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

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1 **ABSTRACT**

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

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

27 1. INTRODUCTION

28 Correct interpretation of stress-induced behaviour is critical for animal welfare (Cook
29 et al., 2000). However, individual differences may confound behavioural measures of
30 stress. Consistent individual differences in behaviour are stable across time and
31 contexts and are mediated by physiological differences (Koolhaas et al., 2010).
32 Included within these variations is the way in which an individual may react, both
33 behaviourally and physiologically, to that of a perceived threat or challenging
34 occurrence. Differing responses to stress, termed coping strategies, exist on a
35 continuum from proactive to reactive (Koolhaas et al., 1999). More proactive
36 individuals attempt to exert control by eliminating the stressor, or removing themselves
37 from the source of stress. Reactive strategies are characterised by freeze responses,
38 emotional blunting and unresponsiveness (Koolhaas et al., 1999). Despite more active
39 behavioural responses to stress in proactive individuals, reactive individuals are
40 known to have more pronounced physiological responses to stress (Koolhaas et al.,
41 2010).

42 Proactivity has been tentatively identified in horses (Ijichi et al., 2013). During a mild
43 handling stressor, subjects were observed showing differences in behavioural
44 response that shared characteristics of proactivity in other species (Koolhaas et al.,
45 2010). Whilst more proactive horses appeared to be more stressed when asked to
46 cross a novel surface, these individuals were just as likely as their more reactive
47 counterparts to eventually cross the bridge. Further, the level of compliance shown by
48 equine subjects during sham clipping procedures (Yarnell et al., 2013) and police
49 horse training (Munsters et al., 2013) is not associated with physiological indicators of
50 stress. In addition, behaviour in a clinical setting was not predictive of actual tissue

51 damage sustained in horses (Ijichi et al., 2014). Taken together, these studies suggest
52 that compliance and behaviour in horses may not accurately reflect underlying
53 affective states in response to aversive procedures or experiences.

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

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

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

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

100 hypothesised that more reactive individuals would show a greater physiological stress
101 response, as observed in other species (Koolhaas et al., 2010).

102 2. METHOD

103 A sample of 46 privately owned horses (26 geldings and 20 mares) were sourced from
104 Hartpury College liverys. Age of subjects ranged between 3 – 20 years (mean = 9.33
105 \pm 4.20) and subjects were of mixed breeds. Subjects were housed and managed as
106 per owner preferences on a large livery yard. In general, subjects were provided forage
107 three times a day with hard-feed dependent on workload and nutritional requirements
108 and constant access to fresh water. They were individually stabled with a minimum of
109 1 hour of exercise each day but limited turn-out at the time of testing. The current study
110 took place within the indoor holding arena at Hartpury College Equestrian Centre,
111 Gloucestershire (UK) during October 2016. Testing took place in the subject's home
112 environment to reduce the effect of environmental novelty (Wolff et al., 1997). Subjects
113 were handled in their own headcollar and a long lead rope was provided. Headcollars
114 with inbuilt pressure mechanisms were not permitted.

115 2.1 Handling Tests

116 Subjects completed two novel handling tests where they were asked to navigate two
117 distinct obstacles. Test order was randomised and horse order was pseudo-random
118 depending on the availability of owners. The start of each test was marked by a
119 horizontal pole placed on the ground 2m in front of the obstacle. A video camera was
120 used to record each attempt to accurately identify crossing time and the subject's
121 refusal behaviour. Task A consisted of a 2.5m x 3m blue tarpaulin secured to the
122 surface of the indoor holding arena by 20 individual tent pegs. To complete this test,

123 the subject walked over the tarpaulin. Test B consisted of two jump wings extended to
124 a height of approximately 2.5m with a 1.6m long pole suspended over-head, from
125 which hung 2m long plastic streamers. To complete this test, the subject walked under
126 the overhead pole, causing the streamers to touch the face and body of the subject as
127 they passed through.

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

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

146 3 minute threshold had been reached the test was ended. A crossing time of 180
147 seconds was given to any horse reaching this time limit.

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

156

157 2.2 Eye Temperature Measurement

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

167 Images were analysed using FLIR Tools software (ver. 5.9.16284.1001) to obtain a
168 measurement for each eye. Eye temperature recordings were the maximum
169 temperature within the palpebral fissure from the lateral commissure to the lacrimal

170 caruncle (Yarnell et al., 2013). A mean of the left and right eyes was calculated for
171 each subject, pre and post-test, for each test. In addition, the temperature of the left
172 eye was subtracted from the right eye to indicate the discrepancy between both eyes,
173 pre and post-test, for each test. A positive score indicates a hotter right eye, whilst a
174 negative score indicates a hotter left eye.

