

Szent Istvan University, Faculty of Veterinary Science,
HAS-SZIU Large Animal Clinical Research Group and
Department and Clinic for Food Animals

**Cranial electrotherapy stimulation for treating
stress-related disorders in horses**

Kate Hannigan

Tutor: Dr. Krisztina Nagy, PhD

Budapest

2013

Contents

1.1. Introduction	3
1.2. A survey of literature	4
2. Materials and methods	11
2.1. Animals	11
2.2. Experimental procedure	11
2.3. Salivary cortisol.....	12
2.4. Heart rate variability.....	13
2.5. Behaviour reaction during treatment.....	16
2.6. Statistical analysis	16
3. Results	17
4. Discussion.....	22
5. Conclusions	28
6. Summary.....	29
7. References	31
8. Acknowledgements	38

1.1. Introduction

The Hungarian National Police Force comprises of a section on horseback. Their work involves having to patrol varied terrain, as well as providing riot control at football matches. They also carry out the tasks of the Hungarian Subsidiary National Horse Ceremonial Unit (Fig. 1). Changeable conditions and competence in a wide range of diverse tasks, see these horses endure much higher stress levels than horses for recreational use only. The animals are under constant supervision but concern has been raised about the possibility that the increased stress of everyday life situations is a common cause of underlying diseases, i.e. colic. Twenty three horses in the last thirty eight years have suffered bouts of colic. This study interested me as I have a keen interest in the equine species and the challenge of decreasing stress amongst horses was one which I find to be of upmost importance.



Figure 1 Hungarian National Police Force

1.2. A survey of literature

Mounted police horses have to cope with challenging, unpredictable situations when on duty and it is essential to gain insight into how these horses handle stress to warrant their welfare. They continue to serve in remote areas and in metropolitan areas where their day-to-day function may be picturesque or ceremonial, but they are also employed in crowd control because of their mobile mass and height advantage and increasingly in the UK for crime prevention and high visibility policing roles. The added height and visibility that the horses give their riders allow officers to observe a wider area, but it also allows people in the wider area to see the officers, which helps deter crime and helps people find officers when they need them (Cooper, 2011).

Stress often produces an alteration in autonomic sympathetic balance, para-sympathetic, resulting in elevations in blood pressure, pulse rate, vasoconstriction in peripheral blood vessels, and increased outputs of stress hormones. In the stress response, skeletal muscle tension may rise along with central nervous system shifts toward increased EEG desynchrony, sleep disturbance, reduced brain serotonin, and eventual declines in cognitive performance (Seelv et al., 1989). Prior work by Zimmerman and Lerner (1989) using CES at 0.5 Hz, random low repetition rate, biphasic square waves, demonstrated CES lowered one component of the stress response beyond that achieved by biofeedback. Specifically, the combined use of CES and EMG feedback was demonstrated to produce a synergistic effect in lowering muscle tension levels in chronic pain patients. Other investigations using EMG measures to assess CES on stress arousal are less conclusive. Weingarten (1981) found that 15, 40-minute CES treatments lowered standardized anxiety scale scores but failed to uniformly lower frontalis EMG without feedback training.

A review of CES literature shows that most double blind, well-controlled studies look at only one measure of the stress response, often in conjunction with some personality or cognitive psychometric measure. This type of research ignores the obvious clinical reality of symptom substitution and individual physiologic variability in stress responding. Each individual responds differently to stress, indeed some persons are classified as musculoskeletal responders, whereas others are seen as autonomic responders. Another shortcoming of current psychophysiology investigations of CES is the failure to assess a single trial treatment effect of

CES. The majority of CES studies use several regularly spaced multiple treatments with CES.

Recent studies describe an effective and drug-free alternative in the treatment of human anxiety disorders, called cranial electrotherapy stimulation or CES. CES is a non-invasive therapeutic device that has recently been used in the treatment of human depression, anxiety and sleeping disorders (Kirsch, 1996). This effective and drug free alternative applies pulsed, alternating micro current (~1-2mA) transcutaneously to the head via electrodes placed on the earlobes, mastoid processes, zygomatic arches, or the maxillo-occipital junction. The U.S. Food and Drug Administration (FDA) granted approval in 1979 for CES for the treatment of insomnia, depression, and anxiety, and it is commercially available for personal use (Zaghi et al, 2010). It can also be called electrosleep therapy and transcranial electrotherapy (TET). The other main form of low-intensity cranial electrical stimulation, transcranial direct current stimulation (tDCS), uses direct current (DC) and is therefore distinguishable from CES which uses alternating current (AC). Available evidence indicates that these differences in electrical stimulation result in significant differences in biological effects (Zaghi et al, 2010). A number of different models have been put forward, proposing to account for the effect of CES on the brain (Kirsch, 2007 and Smith, 2007). A hypothesis which has been popular since the 1960's was described by Krishnan. This monoamine hypothesis (Krishnan, 2008) suggests that depression results from the reduced activity of key neurotransmitters such as serotonin, norepinephrine and dopamine (all monoamine molecules). Anti-depressants on the market these days work in such a way as to increase the availability of these neurotransmitters. In regard to depression, double-blinded studies of psychiatric patients have been inconclusive or negative (Levitt et al., 1975; Feighner et al., 1973). In Feighners studies, four out of six clinically depressed patients dropped out of the study because of worsening of depressive symptoms, with two of them becoming suicidal again. Zaghi et al. (2010) published an article in the journal *The Neuroscientist*, finding that CES increases the production of serotonin, GABA, and endorphins. These neurochemical changes explain any positive effects that might be experienced from CES. Computer modelling predictions using a highly detailed anatomical model show that CES induces significant currents in cortical, sub-cortical structures like thalamus, insula and hypothalamus, and brain-stem structures (Klawansky, 1995). A pilot study showed that CES reduced the symptom burden of generalized anxiety disorder, with a decrease in Hamilton Anxiety Rating Scale (HARS) across a 6 week study, but the study had a small sample of participants and no control group (Bystritsky et al., 2008).

A meta-analysis of eight randomly controlled trial studies assessing the efficacy of CES on anxiety found that CES improved anxiety significantly as compared with placebo treatment (Klawansky, 1995). A systematic review which assessed 34 controlled trials involving a total of 767 CES patients and 867 control patients reported that in 77% of studies (26 of 34), CES was found to significantly reduce anxiety (De Felice, 1997). A 3-week randomized controlled study which looked at insomnia in fibromyalgia patients found significant improvement in sleeping patterns (Lichtbroun et al, 2001) In a longitudinal insomnia study, subjects showed improvement of symptoms during a two-year follow-up (Weiss, 1973). Further studies in dogs have suggested that CES works by increasing levels of dopamine in the central nervous system (Kirsch, 2002). Also an experiment using CES took place on opiate withdrawal in rats.

It was found that CES helped this opiate withdrawal implying that CES may also act on endogenous neurotransmitter which release endorphins. Knowingly, endorphins act on the same neuronal receptors as opiate drugs, thus increasing levels of this substance in the body helps mediate a variety of functions including mood regulation (Smith, 2007). The activity of individual neurons, which is mediated through the neurotransmitters described above, involves generation of electrical impulses between neurons. Electroencephalography or EEG provides another form of interpreting CES effects on brain physiology. EEG analysis can provide information about brain function by analysing regional and global electrical activity of scalp electrodes attached to a computer (Serman, 1996). A study of CES in healthy male volunteers found that CES produced changes in brain electrical activity similar to that produced by meditation (decreases in higher frequency alpha and beta waves replicating findings of earlier trials) (Schroeder, 2001). Many important aspects of the mechanism of action of CES still remain unclear and the question of whether the treatment is effective is not one which there is a simple answer to. In human medicine, the power spectrum analysis (PSA) of the HRV signal is an established method to quantify the activity of the autonomic nervous system (Bernasconi et al., 1998).

FDA-Cleared since 1991, the Fisher Wallace Stimulator, as seen in Figure 2, restores sleep and improves mood by using patented radio frequencies to gently stimulate the brain's production of serotonin, beta-endorphin and other key neurochemicals. It consists of a base unit (battery operated), sponge applicators and a head band. The two yellow disks inside the head band contain sponge applicators which expand when submerged in water. The head band is placed above the level of the ears and sponge applicators applied as close to the skin as possible. There

are 3 levels on the base unit, level two being used to treat insomnia, depression and anxiety. The device is used for 20min/day for a minimum of 30-45 days after which it should be used 2/3 times a week. If severe symptoms are present and prescribed by the practitioner, it can be used twice daily. Generally 5-10 sessions are needed for the diagnosed symptoms to recede. The “Happy Halter” is the same product, produced by Fisher Wallace, applied in horses suffering from anxiety conditions. Horses should be in their familiar stables, accompanied also by someone familiar. The strap is positioned directly over the zygomatic arch and the device is switched on to begin the 20 minute session. During this time the horse may open its mouth, relax its jaw and pretend to chew, paired also with shuffling of the feet. Near the end of the treatment the horse may begin to get sleepy (Happy Halter, n.d.).



