The effect of post-surgical pain on attentional processing in horses

Attentional processing in horses

Louise Dodds, Laura Knight, Kate Allen, Joanna Murrell

School of veterinary sciences, University of Bristol, Langford House, Langford, North Somerset, BS405DU, UK

Correspondence: Dr. Jo Murrell, School of veterinary sciences, University of Bristol, Langford House, Langford, North Somerset, BS405DU.

Email: Jo.Murrell@bristol.ac.uk Tel: 0117 9289458

Attentional processing in horses

Acknowledgements: Louise Dodds was funded by a BBSRC vacation scholarship while she was carrying out the study

Abstract

Objective: Investigate the effect of post-surgical pain on the performance of horses in a novel object and auditory startle task

Study design: Prospective clinical study

Animals: 20 horses undergoing different types of surgery and 16 control horses that did not undergo surgery were studied

Methods: The interaction of 36 horses with novel objects and a response to an auditory stimulus was measured at two time points; the day before surgery (T1) and the day after surgery (T2) for surgical horses (G1), and at a similar time interval for control horses (G2). Pain and sedation were measured using Simple Descriptive Scales (SDSs) at the time the tests were carried out. Total time or score attributed to each of the behavioural categories was compared between groups (G1 and G2) for each test and between tests (T1 and T2) for each group.

Results: The median (range) time spent interacting with novel objects was significantly reduced in G1 from 57.5 (367) seconds in T1 to 12.4 (495) seconds in T2. In G2 the change in interaction time between T1 and T2 was not statistically significant. Median (range) Total Auditory Score was 7 (9) and 10 (11) in G1 and G2 respectively at T1, decreasing to 6 (10) in G1 after surgery and at 9.5 (11) in G2. Similarly, there was a significant decrease in Total Auditory Score within G1 between T1 and T2 ($p=0.003$).

There was a significant difference in Total Auditory Score between G1 and G2 at T2 (p=0.0169), with the score being lower in G1 than G2.

Conclusions and clinical relevance: Post-surgical pain negatively impacts attention towards novel objects and causes a decreased responsiveness to an auditory startle test. Attention demanding tasks in horses and may be useful as a biomarker of pain.

Key words: Pain, attention, horse, novel object, surgery

Introduction

The experience of pain is multidimensional and comprises sensory and affective-motivational elements. The sensory element represents pain intensity and quality, while the affective element encompasses unpleasantness, emotions and cognition. These elements are strongly correlated; in human infants, as pain intensity increases, the more unpleasant it becomes with a greater effect on cognition and emotions (Slater et al. 2008). This has also been demonstrated in rats, whereby pain negatively affected awareness in attention demanding tasks (Boyette Davis et al. 2008; Pais-Vieira et al. 2009). Similar studies of pain in humans (Eccleston et al. 1997; Lorenz et al. 1997) provide evidence that pain and cognition are strongly related (Eccleston et al. 1997; Millecamps et al. 2004), and it is widely accepted that attention can modulate pain and vice versa. Distraction from pain can result in reduced pain perception (Boyette-Davis et al. 2008), while pain can have a negative affect on attention demanding tasks (Millecamps et al. 2004; Pais-Vieira et al. 2009). Recent studies (Moore et al. 2013; Keogh et al. 2014) report preliminary findings that common conditions such as acute headache and menstrual pain lead to an overall dampening of attention, which results in decreased task performance. This is of particular interest as altered performance in experimental tasks is a valid alternative to verbal assessment of pain (Jensen et al. 1992; Rosenfeld et al. 1993) and attention has been indicated as one of the ‘pain-affected complex behaviours’ by which pain may be judged (Mogil 2009). Attention could therefore be used as an indicator of pain, especially in cases where self-reporting is not possible, for example in animals or non-verbal human infants.

Unlike in man, there is currently no ‘gold-standard’ for pain assessment in horses. This is mainly attributed to the difficulties of interspecies communication but also due to the limited knowledge of pain related behaviours in horses. Horses are stoic by nature, having evolved to mask signs of pain from predators, and are reluctant to show signs of pain that humans are able to recognise (Ashley et al. 2005). Although some generic behavioural responses to pain displayed by horses are a useful aid for pain detection (Moloney and kent 1997), those recovering from surgery are least able to display them (Hansen et al. 1997). Despite this, like human infants (Büttner et al. 2000), behavioural and physiological indicators of pain are heavily relied upon to assess pain. There is also evidence to suggest that physiological parameters such as respiration rate and heart rate lack sensitivity for pain (Moloney and Kent 1997; Hansen et al. 1997; Büttner et al. 2000; Price et al. 2003).

Two studies (Price et al. 2003; Ashley et al. 2005) have reported changes in attention type behaviours (decreased exploratory behaviour, distracted demeanour) in post-surgical horses. However, to our knowledge, the direct effect of acute pain on attention in horses has not been previously investigated. However very recently the effect of chronic lower back pain on attention to the environment was investigated in horses (Rochais et al. 2016). This study found that lower attentional engagement and the level of back disorders were correlated suggesting that attentional engagement could become a reliable indicator of chronic pain in the horse. The aim of this study was to investigate if post-surgical pain altered attentional processing in horses. We hypothesized that horses recovering from surgery would have a decreased response to test stimuli compared to control horses that were free from pain. If correct, attention may provide insight into affective state and have the potential as a new biomarker of pain in horses.

Materials and methods

Animals

Thirty six, healthy (ASA I-II) mixed breed horses were included in the study which was carried out at the XXXXXX, XXXX, between August 2013 and March 2014. Twenty horses (3 mares, 15 geldings, 2 stallions) undergoing elective surgery, with minimal or no pre-surgical pain were included in the "Surgery" group (G1). Sixteen horses (10 mares, 6 geldings) admitted for non-painful procedures, such as treadmill evaluation of poor athletic performance, were included in the "Control" group (G2). A power calculation was not carried out prior to the start of the study as there were no preliminary data on which to base such analysis and data collection was bound by the number of eligible horses that presented to the clinic during the time that the study could be carried out.

All horses were stabled individually in standard stables (4m x 3m), bedded with cardboard or shavings. A minimum of three hours post-admittance to the clinic was allowed for the horse to acclimatize to the new environment before the first experimental test session (T1) was carried out. All food was removed from G1 horses a minimum of 6 hours prior to surgery. Control horses had full rations of food during the study. The study was approved by the XXXXX and owner or agent consent was obtained prior to inclusion of horses in the study.

