Poker Face: Discrepancies in behaviour and affective states in horses during stressful handling procedures

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ABSTRACT

Correct assessment of stress in horses is important for both horse welfare and handler safety during necessary aversive procedures. Handlers depend on behaviour when judging how well an individual is tolerating stressful procedures such as loading or veterinary intervention. However, evidence suggests that behaviour may not accurately reflect affective states in horses. This may be explained by individual differences in coping styles, which have tentatively been identified in horses. The current study assessed whether behaviour during two novel handling procedures was associated with physiological indicators of stress. Core temperature, discrepancy in eye temperature and heart rate variability (HRV) were compared with compliance and proactivity shown by horses during two novel handling tests (n = 46). Test A required subjects to cross a large blue tarpaulin on the ground. Test B required subjects to walk through plastic streamers suspended overhead. Physiological indicators of stress did not correlate with time taken to complete the handling tests. This indicates some subjects crossed an object they found aversive. Crossing time may be influenced more by stimulus-control than the level of aversion experienced. The level of proactivity shown was not associated with HRV, HR, core temperature or the discrepancy in temperature between eyes. This suggests that proactive horses, which appear more stressed, show similar stress responses to more reactive individuals. These findings support previous research indicating that behaviour commonly used within the equestrian industry may not provide reliable indicators of a horse's ability to tolerate a stressful procedure. The influence of training and the extent to which a horse is under stimulus-control may over-shadow inherent emotional responses, with implications for handler safety and horse welfare.

KEYWORDS: infrared thermography, heart rate variability, laterality, welfare, coping, handling stress

1. INTRODUCTION

Correct interpretation of stress-induced behaviour is critical for animal welfare (Cook et al., 2000). However, individual differences may confound behavioural measures of stress. Consistent individual differences in behaviour are stable across time and contexts and are mediated by physiological differences (Koolhaas et al., 2010). Included within these variations is the way in which an individual may react, both behaviourally and physiologically, to that of a perceived threat or challenging occurrence. Differing responses to stress, termed coping strategies, exist on a continuum from proactive to reactive (Koolhaas et al., 1999). More proactive individuals attempt to exert control by eliminating the stressor, or removing themselves from the source of stress. Reactive strategies are characterised by freeze responses, emotional blunting and unresponsiveness (Koolhaas et al., 1999). Despite more active behavioural responses to stress in proactive individuals, reactive individuals are known to have more pronounced physiological responses to stress (Koolhaas et al., 2010).

Proactivity has been tentatively identified in horses (Ijichi et al., 2013). During a mild handling stressor, subjects were observed showing differences in behavioural response that shared characteristics of proactivity in other species (Koolhaas et al., 2010). Whilst more proactive horses appeared to be more stressed when asked to cross a novel surface, these individuals were just as likely as their more reactive counterparts to eventually cross the bridge. Further, the level of compliance shown by equine subjects during sham clipping procedures (Yarnell et al., 2013) and police horse training (Munsters et al., 2013) is not associated with physiological indicators of stress. In addition, behaviour in a clinical setting was not predictive of actual tissue damage sustained in horses (Ijichi et al., 2014). Taken together, these studies suggest that compliance and behaviour in horses may not accurately reflect underlying affective states in response to aversive procedures or experiences.

Horses are prey animals and have developed functionally adaptive fear and related flight responses, resulting in increased species fitness (McGreevy et al., 2009). Novel objects, situations and sounds may all induce fear and illicit the motivation to flee (McLean and McGreevy, 2010). Routine procedures such as veterinary intervention, clipping, farriery, loading and travelling, training and aversive objects may trigger this response. As practitioners within the equine industry rely primarily on behaviour when determining whether an individual is coping with a stressor, incorrect interpretation of behaviour presents a potentially significant welfare compromise and may risk human safety.

Stress can also be measured using a number of physiological indicators. Infrared thermography (IRT) has been used to measure increased core temperature as an indicator of stress in a variety of species such as cattle (Stewart et al., 2008), cats (Foster and Ijichi, 2017) and dogs (Travain et al. 2015; Lush & Ijichi, Accepted). Eye temperature has shown promise as a measure of stress in horses when validated against cortisol and may be useful in reducing adverse impacts on their welfare (Yarnell et al., 2013). Overall, core temperature changes in response to emotional arousal (Valera et al., 2012) and pain (Stewart et al., 2008). In addition, there is some evidence for lateralised discrepancy in eye temperature (Lush & Ijichi, Accepted), which may indicate ipsilateral hemispheric dominance. Whilst it is recognised that lateralised cerebral blood flow can be detected via pinnae (Riemer et al., 2016), variation in individual morphology may confound results when using this method.