Figure 2 Fisher Wallace device in use

A cranial electrotherapy stimulator unit, called the Alpha-Stim (Figure 3), has been launched on the U.K. market for commercial use; the effectiveness of the device in treating anxiety, depression and insomnia has been validated elsewhere (Kirsch, 1998). The numerous studies that illustrate the beneficial effects of CES reducing anxiety, are of particular relevance for the purposes of this study. Alpha-Stim has been effective in reducing the anxiety levels of patients suffering from anxiety disorders according to pre and post treatment self rating of anxiety levels as well as objective measures (Overcash, 1999). CES has also been found to effectively decrease levels of anxiety in patients during dental procedures (Winick, 1999) as well as acting as an efficient moderator of fear perception in phobic patients (Smith and Shiromoto, 1992). Kirsch (1999) has conducted a post-marketing survey of some 500 Alpha-Stim CES patients who provided feedback relating to the effectiveness of the CES treatment.

The results indicated that CES displayed the greatest efficacy for treating anxiety and stress, a significant improvement being reported by 90.91% of the anxiety patients and 93.05% of the stress patients.



Figure 3 Alpha Stim in operation

Whilst comparing the two devices mentioned above, the differences are noted in Table 1.

Table 1 Technical parameters of the Fisher Wallace and Alpha Stim devices

Parameter	Fisher Wallace	Alpha-Stim
Output Amplitude	400 μ A (microampere)	10 – 600 μ A
Rate	15/150/15000 Hz	0.5/1.5/100 Hz
Pulse Width	33 microseconds	0.25 – 1 second
Max Charge per pulse	0.13 microcoulombs	1.5 microcoulombs
On time per Burst	50 milliseconds	
Off time per Burst	16.7 milliseconds	

This study was based on three different parameters by which we quantify stress; heart rate, salivary cortisol and heart rate variability. The hypothalamus – pituitary – adrenal (HPA) axis responds rapidly and rather specifically to a wide range of environmental and internal demands often referred to as stress. Following stimulation by ACTH, cortisol is produced and released from the adrenal glands and secreted into the circulating blood where it is bound rapidly to carriers such as corticosteroid-binding globulin (CBG), albumin and erythrocytes. While in blood both bound and free cortisol can be measured, only free cortisol appears in the saliva. Therefore the measurement of salivary cortisol provides an index of the biologically active fraction of this steroid (Kirschbaum and Hellhammer, 2000).

The most immediate stress response is an increase in adreno-medullary and sympathetic nervous activity, leading to a rise in heart rate. In addition, heart rate variability is an indicator for the response of the autonomic nervous system to stress and reflects the oscillatory antagonistic influence of the sympathetic and parasympathetic branch of the autonomous nervous system on the sinus node of the heart (von Borell et al., 2007). Salivary cortisol concentration, and heart rate (HR) and heart rate variability (HRV) were chosen as physiological indicators of 'emotional' stress (Bachmann et al., 2003; Rietmann et al., 2004, Möstl and Palme, 2002). We have chosen police horses for this study, as these horses consist of similar age, breed and gender, are kept in the same stable, receiving similar type and amount of exercise or food, and most importantly, because police horses share special interest regarding stress-related disorders. According to previous studies (Leal et al., 2011) it has been shown that that police horses with abnormal cortisol circadian rhythm (CCR) had more chance to develop colic. The aim of this study was to determine the CCR ratio in horses subjected to different housing and work conditions and to associate abnormal CCR ratio with incidence of colic. A total of 116 police horses belonging to four different groups were studied. In all, 31 were full-time stabled and performed urban patrolling activity, 27 were full-time stabled and performed equine therapy and sports activities, 25 were part-time stabled and performed urban patrolling, and 33 animals were kept full-time on pasture and did not perform any kind of work activity. High incidence of abnormal CCR ratio indicates that police horses were under stressful conditions. Horses with abnormal CCR ratios were more prone to suffer colic episodes, indicating that CCR ratio determination could perhaps be useful in detecting horses at risk of colic.

A study conducted in the University of California, Davis School of Medicine, focused on the effect of CES on cribbing behaviour in horses (Berger et al., 2010). Compulsive behaviours in horses, can also be called stereotypies as they occupy large portions of the horses daily activity, are repetitive and serve no useful function. Crib-biting, or cribbing for short, is a form of oral stereotypy (Merck, 2010). It is thought to have many different contributing factors and has been defined as a type of coping mechanism which has been induced by frustration and/or a central nervous dysfunction (Mason and Rushen, 2006). Using a cross-over design, 8 established cribbing horses were randomly assigned to either a treatment or control group and reversed following a wash-out period. Observations of crib-biting behaviour were made during three 1-hour periods daily. Clinical Trials were started on 20 privately owned horses.

Owners were instructed to observe crib-biting behaviours during daily 15-minute periods over a 1 week baseline and 6 week treatment period. During the treatment period, there was a significant decrease in crib-bite rates of the treatment group when compared to the control group. Horses in the Clinical Trials showed positive responses to treatment according to owner evaluations, however results were inconclusive due to inconsistent data and low sample size of the data returned. The study suggested, much like the suggestions of this study, that the promising preliminary results seen in this study warrant further investigation in a larger group of horses. CES could humanely help decrease this unwanted behaviour in stabled horses. By addressing the motivation, instead of using punishment based techniques, their aim was to increase health and overall welfare in horses.

The aim of this study was to utilize the Happy Halter cranial stimulator described above in conjunction with reducing stress in horses. Police horses were used in collaboration with the Hungarian National Police Force to objectively test the calming effect of the Happy Halter. Such horses are frequently have to deal with stressful situations and have been reported to cope less successful in their stressful environment. The long term objective is to improve equine health and welfare by testing a method that focuses on managing the underlying cause of stress behaviour rather than using disciplinary methods. We hypothesized that the CES treatment would decrease stress rates in treated horses over time when compared with control and placebo horses.

2. Materials and methods

2.1. Animals

Twelve Hungarian Warmblood gelding police horses with a mean±SD age 10.3±3.8 years were selected randomly for the study, out of them six horses received Happy Halter treatment and six horses received placebo treatment. The Happy Halter device was placed on the head of the placebo horses the same way as on the treated horses, but no stimulation was given. Both groups contained three horses showing more and three showing less anxiety during everyday work. Anxiety level was judged by asking the riders about their horse's behaviour reaction to fearful situation in general. Those horses which usually disobey, suddenly stop during an exercise or show fearful reactions several times during serving in field were put into group of horse with larger anxiety level. Horses that usually do not show any fearful reactions, complete every exercise without any problem were put into group of horses with less anxiety level. There was no difference between the treatment and placebo groups regarding age (t-test, p=0.469). The horses were fed separately with concentrates and hay three times per day (4 a.m., 12 p.m. and 6 p.m.) and had water without any limit.

2.2. Experimental procedure

The horses had their normal daily routine on the day of the Happy Halter treatment: horses were cleaned at 6 a.m. and ridden for one and a half hour in all gaits (walk, trot and canter) on a riding court, and after riding horses were cleaned again and rested in the stables afterwards. To prevent the disturbing effect of early morning peak in the cortisol level described in horses (Irvine and Alexander, 2004). Happy Halter treatment took place between 1.p.m and 4 p.m. (at least one hour after the midday meal and one hour before the evening meal). The Happy Halter treatment lasted for 20 minutes with level 1 (weakest intensity, the suggested intensity level for humans) intensity.

2.3. Salivary cortisol

Saliva samples were collected with cotton-based swabs (Salivette, Sarstedt, Nümbrecht-Rommelsdorf, Germany). This process can be seen in Figure 4. Swabs were fixed in straight surgical Pean forceps and were inserted gently into the horses mouth. They were kept in until they became well soaked (approximately 1 min). Saliva was obtained by centrifugation (10 min, 1000 rpm). 1 ml saliva sample was put into clean tubes and kept frozen (-20°C) during transport. Cortisol concentration was determined by an enzyme-immunoassay technique (Schmidt et al., 2010) at the University of Veterinary Medicine Vienna. Processing of the saliva samples is demonstrated in Figure 5.

