Evaluation of the potential efficacy of the Alpha-Stim SCS in the Horse

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Introduction

Cranial electrotherapy stimulation (CES) incorporates the action of applying pulsed electrical currents at a low level to the body. Successive researchers have documented the beneficial effects of such treatment in the alleviation of various physical ailments, pain, diseases, psychiatric and psychological disorders. There are several proposed mechanisms of action through which CES is believed to exert such a beneficial effect. Various researchers have concluded that these effects are essentially mediated through a direct action at several brain areas including the limbic system, the hypothalamus and the reticular activating system (Brotman, 1989; Gibson, 1987 and Madden 1987, all cited in Kirsch, 1999). Following extensive research on the changes in the electroencephalogram (EEG) spectrum, Heffernan (1996) concluded that when an individual is experiencing pain or stress, their EEG profiles develop irregularities in spectral curves. In turn, the application of extremely low frequency (ELF) electrical fields facilitates smoothing and slowing of the EEG and is significantly associated with anxiolytic relaxation responses (Braverman, 1990; Cox, 1975; Krupitsky, 1991; McKensoe, 1976 and Sing, 1971, all cited in Kirsch, 1999) and pain reduction (Heffernan, 1997). However it is also argued that CES affects the auditory nerve, thus explaining the dizziness or nausea that some subjects report during treatment (Kirsch, 1999). Additionally, during the treatment patients have commonly reported synchronised feelings of relaxation and alertness, and sometimes a stinging feeling may be felt at the electrodes (Kirsch, 1999). Other physiological effects that have been found to accompany CES include the lowering of electromyograms (Forster, 1963; Gibson, 1987; Heffernan, 1995; Overcash, 1989 and Voris, 1995 all cited in Kirsch, 1999) and increased peripheral temperature (Brotman, 1989 and Heffernan, 1995 both cited in Kirsch, 1999). Heffernan (1995) studied the effect of CES on multiple stress measures and found there to be reductions in terms of blood pressure, pulse, respiration and heart rate. Importantly, Kirsch (1999) has emphasised that different subjects may experience variable lengths of beneficial effects from CES at differing times either during or after treatment. Also different patients may require the CES treatment to be administered for different lengths of time or receive the treatment several times in order to begin to gain the benefits (Kirsch, 1999). It is therefore clear that there is considerable individual variation in the strength, duration and manifestation of the patient's responses to CES.

Recently a "cranial electrotherapy stimulator" unit, called the Alpha-Stim, 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 elsewhare (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.

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 β-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.

Therefore the objective of this study was to examine the potential of the Alpha-Stim (Cranial Electrotherapy Stimulation) to reduce anxiety in terms of behavioural and physiological symptoms in horses. This study will focus on whether the typical behaviour of the horse changes and, if so, how. However, it is not possible to make definitive inferences about any behavioural changes on the basis of this study alone. It is intended that, as a preliminary test, this study should lay the foundations from which further research may stem to examine the role of CES as a potential future treatment that can be used to improve animal welfare. Despite there being no grounds whatever for suspecting that any procedures within this study would elicit any adverse effects, precautions must be taken as this study is a non-licensed procedure. This involved the completion of a pilot study, which functioned to establish that the CES would not pose any threat to the welfare of the animals, while also helping to determine what level of treatment would be appropriate to use in the controlled study.

In accordance with the findings of Heffernan (1996) it was possible to hypothesise that CES would reduce the heart rate of the horses during, and possibly after, the application of the treatment. It was also hypothesised that if CES reduced anxiety levels of the horses during, and possibly after, the treatment, then the time spent standing alert with the lower lip tense would decrease and the time spent standing dozing would increase. Behavioural indications of the application of treatment might include ear flicking and wobbling of the head.

Materials and Methods

Pilot Study

Experimental Subjects

Six horses, one mare and five geldings, based at De Montfort University Equestrian Centre, Caythorpe, Lincolnshire, UK, were used in this trial (see Table 1). Each horse had the hair above the point of articulation of the lower jaw on both sides of the head trimmed in order to facilitate efficient conduction of a current during the treatment. None of these subjects were used in the later experiments, in order to avoid the effect of any possible interactions associated with multiple application of the treatment.

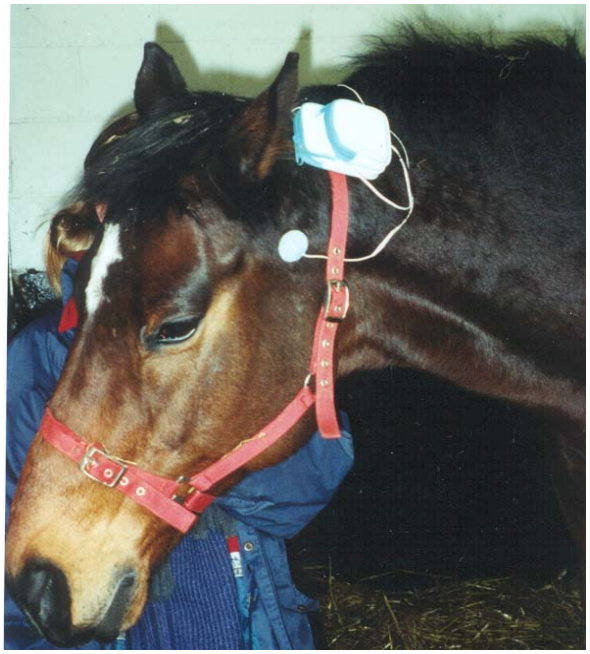
Table 1: The six horses used in the pilot experiment, with their age in years, their sex, height in hands and colour.

Horse	Age (Years)	Sex	Height (HH)	Colour
Bounce	16	Gelding	16.0	Chesnut
Duet	8	Mare	15.2	Bay
Folly	16	Gelding	16.0	Bay
Othello	14	Gelding	16.2	Bay
Red Fred	12	Gelding	16.2	Chestnut
Sausage	12	Gelding	15.2	Bay

Methods

A trial involving the initial application of a non-active Alpha-Stim unit to the headcollar of the horse was conducted first (see Figure 1). This was carried out in order to assess the effect of the use of the Alpha-Stim on individual horses at each potential treatment level without jeopardising the welfare of the test subjects. A standard position above the point of articulation of the lower jaw, just below the ear, was soaked with saline solution prior to electrode application. The behaviour was then assessed before making a decision to activate the device. This was increased incrementally following a period of observation and assessment. Each horse undertook the trial in its own stable, thus maintaining familiar surroundings. It was decided *a priori*, that if there were any signs of aversion their involvement in the study terminated immediately.

Figure 1: Alpha-Stim SCS Unit attached to the headcollar of a test subject



Each test began with the experimenter waiting for signs of acceptance of the apparatus. Two minutes after this, the experimenter re-entered the stable, approached the horse and turned the Alpha-Stim device on to level ‘0’. After leaving the stable, the experimenter used a detailed

ethogram (see Appendix 1) to record behavioural measures for the following 20 minutes, during Alpha-Stim treatment. Duration behaviours such as locomotion, head motion, oral actions and ear positions were instantaneously recorded at 15 second intervals. Event behaviours such as ear flicking, licking and chewing, vocalising, pawing, stall-kicking, wobbling of the head, shaking of the head and elimination were recorded using a one-zero cycle of 15 seconds.

At the end of the 20-minute period the experimenter stopped recording the behavioural measures, re-entered the stable, turned the Alpha-Stim device off, removed the apparatus and left the stable. All six horses proceeded through one trial each in one day and all of the horses received the same treatment on the first day.

It was decided that if a subject had not appeared to be completely habituated to the apparatus or treatment within the twenty minutes of exposure then the procedure would be repeated at the same during the next exposure. For all other subjects, the same procedure was carried out with the treatment level increased by one unit. Therefore on the second day, subjects would receive treatment at level ‘1’ and on the third day treatment at level ‘2’. When horses appeared to be displaying a clear response to the treatment, no further trials at a higher treatment level were completed.

Results

At treatment level 2 there appeared to be a clear effect on at least one behaviour, wobbling head. A general linear model was used to assess the effects of individual horse identity and treatment on each of the behaviours recorded. This confirmed the significant effect of treatment on wobbling head ($F=12.85$, $P=0.02$). No other significant treatment effect on behaviour was found at $P<0.05$, however, the value for quivering lower lip approached significance ($F=3.58$, $P=0.067$) and so should be considered potentially important for the purposes of a pilot study. Individual horse identity had a significant effect on the tendency to walk backwards ($F=4.060$, $P=0.030$) and the number of records of the left ear forward ($F=3.33$, $P=0.050$). The value for the right ear forward also approached significance ($F=2.73$, $P=0.083$). these latter values may indicate different sensitivities of the horses to arousal as ear pricking is an early response to novelty.

Conclusion

On the basis of these results it was decided that treatment level 2 should be used in the main study.