Ocular temperatures are not subject to the same variation such as coat length or thickness. IRT can be paired with changes in heart rate variability (HRV) as indicators of psychological stress. The variability of time between heart beats in animals is not precisely consistent. Evidence from behavioural studies suggest that a reduction in the variation between successive beats may indicate a neurophysiological response to stress, independent of the intensity of physical exertion (Ille et al., 2014; Rietmann et al., 2004). This measure, taken in conjunction with other physiological measures such as eye temperature, indicates a stress response.

Correct assessment of the relationship between behaviour and underlying stress is critical for handler safety and horse welfare. In addition, strategies used to modify behaviour may depend on whether the handler interprets the behaviour as fearful or "stubborn". However, interpreting behaviour may be confounded by individual differences in stress response (Coppens et al., 2010; Ijichi et al., 2014; Lush and Ijichi, Accepted). Whilst previous studies have provided preliminary evidence that behaviour may not reflect internal states in horses (Munsters et al., 2013; Yarnell et al., 2013), they did not investigate whether coping strategies may explain this discrepancy (Ijichi et al., 2013). The aim of the current study was to determine whether behaviour is associated with physiological indicators of stress in horses during novel handling tests and whether this relates to coping strategies. This was achieved by comparing core temperature, discrepancy in eye temperature and heart rate variability in horses with compliance and proactivity shown during two novel handling tests. Two mutually exclusive hypotheses for the relationship between compliance and stress were made. First, that less stressed horses would take less time to complete the tasks, as might be expected. Second, that stress is not associated with compliance as observed by Yarnell et al. (2013) and Munsters et al. (2013). With regards to proactivity, it was hypothesised that more reactive individuals would show a greater physiological stress response, as observed in other species (Koolhaas et al., 2010).

2. METHOD

A sample of 46 privately owned horses (26 geldings and 20 mares) were sourced from Hartpury College liveries. Age of subjects ranged between 3 – 20 years (mean = 9.33 ± 4.20) and subjects were of mixed breeds. Subjects were housed and managed as per owner preferences on a large livery yard. In general, subjects were provided forage three times a day with hard-feed dependent on workload and nutritional requirements and constant access to fresh water. They were individually stabled with a minimum of 1 hour of exercise each day but limited turn-out at the time of testing. The current study took place within the indoor holding arena at Hartpury College Equestrian Centre, Gloucestershire (UK) during October 2016. Testing took place in the subject's home environment to reduce the effect of environmental novelty (Wolff et al., 1997). Subjects were handled in their own headcollar and a long lead rope was provided. Headcollars with inbuilt pressure mechanisms were not permitted.

2.1 Handling Tests

Subjects completed two novel handling tests where they were asked to navigate two distinct obstacles. Test order was randomised and horse order was pseudo-random depending on the availability of owners. The start of each test was marked by a horizontal pole placed on the ground 2m in front of the obstacle. A video camera was used to record each attempt to accurately identify crossing time and the subject's refusal behaviour. Task A consisted of a 2.5m x 3m blue tarpaulin secured to the surface of the indoor holding arena by 20 individual tent pegs. To complete this test, the subject walked over the tarpaulin. Test B consisted of two jump wings extended to a height of approximately 2.5m with a 1.6m long pole suspended over-head, from which hung 2m long plastic streamers. To complete this test, the subject walked under the overhead pole, causing the streamers to touch the face and body of the subject as they passed through.

The current study was part of a wider project which also investigated the effect of familiarity on horse behaviour during handling (Ijichi et al., Submitted). Therefore, horses were handled once by their owner and once by an experimental handler (CI). Handler order was randomised for each subject. There was no difference in behaviour or physiology between familiar and unfamiliar handlers. The handler attempted to lead each horse over the tarpaulin or under the streamer obstacles using only pressure on the lead-rope as a cue to the horse. No additional pressures, verbal commands or further encouragement such as whips were used.