175 2.3 HR / HRV measurement

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

186 Heart rate analysis was carried out using Kubios HRV software (ver. 2.2, Biomedical
187 Signal Analysis and Medical Imaging Group, Department of Applied Physics,
188 University of Eastern Finland, Kuopio, Finland.). Kubios settings were adjusted in line
189 with previous equine studies (e.g. Ille et al., 2014). Specifically, artefact correction was
190 set to custom level 0.3, thus removing RR levels varying by more than 30% from the
191 previous interval. This means that if a single RR interval was more than 30% different
192 from the preceding interval, it is deemed to be an incorrect reading. Trend components
193 were adjusted using the concept of smoothness priors set at 500ms, to avoid the effect

194 of outlying intervals. The STD RR value, being the standard deviation of RR intervals,
195 was used as the HRV figure to reflect both short-term and long-term variation with the
196 series of RR intervals.

197 2.6 Statistical Analysis

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

203 2.7 Ethics

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

210

211 3. RESULTS

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

214

Variable	Test A			Test B		
	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

215

216 *3.2 Physiology & Behaviour*

217 95.5% of subjects crossed Test A and 61.9% of subjects crossed Test B. Crossing
 218 time did not correlate with HR for test A ($r_s = 0.253$, $N = 28$, $P = 0.93$) or test B ($r_s =$
 219 0.222 , $N = 31$, $P = 0.93$). Crossing time did not correlate with HRV for test A ($r_s =$
 220 0.072 , $N = 28$, $P = 0.964$) or test B ($r_s = 0.113$, $N = 31$, $P = 0.93$). Crossing time did
 221 not correlate with mean IRT pre-test A ($r_s = -0.14$, $N = 46$, $P = 0.93$), or pre-test B ($r_s =$
 222 -0.045 , $N = 46$, $P = 0.964$). Crossing time did not correlate with mean IRT post-test
 223 A ($r_s = -0.024$, $N = 46$, $P = 0.964$), or post-test B ($r_s = -0.061$, $N = 46$, $P = 0.964$).
 224 Crossing time did not correlate with the discrepancy between eyes pre-test A ($r_s = -$
 225 0.239 , $N = 44$, $P = 0.93$), or pre-test B ($r_s = 0.041$, $N = 46$, $P = 0.964$). Crossing time
 226 did not correlate with the discrepancy in temperature between eyes post-test A ($r_s = -$
 227 0.13 , $N = 46$, $P = 0.93$), or post-test B ($r_s = -0.231$, $N = 41$, $P = 0.93$).

228 Mean proactivity correlated negatively with HR in Test A ($r_s = -0.85$, $N = 9$, $P = 0.144$)
229 but not Test B ($r_s = 0.193$, $N = 24$, $P = 0.93$). Mean proactivity did not correlate with
230 HRV in Test A ($r_s = 0.217$, $N = 9$, $P = 0.93$) or Test B ($r_s = -0.132$, $N = 24$, $P = 0.93$).
231 Proactivity did not correlate with mean IRT pre-test A ($r_s = -0.014$, $N = 13$, $P = 0.964$),
232 or pre-test B ($r_s = 0.197$, $N = 33$, $P = 0.93$). Proactivity did not correlate with mean IRT
233 post-test A ($r_s = -0.074$, $N = 33$, $P = 0.964$), or post-test B ($r_s = -0.163$, $N = 36$, $P =$
234 0.93). Proactivity did not correlate with the discrepancy in temperature between eyes
235 pre-test A ($r_s = -0.028$, $N = 12$, $P = 0.964$), or pre-test B ($r_s = 0.104$, $N = 36$, $P = 0.93$).
236 Proactivity did not correlate with the discrepancy in temperature between eyes post-
237 test A ($r_s = 0.213$, $N = 13$, $P = 0.93$), or post-test B ($r_s = 0.022$, $N = 36$, $P = 0.964$).

238 4. DISCUSSION

239 The aim of the current study was to investigate whether compliance is a reliable
240 indicator of stress responses in horses, and whether this may relate to coping
241 strategies. Physiological indicators of stress were not associated with compliance,
242 indicated by crossing time. Crossing time did not correlate with either pre-test or post-
243 test eye temperatures or the discrepancy between eyes. Additionally, it did not
244 correlate with heart rate variability. It might be assumed that crossing time is an
245 indicator of willingness to complete the handling test and that this would be associated
246 with how stressful subjects find the procedure. Therefore, it would be expected that
247 subjects that find the handling procedure stressful would not complete it, or would take
248 longer to do so. These results indicate that this is not accurate, with subjects crossing
249 the obstacles despite some exhibiting physiological signs of stress. Others refused to
250 complete the test whilst showing less pronounced physiological indicators of stress.
251 Overall, results support previous findings, in that a horse's behaviour does not

252 necessarily reflect its psychological and physiological response to a handling stressor
253 (Munsters et al., 2013; Yarnell et al., 2013).