Controlled Experiment

Experimental Subjects

Eight horses (two fillies, three mares and three geldings), with varied backgrounds, based at the De Montfort University Equestrian Centre and Field Station at Caythorpe, Lincolnshire, UK were used (see Table 2).

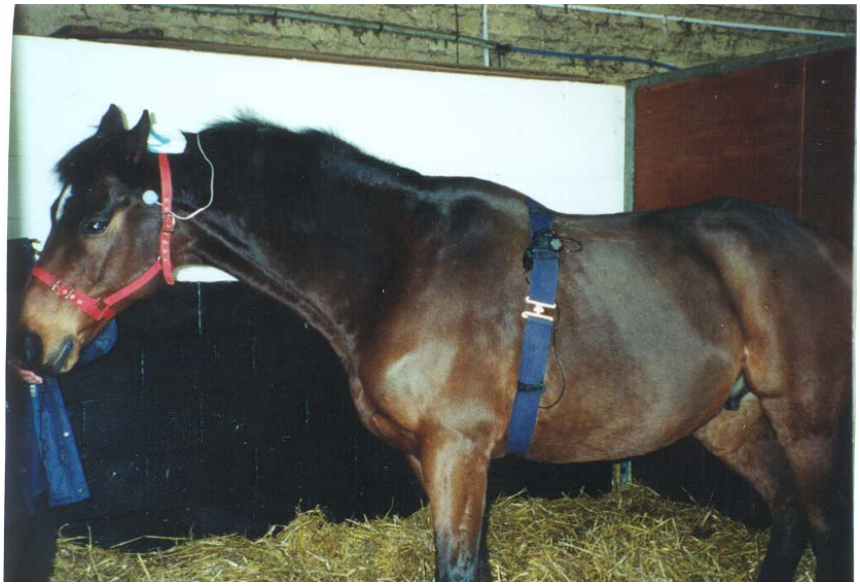
Table 2: The eight horses used in the control experiment, with their age in years, their sex, height in hands, colour, breed and working history.

Horse	Age (Years)	Sex	Height (HH)	Colour	Breed	History
Bassy	11	Gelding	16.3	Bay	Thoroughbred	Flat-racing
Bee	10	Mare	15.3	Chestnut	-	Riding School
Geordie	15	Gelding	17.1	Bay	Thoroughbred	Flat-racing
Honour	9	Mare	16.3	Bay	Thoroughbred	Flat-racing
Olive	1	Filly	15.0	Bay	-	Riding School
Ozzy	1	Filly	16.0	Skewbald	-	Riding School
Stoney	11	Gelding	16.1	Black	Thoroughbred	Flat-racing
Weasel	10	Mare	16.0	Brown	Thoroughbred	Flat-racing

Methods

Following the pilot study, it was established that the treatment level should be set at level '2' for all subjects. The Alpha Stim was connected as before and a heart rate (HR) monitor consisting of a Polar Horse Trainer transmitter belt and a Polar Vantage NV receiver (both Polar Electro Oy, Kempele, Finland) was used to record heart rate data (see Figure 2).

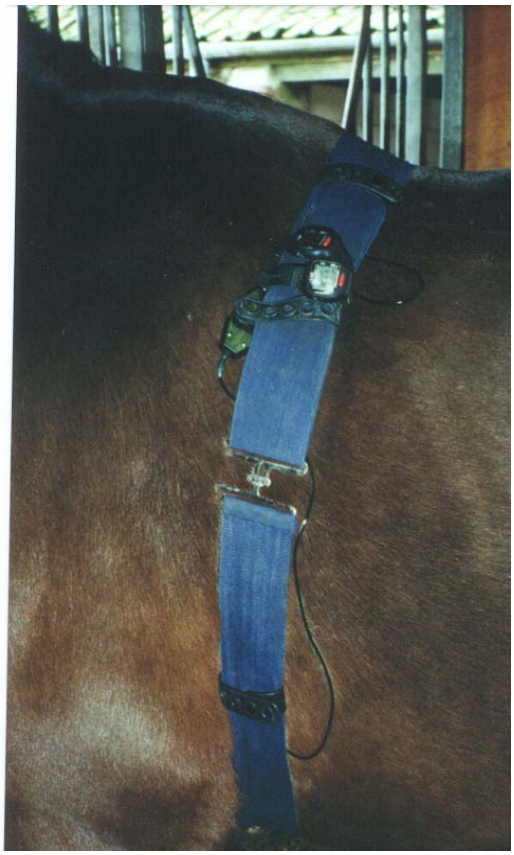
Figure 2: Test subject with Alpha-Stim SCS unit connected to headcollar and Polar Vantage NV attached to surcingle



Prior to HR electrode application, the horse's coat was soaked with water where the electrodes were to be positioned. Both electrodes were also given a layer of salt-free ultra-sound gel (Henleys Medical, Welwyn Garden City, UK) to maximise signal transfer, it was also important

to ensure that the grooves of the electrodes lay flat against the horse's coat. The positive electrode was fixed in a standard position at the left side four inches from the withers by means of an elastic surcingle. The negative electrode, also attached by means of the elastic surcingle, was positioned in a more ventral location. The transmitter was attached to the surcingle and two Vantage NV receivers were attached to the surcingle close to the transmitter. One Vantage receiver was set to record individual inter-beat intervals, in milliseconds. The other receiver was set to average HR over 5 second intervals (see Figure 3).

Figure 3: Receivers for Polar Vantage NV located dorsolaterally on the surcingle between the two transmitter electrodes



As before, the experimenter waited for signs of acceptance of the apparatus. Two minutes later, recording then began with experimental period divided into four phases. In the first phase the experimenter approached the horse and turned the Alpha-Stim on to treatment level '0' (sham approach 1) and started both HR receivers, which continually recorded HR over the entire experimental period. The experimenter then left the stable and recorded behavioural measures for 10 minutes using the ethogram from the pilot study and a check sheet (see Appendix 2), whilst noting 'start' and 'finish' times. After 10 minutes the first phase of the trial ended. The second phase started with the experimenter re-entering the stable, approaching the horse and adjusting exposure to level '2'. Following this, the experimenter left the stable and recorded behavioural measures for a further 10 minutes. The second phase of the trial then ended and the third phase started, with the experimenter re-entering and approaching the horse as before. The experimenter reached out to the horse as if to change the exposure level of the Alpha Stim whilst leaving it at level '2' (sham approach 2). Then, after leaving the stable, the experimenter again made a note of the time and recorded behavioural measures for a

further 10 minutes. The fourth phase began with the experimenter approaching the horse again, changing the treatment level back to '0', leaving the stable, making a note of the time and recording behavioural measures for a further 10 minutes. After this both behavioural and heart rate recording ceased and all of the apparatus was removed from the horse.

Four trials were carried out each day for a total of eight days (four testing days per week). These trials were carried out between 1000h - 1100h, 1400h - 1500h, 1500h - 1600h, and 1800 - 1900h, thus accommodating an hour either side of exercise and feeding times. To control for possible time of day effects across the trials, all subjects were tested once at each trial time, according to the sequence in Table 3.

Table 3: Trial dates and times together with sequence of testing of horses

Day Time	29/11/99	30/11/99	1/12/99	2/12/99	6/12/99	7/12/99	8/12/99	9/12/99
1000h - 1100h	Geordie	Ozzy	Stoney	Olive	Bassy	Bee	Honour	Weasel
1400h - 1500h	Stoney	Olive	Bassy	Bee	Honour	Weasel	Geordie	Ozzy
1500h - 1600h	Bassy	Bee	Honour	Weasel	Geordie	Ozzy	Stoney	Olive
1800h - 1900h	Honour	Weasel	Geordie	Ozzy	Stoney	Olive	Bassy	Bee

It was decided *a priori*, that if any signs of aversion appeared with any subject then use of the individual concerned in the experiment would be terminated immediately.

Behavioural Observations

A number of categories of behaviour were investigated simultaneously including body locomotion, head motion, ear position, oral behaviour and the state of the lower lip. Duration behaviours such as locomotion, head motion, oral actions and ear positions were instantaneously recorded at 15 second intervals. Event behaviours such as ear flicking, licking and chewing, vocalising, pawing, stall-kicking, wobbling of the head, shaking of the head and elimination were recorded using a one-zero cycle of 15 seconds.

Data analysis

The frequency of each behaviour was calculated and divided by the total number of observations made during that phase to give a proportion. Statistical analysis was then performed using Excel 97 and Minitab 12. The mean proportion of each behaviour for each horse in each trial phase across the four time slots was then calculated. Using these values, paired t-tests were employed where necessary.

Heart rate data were transferred to a PC via the Polar interface, the data was then transferred from the Polar HR Analysis Software (Version 5.04.01, Polar Electro Oy, Kempele, Finland) to Microsoft Excel version 97 and Minitab 12.0 for further analysis.

Mean heart rate for each trial phase across each time slot was calculated. These mean values were then analysed by performing a paired t-test which compared each horses mean heart rate for

trial phase one with that of trial phase three and also trial phase four. The significance of particular factors was then further assessed by means of a general linear model.