Salivary cortisol samples were collected seven times, starting 30 (1) and 15 minutes before treatment (2), at the beginning of the treatment (3), 5 minutes after the treatment (4) and also 15 (5) and 30 (6) and 60 (7) minutes later.



Figure 4 Collecting saliva samples to determine cortisol levels. Salivette tubes



Figure 5 Processing of saliva samples

2.4. Heart rate variability

Beat-to-beat (R-R) intervals were recorded by Polar® Equine heart rate monitor (RS800CX and S810i). Flexible girths containing electrodes was placed around the chest (Figure 6a). The negative electrode was placed on the left hand side above the area of the heart, the positive electrode was placed on the right hand side of the chest. The hair and skin of the horse and the electrodes were moistened with water and also with ultrasound gel to reach a better contact during the whole time of the recording.

HR measurements started 15 minutes before and lasted 60 min after Happy Halter treatment. During treatment HR monitors were switched off to prevent disturbing effects of the magnetic/electric field created by both devices.

Prior to HRV analysis, R-R interval data were pre-processed for excluding artefacts by automatic error correction (custom level in the 'Kubios HRV-analysis software 2.0', Figure 6b) and the smoothness priors method was used for detrending of the R-R series (Niskanen et al., 2004). Five minutes intervals were chosen for analysis according to the schedule of the saliva samples: 30 (1) and 15 minutes before treatment (2), at the beginning of the treatment (3), 5 minutes after the treatment (4) and also 10 (4), 15 (5), 20 (6), 25 (7), 30 (8) and 60 (9) minutes later.

To evaluate HRV the ratio of the high-frequency component (HF) of the HRV was chosen. All analyses were carried out with a threshold set at 0.05-0.15 Hz for LF and 0.15-0.4 for HF (Rietmann et al., 2004).



Figure 6a The chest strap and watch

Heart Rate Variability Analysis

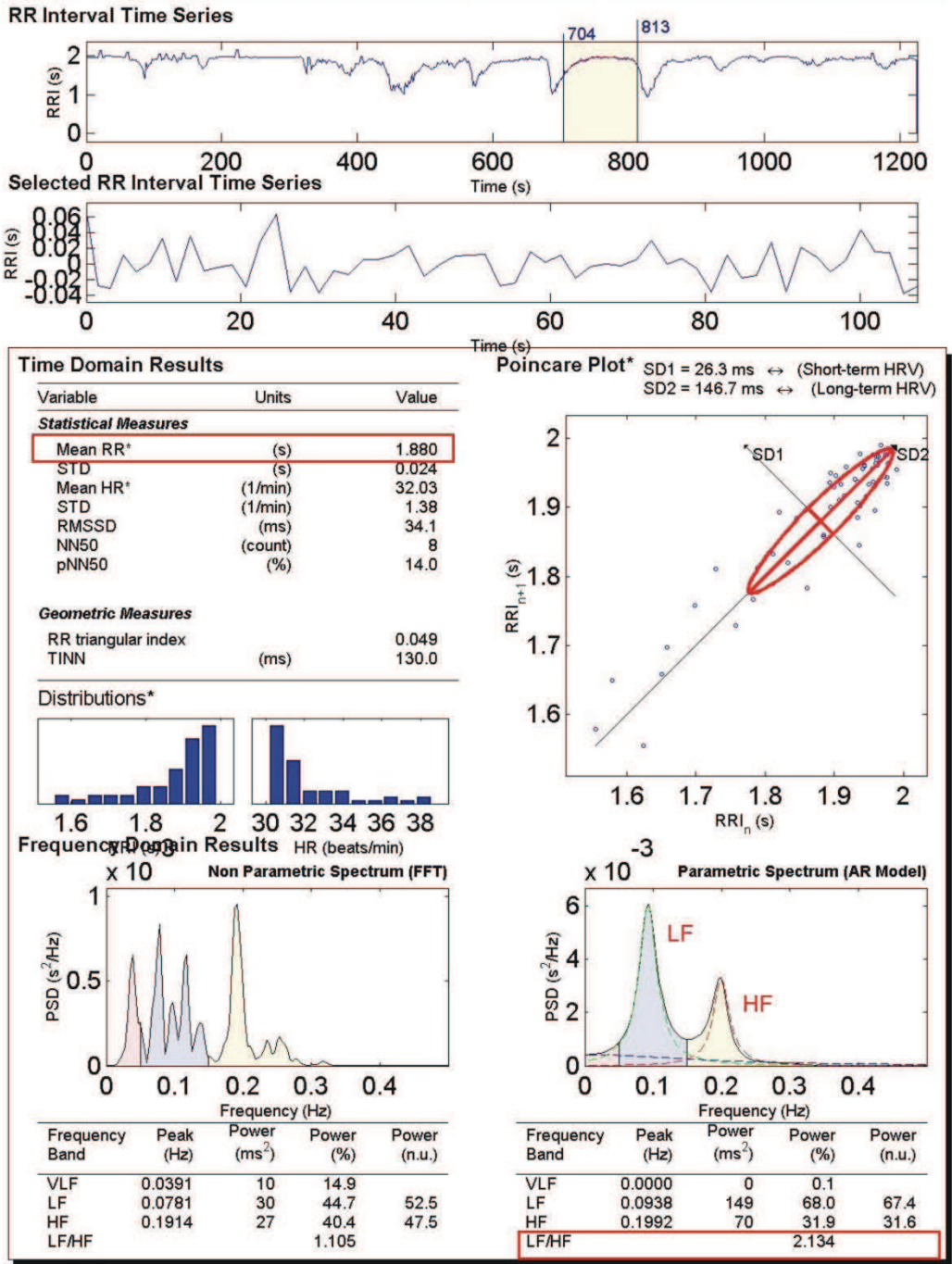


Figure 6b A typical output of the Kubios HRV-analysis software

2.5. Behaviour reaction during treatment

Behaviour reactions were judged visually, and fearful or painful reactions of the horses were noted (Figure 7).



Figure 7 Happy Halter during use (note the behavioural sign of relaxation: ears, head, penis etc.)

2.6. Statistical analysis

For statistical analysis we have divided the time periods into two categories, before treatment (time periods 1-3) and after treatment (time periods 4-7 in case of cortisol and 4-9 in case of HR/HRV parameters). For hypothesis testing, general linear mixed model was fit to the data (Pinheiro and Bates, 2000) with random effects for each horse and fixed effects for the different groups (treatment or placebo), time periods (before or after treatment) and their interaction. This model takes into account that we had several measurements from one individual. The given salivary cortisol concentration, HR, and HF served as response variables in the different models. For post-hoc tests the Tukey-Kramer method was used. For statistical analysis the R 2. 12. 2. statistical software was used. The significance level was set at $p < 0.05$.

3. Results

In one case (horse with higher anxiety level from placebo group) the HR monitor did not record the HR data at all due to technical difficulties. The measured parameters during the time periods (1-9) are visualized in Figure 1-3 and the mean (\pm standard error) values before and after treatment are listed in Table 2.

In case of salivary cortisol concentration and HR none of the response variables (the effect time period, group or their interaction) reached the significance level, meaning that before or after the Happy Halter treatment no differences were found between placebo and treated horses regarding HR or salivary cortisol concentration ($p > 0.1$ in all cases, Table 2, Figure 8 - 9).

In case of HF the time period or group effect was not significant, however, the interaction of time period and group was significant ($p = 0.031$, $F = 4.804$), meaning that the time dependence of HF of treated horses differed significantly from placebo horses. HF values in treated groups increased after the Happy Halter treatment significantly compared to before treatment values, while it did not change among placebo horses (Table 2, Figure 10). None of the horses showed any fearful or pain behavioural reaction except a small head elevation when switching on the Happy Halter device. Usually during treatment, treated horses showed sign of relaxation. Head appears relaxed and ears are flaccid. Penile protrusion is also evident, again indicating relaxation (see Figure 7).

Table 2 Mean baseline values (+ standard error) of the heart rate (HR) and heart rate variability (HF) before and after the Happy Halter treatment.

	Treated horses (N=6)		Placebo horses (N =6)	
	before treatment	after treatment	before treatment	after treatment
Salivary cortisol concentration (ng/mL)	0.293 (0.066)	0.323 (0.044)	0.227 (0.034)	0.366 (0.070)
HR (bpm)	40.703 (1.086)	40.322(0.712)	39.686(1.255)	38.704(0.874)
HF (n.u.)	44.058 (3.779)	49.829(2.505)	39.636(3.274)	37.817(1.734)

HR: heart rate, bpm: beat per minutes, HF: high frequency rate of the heart rate variability, n.u.: normalised units.