Anaesthesia and surgery (G1 horses)

The anaesthetic protocol for G1 was similar for each horse but was not standardised between animals. Pre-anaesthetic medication comprised 0.03 mg kg^{-1} IV acepromazine (ACP Injection, Elanco, UK) administered at least 30 minutes before induction of anaesthesia. Immediately prior to induction of anaesthesia further sedation was provided with an alpha 2 adrenergic agonist (romifidine (Sedivet, Boehringer Ingelheim, UK) 80 µg kg^{-1} or detomidine (Domidine, Dechra Veterinary Products, UK) 10 µg kg^{-1}) administered IV. Anaesthesia was induced with a combination of midazolam (Hypnovel, Roche Products Limited, UK) (30 mg) and ketamine (Narketan, Vetoquinol UK Ltd., UK) (2.2 mg kg^{-1}) IV. Following orotracheal or nasotracheal intubation with a suitably sized cuffed endotracheal tube, anaesthesia was maintained with isoflurane (IsoFlo, Zoetis UK Ltd, UK) vaporised in oxygen delivered via a large animal circle system (Tafonius, Vetronic Services and Hallowell EMC), the concentration of isoflurane was adjusted to maintain an adequate depth of anaesthesia for surgery. Respiration was supported with Intermittent Positive Pressure Ventilation (IPPV). Episodes of inadequate anaesthesia, signalled by gross purposeful movement, were treated either with an IV bolus of ketamine (100 mg) and midazolam (10 mg) or thiopental (Thiopental sodium, Archimedes Pharma UK Ltd., UK) (500 mg). Standard monitoring during anaesthesia included pulse rate, electrocardiogram (ECG), direct arterial blood pressure measured using a catheter placed in the facial artery, end tidal carbon dioxide and isoflurane concentrations and SpO2, using a multiparameter monitor (Tafonius, Vetronic Services and Hallowell EMC). On the day of surgery analgesia was provided with a single dose of either morphine (Morphine Sulphate, Martindale Pharmaceuticals Ltd., UK) (0.2 mg kg^{-1}) or buprenorphine (Buprenodale, Dechra Veterinary Products Ltd, UK) (10 µg kg^{-1}) administered intravenously at the time of induction of anaesthesia. In addition a non-steroidal anti-inflammatory drug (NSAID) was administered prior to surgery and for a minimum of two days after surgery at the licensed dose. Control horses did not undergo anaesthesia and surgery and no animal experienced anaesthesia or surgery purely for the purpose of this study.

Conduct of the study

Interaction of horses with novel objects and response to an auditory stimulus were assessed at two time points in all horses; In G1 horses test 1 (T1) was the day before surgery and the second experimental test session (T2) was the day after surgery. No tests were carried out on the day of anaesthesia and surgery itself. A similar time interval was used for control horses. All tests were carried out by one of two investigators who were not blinded to treatment group.

The same protocol was followed for the sequence of tests carried out at T1 and T2 with all tests carried out while the horse was in it's own stable. At the start of each test session (T1, T2) sedation was scored using a Simple descriptive Scale (SDS) (Table 1) (Love et al. 2013), the horse's personality was scored using an SDS (Table 2) (adapted from Wulf 2103) and pain was scored using a composite pain scale (CPS (Bussieres et al. 2008)), Table 3. Subsequently two cameras (Legria HFM406, Canon Inc, UK) were mounted in the stable to ensure that the whole stable was under surveillance during video recording of the novel object test.

Novel object test

The novel objects, a swimming noodle, approximately 1 m long and 10 cm in diameter, (Kandytoys, 892026, Figure 1) and a diving flipper, approximately 50 cm long, 20 cm wide and 5 cm deep (Hot Tuna, 881008) (Figure 1) were placed in the stable at the positions shown in Figure 2. The investigator then left the stable, the lower door of the stable was closed and ten minutes was timed from the moment the observer exited the stable. The observer remained out of visual contact with the horse during this 10 minute period. For the second test session (T2) the position of the novel objects was switched, so that the noodle was placed where the flipper had been positioned and vice versa to maintain novelty (Figure 2). The video footage recorded during the novel object test was analysed after the end of experiment and interactive attention and non interactive attention with the novel objects was recorded (see tables 5a,b,c).

Auditory test

The auditory test was conducted immediately after the novel object test before the novel objects had been removed from the stable. The investigator stood directly outside the stable door in the middle, facing the horse. The lower half of the stable door was closed, and the upper half of the stable door was open so that the investigator was in direct visual and auditory contact with the horse. A hairdryer was then blown at the horse for five seconds at each power setting; low (98 dB), medium (112 dB) and high (116 dB) with a 40 second break between each stimulus. The noise levels produced by the hairdryer was confirmed once using a sound level meter positioned close to a horse while the hairdryer was held outside the stable in the same position as during testing. The horse’s reaction to each setting was recorded using a SDS (Table 4). The score from each setting was added to give a total score ranging from 0 to 12. Video recording was stopped at the end of the auditory test and the objects were removed from the stable.

Data analysis

A single non-blinded researcher analysed the video recordings using The Observer XT 11 software (Noldus Information Technology bv, The Netherlands). Footage from camera 1 was coded first and adjusted accordingly using camera 2 footage. The total time each horse spent performing the behaviours defined in Tables 5 a,b,c were calculated for each 10 minute period. For the auditory test the “total auditory score” (the sum of the auditory scores from each setting of the hairdryer) was compared within and between groups. The “average personality score” (average score during T1 and T2) was used to investigate correlations between novel object test and auditory score data and horse personality.

Statistical analyses

Statistical analysis was performed using the statistical package SPSS for Windows (IBM, Version 21.0). Behaviours were divided into two different categories: “interactive attention” (total time spent interacting with objects) and “non-interactive attention” total time looking at, but not interacting with, the objects). Total time attributed to each of these behavioural categories was compared between groups (G1 and G2) for each test and between tests (T1 and T2) for each group. Data were found to be non-normally distributed, therefore nonparametric tests were used throughout. A Mann-Whitney U test was used to evaluate the statistical significance of differences in scores between groups and a Wilcoxon Signed Rank test was performed for within group comparisons. A 2-tailed Spearman’s Coefficient of Rank was used to assess correlations. The level of significance was set at $P < 0.05$.

Results

Demographics

There was a significant difference (U=47, p=0.002) in age between groups; G1 horses had a mean age of 6 years (range 0.5-20 years) compared to the mean age of 10.8 years (range 5-21 years) in G2. Thoroughbreds (TB) and thoroughbred cross (TB x) horses were over represented within the study (n=16), due to the nature of the hospital caseload. The surgical procedures that G1 horses underwent are described in Table 6a, as are the reasons for admitting the G2 horses to the clinic (Table 6b).

Horses were stabled for between 0 and 15 days (median = 0) prior to the start of Test 1 and there was no significant difference in this time period between groups. All testing was carried out between the hours of 07.30 - 15.30, or 19.30 -21.30. There was a significant difference (p<0.001) between the time of day of T1 and T2 for the surgery group, with more testing performed in the evening for T1 and in the morning for T2. This can be explained by the arrival and departure times for horses in G1. There was no significant timing variation within G2.

Sedation and Composite Pain Scores

With the exception of one horse, all horses scored 0 for sedation score, indicating that they were not sedated during T1 and T2. One horse in G1 was awarded a sedation score of 1, indicating that it was mildly sedated during T1. Composite Pain Scale scores for T1, ranged from 0 to 3 (median = 0) in groups G1 and G2, with no horses scoring greater than 0 in G2. There was no significant difference in CPS between each group at timepoint T1. Composite Pain Scale scores for T2 ranged 0 to 14 (median = 3) in G1 and from 0 to 4 (median = 0) in G2. There was a significant increase in CPS score within G1 between T1 and T2 ($p<0.001$).