Results

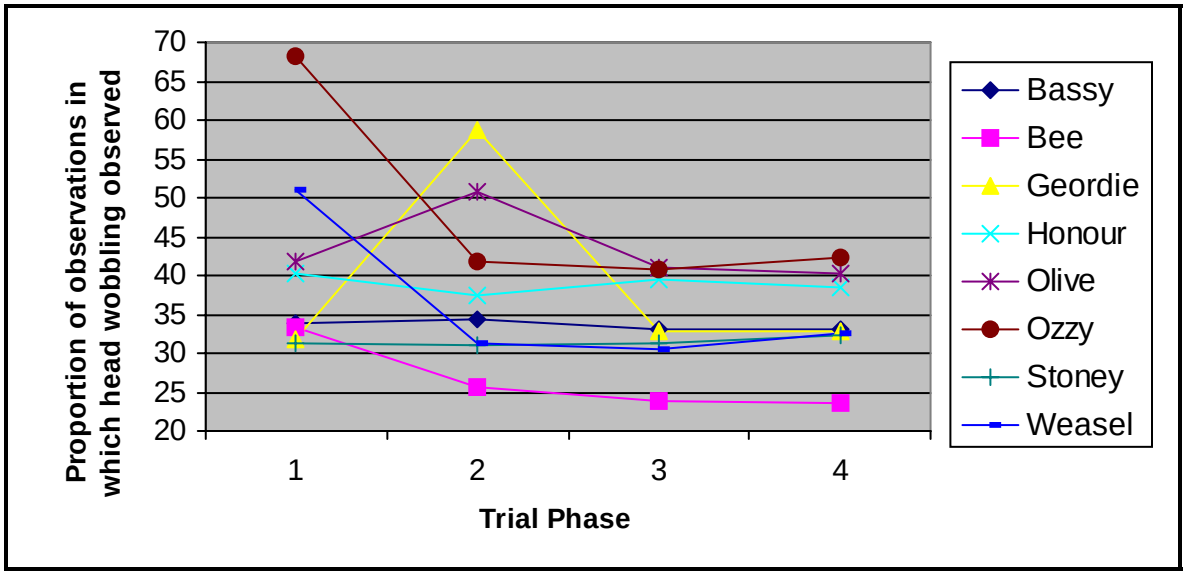
Heart Rate

Mean HR values are shown in Table 4 and Figure 4. Paired t-tests revealed there to be no significant differences between each horse's mean HR in trial phase one and three ($n=8$, $t=1.95$, $p>0.05$) or between trial phase one and four ($n=8$, $t=1.95$, $p>0.05$). However, there appears to be a trend in which the variation in mean HR values decreases from trial phase one through trial phases three and four.

Table 4: The mean HR through out the four trial phases for each horse.

Horse	Trial Phase One	Trial Phase Two	Trial Phase Three	Trial Phase Four
Bassy	33.90	34.43	32.95	33.14
Bee	33.40	25.76	23.73	23.68
Geordie	31.92	58.70	32.80	32.80
Honour	40.16	37.47	39.50	38.41
Olive	41.78	50.68	41.05	40.35
Ozzy	68.10	41.73	40.83	42.25
Stoney	31.20	31.10	31.21	32.37
Weasel	51.05	31.40	30.42	32.62

Figure 4: Mean HR for each horse across the four phases for each horse



In Figure 4, there appears to be three different trends in the data according to each horses individual mean values. The mean heart rate of three horses (Bee, Ozzy and Weasel) decreases sharply between trial phases one and two, while remaining relatively constant from trial phase two, through trial phase three to trial phase four. The mean heart rate of three horses (Bassy, Honour

and Stoney) appears to remain relatively constant through trial phases one to four. Lastly, the mean heart rate of the two remaining horses (Geordie and Olive) rises between trial phase one and two but then decreases between trial phase two and three. The mean heart rate then remains consistent between trial phases three and four at a level close the value trial phase one.

A general linear model (GLM) was used to assess the effects of individual subject identity, trial phase and time of trial (trial slot) on mean heart rate values. This not surprisinngly, revealed that individual horse was a highly significant factor (F=3.03, P=0.006) in determining variation in heart rate. Time of day was not significant (F=2.03, P=0.115), but there was a suggestion that trial phase may be having an effect (F=2.27, P=0.085), but this was masked by the high level of individual variation. The data were therefore re-analysed with mean heart rate in phase one for each individual horse used as a covariate in the model which also included the other trial phases and time of day. This suggested a highly significant effect of trial phase on heart rate (F=4.33, P=0.007). Thus it would seem that when individual baseline heart rate values are taken into consideration there is a significant effect of trial phase. Interestingly, inspection of the basic statistics collated by trial phase reveal a fall in the mean heart rate from phase 2 (39.00 bpm) to a relatively consistent value in the later phases (34.32 bpm and 34.83 bpm respectively). There is also a much more marked change in the standard deviation, (18.32 in phase 2, 7.60 in phase 3 and 7.01 in phase 4) across this time span, which might suggest some physiological normalisation.

Behavioural Measures

The pooled mean data from all subjects across all trials provides the observed time budget below:

Behaviour	**Proportion of Observations**
Locomotion	
Alert	0.847
Dozing	0.120
Circling	0.002
Moving forward	0.021
Moving backward	0.003
Head movement	
Up and down	0.028
Side to side	0.025
Backwards and forwards	0.001
No movement	0.943
Oral activity	
Drinking	0.009
Eating bedding	0.137
Eating forage	0.321
Tense lower lip	0.385
Relaxed lower lip	0.117
Lower lip quivering	0.021
Others	0.000

Ear position

	Left	Right
Forwards	0.367	0.366
Sideways	0.598	0.600
Back	0.034	0.033

Proportion of observations in which behaviour observed:

Behaviour	Proportion of Observations
Ear flicking	0.039
Licking and chewing	0.102
Wobbling head	0.038
Vocalising	0.015
Stall kicking	0.001
Pawing	0.001
Shaking head	0.024
Eliminating	0.006
Weaving	0.001

A correlation matrix was calculated for assessment of the Pearson correlation coefficients within and between the various behaviour categories in each trial phase. The statistical analysis revealed that amount of time spent engaged in several behaviours varied significantly between certain trial phases. Therefore only the results relating to these behaviours are summarised below by category.

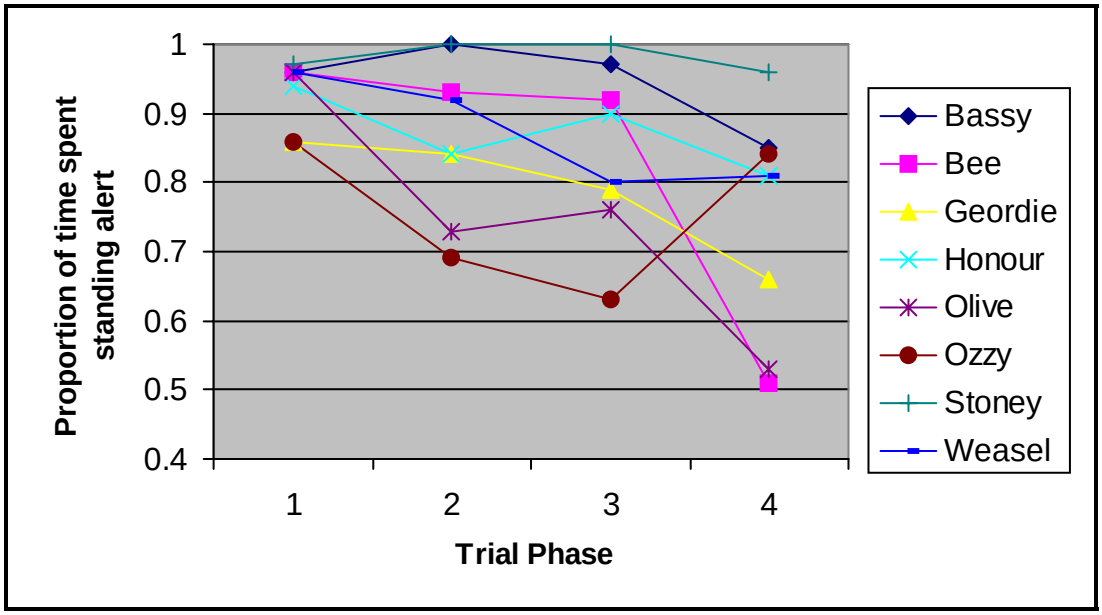
Standing Alert

The proportion of time spent standing alert through the four trial phases based on the data recorded, are shown in Table 5 and Figure 5. The data reveal, for the majority of horses, that the mean values decrease between trial phase one and two. The mean values for during trial phase three are lower than the mean value in phase one, except for those horses whose value does not fall between the first and second phases. A paired t-test comparing the mean values during trial phase one with those of trial phase three shows there to be a significant difference ($t=2.50$, $p<0.05$). All of the horses' mean values during trial phase four are lower than those during trial phase one. A paired t-test comparing the mean values during trial phase one and four confirms the significant difference ($t=3.10$, $p<0.05$).