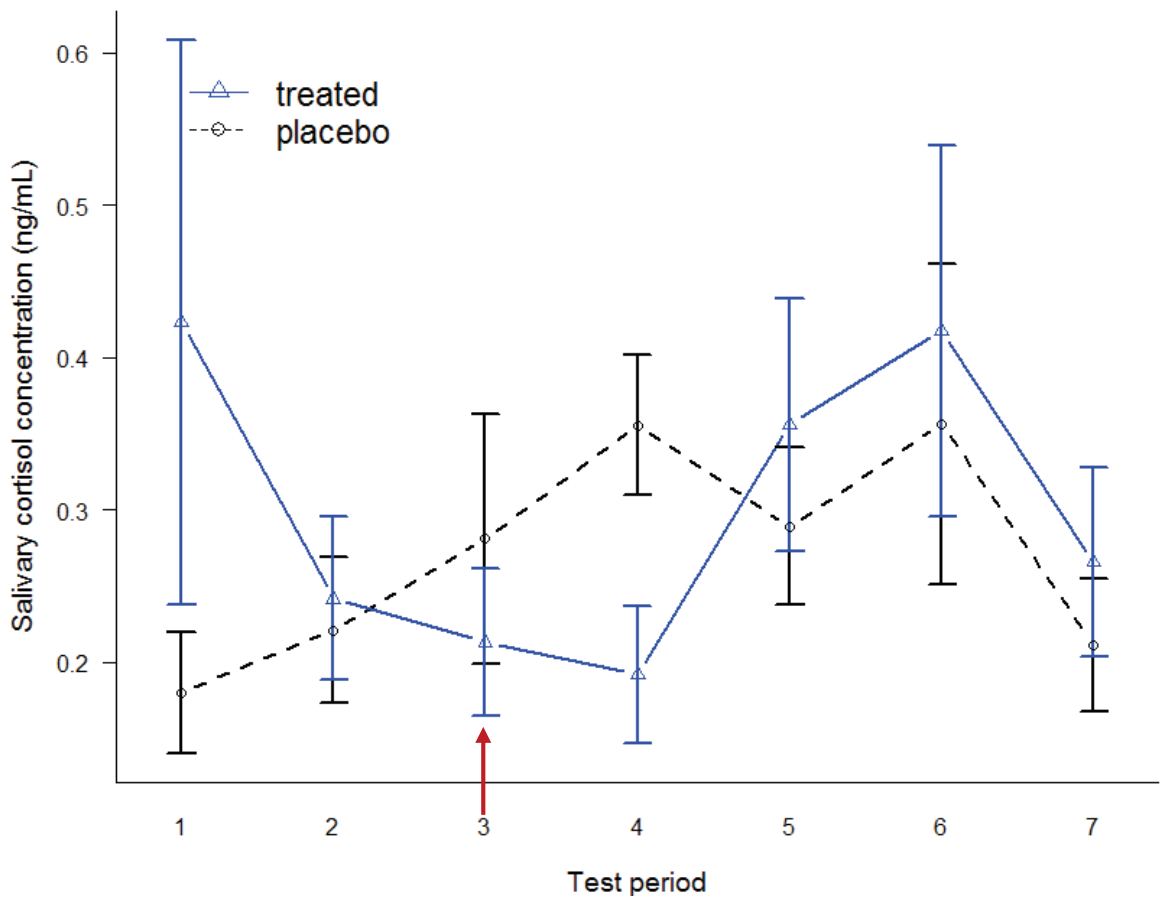


Figure 8 Mean value (arrows indicating 95% CI) of salivary cortisol concentration (ng/ml) in the placebo (black) and treatment (blue) groups during the test periods. Salivary cortisol samples were collected seven times: 30 (Test period 1) and 15 minutes before treatment (Test period 2), at the beginning of the treatment (Test period 3), 5 minutes after the treatment (Test period 4) and also 15 (Test period 5) and 30 (Test period 6) and 60 (Test period 7) minutes later. The red arrow symbolises the start of the Happy Halter treatment.

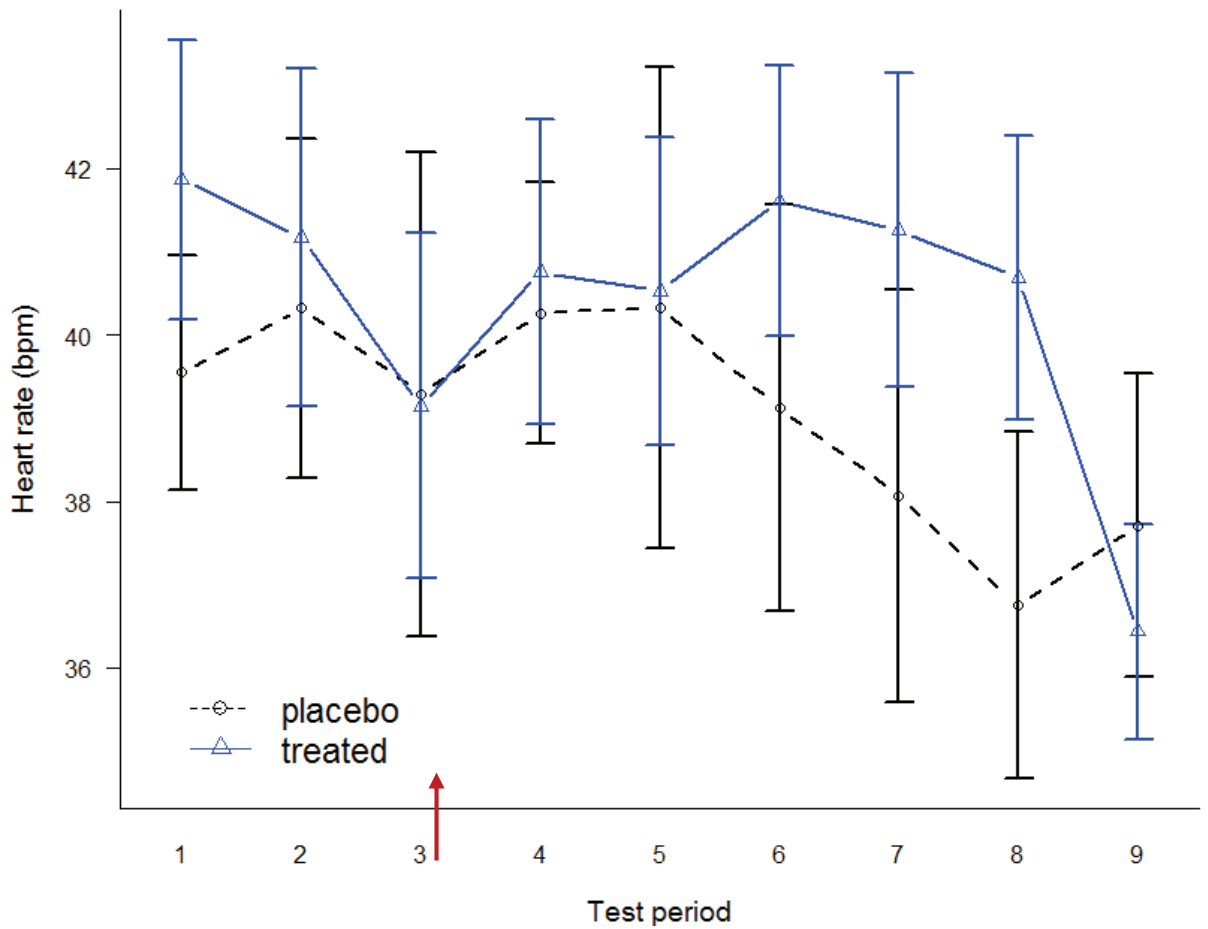


Figure 9 Mean value (arrows indicating 95% CI) of heart rate (bpm) in the placebo (black) and treatment (blue) groups during the test periods. Regarding heart rate parameters five minutes intervals were chosen for analysis according to the schedule of the saliva samples: 30 (Test period 1) and 15 minutes before treatment (Test period 2), at the beginning of the treatment (Test period 3), right after the treatment (Test period 4) and also 5 (Test period 5), 10 (Test period 6), 15 (Test period 7), 30 (Test period 8) and 60 (Test period 9) minutes later. The red arrow symbolises the start of the Happy Halter treatment.