Novel object test

The median (range) time that horses spent interacting with novel objects was 57.5 (367) seconds in G1 and 30 (246.05) seconds in G2 at T1. The median time was significantly decreased in G1 at T2 (12.4 (495) seconds) ($p=0.0005$), (Figure 3), but remained the same in G2 (G2 T2 24 (452) seconds) ($p=0.532$). No statistically significant differences in total interaction time between groups for either T1 or T2 were found.
Similarly, G1 horses spent less time looking at the objects in T2 compared with T1 ($p=0.0006$); 103.6 (407.6) and 28.3 (540) seconds for T1 and T2 respectively (Figure 4). No difference between tests was found for the G2 and no significant differences were found between groups for T1 or T2. Point behaviours were rarely observed during the novel object test and were not analysed statistically between or within groups.

Auditory test

Overall the behavioural reaction to the auditory stimulus was mixed and specific to the individual horse. Median (range) Total Auditory Score was 7 (9) and 10 (11) in G1 and G2 respectively at T1, decreasing to 6 (10) in G1 after surgery and at 9.5 (11) in G2. There was a significant decrease in Total Auditory Score within G1 between T1 and T2 ($p=0.003$). There was a significant difference in Total Auditory Score between G1 and G2 at T2 ($p=0.0169$), with the score being lower in G1 than G2.

Relative change in total interaction time and CPS between T1 and T2

The relative change in interactive attention, CPS score and auditory score were calculated to account for individual variance, by subtracting the values at T2 from the values at T1. A significant difference, in the individual relative change, between the G1 and G2 was found for change in interaction time (p=0.004), change in CPS score (p<0.001) and difference in auditory score (p=0.007). A significant negative correlation was found between the difference in CPS and difference in interactive attention (p=0.006) implying, the greater the increase in CPS the greater the decrease in attention (Figure 6).

Effect of horse personality

Most horses were easily approachable with average personality score ranging between 0 and 3.5 (median = 1), there was no significant difference in score between groups (p=0.683) or tests. There was no correlation between average personality score and non-interactive attention (p=0.510), interactive attention (p=0.655), or auditory score (p=0.065).

Discussion

This study investigated the effects of post-surgical pain on attention modulation, using two experimental paradigms in which attention was measured. There are no recognized standardized tests of attention in horses therefore the novel object test was adapted from similar types of test described in the laboratory animal literature (e.g. Aloisi et al. 1995). The auditory startle test was developed following discussions with experts in animal behavior and was loosely based on the principle of the acoustic startle test that is commonly used in laboratory animals (Crawley 1999). The results indicate that post-surgical pain negatively impacts non-sustained, non-selective attention towards novel objects. As predicted, post-surgical pain also decreased responsiveness to an auditory startle test. Together, these results demonstrate that post-surgical pain has an interruptive effect on attention demanding tasks in horses.

Novel object paradigm

In horses undergoing surgery a significant overall reduction in both interactive and non-interactive attention towards novel objects was found after surgery compared with before surgery. This is consistent with the findings of similar studies investigating the effect of pain on attention in rats and humans. It is also noteworthy that although all surgical horses experienced some degree of post-surgical pain, the CPS scores were not particularly high, yet an effect on attention was still apparent. Pain is intrinsically threatening, thereby disrupting attention (Johansen et al. 2001) and leading to prioritisation of behavioural actions that are important for escape or avoidance (Fields 2000). The mechanisms behind attentional modulation of pain are not fully understood and are likely to involve several areas of the central nervous system (CNS) (Villemure and Bushnell 2002). For example the frontal cortex, amygdala, periaqueductal gray (PAG), rostral ventral medulla, spinal cord dorsal horn, anterior cingulate cortex (ACC) and the thalamus, have all been shown to be associated with pain in man and other mammals (Villemure and Bushnell 2002) and are also involved in control of attention (Eccleston and Crombez 1999; Tracey et al. 2002; Gatzounis et al. 2014).

Responses to the novel object tests did not differ in G2 horses at the two time points supporting the contention that the observed decrease in attention in horses undergoing surgery was due to pain rather than habituation to the novel objects alone. As predicted there was no significant difference between G1 and G2 horses in attention times before surgery, which probably reflects the very low or no pain levels in G1 before surgery. However it was predicted that there would be a difference in attention times between groups after surgery. The lack of a statistically significant difference between groups after surgery may be attributed to the great variability between individual horses in attention levels. Contrary to expectations no relationship between horse personality score and attention to the novel objects was found. A personality test was included in the study as it is suggested (Lansade et al. 2008) that the response to a novel object may be affected by a horse's general temperament. However, this was not proven to be the case in this study. It is possible that the sample size included in the study was too small to detect any correlations between personality score and attention to novel objects, particularly because the range of personality scores was narrow in the test population of horses.

Auditory startle test

Reinforcing the findings of the novel object test, there was also a statistically significant decrease in response to the auditory scores, between tests, for horses undergoing surgery (G1) but not in the control (G2) horses. However, in contrast to the novel object test, there was also a significant difference between groups at T2. It is reasonable to attribute this decrease in responsiveness to the mechanisms outlined above, assuming that pain is an attention-demanding modality. If pain is distracting from the auditory stimulus, the sound may appear less startling than for those animals free from pain and thus able to fully attend to the stimuli and react with more vigor. In pain free humans, a similar decreased responsiveness to auditory stimuli has been demonstrated when attention was diverted to other cognitively demanding tasks (Valls-Solé et al. 1997). The startle reflex consists of a rapid response with the likely purpose of facilitating the flight reaction in a threatening environment. This reflex is a cross-species response to sudden and intense stimulation (Grillon and Baas 2003).

Previous studies investigating auditory startle reflexes and pain have shown mixed results; from no comparable difference between painful and non-painful subjects, to a hyper-vigilant response in painful subjects. For example, Combez et al. (Crombez et al. 1996, Crombez et al. 1997) found startle intensification associated with phasic pain in people. Whereas, Horn et al. (Horn et al. 2012a,b), also in man, failed to find an association between potentiation of the startle response and tonic pain. The main differences between these studies lies in the predictability of the painful stimuli. Crombez et al. (1996) applied short (5 seconds) phasic heat pulses of different (painful and non-painful) intensities, in a random order, so subjects were unable to predict the painfulness of the impending stimulus. In contrast Horn et al. (Horn et al. 2012 a,b) delivered tonic, predictable stimulation with regards to intensity and time course. These results could suggest that phasic pain elicits a rapid flight response to enable immediate escape from threat, therefore amplifying a startle response. In contrast, tonic pain, which can be defined as a continuous challenge of bodily function managed by persisting stress responses (Horn et al. 2012a), was associated with an unchanged or decreased startle. The latter fitting with the results of the present study.