Table 5: The proportion of time spent standing alert throughout the four trial phases, for each horse.

Horse	Trial Phase One	Trial Phase Two	Trial Phase Three	Trial Phase Four
Bassy	0.96	1.00	0.97	0.85
Bee	0.96	0.93	0.92	0.51
Geordie	0.86	0.84	0.79	0.66
Honour	0.94	0.84	0.90	0.81
Olive	0.96	0.73	0.76	0.53
Ozzy	0.86	0.69	0.63	0.84
Stoney	0.97	1.00	1.00	0.96
Weasel	0.96	0.92	0.80	0.81

Figure 5: The proportion of time spent standing alert throughout the four trial phases, for each horse.



Time spent standing alert across the four phases was found to correlate positively with two factors: time spent eating bedding (r=0.184, p=0.037) and time spent eating forage (r=0.363, p=0.002).

Time spent standing alert across the trial phases was found to have a negative correlation (inverse relationship) with eight factors. These were; trial phase (r=-0.244, p=0.006), time spent standing dozing (r=-0.945, p<0.001), time spent walking in circles (r=-0.197, p=0.026), time spent with lower lip relaxed (r=-0.575, p<0.001), time spent with lower lip quivering (r=-0.455, p<0.001), time spent with the left ear back (r=-0.241, p<0.006), time spent with the right ear back (r=-0.242, p<0.006) and wobbling of the head (r=-0.312, p<0.001).

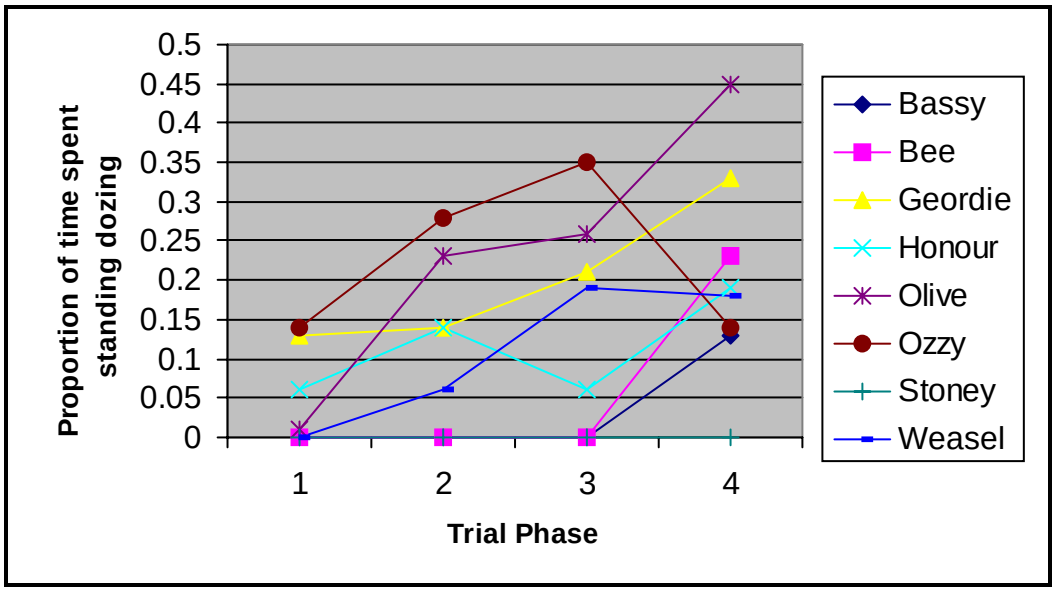
Standing Dozing

Table 6 and Figure 6 show the proportion of time spent standing dozing throughout the four trial phases, based on the data gathered. In phase three, values are higher (Geordie, Olive, Ozzy and Weasel) or equal to (Bassy, Bee, Honour and Stoney) the values in phase one. A paired t-test comparing the mean values during these phases one and three suggests the difference is significant (t=-2.44, p<0.05). In phase four, the same trend is seen with each horse's value higher (n=6) or equal (n=2) to that in phase one. A paired t-test confirms the significance of this difference (t=-3.29, p<0.05).

Table 6: The proportion of time spent standing dozing throughout the four trial phases, for each horse.

Horse	Trial Phase One	Trial Phase Two	Trial Phase Three	Trial Phase Four
Bassy	0.00	0.00	0.00	0.13
Bee	0.00	0.00	0.00	0.23
Geordie	0.13	0.14	0.21	0.33
Honour	0.06	0.14	0.06	0.19
Olive	0.01	0.23	0.26	0.45
Ozzy	0.14	0.28	0.35	0.14
Stoney	0.00	0.00	0.00	0.00
Weasel	0.00	0.06	0.19	0.18

Figure 6: The proportion of time spent standing dozing throughout the four trial phases, for each horse.



The proportion of time spent standing dozing across the phases was found to have positive correlation with trial phase ($r=0.220$, $p=0.013$), time spent with lower lip relaxed ($r=0.620$, $p<0.001$), time spent with lower lip quivering ($r=0.484$, $p<0.001$), the time spent with the left ear back ($r=0.265$, $p=0.002$) time spent with the right ear back ($r=0.268$, $p=0.002$) and head wobbling ($r=0.353$, $p<0.001$).

Time spent standing dozing across the trial phases was also found to have a negative correlation with time spent standing alert ($r=-0.945$, $p<0.001$), time spent eating bedding ($r=-0.205$, $p=0.05$) and time spent eating forage ($r=-0.331$, $p<0.001$).

Lower Lip Tense

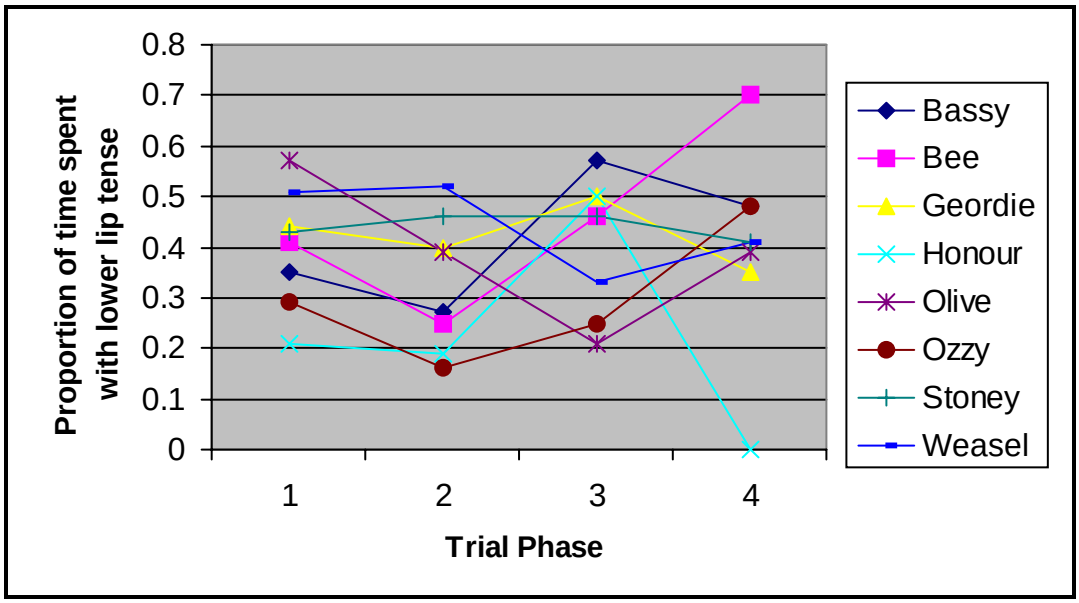
Table 7 and Figure 7 show the proportion of time spent with lower lip tense throughout the four trial phases, based on the data gathered. The mean values for most horses (n=6) decreases between phase one and two decrease. A paired t-test revealed that the difference between the mean values during these phases are significant level ($t=2.49$, $p<0.05$). The values in trial phase three are

higher compared to those in trial phase one for five subjects, but a paired t-test suggests that this difference is not significant. Between phases three and four, half of the horses' values decrease (Bassy, Geordie, Honour and Stoney) while the other half of the horses' values increase (Bee, Olive, Ozzy and Weasel). A paired t-test suggests that there is no significant difference between the mean values in trial phases one and four.