Crossing time for each test began when the subject's second front hoof crossed over the pole and bore weight on the ground. For Test A, time stopped when the last rear hoof bore weight on the tarpaulin. Horses engage their rear legs first when transforming into faster gaits. Therefore, horses that showed a flight response on the tarpaulin were not give faster crossing times. For the attempt to be classed as a successful crossing all four hooves must have, at some stage, been placed onto the tarpaulin. Crossing Time for Test B stopped once the whole body of the subject passed between the jump wings supporting the streamers. A time limit of 3 minutes was allotted for each attempt as previous research indicated that subjects which had not completed the test within this time were unlikely to do so (Ijichi et al., 2013). Once the

3 minute threshold had been reached the test was ended. A crossing time of 180 seconds was given to any horse reaching this time limit.

Refusal behaviour was defined as any behaviour which did not contribute to crossing the object. This included moving backwards, sideways, forwards but away from the tarpauling, rearing or remaining stationary. Refusal that lasted for 10 seconds or more was analysed to determine how proactive that refusal was (Test A: N = 13, Test B: N = 36). Proactive refusal was defined as any refusal behaviour that involved movement. Proactive refusal was then recorded as the percent of total refusal time for any individual which showed refusal behaviour (which included remaining stationary). A higher value indicated a greater amount of proactive behaviour (Ijichi et al., 2013).

2.2 Eye Temperature Measurement

A FLIR E4 thermal imaging camera (FLIR Systems, USA.) was used to record eye temperature. Images were taken at a distance of approximately 1m from the subject and at an angle of 90º (Travain et al., 2015; Yarnell et al., 2013). Eye temperature images of each subject's left and right eyes were taken on entering the arena prior to each test and immediately after testing. All images were taken by the same researcher each time (KS). Subjects were positioned between two parallel jump poles in the same position and direction within an enclosed arena without direct sunlight. This was to reduce the potential confounding effects of environmental factors, which may confound the accuracy of infrared thermography readings (Church et al., 2014).

Images were analysed using FLIR Tools software (ver. 5.9.16284.1001) to obtain a measurement for each eye. Eye temperature recordings were the maximum temperature within the palpebral fissure from the lateral commissure to the lacrimal caruncle (Yarnell et al., 2013). A mean of the left and right eyes was calculated for each subject, pre and post-test, for each test. In addition, the temperature of the left eye was subtracted from the right eye to indicate the discrepancy between both eyes, pre and post-test, for each test. A positive score indicates a hotter right eye, whilst a negative score indicates a hotter left eye.

2.3 HR / HRV measurement

Polar Equine V800 equipment was used (Polar Electro Oy, Kempele, Finland) to monitor the heart rate of thirty-five subjects. Prior to entering the arena the Polar elasticated adjustable surcingle was attached to the girth area of the subject by the same researcher each time (KG). This was moistened with water to aid conductivity and checked to ensure it was detecting HR. Subjects had a minimum of 5 minutes to habituate to the surcingle which was deemed to be sufficient as all subjects had previously worn girths and/or lunging rollers. The receiving Polar watch was worn by the handler to ensure it remained within connectivity limits at all times. HR data was measured from the point of the pre-test IRT measurement to the post-test IRT measurement.

Heart rate analysis was carried out using Kubios HRV software (ver. 2.2, Biomedical Signal Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern Finland, Kuopio, Finland.). Kubios settings were adjusted in line with previous equine studies (e.g. Ille et al., 2014). Specifically, artefact correction was set to custom level 0.3, thus removing RR levels varying by more than 30% from the previous interval. This means that if a single RR interval was more than 30% different from the preceding interval, it is deemed to be an incorrect reading. Trend components were adjusted using the concept of smoothness priors set at 500ms, to avoid the effect of outlying intervals. The STD RR value, being the standard deviation of RR intervals, was used as the HRV figure to reflect both short-term and long-term variation with the series of RR intervals.

2.6 Statistical Analysis

Statistical analysis was carried out using R (*RStudio Team, 2015).* Data normality was tested using Shapiro-Wilks, Spearman Rank correlations used, as appropriate for normality (Field, 2009). Due to the number of correlations, the False Discovery Rate was used (Benjamini and Hochberg, 1995) to adjust p-values to remove likely false discoveries (Field, 2009).

2.7 Ethics

Each owner provided informed consent for each subject via the completion of a participant information form. All data provided will be held in accordance with the Data Protection Act (1998). Both researchers and owners had the right to withdraw a subject at any time for any reason until the point of data analysis. Prior to commencement, this current study was authorised by the Hartpury College Ethics Committee (reference ETHICS2015-34).