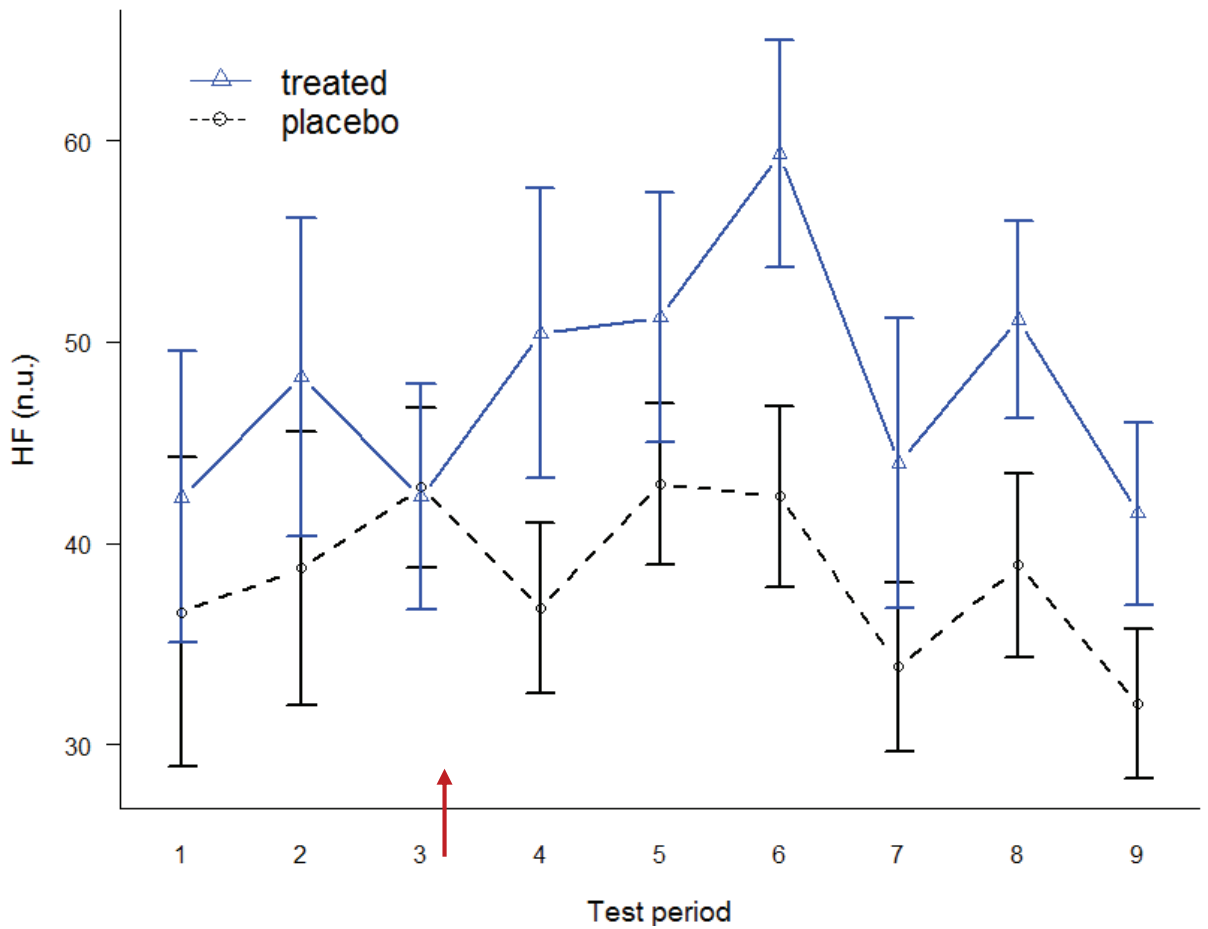


Figure 10 Mean value (arrows indicating 95% CI) of HF (n.u.) in the placebo (black) and treatment (blue) groups during the test periods. Regarding heart rate parameters five minutes intervals were chosen for analysis according to the schedule of the saliva samples: 30 (Test period 1) and 15 minutes before treatment (Test period 2), at the beginning of the treatment (Test period 3), right after the treatment (Test period 4) and also 5 (Test period 5), 10 (Test period 6), 15 (Test period 7), 30 (Test period 8) and 60 (Test period 9) minutes later. The red arrow symbolises the start of the Happy Halter treatment.

4. Discussion

Interpretation of results based on Heart Rate Variability (HRV) and Heart Rate (HR)

The lower vagal tone (HF, parasympathetic tone) of the more anxious horses implies that these horses have a lower capability to react as competently to an external stimulus as a normal horse who possess a higher vagal tone. A higher vagal tone provides greater flexibility when faced with a stressor (Porges, 1995, Bachmann et al., 2003).

In accordance with previous results, police horses seem to have a significantly lower basal parasympathetic activity compared to horses used for other purposes. Munsters et al., (2013) study showed the normal HRV of a police horse to be 33n.u. This compared to the current study under discussion (HRV ranged from 39.636 – 44.058 before treatment), shows even lower results, confirming the significantly lower parasympathetic activity of police horses. For the purpose of comparison, we could use Rietmann et al., (2004) study on HRV in warmblood animals. HRV measured in rest groups of warmblood horses was significantly higher at 50.6n.u. Bachmann et al., (2003) compared HRV of crib-biting and non crib-biting horses. Again, HF results from both the crib-biting and control groups differed significantly. HF in crib-biters (who we could hypothesize are experiencing similar stress levels to the police horses in our study) was found to be 31.6n.u., whereas HF in control horses was 49.6n.u. These results are again coinciding with the thought that a lower vagal tone (HF) is present in more anxious horses. This indicates that police horses were not in a normal state of rest during the pre-interval to our experiment. Due to the lower basal parasympathetic and higher sympathetic activity, the reactivity of the autonomic nervous system of stressed horses may not be capable to react as competent to an external stimulus as in normal horses. According to Friedmann and Thayer (1998) the vagal tone controls physiological and psychological flexibility. Thus, horses with a low basal vagal tone may be regarded as very stress sensitive and less flexible when coping with stress than control horses. The HF component relates the vagal tone, whereas the LF component is primarily a general indicator of the sympathetic activity but also a feedback influenced by the vagal activity (Bernasconi et al., 1998; Kuwahara et al., 1996). Consequently, changes in the LF/HF ratio are an indicator of alterations in the sympathetic-vagal balance. The normal horse in a state of rest is predominately in parasympathetic nervous activity (Kuwahara et al., 1996). In stressful situations, the parasympathetic will decrease in favour of an increase in sympathetic tone, enabling the animal to react appropriately (Porges, 1995).

During the first treatment an increase in parasympathetic tone (HF) could be observed in the treated group, but not in the placebo group. The HRV in the treated group went from 44.058 to 49.829. This indicated promising results, as the placebo horses showed the opposite trend and HRV dropped from 39.636 to 37.817 (Table 2).

Interpretation of results based on Salivary Cortisol

There was no differences in salivary cortisol concentration at the start of the experiment. There was no changes in salivary cortisol concentration, however its base level is very low compared to other horse studies (Bohak et al., 2013). Salivary cortisol results in Bohaks study revealed higher results than in ours. Between the times of 12a.m to 4p.m, salivary cortisol in the twenty horses that were sampled, were between 0.475 and 0.5ng/ml. This in contrast with the results obtained above. Our study revealed levels ranging from 0.227 – 0.366ng/ml (Table 2). The potential reason for this was investigated in a study carried out by Ayala et al. (2012). They studied the cortisol concentrations in relation to disease and stress in the horse. They found that cortisol concentrations rose to a lesser degree in groups with chronic diseases. A review of literature has shown that total cortisol concentrations may increase or decrease during chronic stress, which suggests that variation may be species or stimuli dependent (Mills et al., 1997). In the horse, chronic social stress, which is probably being suffered by many police horses, has been shown to reduce plasma cortisol concentrations by 50% within one week of the introduction of passive horses to an established social group (Alexander et al., 1988).

Interpretation of results based on Behavioural changes

Regarding behavioural changes in relation to the application of the Happy Halter itself, there was little or no behaviour reaction to the device. Although it must have been strange to the horses (as HR increased a bit in both placebo and treated groups), but probably not painful. This was seen as changes in HR was not significant and HF increased significantly with the treatment, indicating a relaxation or calmness of the treated horses. As shown in Figure 7, the horses displayed relaxed ears, head and penis.