In a more recent study, Horn and Lautenbacher (2014) suggested that the threat level associated with a painful stimulus, which is also determined by previous experiences, is critical for triggering startle intensification. This theory provides a rational explanation of why a hyper-vigilant state was not detected in G1 after surgery in the present study. During T1 horses encountered the auditory stimulus free from any pain-associated threat. Therefore during T2, G1 horses did not experience any anticipatory fear response to provoke a startle potentiation. Some reduction in response to the second auditory stimulus attributable to habituation cannot be discounted as a similar, but not statistically significant decrease was also found between T1 and T2 for the Control (G2) group.

Study limitations

There are several limitations to this study. First, observer bias during video analysis and assessment of response to the auditory stimulus cannot be totally excluded as the observer was not blinded to treatment group. However, descriptions of each scoring system and definitions of each behaviour dictating both interactive and non interactive attention were clearly specified to minimise any potential bias. It would have been preferable to recruit a new researcher to analyse the videos who was blinded to treatment group but this was not possible for the present study. Horses in G1 and G2 were also not individually matched for age, sex, breed, or test time, due to the limited numbers of cases available. There have been reports of gender differences in nociception in rodents (Mogil et al. 1993) and humans (Fillingim et al. 2009), with women reported to have lower pain thresholds and less pain tolerance than men (Berkley 1997) due to multiple factors including genetics and hormonal influences (Craft et al. 2004). Similar studies in horses have not been carried out, but it is plausible that stallions, geldings and mares could all have differing sensitivities to pain and this is an important consideration if further research is carried out in this area. Hormones that contribute to both pain perception and alertness also fluctuate throughout the day. For example, melatonin, a hormone that has been shown to contribute to nociceptive responses in animals and humans (Wilhelmsen et al. 2011) has a circadian rhythm of secretion. Cortisol also has the potential to suppress pain responses, which is attributed to its involvement with endogenous opioids and activity of the proopiomelanocortin peptide, which enhance analgesia (Flier et al. 1995). Therefore it would have been preferable to standardize the times that T1 and T2 were carried out for both G1 and G2 horses so that there were no differences in the times of the tests both within and between groups. Unfortunately due to the times of arrival at the hospital and discharge of the horses this was not possible and should be considered a potential confounder in the study. Six of the G2 horses were ‘teaching horses’ that reside at the study location and are exposed to a variety of situations and stimuli. It is therefore impossible to exclude the possibility of a reduced response to testing procedures due to desensitisation in this cohort of horses, limiting the likelihood of detecting differences in responses between the two testing time points T1 and T2. Ideally horses in G1 and G2 would have come from similar backgrounds with similar experiences of the yard environment before testing. However, probably the most important limitation of the study was that the G2 horses did not undergo anaesthesia; therefore the confounding effect of anaesthetic agents on attentional processing in G1 cannot be excluded. For this reason sedation was scored before testing and the second test was carried out on the day after surgery when any sedative effects of anaesthetic agents would have likely waned (Price et al. 2003). This was confirmed by average sedation scores of zero, which were constant between groups and time points. However it must be considered that detection of residual sedation can be challenging and a possible carry over effect of sedation to influence the results of T2 in the G1 horses cannot be ruled out. Future investigation could ensure that the control group undergoes anaesthesia without any surgical procedure, to rule out the effects of anaesthesia more confidently. Another potential limitation of the study was that the data were very variable between individual horses and non parametric statistics were used to analyse the data, therefore there is a risk of a Type 1 error in the statistical analysis. Finally, due to the caseload of the hospital it was not possible for all of the horses in G1 to undergo the same surgical procedure. However, a CPS was performed on each animal before each test, and a positive correlation between change in CPS and change in interaction was found. This suggests that despite the lack of a standardised surgical procedure, and analgesia, surgical horses did experience a similar level of pain, which affected attention.

Significance and future directions

The present results have important implications for the study of attention modulation associated with post-surgical pain in horses. For instance, the linear correlation between the difference in attention and difference in pain scores, suggests that attention modulation is a sensitive method of pain assessment. Further, the correlation also displayed a gradated difference in the change in interaction associated with the degree of pain at an individual level, instead of just an overall decrease at a population level. With further refinement, this change in attentional behaviour may potentially be used as the basis for a novel multidimensional approach to evaluating pain in horses, which incorporates cognitive and sensory aspects of discomfort. In conclusion, the current study is one of the first to show that post-surgical pain interrupts attention in horses. Testing of a control group confirmed that the decreased attention observed within the horses undergoing surgery were not due to habituation. While the findings of this preliminary study are exciting and offer the potential as a future biomarker of pain in equines, further research is required.

References

Aloisi AM, Albonetti ME, Carli G 91995). Behavioural effects of different intensities of formalin pain in rats. Physiol Behav 58, 603-610.

Ashley FH, Waterman-Pearson AE, Whay HR (2005). Behavioural assessment of pain in horses and donkeys: application to clinical practice and future studies. Equine Vet J 37, 565–575.

Berkley K (1997) Sex differences in pain. Behav Brain Sci 20, 371–380.

Boyette-Davis JA, Thompson CD, Fuchs PN (2008). Alterations in attentional mechanisms in response to acute inflammatory pain and morphine administration. Neuroscience 151, 558–563.

Bussieres G, Jacques C, Lainay O et al. (2008). Development of a composite orthopaedic pain scale in horses., Res Vet Sci, 85, 294-306.

Büttner W, Finke W (2000). Analysis of behavioural and physiological parameters for the assessment of postoperative analgesic demand in newborns, infants and young children: a comprehensive report on seven consecutive studies. Pediatr Anesth 10, 303–318.

Craft RM, Mogil JS, Maria Aloisi A (2004) Sex differences in pain and analgesia: the role of gonadal hormones. Eur J Pain 8, 397–411.

Crawley JN (1999). Behavioural phenotyping of transgenic and knockout mice: experimental design and evaluation of general health, sensory functions, motor abilities and specific behavioural tests. Brain Res 835, 18-26.

Crombez G, Baeyens F, Vansteeenwegen D et al (1997). Startle intensification during painful heat. Eur J Pain 1, 87–94.

Crombez G, Eccelston C, Baeyensl F et al (1996). The disruptive nature of pain: An experimental investigation. Behav Res Ther 34, 911–918.

Eccleston C, Crombez G (1999). Pain Demands Attention: A cognitive-Affective Model of the Interruptive Function of Pain. Psychol Bull 125, 356-366.

Eccleston C, Crombez G, Aldrich S et al (1997). Attention and Somatic Awareness in Chronic Pain. Pain 72, 209–215.

Fields HL (2000). Pain modulation: expectation, opioid analgesia and virtual pain. Prog Brain Res 122, 245–253.

Fillingim RB, King CD, Ribeiro-Dasilva MC et al (2009). Sex, gender, and pain: a review of recent clinical and experimental findings. J Pain 10, 447–485.

Flier JS, Underhill LH, Chrousos GP (1995). The hypothalamic-pituitary-adrenal axis and immune mediated inflammation. N Engl J Med 332, 1351-1363.

Gatzounis R, Schrooten M, Crombez G et al (2014). Interrupted by pain: An anatomy of pain-contingent activity interruptions. Pain 155 1192-1195.

