having a one-week wash-out period due to time constraints. Carry over effects were apparent in this trials results therefore a longer wash-out of two weeks would be suggested.

Although algometry provides a repeatable objective assessment of back pain, individual variability between subjects present as an issue within this trial, therefore using this tool alongside kinematics and veterinary palpation could be more reliable.

It would be possible that the use of horses with diagnosed painful conditions might have better highlighted differences between groups. For example, for deeper orthopaedic conditions PEMFT may have been beneficial due its deeper penetration depths. Whereas laser is thought to be more optimal for superficial musculoskeletal issues.

7.3 Recommendations for further research

To further develop the use of laser acupuncture on horses in the veterinary physiotherapy profession more clinical trials are needed. Further research needs to concentrate on finding the optimum dose of laser to treat musculoskeletal back pain. This study provides one lot of evidence to suggest that laser acupuncture is able to increase MNTs and maintain analgesia over three hours with little variation, therefore aiding in reducing back pain. Furthermore, not only is the laser dosage and reporting the parameters important, locating the best acu-points to treat back pain will also be useful for veterinary physiotherapists. The points used in this study provides grounds for treatments but more studies using different acu-points are needed.

Recommendations to further extend this research would be to use horses that have been diagnosed with back pain by a veterinarian. Additionally, using not only algometry readings throughout the study but also have two vets to test MNTs using palpation and scoring on a pain scale to provide further reliability in the results and relate to common practice. To progress on from this study measuring kinematics of the spine after the treatment of back pain using the same protocol could be beneficial to identify if there is any improvement in movement and performance (Dunkel *et al*., 2017).

Future studies should also look at the long term benefits, this study provided evidence that a single treatment of laser acupuncture or both laser acupuncture and PEMFT increases MNTs and is maintained over three hours. Furthermore, using a longer treatment plan such as three treatments a week over four weeks and have long term follow up, might be more beneficial for physiotherapists and relatable to current practice.

8 Conclusion

Back pain in horses has been a problem for many years. Studies suggest it is a secondary problem to dysfunction from hindlimb lameness, however primary back pain is becoming more of an issue. Veterinarians are able to diagnose and treat back pain however, it is becoming more common for vets to refer horses for physiotherapy and rehabilitation. Physiotherapy provides a multimodal approach for rehabilitation from injury or dysfunction. Therefore, the use of laser acupuncture along with other manual techniques will be hugely beneficial for treating horses with back pain. This study provides evidence that the stimulation of acu-points with PBM therapy is a clinically useful tool for treating back pain in horses. One disadvantage of this therapy is the initial cost of the equipment. However, the main advantage of laser acupuncture over electro-acupuncture or dry needling is that it is a painless and non-invasive tool, available to physiotherapists to aid in restoring musculoskeletal function.

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10 Appendices

10.1 Appendix i.

Name:		**Age:**		**Date:**
Skin	Hair	Sex	Breed	
Temp:				
Comments before:	Left		Right	

Base Reading	Time:					Temp:			
Points	Point 1		Point 2		Point 3		Point 4		Comments
	Left	Right	Left	Right	Left	Right	Left	Right	
1									
2									
3									
Mean									
Straight after Treatment 4:		Time:				Temp:			
Points	Point 1		Point 2		Point 3		Point 4		Comments
	Left	Right	Left	Right	Left	Right	Left	Right	
1									
2									
3									
Mean									

60 mins after Treatment 4:			Time:				Temp:		
Points	Point 1		Point 2		Point 3		Point 4		Comments
	Left	Right	Left	Right	Left	Right	Left	Right	
1									
2									
3									
Mean									
120 mins after Treatment 4:			Time:				Temp:		
Points	Point 1		Point 2		Point 3		Point 4		Comments
	Left	Right	Left	Right	Left	Right	Left	Right	
1									
2									
3									
Mean									
180 mins after Treatment 4:			Time:				Temp:		
Points:	Point 1		Point 2		Point 3		Point 4		Comments
	Left	Right	Left	Right	Left	Right	Left	Right	
1									
2									
3									
Mean									

10.2 Appendix ii

Owner consent for participating horse
This form is an agreement that you allow permission for the use of your horse within the proposed study.
Owner name:

Contact details:

Name of Horse:

Age of Horse:

Breed of Horse:

Veterinary Practice details:

Veterinary Surgeon:

You **must** clear the horse of the following and should be within the criteria of;

1. **Ensure the horse is healthy and non-lame**
2. **Ensure the horse has had no tumours/heart conditions**
3. **The horse has no skin conditions/infections and are not on NSAIDS (e.g. bute)**
4. **The horse is well behaved**

By signing this agreement, you confirm that your vet has given confirmation that the horse participating in the experiment has been cleared from the above criteria and is suitable.

…………...………………………………………………………………………………………………
(print/signed signature)