Table 7: The proportion of observations with lower lip tense throughout the four trial phases, for each horse

Horse	Trial Phase One	Trial Phase Two	Trial Phase Three	Trial Phase Four
Bassy	0.35	0.27	0.57	0.48
Bee	0.41	0.25	0.46	0.70
Geordie	0.44	0.40	0.50	0.35
Honour	0.21	0.19	0.50	0.00
Olive	0.57	0.39	0.21	0.39
Ozzy	0.29	0.16	0.25	0.48
Stoney	0.43	0.46	0.46	0.41
Weasel	0.51	0.52	0.33	0.41

Figure 7: The proportion of observations with lower lip tense throughout the four trial phase, for each horse



The proportion of time spent with the lower lip tense across the trial phases was found to correlate positively with the time spent walking in circles (r=0.249, p=0.005), time spent moving the head up and down (r=0.346, p<0.001), time spent moving the head from side to side (r=0.287, p<0.001), time spent with the left ear forwards (r=0.545, p=0.001), time spent with the right ear forwards (r=0.546, p=0.001), licking and chewing r=0.687, p<0.001) and weaving (r=0.224, p=0.01).
Time spent with the lower lip tense across the trial phases was found to have a significant negative correlation with several factors: time spent with head still (r=-0.405, p<0.001), time spent drinking (r=-0.180, p<0.042), time spent eating bedding (r=-0.226, p=0.01), time spent eating forage (r=-0.621, p<0.001), time spent with the lower lip relaxed (r=-0.183, p<0.038), time spent with the left

ear positioned sideways (r=0.545, p<0.001) and time spent with the right ear positioned sideways (r=-0.552, p<0.001).

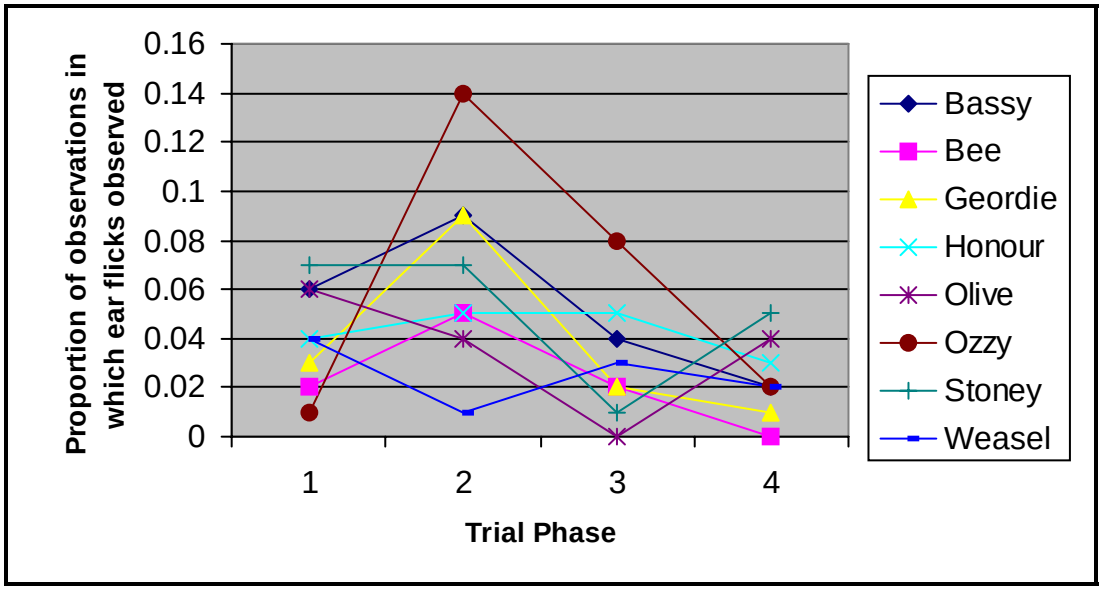
Ear Flicking

Table 8 and Figure 8 show the proportion of observations where ear flicking was recorded throughout the four trial phases, based on the data gathered. Between trial phases one and two, the mean values increase for the majority of the horses (n=5) then between phases two and three, the values decrease, (n=6). The mean values during phase three are lower than in trial phase one in six subjects, but a paired t-test suggests no significant difference. Between phase three and four the mean values decrease in six subjects. The mean values during trial phase four are lower than those during trial phase one, for seven subjects. A paired t-test suggests that this difference is significant (t=2.78, p< 0.05).

Table 8: The proportion of observations with ear flicking throughout the four trial phases, for each horse.

Horse	Trial Phase One	Trial Phase Two	Trial Phase Three	Trial Phase Four
Bassy	0.06	0.09	0.04	0.02
Bee	0.02	0.05	0.02	0.00
Geordie	0.03	0.09	0.02	0.01
Honour	0.04	0.05	0.05	0.03
Olive	0.06	0.04	0.00	0.04
Ozzy	0.01	0.14	0.08	0.02
Stoney	0.07	0.07	0.01	0.05
Weasel	0.04	0.01	0.03	0.02

Figure 8: The proportion of observations with ear flicking throughout the four trial phases, for each horse.



The proportion of observations with ear flicking across the phases was found to correlate positively with time spent walking forwards (r=0.248, p=0.005), time spent with the left ear forward (r=0.192,

p=0.03) and time spent with the right ear forward (r=0.191, p<0.05). A negative correlation was found with time of day (r=-0.225, p=0.011).

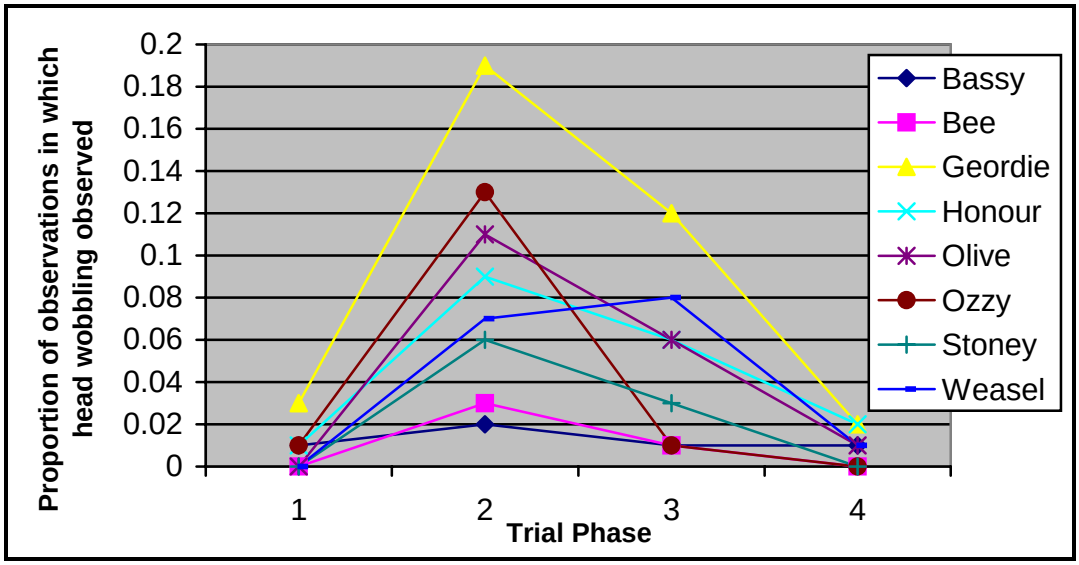
Wobbling of the Head

Table 9 and Figure 9 show how the proportion of observations with wobbling of the head changes through the four trial phases. A very low proportion of the time was spent head wobbling during trial phase one, with the mean values residing close to zero. However, in trial phase two these mean values increase for all horses. A paired t-test suggests that this increase is highly significant (t=-4.83, p<0.01). It is evident in Figure 9 that the mean values peak for all but one of the horses in phase two; Weasel peaks in phase three. Despite the decrease in phase three, the values are still significantly higher than that during phase one, (t=-3.41, p<0.05). Between trial phases three and four, subsequent decreases in all the horses mean values are evident, with the values appearing to return to pre-exposure levels. A paired t-test comparing the mean values of phase one and four revealed no significant difference.

Table 9: The proportion of observations with wobbling of the head throughout the four trial phases, for each horse.

Horse	Trial Phase One	Trial Phase Two	Trial Phase Three	Trial Phase Four
Bassy	0.01	0.02	0.01	0.01
Bee	0.00	0.03	0.01	0.00
Geordie	0.03	0.19	0.12	0.02
Honour	0.01	0.09	0.06	0.02
Olive	0.00	0.11	0.06	0.01
Ozzy	0.01	0.13	0.01	0.00
Stoney	0.00	0.06	0.03	0.00
Weasel	0.00	0.07	0.08	0.01

Figure 9: The proportion of observations with wobbling of the head throughout the four trial phases for each horse.



The proportion of observations with head wobbling across the trial phases was found to correlate positively with treatment ($r=0.430$, $p<0.001$), time spent standing dozing ($r=0.353$, $p<0.001$), time spent with lower lip relaxed ($r=0.228$, $p=0.01$), time spent with lower lip quivering ($r=0.339$, $p<0.001$), licking and chewing ($r=0.434$, $p<0.001$) and vocalising ($r=0.322$, $p<0.001$).
The proportion of time spent wobbling the head across the trial phases was found to have a negative correlation with time spent standing alert ($r=-0.312$, $p<0.001$) and time spent eating bedding ($r=-0.191$, $p=0.031$).