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

264 It is possible that individuals that completed the handling test, despite experiencing
265 stress, were under greater stimulus control than those that refused but displayed lower
266 levels of stress. Whilst this is beneficial to handler safety (Thompson et al., 2015) and
267 the reduction of conflict due to a lack of clarity on the desired response (McLean and
268 McGreevy, 2010), it should be noted that horses may be completing tasks they find
269 aversive due to stimulus control. It is known that stimulus control based training
270 practices, such as over-shadowing, are effective in training horses to tolerate aversive
271 procedures (McLean, 2008). Previous research indicates that negative reinforcement
272 is more effective in getting horses to approach and habituate to aversive objects
273 (Christensen, 2013), as measured using behavioural indicators. However, it is
274 important to explore whether completing the task due to stimulus-control results in a
275 reduced physiological stress response on subsequent attempts. A study of police

276 horses indicates that significant habituation does not occur with repeated exposure to
277 stressful stimuli (Munsters et al., 2013) and supports our findings that compliance in
278 novel tests is not associated with lower physiological indicators of stress. Therefore, it
279 is possible that horses are being subjected to aversive procedures due to their own
280 compliance, which may result in conflict between the motivation to give the reinforced
281 response and the unconditioned response to avoid a stressor.

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

293 Incorrect interpretation of the behaviour of individuals that become unresponsive may
294 impact upon welfare if they are ascribed adjectives such as “stubborn” or “defiant”.
295 This may be associated with punishment to reduce the expression of the behaviour,
296 without rectifying the source of stress, or reinforcing the correct training aid (Goodwin
297 et al., 2009). In the current study, these individuals had a similar stress response to
298 more proactive subjects. Being unaware of stress levels in these circumstances and
299 forcing the animal to complete a task may cause negative welfare and, in extreme

300 cases, exposure to regular repeated aversive stimuli may lead to the development of
301 learned helplessness (McGreevy et al., 2009). Such a development is undesirable as
302 the animal abandons its attempts to cope and develops a 'dullness' related to a decline
303 in motivation and emotional response.

304 5. CONCLUSION

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

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330 REFERENCE LIST

- 331 Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical
332 and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* 57, 289–300.
- 333 Christensen, J.W., 2013. Object habituation in horses: The effect of voluntary versus
334 negatively reinforced approach to frightening stimuli. *Equine Vet. J.* 45, 298–
335 301. doi:10.1111/j.2042-3306.2012.00629.x
- 336 Church, J.S., Hegadoren, P.R., Paetkau, M.J., Miller, C.C., Regev-Shoshani, G.,
337 Schaefer, A.L., Schwartzkopf-Genswein, K.S., 2014. Influence of environmental
338 factors on infrared eye temperature measurements in cattle. *Res. Vet. Sci.* 96,
339 220–226. doi:10.1016/j.rvsc.2013.11.006
- 340 Cook, C., Mellor, D., Harris, P., Ingram, J., Matthews, L., 2000. Hands-on and
341 Hands-off Measurement of Stress, in: Moberg, G., Mench, J.A. (Eds.), *The*
342 *Biology of Animal Stress: Basic Principles and Implications for Animal Welfare.*
343 CABI, Wallingford, UK, pp. 123–147.
- 344 Coppens, C.M., de Boer, S.F., Koolhaas, J.M., 2010. Coping styles and behavioural
345 flexibility: Towards underlying mechanisms. *Philos. Trans. R. Soc. B Biol. Sci.*
346 365, 4021–4028.

347 Field, A., 2009. *Discovering Statistics using SPSS*, Third. ed. SAGE Publications Ltd,
348 London.

349 Foster, S., Ijichi, C., 2017. The association between infrared thermal imagery of core
350 eye temperature, personality, age and housing in cats. *Appl. Anim. Behav. Sci.*
351 189, 79–84. doi:10.1016/j.applanim.2017.01.004

352 Goodwin, D., McGreevy, P., Waran, N., McLean, A., 2009. How equitation science
353 can elucidate and refine horsemanship techniques. *Vet. J.* 181, 5–11.

354 Ijichi, C., Collins, L.M., Creighton, E., Elwood, R.W., 2013. Harnessing the power of
355 personality assessment: Subjective assessment predicts behaviour in horses.
356 *Behav. Processes* 96, 47–52. doi:10.1016/j.beproc.2013.02.017

357 Ijichi, C., Collins, L.M., Elwood, R.W., 2014. Pain expression is linked to personality
358 in horses. *Appl. Anim. Behav. Sci.* 152, 38–43.
359 doi:10.1016/j.applanim.2013.12.007

360 Ijichi, C., Griffin, K., Squibb, K., Favier, R., Submitted. Stranger Danger: An
361 investigation into the influence of human-horse bond on stress and behaviour.