Grillon C, Baas J (2003). A review of the modulation of the startle reflex by affective states and its application in psychiatry. Clin Neurophysiol 114, 1557-1579.

Hansen BD, Hardie EM, Carroll GS (1997). Physiological measurements after overiohysterectomy in dog: What is normal? Appl Anim Behav Sci 51, 101– 109.

Horn C, Blischke Y, Kunz M et al (2012). Does pain necessarily have an affective component? Negative evidence from blink-reflex experiments. Pain Res Manag17, 1–10.

Horn C, Schaller J, Lautenbacher S (2012). Investigating the affective component of pain: No startle modulation by tonic heat pain in startle responsive individuals. Int J Psychophysiol 84, 254–259.

Horn C, Lautenbacher S (2014) Modulation of the startle reflex by heat pain: Does threat play a role? Eur J Pain 19, 216-24.

Jensen MP, Karoly P (1992) Self-report scales and procedures for assessing pain in adults. In: Handbook of Pain Assessment Turk DC, Melzack R (eds) Guilford Press, New York, USA pp19–44.

Johansen JP, Fields HL, Manning BH (2001). The affective component of pain in rodents: direct evidence for a contribution of the anterior cingulate cortex. P Natl Acad Sci USA, 98, 8077–8082.

Keogh E, Cavill R, Moore DJ et al (2014) The effects of menstrual-related pain on attentional interference. Pain, 155, 821-827.

Lansade L, Bouissou MF, Erhard HW (2008). Fearfulness in horses: A temperament trait stable across time and situations. Appl Anim Behav Sci 115, 182– 200.

Lorenz J, Beck H, Bromm B (1997). Cognitive performance, mood and experimental pain before and during morphine-induced analgesia in patients with chronic non-malignant pain. Pain 73, 369–375.

Love EJ, Taylor PM, Whay HR et al (2013). Postcastration analgesia in ponies using buprenorphine hydrochloride. Vet Rec, 172, 635.

Millecamps M, Etienne M, Jourdan D et al. (2004). Decrease in non- selective, non-sustained attention induced by a chronic visceral inflammatory state as a new pain evaluation in rats. Pain, 109, 214–224.

Mogil JS, Sternberg WF, Kest B et al. (1993) Sex differences in the antagonism of swim stress-induced analgesia: effects of gonadectomy and estrogen replacement. Pain 53, 17–25.

Mogil J (2009). Animal models of pain: progress and challenges. Nat Rev Neurosci 10, 283-94.

Molony V, Kent JE (1997). Assessment of Acute Pain in Farm Animals Using Behavioural and Physiological Measurements. J Anim Sci 75, 266–272

Moore DM, Keogh E, Eccleston C (2013) Headache impairs attentional performance. Pain 154, 1840-1845.

Pais-Vieira M, Lima D, Galhardo V (2009). Sustained attention deficits in rats with chronic inflammatory pain. Neurosci Lett, 463 98–102.

Price J, Catriona S, Welsh EM et al (2003). Preliminary evaluation of a behaviour-based system for assessment of post-operative pain in horses following arthroscopic surgery. Vet Anaesth Analg 30, 124–137.

Rochais C, Fureix C, Lesimple C, Hausberger M (2016). Lower attention to daily environment: a novel cue for detecting chronic horses' back pain? Sci Rep 6:20117.

Rosenfeld GP, Johnson MM, Koo J (1993). Ongoing ischemic pain as a workload indexed by P3 amplitude and latency in real-versus feigned-pain conditions. Psychophysiology 30, 253–260.

Slater R, Cantarella A, Franck L et al. (2008) How Well Do Clinical Pain Assessment Tools Reflect Pain in Infants? PLoS Med 5, 0928-0933.

Tracey A, Ploghaus JS, Gati S et al. (2002). Imaging attentional modulation of pain the periaqueductal gray in humans. J Neurosci 22, 2748-52.

Valls-Solé J, Valldeoriola F, Tolosa E et al. (1997). Habituation of the auditory startle reaction is reduced during preparation for execution of a motor task in normal human subjects. Brain Res 751, 155–159.

Villemure C, Bushnell CM (2002). Cognitive modulation of pain: how do attention and emotion influence pain processing? Pain 95, 195-199.

Wilhelmsen M, Amiriab I, Reiter RJ et al. (2011) Analgesic effects of melatonin: A review of current evidence from experimental and clinical studies. J Pineal Res 51, 27-277.

Wulf M, Aurich J, May AC et al. (2013) Sex differences in the response of yearling horses to handling by unfamiliar humans. Journal of Veterinary Behaviour: Clinical Applications and Research. 8, 238-244.

.

List of tables

Table 1: Simple descriptive scale used to score sedation in horses

Behavioural response	Score
Fully conscious	0
Reduced response to local activity	1
Standing, ataxic and uncaring about stimulation from handling	2
Very ataxic, would fall if moved, oblivious to local surroundings	3

Table 2: Simple descriptive scale used to score horse personality; the passive test involved the observer entering the stable and standing motionless by the door. The active test involved approaching the horse with arm outstretched and hand flat.

Passive Observer - Behavioural Response	Score
Approaches readily, constant sniffing and nibbling	0
Shows interest, some interaction	1
No interest in interacting	2
Fear or anxiety response	3
Active observer - Behavioural response	**Score**
Accepts touching, moves towards observer	0
Accepts touching, some reluctance to remain in contact	1
Moves away from observer, eventually allows contact	2
Moves away from observer, cannot make contact	3

Table 3: Composite pain score used to quantify pain in horses (Bussieres et al. 2008)

Behaviour	Criteria	Score
Heart Rate	Normal compared to baseline value (increase < 10%)	0
	11-20% increase	1
	31-50% increase	2
	>50% increase	3
Respiration rate	Normal compared to baseline value (increase < 10%)	0
	11-20% increase	1
	31-50% increase	2
	>50% increase	3
Digestive sounds	Normal motility	0
	Decreased motility	1
	No motility	2
	Hypermotility	3
Rectal temperature	Normal compared to baseline value (<0.5°C variation)	0
	Variation < 1°C	1
	Variation <1.5°C	2
	Variation <2°C	3

Response to treatment	Criteria	Score
Interactive behaviour	Pays attention to people	0
	Exaggerated response to an auditory stimulus	1
	Excessive to aggressive response to an auditory stimulus	2
	Stupor, prostration, no response to auditory stimulus	3
Response to palpation	No reaction	0
	Mild reaction	1
	Resistance	2
	Violent reaction	3

Appearance	Criteria	Score
Reluctance to move/anxiety/agitation	Bright, lowered head and ears, no reluctance to move	0
	Bright and alert, occasional head movements, no reluctance to move	1
	Restlessness, pricked ears, abnormal facial expression, dilated pupils	2
	Excited, continuous body	3

	movement, abnormal facial expressions, dilated pupils	
Sweating	No obvious signs of sweat	0
	Damp to touch	1
	Wet to touch, beading apparent	2
	Excessive dripping	3