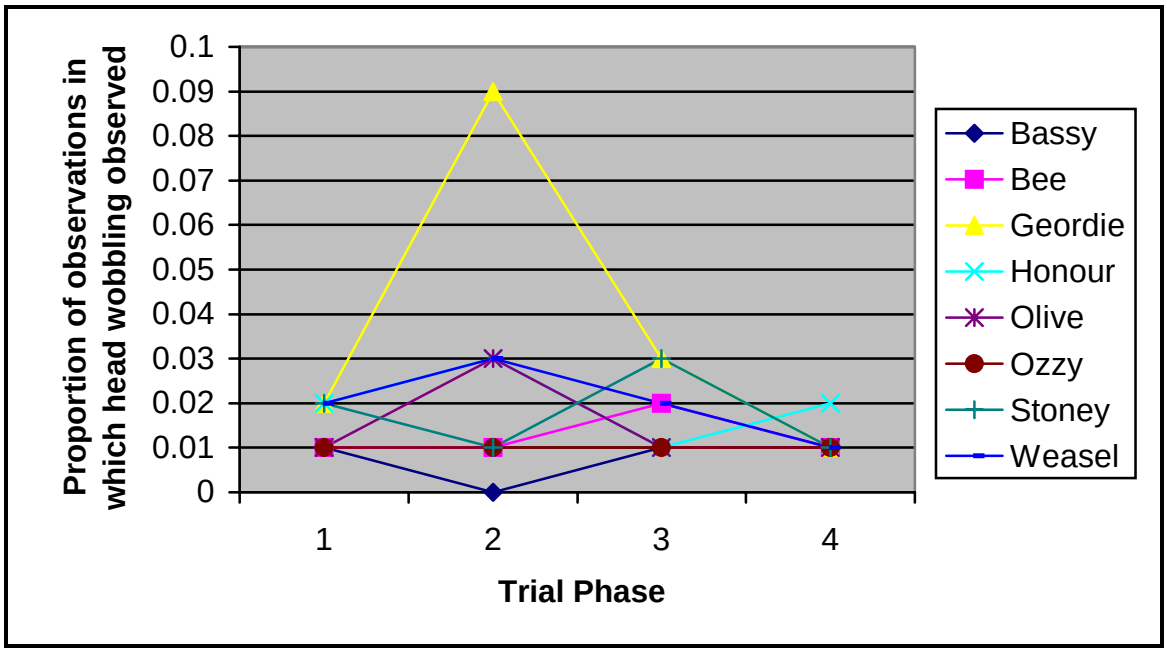
Vocalisation

Table 10 and Figure 10 display the proportion of observations with vocalising through the four trial phases. Vocalisation can clearly be seen to be highly variable between horses and trial phase. However, a paired t-test comparing the mean values during trial phase one and four revealed there to be a significant difference ($t=2.50$, $p<0.05$).

Table 10: The proportion of observations with vocalising through out the four trial phases, for each horse

Horse	Trial Phase One	Trial Phase Two	Trial Phase Three	Trial Phase Four
Bassy	0.01	0.00	0.01	0.01
Bee	0.01	0.01	0.02	0.01
Geordie	0.02	0.09	0.03	0.01
Honour	0.02	0.03	0.01	0.02
Olive	0.01	0.03	0.01	0.01
Ozzy	0.01	0.01	0.01	0.01
Stoney	0.02	0.01	0.03	0.01
Weasel	0.02	0.03	0.02	0.01

Figure 10: The proportion of observations with vocalising through out the four trial phases, for each horse



The pattern of observations with vocalising across the trial phases was found to have a negative correlation with the time slot (time of day) (r=-0.199, p=0.024).

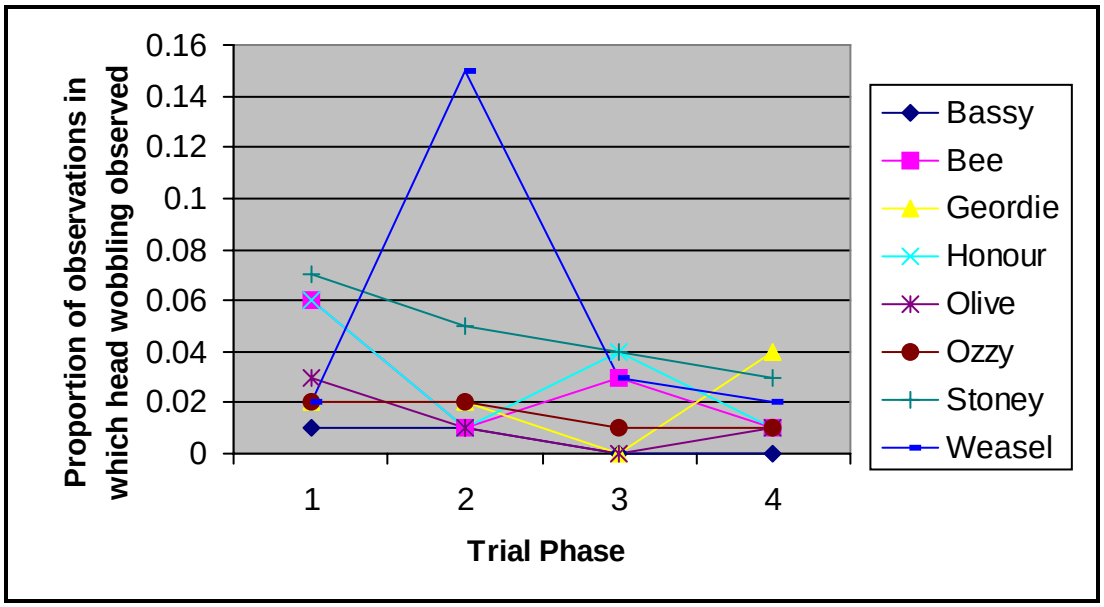
Shaking of the Head

Table 11 and Figure 11 display the mean values through the four trial phases. No significant difference between the values in trial phase one and two is found. Between trial phases two and three the mean values decrease for six of the horses. Compared with the mean values in trial phase one, the mean values in phase three are lower, for seven subjects. A paired t-test suggests that this difference is significant (t=2.94, p<0.05). There is no significant difference between the mean values in trial phase one and four.

Table 11: Proportion of observations with shaking of the head throughout the four trial phases, for each horse

Horse	Trial Phase One	Trial Phase Two	Trial Phase Three	Trial Phase Four
Bassy	0.01	0.01	0.00	0.00
Bee	0.06	0.01	0.03	0.01
Geordie	0.02	0.02	0.00	0.04
Honour	0.06	0.01	0.04	0.01
Olive	0.03	0.01	0.00	0.01
Ozzy	0.02	0.02	0.01	0.01
Stoney	0.07	0.05	0.04	0.03
Weasel	0.02	0.15	0.03	0.02

Figure 11: Proportion of observations with shaking of the head throughout the four trial phases, for each horse



The pattern of time spent shaking the head was found to correlate positively with the time spent moving the head from side to side (r=0.330, p<0.001). There was a negative correlation with the trial phase (r=-0.195, p=0.027) and time spent not moving the head (r=-0.269, p=0.002).

In order to examine the potential effects of individual horse, time of day and trial phase on each behaviour recorded, the data were analysed using a general linear model. With the multiple use of t-tests described above, type I errors (rejection of a null hypothesis when the results are really due to chance) are obviously likely. Nonetheless these tests are useful in exploring potential relationships in the data of a preliminary study such as this. The subsequent finding of a trial phase effect in the GLM provides further evidence that such relationships are real and not due to chance. The results for behaviours with significant factors are given in Table 12.

Table 12: Results of analysis of behaviour data with a general linear model

	F value and probability: * P = 0.05, ** P = 0.01, *** P = 0.001		
Behaviour	**Individual horse**	**Time of day**	**Trial phase**
Locomotion			
alert	2.25*	10.87***	3.64*
dozing	2.58*	12.72***	3.00*
circling	3.04**	3.74*	0.75
walking forwards	2.96**	3.33*	0.12
walking backwards	1.16	2.82*	0.34
Head movement			
up & down	3.30**	3.11*	0.95
side to side	5.12***	3.04*	1.08
none	4.33***	3.42*	1.41
Oral activity			
Eating bedding	3.78***	6.07***	0.70
Eating forage	3.95***	39.58***	0.12
Tense lower lip	1.20	29.84***	0.50
Relaxed lower lip	3.04**	2.44	0.87
Quivering lower lip	5.58***	5.80***	0.80
Ear position			
Left forward	2.10*	11.54***	1.55
Left sideways	0.96	8.43***	0.33
Left back	1.77	3.83*	0.84
Right forward	2.08	11.55***	1.54
Right sideways	0.98	8.48***	0.36
Right back	1.82	3.95**	0.84
Events			
Ear flicking	1.06	3.56*	3.70*
Licking & chewing	4.34***	20.39***	1.80
Head wobbling	3.00**	2.27	13.80***
Pawing	2.28*	2.84*	0.67
Headshaking	3.04**	2.18	2.06
Eliminating	0.87	2.23	2.77*
Weaving	3.12**	3.12*	0.52

These results also emphasise individual differences and the importance of standardising the time of day of multiple treatments if comparable data are required. A second analysis using treatment on versus treatment off instead of trial phase was conducted and this revealed significant effects

($P<0.05$) of treatment on licking and chewing and head wobbling only. Inspection of the summary statistics reveals that both of these behaviours increased when the treatment was applied.