Interpretation of results based on other findings published previously

Rebecca Thomas (2010), reported the importance of keeping police horses as an integral part of our police agencies. Police horses are mainly used to monitor metropolitan areas and for specialised duties like managing riots, where the horses serve to control crowds and deter violence, Police horses must be able to remain calm in challenging and unpredictable situations, such as fires and boisterous crowds. She suggested the power of using the learning theory in such horses to improve their capability to cope with the stressful situations that they encounter as part of their job. Horses are fight or flight animals, meaning that the horses fear response may be active or passive (McGreevy and McLean, 2010). Limiting stress and fear responses in police horses and keeping them calm are real challenges throughout their careers.

Many studies have also been conducted in relation to stress coping abilities of police dogs who are subjected to similar such stressful situations. A study conducted in South Africa on the early prediction of adult police dog efficiency proved very successful in selecting dogs who had the aptitude to deal with the stresses of being a police dog. The problem at the South African Police Service Dog Breeding Centre being that up to 70% of the dogs were unsuitable for training. Since the cost of training a police dog is high, it was readily understood that no time could be wasted on attempting to train a doubtful case. Also to allow a police dog on the street that was unready and unable would be extremely dangerous to the general public. Thus Slabbert and Odendaal (1999), demonstrated the importance of performing behaviour test on dogs as young as 8 weeks old in order to predict their suitability for the job. The conclusion of these reliable behavioural tests save unnecessary training and costs on unsuccessful dogs. Wilkes (1997) said that developing a test to predict a puppy's future behaviour was obviously a desirable goal. The use of the specific puppy tests for police dogs provides a reliable tool which enables dog handlers to distinguish between puppies that would succeed as efficient police dogs and those who wouldn't. I think the same approach could be taken in relation to police horses. Aptitude tests on their ability to cope with stressful situations could avoid these possible stress and fear induced reactions from occurring.

A similar study was conducted in the Netherlands by Munsters et al (2013). The aim of the study was to evaluate stress and to assess future capability of twelve police horses; six experienced and six non-experienced. The study suggested that stress and therefore the physiological and psychological state of the horse is not always reflected in its behaviour. They suggested that experience was not a key factor in how police horses handle stress, hence relating back to the importance of aptitude tests in the process of preselecting horses for this type of work. They explained how quantifying and evaluating police work is essential to avoid behavioural problems, minimise wastage and maximise welfare of police horses. In this study, in order to distinguish between an increase in HR caused by physical activity and an increase in HR due to emotional reactivity (Baldock and Sibly, 1990; Visser et al., 2002), only the HRs of horses during identical physical activity were compared. The HR was consistently low during all physical activities of the night patrols, supporting the suggestion that habituation leads to a substantial decrease in stress (McGreevy and McLean, 2010). The study conducted by Munsters concluded that further research into horse – rider interaction in relation to occurrence of behavioural problems seemed warranted. The workload of police horses is low compared to that of sport horses. The use of behavioural score to assess stress in ridden police horses is only useful if the situation is challenging enough to provoke a more than moderate behavioural response. If the situation is less challenging, HR seems to be a more valuable tool.

Another study conducted by Munsters et al., (2012), investigated the influence of horse – rider matching using some of the same parameters as this experiment. Evaluation of the horse's HR can facilitate in the assessment of the horse – rider relationship with attendant benefits to both. In this study, all non-compliant horses were deemed as 'mismatches' with all three of their test riders. It is therefore likely that these mismatches reflected conflict on the part of the animal rather than on the horse – rider interaction. In the context of horses challenged by object tests, HR can be used as a measure of the horses' temperament (Visser et al., 2002). This study indicated that measuring horse HR may prove to be a valuable tool in assessing horse – rider matching and the degree of compliance that may in turn contribute to the welfare of the ridden animal. This strategy could be adopted with police horses and their riders. If the partnership between horse and rider was to be more compatible, perhaps the animals would cope better in the stressful situations in which they find themselves. Further research into the horse – rider interaction in relation to the occurrence of behavioural problems in police horses seems warranted.

Bergamasco et al, (2010) measured HRV in shelter dogs. This study focussed on the relationship between HRV and behaviour. Twenty dogs were behaviourally and clinically pre tested and then matched in two homogenous groups. Ten dogs were submitted to a human interaction program while the other ten served as controls. Both sympathetic arousal and vagal tone alterations resulting from confrontational environmental challenges can influence cardiovascular function and subsequent HR. In farm animals and horse cardiac changes are used as a marker of psycho-physiological stress (von Borell et al., 2007), mental stress (Rietmann et al., 2004), and temperamental traits (Visser et al., 2002). In this study HRV data showed very little correlation with behavioural data, but post-test cortisol levels did however correlate with behavioural data. These data suggest that human interaction supplement sessions have a positive effect upon the behaviour and they could affect the physiological indicators of animal welfare.

Despite the undisputed beneficial effects found in numerous studies involving human subjects, there has been little research concerning the clinical effects of CES in animals. Of the little research that has been completed, the objective has tended to be to investigate any potentially hazardous side effects. Such studies have also tried to establish the mechanism through which CES exerts an impact. Some conclusions from these studies however implicate that there is a potential for CES to elicit a beneficial clinical effect among animals.

In one study, comparing the neurophysiological, cardiorespiratory and physiological gastric secretory response of both squirrel monkeys and humans to electrical currents, both the EMG and ECG results suggested variations consistent with relaxation in the primates while respiration and EKG remained stable in both species. Total gastric acid output was also found to be significantly reduced by CES (Reigel et al., 1970). Another study has revealed that CES is an effective treatment for rats with induced Alcohol Withdrawal Syndrome and significantly increased plasma beta-endorphin concentrations (Krupisky et al., 1991). Importantly, a study conducted by Stinus et al. (1990) concluded that CES could be administered to rats for several consecutive days without inducing abnormal behaviour or adverse reactions.

Therefore it is possible that CES may be an effective treatment for animals with equivalent clinical problems manifested by comparable physiological or behavioural changes. If effective, CES could provide beneficial treatment of certain behaviour problems and improve the welfare of the animals concerned. In horses this might include during transport or pre-feeding anxiety. CES could even potentially be used to enhance the effectiveness of other treatments and therapies whether they be of a pharmaceutical, behavioural or holistic nature.

In 2010, Nancy Clarke et al., performed a study evaluation of the potential efficacy of the Alpha-Stim device in the horse. The study was focused on whether the typical behaviour of the horse changes and, if so, how. Horses were treated for a twenty minute period and thereafter, their behaviour was noted. The study concluded that effects were seen on the behaviours of greatest relevance to assessing anxious arousal in the given circumstances, namely time spent alert and dozing, and a number of other parameters consistent with relaxation. Specifically, there was no significant increase in any parameter associated with excitement nor is there any evidence that CES (as used in this study) has any detrimental effects on the horse's wellbeing. A number of parameters, which may also be indication of relaxation, were not significantly affected by the Alpha-Stim SCS but this could be explained by their rarity. The results further suggested that if CES is responsible for the changes in behaviour, then its effects extend beyond the period of immediate stimulation. Further work would be needed to evaluate this potential therapy further and perhaps the most logical next stage is to conduct some form of blind, placebo controlled study on putatively anxious behaviours (Clarke, N., et al., 2010)

5. Conclusions

Our preliminary results suggest that Cranial Electrotherapy Stimulation for treating stress-related disorders in horses may be promising. However, further studies involving treatment of longer period including more horses with extreme anxiety levels and perhaps treatment with greater intensity may be needed to support or reject the effectiveness of Happy Halter treatment in horses. A possible practical limitation with the continuation of such studies in real life situations does arise. Unfortunately not every rider has 20-30 min/day to undertake the Happy Halter procedure. It is a tedious task and most riders may only visit their horses 2-3 times/week and they are then relying on other staff members to carry out the task and to carry it out properly. However, one may consider behaviour therapy with or without the happy halter treatment as well, as previous studies indicated. Aptitude tests on police horses ability to cope with stressful situations could eliminate the high stress levels of these horses to begin with.

6. Summary

The Hungarian National Police Force comprises of a section on horseback. Their work involves having to patrol varied terrain, as well as providing riot control at football matches. They also carry out the tasks of the Hungarian Subsidiary National Horse Ceremonial Unit. Changeable conditions and competence in a wide range of diverse tasks, see these horses endure much higher stress levels than horses for recreational use only.

Recent studies describe an effective and drug-free alternative in the treatment of human anxiety disorders, called cranial electrotherapy stimulation or CES. CES is a non-invasive therapeutic device that has recently been used in the treatment of human depression, anxiety and sleeping disorders. Such cranial stimulator exists also for horses (Happy Halter, Fisher Wallace Laboratories).