Behaviour	Criteria	Score
Kicking at abdomen	Quietly standing, no kicking	0
	Occasional (1-2 times / 5 mins)	1
	Frequent (3-4 times / 5 mins)	2
	Excessive (> 5 times / 5 mins)	3
Pawing at floor, hanging limbs	Quietly standing no kicking	0
	Occasional (1-2 times / 5 mins)	1
	Frequent (3-4 times / 5 mins)	2
	Excessive (> 5 times / 5 mins)	3

Posture, weight distribution, comfort	Stands quietly, normal walk	0
	Occasional weight shift, slight muscle tremor	1
	Non weight bearing abnormal weight distribution	2
	Analgesic posture, attempts to urinate, prostration, muscle tremor	3
Head movement	Head straight ahead, no discomfort	0
	Intermittent head movement, laterally or vertically, flank watching 1-2 times/5 mins, lip curling 1-2 times /5 mins	1
	Rapid head movement, laterally or vertically, flank watching 1-2 times/5 mins, lip curling 1-2 times /5 mins	2
	Continuous head movement laterally or	3

	vertically, flank watching laterally or vertically, flank watching >5 times/5 mins, lip curling >5 times /5 mins	
Appetite	Eats hay readily	0
	Hesitates to eat hay	1
	Shows little interest, takes some in mouth	2
	Neither shows interest in nor eats hay	3

Table 4: Simple descriptive scale used to measure the startle response to a novel auditory stimulus in horses

Reaction –low/medium/high setting	Score
No reaction	0
Ear movements (turned one or both ears)	1
Lateral head movement (turned head to look to sound’s origin)	2
Head straightened in a vigilance position	3
Startled – move/jump away	4

Table 5a: Interactive behaviours recorded during the observation period for the novel object test in horses

Behaviour	Definition
Sniffing flipper/noodle	No contact with objects, but with muzzle in close proximity, nostril movement seen
Nuzzling flipper/noodle	Muzzle in contact with the object, potentially moving the object around
Licking flipper/noodle	Tongue seen and/or licking noises heard
Biting flipper/noodle	Mouth opened and/or biting noises heard
Picking up flipper/noodle	Object lifted from the ground or moved around using teeth
Pawing flipper/noodle	Pawing directed towards an object or while the head was interacting with an object

Table 5b: Non-interactive behaviours recorded during the observation period for the novel object test in horses

Behaviour	Definition
Direction of gaze	Looking at the flipper / noodle
Ignoring flipper / noodle	No interaction with the flipper or noodle

Table 5c: Point behaviours recorded during the observation period for the novel object test in horses

Behaviour	Definition
Snort	Loud exhalation of air from the nostrils
Sniffing air	Nostrils flared or loud exhalation directed at any object
Flehmen	Lip curled upwards, head raised
Pawing ground	Pawing not directed towards or in association with any object

Table 6a: Summary of surgery horses (G1)

Horse number	Breed	Sex	Age (years)	Type of surgery
1	TB	Gelding	4	Prosthetic Laryngoplasty
2	Irish Sportshorse	Gelding	9	Prosthetic Laryngoplasty
3	TB	Gelding	2	Prosthetic Laryngoplasty
4	Clydsedale	Mare	1	Tarso-Crural Arthroscopy
5	TB	Gelding	Unknown	Ventriculocordectomy
6	Irish Sportshorse	Stallion	0.5	Mass resection and Castrate
7	New Forest	Stallion	4	Castration
8	Warmblood x	Gelding	14	Penile amputation
9	TBx	Mare	5	Wound debridement
10	TBx	Gelding	5	Neurotomy and Fasciotomy
11	Welsh Section B	Gelding	6	Cystolith removal
12	TB	Gelding	4	Ventriculocordectomy
13	Cob x	Gelding	5	Sarcoid Removal
14	TB	Gelding	5	Soft Palate Cautery
15	Anglo Arab	Gelding	20	Mass removal

16	TB x	Gelding	14	Frontal Sinus Flap
17	TB		4	Soft Palate Cautery
18	TB	Gelding	4	Soft Palate Cautery
19	TB	Gelding	6	Soft Palate Cautery and Ventriculocordectomy
20	TB	Gelding	8	Soft Palate Cautery

TB = thoroughbred

x =cross breed

6b: Summary of control horses (G2)

Horse number	Breed	Sex	Age (years)	Procedure
1	Cob x Connemara	Mare	6	Diagnostic investigations for headshaking
2	Cob	Gelding	11	Diagnostic investigations for headshaking
3	TB	Gelding	7	Diagnostic investigations for poor athletic performance
4	TB	Gelding	5	Diagnostic investigations for poor athletic performance
5	Cob	Mare	17	Teaching horse
6	TB	Gelding	6	Diagnostic investigations for poor

				athletic performance
7	Irish Draught horse	Mare	19	Teaching horse
8	Unknown	Mare	5	Diagnostic investigations for poor athletic performance
9	Warmblood	Gelding	7	Boarding
10	Unknown	Mare	Unknown	Teaching horse
11	Appaloosa	Mare	21	Teaching horse
12	Cob x	Mare	17	Teaching horse
13	Irish Sportshorse	Mare	12	Re-examination
14	TB	Gelding	9	Re-examination
15	Pony	Mare	Unknown	Teaching horse
16	Welsh mountain pony x	Mare	9	Boarding

TB = Thoroughbred

x = cross breed

List of figures

Figure 1: Photo of the swimming noodle and flipper used as novel objects in the study

Figure 2: Schematic diagram to show the layout of stable for each test. Each camera recorded footage from the object in the opposite corner. Different sections of the stable used to code the position of the horse throughout the observation period are outlined.

Figure 3: Median, IQR and range of total time spent interacting with novel objects, before and after surgery in G1 (n=20) and G2 (n=16) horses. A significant decrease in interaction time was found for Surgery Group (G1), between T1-and T2 ($p = 0.0005$), Wilcoxon Signed Ranks test.

Figure 4: Median, IQR and range of total time spent looking at, but not interacting with, novel objects before and after surgery in G1 (n=20) and G2 (n=16) horses. A significant decrease in time spent looking at the objects was found for G1, between T1-and T2 ($p=0.0006$) Wilcoxon Signed Ranks test.

Figure 5: Median, IQR and range of Total Auditory Scores, before and after surgery in G1 (n=20) and G2 (n=16) horses. A significant decrease in Total Auditory Score was found for G1 between T1-and T2 ($p=0.003$), Wilcoxon Signed Ranks test. In T2, there was a significant difference between groups, with G1 scoring lower than G2 ($p=0.0169$) Mann-Whitney U test.

Figure 6: Analysis of relative change in interactive attention, CPS score and auditory score were calculated to account for individual variance, by subtracting the values in T2 from the values in T1. Data show a negative correlation between the difference in CPS and difference in interactive attention ($p= 0.006$, $r_s = -0.451$), implying, the greater the increase in CPS the greater the decrease in attention.