Discussion

Pilot study

This part of the study was used as a preliminary screen to determine whether or not the Alpha-Stim SCS had any noticeable effect on the behaviour of horses and at what level. Field observations concerning an effect on head wobbling were confirmed statistically. Thus it would seem reasonable to conclude that the Alpha-Stim SCS does have an effect on equine behaviour. The main study, which followed, was used to try to elucidate the nature of this effect further.

Main study

In order to examine the potential effects of the Alpha-Stim SCS, it is important to recognise the different categories of response. Some effects will be associated with the application of the treatment *per se*. These may be expected to arise during phases 2 and 3 of the trial, and are a direct response to the applied current. Others may be a consequence of treatment. These may not be immediately apparent, but may extend beyond the treatment period. These effects may therefore be seen in trial phase 3 and either or both of 2 and 4. Those effects seen to increase during the trial are inevitably confounded by an order effect relating to habituation. Each trial consisted of four replicate approaches by the experimenter. The first of these may be perceived as more novel than the next and the second more than the third and so forth. Thus with some results it is not possible to distinguish this effect from one due to the Alpha-Stim SCS, which may also be expected to reduce the reaction to novelty if it has a calming effect. In order to do this a blind, placebo controlled study would be necessary and this may be the object of future work. It should be remembered that the aim of this study was to examine in a safe and ethical way whether or not the Alpha-Stim SCS was *potentially* useful. In this regard, at this stage, we are not so much concerned with rejecting specific null-hypotheses, but rather evaluating whether or not the results are consistent with potential beneficial effect.

Heart Rate

The Alpha-Stim SCS treatment was potentially associated with a fall in heart rate and reduction in heart rate variability. The R-R interval data gathered during this trial have yet to be analysed, but may potentially reveal whether or not an increase in vagal tone is apparent in the latter stages. Heart rate data therefore appear to be a potentially useful adjunct to the evaluation of the effect of the Alpha-Stim SCS. Figure 4 would seem to suggest that any effect may be differential, being greatest in those horses with a higher mean heart rate (Bee, Ozzy and Weasel). In future studies it may prove valuable to categorise the horses according to whether they have a high, moderate or low mean heart rate and see if there is a significant difference between the strength of the different groups' heart rate response to the Alpha-Stim SCS treatment. Additionally horses could be categorised according to what characteristics (personality or individual difference factors) that may relate to their mean heart rate. It should also be remembered that an arbitrary value of 2 was chosen for treatment of all subjects on the basis of the pilot study, but this may not be adequate for all subjects. The use of easily discernible behaviour measures, such as head wobbling rate, may be a useful guide if the significance of this can be validated.

The response of Geordie and Olive (Figure 4), whose heart rate initially increased, could also be attributed to an immediate effect of the Alpha-Stim SCS treatment. The horses may initially

experience a prickling or sharp tingling sensation when the CES began, similar to that reported by human subjects. With time, the horses may have habituated to such a sensation, and so their heart rate would be expected to fall again.

Behavioural measures

Those behaviours in which a trial phase effect was found in the GLM analysis and the t-test comparisons are discussed first, Other behaviour categories, in which there was a suggestion of a difference between certain phases from the t-tests only are discussed afterwards

Standing Alert

The results suggest that the Alpha-Stim SCS treatment may have an effect on the proportion of time spent standing alert especially during trial phases three and four, compared to that during trial phase one (Value for phase 1 = 0.93, 2 =0.87, 3 = 0.84, 4 =0.74). The reduction in time spent alert is consistent with any anxiolytic effect which the device may have, but the order effect discussed above must also be considered a potential explanation. As the proportion of time spent standing alert continues to decrease after the treatment has stopped, this would suggest that if the Alpha-Stim SCS is responsible for the effect then the effect is not dependent upon continued stimulation. This is consistent with the findings of other studies in humans (Kirsch 1999).

Future studies should perhaps establish the nature and duration of any continued effect following an initial bout of treatment, whilst accepting individual variability in responsiveness. Little effect on this parameter or heart rate was seen in Stoney. His heart rate was consistently within normal resting values, perhaps indicating that no therapeutic effect was possible, alternatively, a higher level of CES may be necessary. However, this individual did show an increase in the head wobbling parameter used to determine effective level in the pilot study. If anything, the amount of time spent standing alert by Stoney increased during the Alpha-Stim SCS treatment, taken together this might suggest that at certain levels, the direct effect of the Alpha-Stim SCS may cause a paradoxical increase in arousal as the animal resists its effect. This potential complication needs to be born in mind when assessing clinical efficacy in later trials.

Standing Dozing

The majority of the horses became more dozey during the latter trial phases and this is again consistent with an anxiolytic effect, however the confounding factor discussed above must also be considered a viable explanation. Not surprisingly this behaviour had a strong inverse relationship with the amount of time spent alert. Positive correlations were found with lower lip relaxation, lip quivering and the laying back of the ears, which are all consistent with the classic view of a dozing horse. However, perhaps of more significant note is the positive correlation with head wobbling which was used as the index of effect by the device. Whilst this is an encouraging result as far as exploring the potential efficacy of the Alpha-Stim SCS, it must be remembered that correlation does not equate with causation.

One horse, Bee, only dozes after the Alpha-Stim SCS treatment has stopped. This could be due to Bee failing to experience any sensation or treatment effect during stimulation or could reflect a combination of arousal from the current during treatment and extended CES effect beyond direct application. Again, this emphasises that individual variation in sensitivity is likely to be an important consideration in future trails.

Wobbling of the Head

The proportion of time spent wobbling the head appeared to increase significantly between trial phase one and when the Alpha-Stim device was switched on at the Alpha-Stim SCS treatment level two (trial phase two). Despite decreasing between trial phases two and three, the mean values during trial phase three were still significantly higher than those during trial phase one. As there was no significant difference between the percentage of time spent wobbling the head between trial phase one and four we can conclude that the wobbling of the head returns to a value not dissimilar to the original value in trial phase one, when direct stimulation ceased. Therefore it seems likely, especially in the light of the pilot study, that the Alpha-Stim SCS treatment induced the head wobbling and this effect is limited to the time of current flow. This effect could be due to horses experiencing a treatment- related sensation such as a slight feeling of dizziness similar to that reported in human subjects (Kirsch 1999). It may therefore be appropriate to use time spent head wobbling as a potential indicator of the extent to which each horse is experiencing sensations relating to the level of CES treatment, or the individual differences in sensitivity to the sensations associated with the CES treatment.

Whilst head wobbling appears largely restricted to the time of direct Alpha-Stim SCS treatment it is interesting to note the putative correlates, which include vocalisation, licking and chewing and those associated with relaxation described above in the section on dozing. The latter behaviours appear to change for a longer period than the head wobbling. The GLM analysis would suggests that both head wobbling and licking and chewing are significant behaviour changes restricted to the time of direct stimulation. This is particularly interesting as licking and chewing is widely used as a sign of impending relaxation by horsemen using non-physical training methods. It has also been suggested that this behaviour is a displacement reaction, exhibited at the time of motivational conflict, but its true biological significance remains largely unknown.

Ear Flicking

A significant difference appears to exist between the mean values for the proportion of time spent ear flicking during trial phases one and four. Specifically, the mean values were lower after the Alpha-Stim SCS treatment. One explanation for this result may be that the horses had not fully habituated to the presence of the Alpha-Stim apparatus before trial phase one started and by trial phase four, habituation to the apparatus had fully occurred. Despite there being no significant differences between the mean values during trial phase one and two, or between one and three, trends in the data suggest that the amount of ear flicking increased during the initial ten minutes of the Alpha-Stim SCS treatment. This could be an indication of the Alpha-Stim SCS treatment causing some sensation at the point of contact of the electrodes. Reduction in ear flicking may again be a measure suggestive of relaxation as ear flicking is commonly interpreted as a sign of mild irritation or frustration in the horse.

The following factors were not found to be significant in the GLM analysis by paired t-tests revealed a potential difference in the trial phases, and so they are considered here, in the context of potentially important factors that could be worthy of future investigation. However the main focus should initially focus on those parameters discussed above.