Twelve Hungarian Warmblood gelding police horses with a mean \pm SD age 10.3 \pm 3.8 years were selected randomly for the study, out of them six horses received Happy Halter treatment and six horses received placebo treatment. The Happy Halter device was placed on the head of the placebo horses the same way as on the treated horses, but no stimulation was given. The Happy Halter treatment lasted for 20 minutes with level 1 intensity (weakest intensity, the suggested intensity level for humans). Saliva samples were collected with cotton-based swabs and cortisol concentration was determined by an enzyme-immunoassay technique. Beat-to-beat (R-R) intervals were recorded by Polar® Equine heart rate monitor. Behaviour reactions were judged visually, and fearful or painful reactions of the horses were noted. For statistical analysis we have divided the time periods into two categories, before treatment (time periods 1-3) and after treatment.

Before or after the Happy Halter treatment no differences were found between placebo and treated horses regarding HR or salivary cortisol concentration. During the first treatment a significant increase in parasympathetic tone (HF) could be observed in the treated group, but not in the placebo group. The lower vagal tone (HF, parasympathetic tone) of the more anxious horses implies that these horses have a lower capability to react as competently to an external stimulus as a normal horse who possess a higher vagal tone. A higher vagal tone provides greater flexibility when faced with a stressor. Regarding behavioural changes in relation to the application of the Happy Halter itself, there was little or no behaviour reaction to the device.

Although it must have been strange to the horses (as HR increased a bit in both placebo and treated groups), but probably not painful. Our preliminary results suggest that Cranial Electrotherapy Stimulation for treating stress-related disorders in horses may be promising. Further studies involving more horses with extreme anxiety levels and perhaps treatment with greater intensity and for longer period may be needed to support or reject the effectiveness of Happy Halter treatment in horses

7. References

- Alexander, S.L., Irvine, C.H.G., Livesey, J.H., Donald, R.A., 1988. Effects of isolation stress on concentrations of arginine vasopressin, alpha-melanocyte-stimulating hormone and ACTH in the pituitary venous effluent of the normal horse. *Journal of Endocrinology* 116, 325 – 334.
- Archer, D.C., Pinchbeck, G.K., French, N.P., Proudman, C.J., 2008. Risk factors for epiploic foramen entrapment colic: an international study. *Equine Vet. J.* 40, 224-230.
- Ayala, I., Martos, N.F., Silvan, G., Gutierrez-Panizo, C., Clavel, J.G., Illera, J.C., 2012. Cortisol, adrenocorticotrophic hormone, serotonin, adrenaline and noradrenaline serum concentrations in relation to disease and stress in the horse. *Research in Veterinary Science*, 93 (1) 103–107.
- Bachmann, I., Bernasconi, P., Hermann, R, Weishaupt, M.A., Stauffacher, M., 2003. Behavioural and physiological responses to an acute stressor in crib-biting and control horses. *Appl. Anim. Behav. Sci.* 82, 297-311.
- Baldock, N.M., Sibly, R.M., 1990. Effects of handling and transportation on the heart rate and behaviour of sheep. *Appl. Anim. Behav. Sci.* 28, 15 – 39.
- Bergamasco, L., Osella, M.C., Savarino, P., Larosa, G., Ozella, L., 2010. Heart rate variability and salivary cortisol assessment in shelter dog: Human – animal interaction effects. *Appl. Anim. Behav. Sci.*, 125, 56 – 68.
- Berger, J., San Jose, N., Holcomb, K., Toth, B., Spier, S., 2010. The Effect of Cranial Electrotherapy Stimulation on Cribbing Behaviour in Horses.
- Bernasconi, P., Messmar, E., Bernasconi, A., Tholen, A., 1998. Assessment of the sympathico-vagal interaction in central serous chorioretinopathy measured by power spectrum analysis of heart rate variability. *Graefe's Arch. Clin. Ecp. Ophthalmol.* 236, 571-576.

Bohak, Z., Szabo, F., Beckers, J.F., Melo de Sousa, N., Kutasi, O., Nagy, K., Szenci, O. 2013. Monitoring the circadian rhythm of serum and salivary concentrations in the horse. *Domest. Anim. Endocrinol.* 45, 38 – 42.

Bystritsky, A., Kerwin, L., Feusner, J., 2008. A pilot study of cranial electrotherapy stimulation for generalized anxiety disorder. *Journal of Clinical Psychiatry* 69, 412–417.

Cacioppo, J., Malarkey, W., Kiecolt – Glasser, J., Unchino, B., Sgoutas – Emch, S., Sheridan, J., Berntson, G., Glasser, R., 1995. Heterogeneity in neuroendocrine and immune responses to brief psychological stressors as a function of autonomic cardiac activation. *Psychosom. Med.* 57, 154 – 164.

Clarke, N., Mills, D., Marchant, J., 2010. Evaluation of the potential efficacy of the Alpha-Stim SCS in the Horse.

Cooper, M., 2001. Police Horses are Diminished in Number, but Not Presence. *New York Times*.

Datta, A., Dmochowski, J.P., Guleyupoglu, B., Bikson, M., Fregni, F., 2012. Cranial electrotherapy stimulation and transcranial pulsed current stimulation: A computer based high-resolution modelling study. *Neuroimage*.

DeFelice, E.A., 1997. Cranial electrotherapy stimulation (CES) in the treatment of anxiety and other stress-related disorders: A review of controlled clinical trials. *Stress Medicine*, 13(1):31-42.

Feighner, J.P., Brown, S.L., Olivier, J.E., 1973. Electrosleep therapy. A controlled double blind study. *J. Nerv. Ment. Dis.* 157 (2), 121–128.

Friedmann, B.H., Thayer, J.F., 1998. Anxiety and autonomic flexibility: a cardiovascular approach. *Biol. Psychol.* 47, 243 – 263.

Gunnar, M., Porter, F., Wolf, C., Rigatuso, J., Larson, M., 1995. Neonatal stress reactivity: predictions to later emotional temperament. *Child Dev.* 66, 1 – 13.

Happy Halter [http:// http://www.happyhalter.com/](http://www.happyhalter.com/) Downloaded: 1/11/13.

Irvine, C.H.G., Alexander, S.L., 1994. Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domest. Anim. Endocrinol.* 11 (2), 227 – 238.

Kahn, C.M. (ed.) 2010: *The Merck veterinary manual*. Whitehouse Station, N.J., USA. Merck & Co., Inc. p.1432-1433

Kirsch D, 2002: *The Science Behind Cranial Electrotherapy Stimulation*. Edmonton, Alberta, Canada: Medical Scope Publishing Corporation.

Kirsch DL, 1996. Cranial electrotherapy stimulation: a safe and effective treatment for anxiety. *Med. Scope Mon. (Alberta)*; 3:1–26.

Kirsch DL, Gilula MF. 2007: CES in the treatment of depression, part 2. *Practical Pain Management*, 6:32-40.

Kirsch, D., 1999. Cranial Electrotherapy Stimulation (CES): An Overview of its Application for the treatment of Pain, Anxiety, Depression, Insomnia and Other Stress-Related Disorders. Tenth International Montreux Congress on Stress, Montreux Switzerland, Wednesday, March 3, 1999.

Kirsch, D., 1999. Postmarketing survey of Alpha-Stim CES patients. In Kirsch, D. *The Science Behind CES*, Medical Scope Publications, Edmonton.

Klawansky, S., 1995. Meta-Analysis of Randomized Controlled Trials of Cranial Electrostimulation Efficacy in Treating Selected Psychological and Physiological Conditions. *Journal of Nervous & Mental Disease* 183 (7), 478–484.

Krishnan V, Nestler EJ. 2008: The molecular neurobiology of depression. *Nature*; 455(16):894-902.

Krupisky, E.M., Katznelson, Y.S., Lebedev, V.P., Flood, N.V., Patterson, M.A., Lipton, J.M., Kozlowski, G.P., 1991. Transcranial electrostimulation (TES) of brain opioid structures (BOS):

experimental treatment of alcohol withdrawal syndrome (AWS) and clinical application. Presented at the Society for Neuroscience Annual Meeting, New Orleans, November 10-15, 1991.

Kuwahara, M., Hashimoto, S., Ishii, K., Yagi, Y., Hada, T., Hiraga, A., Kai, M., Kubo, K., Oki, H., Tsubone, H., Surgano, S., 1996. Assessment of autonomic nervous function by power spectrum analysis of heart rate variability in the horse. *J. Auton. Nerv. Syst.* 60, 43-48.