Figure 1

Figure 2

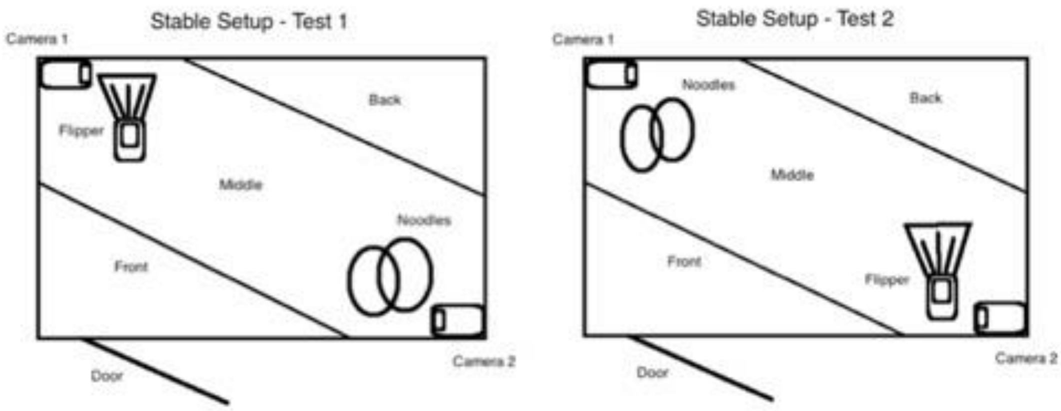


Figure 3

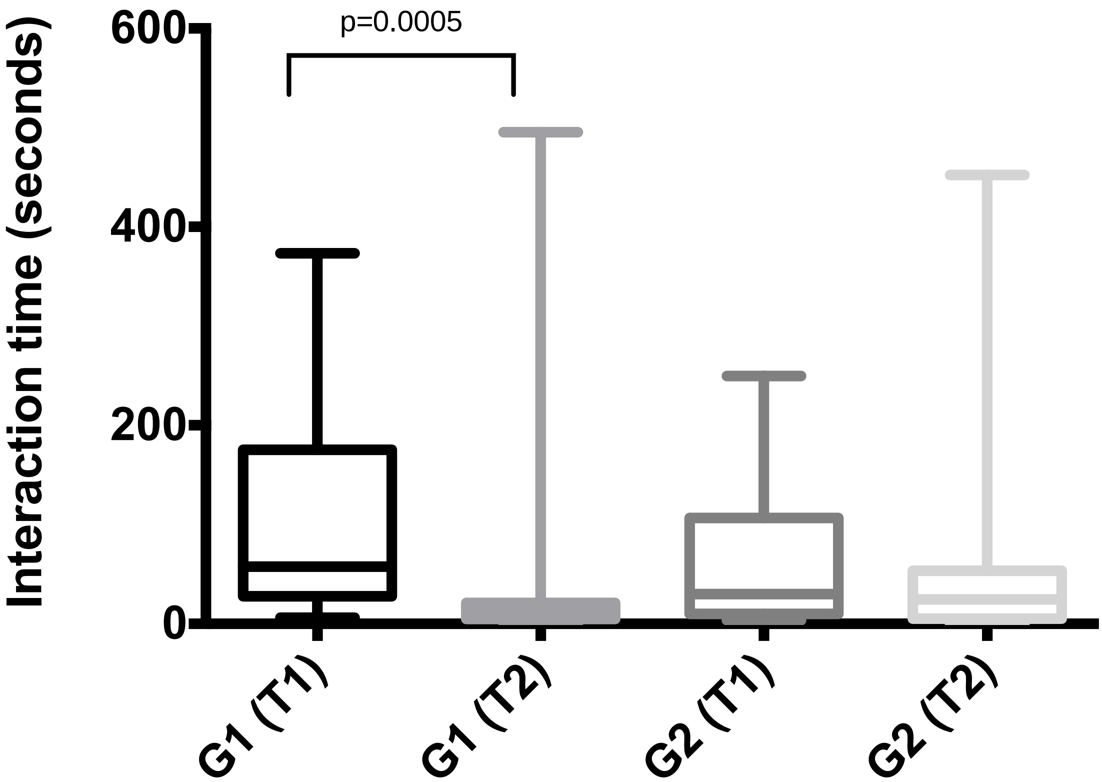
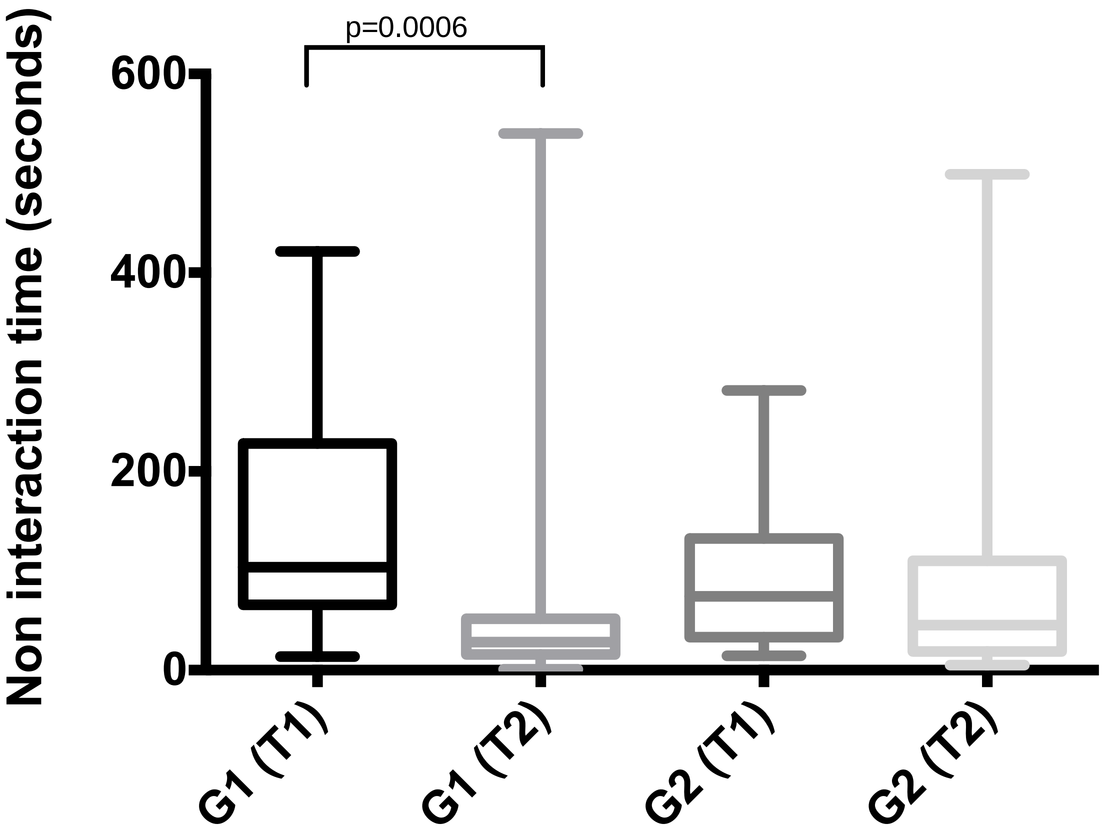
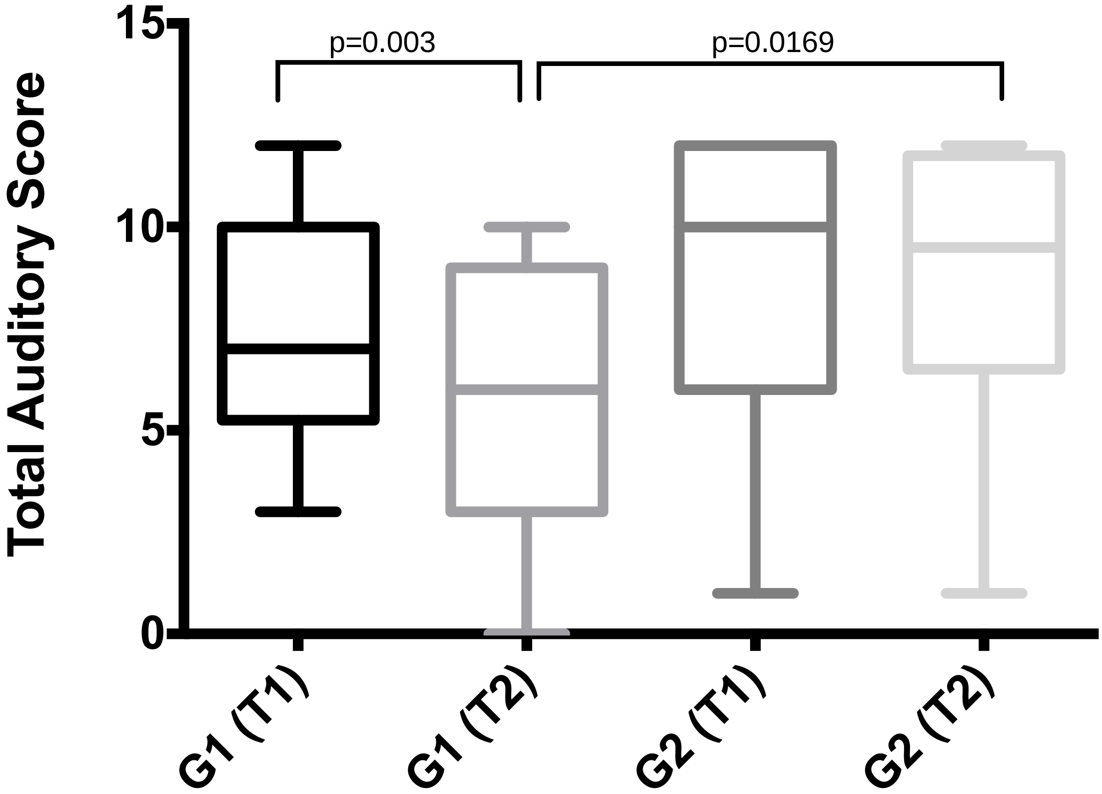