3. RESULTS

Table 1. Mean values for measured variables with standard deviation (SD) or interquartile ranges (IQR), depending on normality.

	Test A			Test B		
Variable	**N=**	**Mean**	**IQR/SD**	**N=**	**Mean**	**IQR/SD**
Crossing Time (secs)	46	19.93	4.04 - 17.09	46	92.97	20.8 - 180
Proactivity (%)	13	66.03	46.87 - 86.42	36	16.34	1.36 - 24.33
HR	28	82.79	54.67 - 115.88	31	69.07	55.39 - 79.64
HRV	29	103.23	±47.92	31	107.66	±39.37
Pre-Test average IRT	46	33.34	±1.14	46	33.23	±1.10
Post-Test average IRT	46	33.10	±1.01	46	33.04	±0.83
Pre-Test IRT discrepancy	44	0.25	±0.86	46	0.13	±0.86
Post-Test IRT discrepancy	41	0.11	±0.75	44	0.18	-0.53

3.2 Physiology & Behaviour

95.5% of subjects crossed Test A and 61.9% of subjects crossed Test B. Crossing time did not correlate with HR for test A (r_s = 0.253, N = 28, P = 0.93) or test B (r_s = 0.222, N = 31, P = 0.93). Crossing time did not correlate with HRV for test A (r_s = 0.072, N = 28, P = 0.964) or test B (r_s = 0.113, N = 31, P = 0.93). Crossing time did not correlate with mean IRT pre-test A (r_s = -0.14, N = 46, P = 0.93), or pre-test B (r_s = -0.045, N = 46, P = 0.964). Crossing time did not correlate with mean IRT post-test A (r_s = -0.024, N = 46, P = 0.964), or post-test B (r_s = -0.061, N = 46, P = 0.964). Crossing time did not correlate with the discrepancy between eyes pre-test A (r_s = -0.239, N = 44, P = 0.93), or pre-test B (r_s = 0.041, N = 46, P = 0.964). Crossing time did not correlate with the discrepancy in temperature between eyes post-test A (r_s = -0.13, N = 46, P = 0.93), or post-test B (r_s = -0.231, N = 41, P = 0.93).

Mean proactivity correlated negatively with HR in Test A (r_s = -0.85, N = 9, P = 0.144) but not Test B (r_s = 0.193, N = 24, P = 0.93). Mean proactivity did not correlate with HRV in Test A (r_s = 0.217, N = 9, P = 0.93) or Test B (r_s = -0.132, N = 24, P = 0.93). Proactivity did not correlate with mean IRT pre-test A (r_s = -0.014, N = 13, P = 0.964), or pre-test B (r_s = 0.197, N = 33, P = 0.93). Proactivity did not correlate with mean IRT post-test A (r_s = -0.074, N = 33, P = 0.964), or post-test B (r_s = -0.163, N = 36, P = 0.93). Proactivity did not correlate with the discrepancy in temperature between eyes pre-test A (r_s = -0.028, N = 12, P = 0.964), or pre-test B (r_s = 0.104, N = 36, P = 0.93). Proactivity did not correlate with the discrepancy in temperature between eyes post-test A (r_s = 0.213, N = 13, P = 0.93), or post-test B (r_s = 0.022, N = 36, P = 0.964).

4. DISCUSSION

The aim of the current study was to investigate whether compliance is a reliable indicator of stress responses in horses, and whether this may relate to coping strategies. Physiological indicators of stress were not associated with compliance, indicated by crossing time. Crossing time did not correlate with either pre-test or post-test eye temperatures or the discrepancy between eyes. Additionally, it did not correlate with heart rate variability. It might be assumed that crossing time is an indicator of willingness to complete the handling test and that this would be associated with how stressful subjects find the procedure. Therefore, it would be expected that subjects that find the handling procedure stressful would not complete it, or would take longer to do so. These results indicate that this is not accurate, with subjects crossing the obstacles despite some exhibiting physiological signs of stress. Others refused to complete the test whilst showing less pronounced physiological indicators of stress. Overall, results support previous findings, in that a horse’s behaviour does not necessarily reflect its psychological and physiological response to a handling stressor (Munsters et al., 2013; Yarnell et al., 2013).