Leal, B.B., Alves, G.E.E., Douglas, R.H., Gringel, B., Young, R.J., Haddad, J.P.A., Viana, W.S., Faleiros, R.R., 2011. Cortisol Circadian Rhythm Ratio: A Simple Method to Detect Stressed Horses at Higher Risk of Colic? *Journal of Equine Veterinary Science*, 31. 188 – 190.

Levitt, E.A., James, N.M., Flavell, P., 1975. A clinical trial of electrosleep therapy with a psychiatric inpatient sample. *Aust N Z J Psychiatry* 9 (4), 287–290. doi:10.3109/00048677509159864.

Lichtbroun, A.S., Raicer, M.M.C. and Smith, R.B., 2001. The treatment of fibromyalgia with cranial electrotherapy stimulation. *Journal of Clinical Rheumatology*. 7(2):72-78.

Mason, G., Rushen, J. (Eds.) 2006: *Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare*, second ed. CAB International, Wallington OXON.

Mills, P.C., Kramer, H., Auer, D.E., 1997. Stress response to chronic inflammation in the horse. *Equine Veterinary Journal* 6, 483 – 486.

Moeller, B.A., McCall, C.A., Silverman, S.J., McElhenny, W.H., 2008. Estimation of saliva production in crib biting and normal horses. *J. Equine.Vet.Science*.28 (20) 85-90.

Mostl, E., Palme, R., 2002. Hormones as indicators of stress. *Domest. Anim. Endocrinol.* 23, 67 – 71.

Munsters, C., Visser, K., van den Broek, J., Sloet van Oldruitenborgh-Osterbaan, M.M., 2013. Quantifying stress in experienced and inexperienced mounted police horses, using heart rate,

heart rate variability, behaviour score and suitability score. *Journal of Veterinary Behaviour: Clinical Applications and Research*, 8(2), e16-e17.

Munsters, C.C.B.M., Visser, K.E.K., van den Broek, J., Sloet van Oldruitenborgh Oosterbaan, Marianne, M., 2012. The influence of challenging objects and horse-rider mismatching on heart rate, heart rate variability, and behavioural score in riding horses. *The Veterinary Journal* 192, 75 – 80.

Nicol, C.J., Waters, A.J., Wilson, A.D., Davidson, H.P.D., Harris, P.A., 2002. Study of crib-biting and gastric inflammation and ulceration in young horses. *Vet. Rec.* 151, 658-662.

Niskanen, J.P., Tarvainen, M.P., O Ranta-aho, P., Karjalainen, P.A., 2004. Software for advanced HRV analysis. *Computer Methods and Programs in Biomedicine* Volume 76, (1)73–81

Overcash, S., Siebenthal, A., 1989. The effects of cranial electrotherapy stimulation and multisensory cognitive therapy on personality and anxiety levels of substance abuse patients. *American Journal of Electromedicine*, 6(2), 105-111.

Overcash, S.J.A., 1999. A retrospective study to determine the effect of cranial electrotherapy stimulation (CES) on patients suffering from anxiety disorders. *American Journal of Electromedicine*.

Pinheiro, J., Bates, D., 2000, *Mixed-Effects Models in S and S-PLUS*. 1st ed.

Porges, S. W., 1995. Cardiac Vagal tone: a physiological index of stress. *Neurosci. Biobehav. Rev.* 19 (2), 225-233.

Reigel, D.H., Dallmann, D.E., Christman, N.T., Hamilton, L.H., Zuperku, E.J., Henschel, E.O., Larson, S.J., Sances Jr, A., 1970. Physiological effects of electrotherapeutic currents in the primate and man. Wadeneder, F.M. & St. Schuuy (Eds.), *Electrotherapeutic Sleep and Electroanesthesia*. Vol.II, Amsterdam, Excerpta Medica, pp. 158-165.

Rietmann, T.R., Stuart, E.A.E., Bernasconi, P., Stauffacher, M., Auer, J.A., Weishaupt, M.A., 2004. Assessment of mental stress in warmblood horses: heart rate variability in comparison to heart rate and selected behavioural parameters. *Appl. Anim. Behav. Sci.* 88, 121 – 136.

Schmidt, A., Möstl, E., Wehnert, C., Aurich, J., Müller, J., Aurich, C., 2010. Cortisol release and heart rate variability in horses during road transport. *Horm. Behav.* 57, 209–215.

Schofield, W.L., Mulville, J.P., 1998. Assessment of the modified Forssell's procedure for the treatment of oral stereotypies in 10 horses. *Vet. Rec.* 142, 572-575.

Schroeder MJ, Barr RE. 2001: Quantitative analysis of the electroencephalogram during cranial electrotherapy stimulation. *Clinical Neurophysiology.* 12:2075-83.

Seely, R., Stephens, T., Tate, P., 1989. *Anatomy and Physiology, Times Mirror: St. Louis*, 449.

Slabbert, J.M., Odendaal, J.S.J., 1999. Early prediction of adult police dog efficiency – a longitudinal study. *Appl. Anim. Behav. Science* 64, 269-288.

Smith RB. 2007. *Cranial Electrotherapy Stimulation: Its first fifty years, plus three: a monograph.* Oklahoma City: Tate Publishing & Enterprises, LLC.

Smith, R.B., Shiromoto, F.N., 1992. The use of cranial electrotherapy stimulation to block fear perception in phobic patients. *Journal of Current Therapeutic Research*, 51(2), 249-253.

Sterman MB. 2007: Physiological origins and functional correlates of EEG rhythmic activities: implications for self-regulation. *Biofeedback and Self-Regulation* 1996;21(1):3-33.

Stinus, L., Auriacombe, M., Tignol, J., Limoge, A., LeMoal, M., 1990. Transcranial electrical stimulation with high frequency intermittent current (Limoge's) potentiates opiate-induced analgesia: blind studies. *Pain*, 42(3), 351-363.

Thomas, R., 2010. Predictability in an unpredictable environment: Training the police horse using learning theory. *Journal of Veterinary Behaviour: Clinical Applications and Research.* Vol.5 (4) p 218.

Turner, A.S., White, N., Ismay, J., 1984. Modified Forssell's operation for cribbing in the horse. *J. Am. Vet. Med. Assoc.* 184, 309.

Visser, E.K., Van Reenen, C.G., Van der Werf, J.T.N., Schilder, M.B.H., Knaap, J.H., Barneveld, A., Blokhuis, H.J., 2002. Heart rate and heart rate variability during a novel object test and a handling test in young horses. *Physiology and Behaviour* 76, 289 – 296.

von Burell, E., Langbein, J., Despres, G., Hanean, S., Letterier, C., Marchand-Forde, J., Marchand-Forde, R., Minero, M., Mohr, E., Prunier, A., Valance, D., Vessier, I., 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals – A review. *Physiology and Behaviour* 92, 293 – 316.

Walters, A.J., Nicol, C.J., French, N.P., 2002. Factors influencing the development of stereotypic and redirected behaviour in young horses: the findings of a four year prospective epidemiological study. *Equine Vet. J.* 34, 572-579.

Weingarten, E., 1981. The effect of cerebral electrostimulation on the frontalis electromyogram. *Biological Psychiatry* 160, 61.

Weiss, M.F., 1973. The treatment of insomnia through use of electrosleep: an EEG study. *Journal of Nervous and Mental Disease.* 157(2):108 - 120.

Wilkes, G., 1997. On good behaviour: Testing's a limited tool. In: *Proceedings of the first International Conference on Veterinary Behavioural Medicine*, Birmingham, UK.

Winick, R.L., 1999. Cranial electrotherapy stimulation (CES): A safe and effective low cost means of anxiety control in dental practice. *General Dentistry*, 47(1).

Zaghi S, Acar M, Hultgren B, Boggio PS, Fregni F. 2010. Noninvasive brain stimulation with low-intensity electrical currents: putative mechanisms of action for direct and alternating current stimulation. *Neuroscientist.* 16: 285–307.

Zimmerman, S. and Lerner F., 1989. Biofeedback and Electromedicine, Reduce the Cycle of Pain-Spasm-Pain in Low-Back Patients. *American Journal Of Electro medicine*, 108.

8. Acknowledgements

I would like to extend my deepest gratitude to Dr. Krisztina Nagy, as my supervisor, for her help and guidance throughout my thesis writing. I would also like to thank for Dr. Rezső Kovács, Dr. Regina Erber and Dr. Krisztina Nagy for their contribution regarding collecting and analysing the data. Special thanks for József Farkas, Dr. Mónika Garamszegi and the Hungarian National Police Force for allowing to participate their horses in the study. This study was supported by the Hungarian International Relations Committee (NKB) 2010/15935 grant.