Lower Lip Tense

The majority of the horses' mean values decreased between trial phases one and two and it might be inferred from this that the Alpha-Stim SCS treatment reduced tension, however, values increase again in phase three when treatment is still being applied. Only if this Alpha-Stim SCS effect is

transitory is this likely to be the explanation. We consider this unlikely, but lip tension is not a direct correlate with arousal or anxiety as many other factors can influence this behaviour. Relaxation is perhaps a more important corollary of the opposite

Vocalisation

Vocalisation was generally a rare event but mean values were lower after the Alpha-Stim SCS treatment had been administered (trial phase four) than before the Alpha-Stim SCS treatment was initiated (trial phase one). This is consistent with a reduction in arousal, as vocalisation is an energetically expensive event in the horse, used at times of high arousal

Head Shaking

A significant difference was found between the proportion of this behaviour exhibited in trial phases one and three. Specifically, the mean values are all lower in trial phase three, with the exception of Weasel. The mean values for most of the horses continued to decrease during trial phase four, but the difference with phase one was not significant. Like ear flicking, headshaking is often a sign of frustration and so the effect may not be entirely coincidental.

Again another theoretical explanation for the data relates to incomplete habituation to the Alpha-Stim apparatus, although a subjective evaluation of this was made by the experimenter in order to control for the effect.

Conclusion

Taken together these results are consistent with a potential beneficial effect for the Alpha-Stim SCS in the horse, although other explanations are theoretically possible for the current data. In the light of the pilot study and the correlates of the behaviour used to assess stimulation (head wobbling), we consider CES to be the more likely explanation of the results as a whole. 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 effected by the Alpha-Stim SCS but this could be explained by their rarity. The results further suggest that if CES is responsible for the changes in behaviour, then its effects extend beyond the period of immediate stimulation. Further work is 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.

References

Brotman, P. Low-intensity transcranial electrostimulation improves the efficacy of thermal biofeedback and quieting reflex training in the treatment of classical migraine headache. *American Journal of Electromedicine*, 6(5):120-123, 1989. Doctoral thesis, City University Los Angeles, 117 pages, 1986.

Cox, A. & Heath, R.G. (1975) Neurotone therapy: A preliminary report of its effect on electrical activity of forebrain Structures. *Diseases of the Nervous System*, **36(5):** 245-247.

Gibson, T. H. & O'Hair, D.E. (1987) Cranial application of low level transcranial electrotherapy vs. relaxation instruction in anxious patients. *American Journal of Electromedicine*, **4(1):** 18-21. Doctoral dissertation (TG), California School of Professional Psychology, 152 pages, 1983.

Heffernan, M. (1995) The effect of a single cranial electrotherapy stimulation on multiple stress measures. *The Townsend Letter for Doctors and Patients*, **147:** 60-64. Eighth International Montreux Congress on Stress, Montreux Switzerland, February 1996.

Heffernan, M. (1996) Comparative effects of microcurrent stimulation on EEG spectrum and correlation dimension. *Integrative Physiological and Behavioural Science*, **31(3):** 202-209.

Heffernan, M. (1997) The effect of variable microcurrents on EEG spectrum and pain control. *Canadian Journal of Clinical Medicine*, **4(10):** 4-11.

Kirsch, D. (1999) How to Achieve Optimum Results Using Microcurrent Electrical Therapy For Pain Management: A Basic Clinical Protocol. Electromedicine, Medical Scope Monthly, Vol. 3. No.9.

Kirsch, D. (1999) Cranial Electotherapy 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.

Kirsch, D. (1999) The Science Behind CES, Medical Scope Publications, Edmonton.

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.

Overcash, S. and Siebenthall, 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):** 105-11.

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

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 electrotherepeutic currents in the primate and man. In Wadeneder, F.M.. & St. Schuuy (Eds.), *Electrotherapeutic Sleep and Electroanesthesia*. Vol.II, Amsterdam, Excerpte Medica, pp. 158-165.

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.

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.

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).

Voris, M.D. (1995) An investigation of the effectiveness of cranial electrotherapy stimulation in the treatment of anxiety disorders among outpatient psychiatric patients, impulse control parolees and paedophiles. Delos Mind/Body Institute, Dallas and Corpus Cristi, Texas.

Appendix 1

Ethogram used for behaviour studies
Behaviour states

Behaviour Term	Symbol	Behaviour Type	Behaviour Category	Description
Alert	A	Duration	Locomotor	Horse standing with alert body posture and eyes completely open.
Dozing	D	Duration	Locomotor	Horse standing static with body outline showing a minimum of excitement. Eyes are half closed or closed.
Walking Forwards	F	Duration	Locomotor	Horse travels forwards without obviously investigating it's environment.
Walking Backwards	B	Duration	Locomotor	Horse travels backwards without obviously investigating it's environment.
Walking Circles	C	Duration	Locomotor	Horse travels forwards in a circular direction, halting when having completed a circuit of the stable.
Head Not Moving	N	Duration	Head Motion	Horse's head remains motionless.
Head Moving Up and Down	UD	Duration	Head Motion	Horse moves head and neck either upwards or downwards along a vertical axis.
Head Moving To Either Side	S	Duration	Head Motion	Horse moves head and neck either to the left or right side along a horizontal axis.
Head Moving Forwards and Backwards	FB	Duration	Head Motion	Horse either extends head and neck forwards or shortens head and neck in towards it's body.
Eating Concentrate	C	Duration	Oral	Horse eats concentrate feed.

Eating Bedding	B	Duration	Oral	Horse eats bedding.
Eating Forage	F	Duration	Oral	Horse eats forage.
Drinking	D	Duration	Oral	Horse drinks water.
Lower Lip Tense	T	Duration	Oral	Horse's lower lip is tightly wrinkled and tense.
Lower Lip Relaxed	R	Duration	Oral	Horse's lower lip is loose and may be hanging.
Lower Lip Quivering	Q	Duration	Oral	Horse's lower lip displays a rapid quivering movement.
Cribbing	CR	Duration	Oral	Horse uses teeth to take hold of some form of projection and sucks in air.
Windsucking	W	Duration	Oral	Horse arches neck and swallows air without clamping teeth on to any projection.
Left Ear Forwards	F	Duration	Left Ear Position	The horse's left ear is orientated towards the front.
Left Ear Sideways	S	Duration	Left Ear Position	The horse's left ear is orientated towards the left-side.
Lefts Ear Backwards	B	Duration	Left Ear Position	The horse's left ear is orientated towards the back.
Right Ear Forwards	F	Duration	Right Ear Position	The horse's right ear is orientated towards the front.
Right Ear Sideways	S	Duration	Right Ear Position	The horse's right ear is orientated towards the right-side.
Right Ear Backwards	B	Duration	Right Ear Position	The horse's right ear is orientated towards the back.

Behaviour events

Behaviour Term	Symbol	Behaviour Type	Behaviour Category	Description
Ear Flicking	EF	Event	Head	Ears twitch rapidly
Licking and Chewing	LC	Event	Head	Horse lick and chews repetitively in the absence of any food in mouth.
Wobbling Head	WH	Event	Head	Without moving the neck, the horse discretely rocks the head horizontally in an arc like shape.
Vocalisation	V	Event	Head	Horse makes a vocal call or expression.
Stall-Kicking	SK	Event	Stereotypies	Horse kicks the either the door or sides of the stable.
Pawing	P	Event	Stereotypies	Horse paws or stamps at the floor with a leg.
Shaking Head	SH	Event	Other	Horse vigorously shakes both head and neck.
Weaving	W	Event	Other	Swinging head from side to side while also shifting weight alternatively between both forelegs.
Elimination	E	Event	Other	Horse excretes droppings or urinates.

Appendix 2

Sample behaviour record sheet

Horse Identity……Time Slot……

Trial Phase……1……Time Started……Time Finished……..

Time (min.sec)	Loco	Head	Oral	LE Pos.	RE Pos.	Ear Flick	Lick chew	Wob Hd	Vocal	Stall Kick	Pawing	Other	Time
0 (0.0)													0
0.25 (0.15)													0.25
0.5 (0.30)													0.5
0.75 (0.45)													0.75
1.0 (1.00)													1.0
1.25 (1.15)													1.25
1.5 (1.30)													1.5
1.75 (1.45)													1.75
2.0 (2.00)													2.0
2.25 (2.15)													2.25
2.5 (2.30)													2.5
2.75 (2.45)													2.75
3.0 (3.00)													3.0
3.25 (3.15)													3.25
3.5 (3.30)													3.5
3.75 (3.45)													3.75
4.0 (4.00)													4.0
4.25 (4.15)													4.25
4.5 (4.30)													4.5
4.75 (4.45)													4.75
5.0 (5.00)													5.0
5.25 (5.15)													5.25
5.5 (5.30)													5.5
5.75 (5.45)													5.75
6.0 (6.00)													6.0
6.25 (6.15)													6.25
6.5 (6.30)													6.5
6.75 (6.45)													6.75
7.0 (7.00)													7.0
7.25 (7.15)													7.25
7.5 (7.30)													7.5
7.75 (7.45)													7.75
8.0 (8.00)													8.0
8.25 (8.15)													8.25