Interaction time (seconds)
600
400
200
0
p=0.0005
G1 (T1)
G1 (T2)
G2 (T1)
G2 (T2)

Figure 4



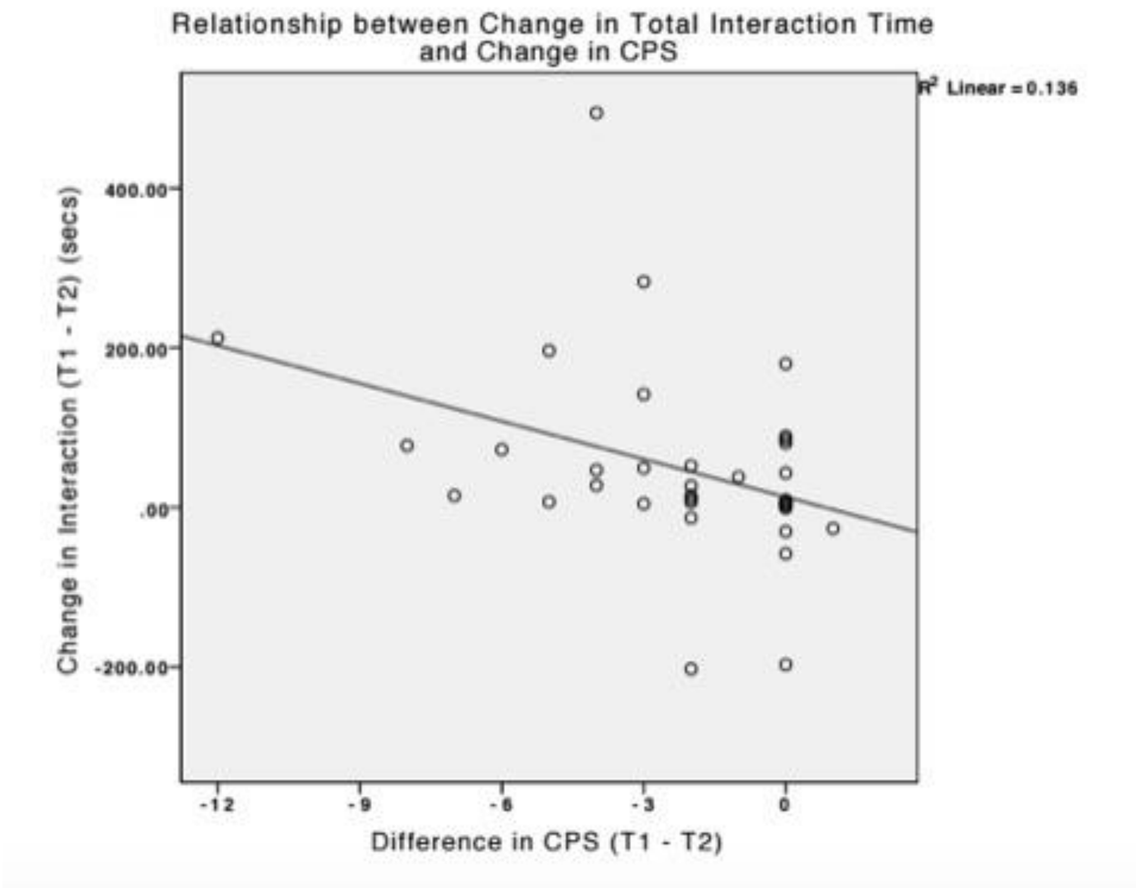
p=0.0006
600
400
200
0
Non interaction time (seconds)
G1 (T1)
G1 (T2)
G2 (T1)
G2 (T2)

Figure 5



p=0.003
p=0.0169
15
10
5
0
Total Auditory Score
G1 (T1)
G1 (T2)
G2 (T1)
G2 (T2)

Figure 6


Relationship between Change in Total Interaction Time and Change in CPS
R² Linear = 0.136
Change in Interaction (T1 - T2) (secs)
400.00
200.00
.00
-200.00
-12
-9
-6
-3
0
Difference in CPS (T1 - T2)