362 Ille, N., Erber, R., Aurich, C., Aurich, J., 2014. Comparison of heart rate and heart
363 rate variability obtained by heart rate monitors and simultaneously recorded
364 electrocardiogram signals in nonexercising horses. *J. Vet. Behav. Clin. Appl.*
365 *Res.* 9, 341–346. doi:10.1016/j.jveb.2014.07.006

366 Koolhaas, J.M., de Boer, S.F., Buwalda, B., van Reenen, K., 2007. Individual
367 variation in coping with stress: a multidimensional approach of ultimate and
368 proximate mechanisms. *Brain. Behav. Evol.* 70, 218–26.

369 Koolhaas, J.M., de Boer, S.F., Coppens, C.M., Buwalda, B., 2010.
370 Neuroendocrinology of coping styles: Towards understanding the biology of

371 individual variation. *Front. Neuroendocrinol.* 31, 307–321.

372 Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Veegt, B.J., Van Reenen, C.G.,
373 Hopster, H., De Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping styles in
374 animals: current status in behavior and stress-physiology. *Neurosci. Biobehav.*
375 *Rev.* 23, 925–935.

376 Lush, J., Ijichi, C., Accepted. A preliminary investigation into personality and pain in
377 dogs. *J. Vet. Behav. Clin. Appl. Res.*

378 McGreevy, P.D., Oddie, C., Burton, F.L., McLean, A.N., 2009. The horse–human
379 dyad: Can we align horse training and handling activities with the equid social
380 ethogram? *Vet. J.* 181, 12–18.

381 McGreevy, P.D.P., McLean, A.N., 2009. Punishment in horse-training and the
382 concept of ethical equitation. *J. Vet. Behav. Clin. Appl. Res.* 4, 193–197.

383 McLean, A.N., 2008. Overshadowing: A silver lining to a dark cloud in horse training,
384 in: *Journal of Applied Animal Welfare Science.* pp. 236–248.
385 doi:10.1080/10888700802101064

386 McLean, A.N., McGreevy, P.D.P., 2010. Horse-training techniques that may defy the
387 principles of learning theory and compromise welfare. *J. Vet. Behav. Clin. Appl.*
388 *Res.* 5, 187–195.

389 Munsters, C., Visser, K., van den Broek, J., Sloet van Oldruitenborgh-Oosterbaan,
390 M.M., 2013. Quantifying stress in experienced and inexperienced mounted
391 police horses, using heart rate, heart rate variability, behavior score and
392 suitability score. *J. Vet. Behav. Clin. Appl. Res.* 8, e16–e17.
393 doi:10.1016/j.jveb.2012.12.037

394 Riemer, S., Assis, L., Pike, T.W., Mills, D.S., 2016. Dynamic changes in ear

395 temperature in relation to separation distress in dogs. *Physiol. Behav.* 167, 86–
396 91. doi:10.1016/j.physbeh.2016.09.002

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

401 Stewart, M., Stafford, K.J., Dowling, S.K., Schaefer, A.L., Webster, J.R., 2008. Eye
402 temperature and heart rate variability of calves disbudded with or without local
403 anaesthetic. *Physiol. Behav.* 93, 789–797. doi:10.1016/j.physbeh.2007.11.044

404 Thompson, K., McGreevy, P., McManus, P., 2015. A critical review of horse-related
405 risk: A research agenda for safer mounts, riders and equestrian cultures.
406 *Animals* 5, 561–575.

407 Travain, T., Colombo, E.S., Heinzl, E., Bellucci, D., Prato Previde, E., Valsecchi, P.,
408 2015. Hot dogs: Thermography in the assessment of stress in dogs (*Canis*
409 *familiaris*)-A pilot study. *J. Vet. Behav. Clin. Appl. Res.* 10, 17–23.
410 doi:10.1016/j.jveb.2014.11.003

411 Valera, M., Bartolomé, E., Sánchez, M.J., Molina, A., Cook, N., Schaefer, A., 2012.
412 Changes in Eye Temperature and Stress Assessment in Horses During Show
413 Jumping Competitions. *J. Equine Vet. Sci.* 32, 827–830.
414 doi:10.1016/j.jevs.2012.03.005

415 Wolff, A., Hausberger, M., Le Scolan, N., 1997. Experimental tests to assess
416 emotionality in horses. *Behav. Processes* 40, 209–221.

417 Yarnell, K., Hall, C., Billett, E., 2013. An assessment of the aversive nature of an
418 animal management procedure (clipping) using behavioral and physiological

419 measures. *Physiol. Behav.* 118, 32–39. doi:10.1016/j.physbeh.2013.05.013

420