Horses are trained to carry out desired behaviours by stimulus control (McGreevy and McLean, 2009). This provides a possible explanation for horses crossing the test whilst stressed. Training the horse to respond reliably to stimuli from a rider or handler, rather than react to environmental stimuli, is essential within horse training to reduce conflict for the horse (McGreevy and McLean, 2009) and improve safety for the rider or handler by reducing unpredictability. A major element of stimulus control is the use of the head collar and/or bridle. Pressure on the head collar, usually via a rope or rein, is used to initiate a lead response within the horse (McGreevy and McLean 2010). This acts by a means of negative reinforcement where the horse will seek comfort by moving in an attempt to release the pressure applied (McGreevy and McLean, 2009).

It is possible that individuals that completed the handling test, despite experiencing stress, were under greater stimulus control than those that refused but displayed lower levels of stress. Whilst this is beneficial to handler safety (Thompson et al., 2015) and the reduction of conflict due to a lack of clarity on the desired response (McLean and McGreevy, 2010), it should be noted that horses may be completing tasks they find aversive due to stimulus control. It is known that stimulus control based training practices, such as over-shadowing, are effective in training horses to tolerate aversive procedures (McLean, 2008). Previous research indicates that negative reinforcement is more effective in getting horses to approach and habituate to aversive objects (Christensen, 2013), as measured using behavioural indicators. However, it is important to explore whether completing the task due to stimulus-control results in a reduced physiological stress response on subsequent attempts. A study of police horses indicates that significant habituation does not occur with repeated exposure to stressful stimuli (Munsters et al., 2013) and supports our findings that compliance in novel tests is not associated with lower physiological indicators of stress. Therefore, it is possible that horses are being subjected to aversive procedures due to their own compliance, which may result in conflict between the motivation to give the reinforced response and the unconditioned response to avoid a stressor.

Proactivity during testing did not correlate with any physiological measures of stress. Previous research indicates that reactive individuals have greater physiological stress responses than more proactive individuals (Koolhaas et al., 2007). The results of this study suggest that the magnitude of stress response is not associated with a coping strategy in horses. Behaviour observed in horses that do not immediately complete the tasks may not be comparable with coping strategies, as identified by Koolhaas et al. (1999). Instead they may be learnt behaviour which has proven successful in mitigating human influences in past experiences. Previous handlers may have aborted attempts to influence these individuals if they became intimidated by extreme activity or frustrated at complete unresponsiveness. Previous work has shown that both of these strategies are equally successful in avoiding the task (Ijichi et al., 2013).

Incorrect interpretation of the behaviour of individuals that become unresponsive may impact upon welfare if they are ascribed adjectives such as “stubborn” or “defiant”. This may be associated with punishment to reduce the expression of the behaviour, without rectifying the source of stress, or reinforcing the correct training aid (Goodwin et al., 2009). In the current study, these individuals had a similar stress response to more proactive subjects. Being unaware of stress levels in these circumstances and forcing the animal to complete a task may cause negative welfare and, in extreme cases, exposure to regular repeated aversive stimuli may lead to the development of learned helplessness (McGreevy et al., 2009). Such a development is undesirable as the animal abandons its attempts to cope and develops a 'dullness' related to a decline in motivation and emotional response.

5. CONCLUSION

The current study explored the relationship between stress, coping strategy and compliance behaviour in horses. Physiological indicators of stress did not correlate with the time taken to complete two handling tests. This indicates some subjects that found the handling tests stressful still completed them and were compliant. It is possible that crossing time is influenced more by the extent to which the subject is under stimulus-control, rather than their level of aversion. Important considerations remain regarding the effect this has on equine welfare. Further, the level of proactivity shown as a strategy to avoid completing the tests was not associated with stress. This suggests that proactive horses, which anecdotally appear to be more stressed, are in fact showing similar stress responses to more reactive individuals. Physiological responses measured here do not follow the same profile noted in other species. Therefore, it is possible that the refusal behaviour originally noted by Ijichi et al. (2013) is not comparable to consistent and stable coping strategies documented in other species by Koolhaas et al. (2010). Instead, it might be that both compliance, and the strategies used to avoid human influences, are learnt from previous handling experiences. Regardless, this suggests that behavioural indicators commonly used with the equestrian industry may not be reliable indicators of a horse's ability to tolerate a stressful procedure. The influence of training and the extent to which a horse is under stimulus-control may over-ride inherent emotional responses.

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