Stranger Danger? An investigation into the influence of human-horse bond on stress and behaviour.

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1 ABSTRACT

2 Human-animal bond is receiving increasing attention and is thought to confer benefits on well-3 being and performance in working animals. One important benefit of bonding is the "safe base" an attachment figure provides, which manifests in better coping and increased exploration 4 during potential threat. However, there is limited research exploring the existence or benefits of 5 6 human-horse bonds, though bonding is sought after by both pleasure and elite riders. The 7 purpose of the current study was to determine whether the presence of horses' owners confers 8 a safe-base, therefore improving horse behaviour and physiological stress responses during 9 novel handling tests. Horses completed two different handling tests, one with their owner and 10 the other with an unfamiliar experimental handler (n = 46). Test and handler order was randomised and handlers were double blind to the performance of the horse with the alternate 11 12 handler. Time taken to complete the tests and proactive behaviour were measured as indicators of performance and compliance. Core temperature, discrepancy in eye temperature, heart rate 13 and heart rate variability were recorded to assess stress responses. If horses experience a 14 15 "safe base" effect in the vicinity of their owner, they would be expected to show lower stress responses and greater behavioural compliance, compared to being handled by a stranger. 16 17 There was no difference in behaviour or any physiological stress response between the handlers. This indicates that a calm, competent, but unknown handler may be equally effective 18 19 to an owner during stressful procedures as neither equine performance nor affective state 20 supported a safe-base effect. This supports previous research suggesting that the level of bond between human and horse may not be the most salient factor in coping or compliance during 21 training and handling. These findings have implications for veterinary and clinical behaviour 22 23 counselling, where novel human handlers must modify behaviour under potentially stressful 24 circumstances.

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26 KEY TERMS: infrared thermography; heart rate variability; bond; trust; horse; handling;

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28 1. INTRODUCTION

29 Human-animal bond has received increasing interest in recent years (e.g. Payne et al. 2016; 30 Payne et al. 2015). Attachment Theory is concerned with the development of bonds between infants and their caregivers both within humans (Cassidy, 1999) and other mammalian species 31 (Newberry and Swanson, 2008). It is theorised that appropriate bonds aid in survival because 32 33 vulnerable offspring keep close to their mothers in such species. Since domestic animals 34 depend on human caregivers to a certain extent, some level of attachment-type bond may exist. A fully developed relationship bond is characterised by proximity seeking, secure base, safe 35 36 haven and separation distress (Cassidy, 1999). Secure base refers to reduced stress under 37 perceived threat and increased exploration in the presence of the attachment figure (Mikulincer 38 and Shaver, 2003). It is therefore, a suitable construct of bonding to investigate objectively in 39 human-animal bonds.

40 Bonding between animals and their human caregivers is highly desirable as it is purported to 41 improve human well-being (Walsh, 2009) and is anecdotally reported to affect training outcomes 42 in horses (e.g. Parelli 1993; Roberts 1997). Within competitive equestrianism, human-horse bonds are thought to be integral to the success of partnerships during challenging and highly 43 pressurised situations (Fallis, 2013). However, due to this perceived importance, and the fact 44 45 that many human carers feel strong bonds towards their animal companions, it may be that reciprocal bonds are incorrectly perceived. Species that are highly dependent upon their care-46 giver, such as dogs, may be presumed to have more opportunities to bond. Indeed, the safe 47 base effect has been observed in dogs (Gácsi et al., 2013), whilst separation anxiety is a 48 49 relatively commonly recognised phenomenon in this species when isolated from their owners

(Riemer et al., 2016) Horses do not live as inter-dependently with their carers, yet studies
indicate that horses can discern the difference between familiar and unfamiliar humans and that
this elicits different cognitive responses (Proops and McComb, 2012). Therefore, it is possible
that such bonds do form in a species that does not live in such close proximity with their carers,
though this has not yet been investigated to our knowledge.

55 Whilst familiarity is known to have positive influences on behaviour during handling in horses 56 (Marsbøll and Christensen, 2015), the effect of more complex bonds that may result from longer 57 term interactions has not been assessed. Therefore, the current study aims to determine 58 whether horses respond differently to novel handling challenges, depending on whether they 59 are with their owner or a stranger. To this end, horses completed two novel handling tests, one 60 with their owner and the other with an unknown experimental handler. Time taken to complete 61 the task and proactivity during refusal were measured as indicators of compliance and performance. Heart rate, heart rate variability, core temperature and the discrepancy between 62 eye temperatures were measured as physiological indicators of stress and affective states. If an 63 64 owner provides a safe base as the result of a human-horse bond (Cassidy, 1999), horses would be expected to take less time to complete the tasks, show less potentially dangerous proactive 65 66 behaviour and have lower physiological indicators of stress, compared to when handled by an unfamiliar person. 67

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69 2. METHOD

The current experiment was conducted within an indoor arena at Hartpury College Equestrian Centre, Gloucestershire (UK) in October 2016. Subjects were liveries at this facility which allowed testing to occur in a home arena, reducing the effects of environmental novelty (Wolff et al., 1997). Forty-six horses of mixed breeds and genders (26 geldings and 20 mares) took part. Age ranged from 3 - 20 years (mean = 9.33 ± 4.20). All subjects had completed at least 75 preliminary work under saddle. 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-76 77 feed dependent on workload and nutritional requirements and constant access to fresh water. 78 They were individually stabled with a minimum of 1 hour of exercise each day but with limited or 79 no turn-out at the time of testing. The typical method of training was not known and will depend 80 on owner preference, temperament and knowledge. Therefore, subjects are likely to have been 81 trained differently regarding positive and negative reinforcement. Subjects were handled in their 82 own headcollar, providing it did not include inbuilt pressure mechanisms.

83 2.1 Handlers

The familiar handler was the owner and daily care-giver of the subject. The unfamiliar handler was the same for all subjects (C.I.) and had not made contact with any subject prior to testing. This individual was a competent, experienced handler and had completed similar handling tests before (Ijichi et al., 2013). The experimental handler wore the same clothing for all tests, whilst owners were free to choose their own attire. This was to reduce the potential effect of clothing on how subjects perceived the unfamiliar handler (Hausberger et al., 2008). Both the owner and experimental handler wore gloves, a riding helmet and protective footwear.

91 2.2 Handling Tests

Tests required subjects to navigate novel objects in response to leadrope pressure, which is an aid used to indicate that the horse should step forward (McGreevy and McLean, 2007). Each test was sufficiently different to prevent habituation, which might alter behaviour between the first and the second test. 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 (Ijichi et al., 2013). To complete this test, the subject walked over the tarpaulin. Test B consisted of a frame that was 2.5m high and 1.6m wide, from which hung 2m long coloured plastic streamers (Squibb et al., 2018). To complete this test, the subject walked through the frame, causing the streamers to touch the face andbody of the subject as they passed through.

101 Both objects were present within the test arena and faced the exit and conspecifics, because 102 differing directions could have affected the motivation to complete the test. A standard jump pole was placed 2m in front of each test, which the subject walked over to mark the start of the 103 104 test. Handlers indicated that the horse should walk towards the obstacle using leadrope 105 pressure but no verbal or additional tactile cues were permitted. Horses had a maximum of 3 106 minutes to complete each handling test, as previous research indicates that horses that have 107 not completed the test within this time do not do so (ljichi et al., 2013). Tests were recorded on 108 video for post-hoc analysis.

109 2.3 Experimental Design

Upon arrival at the testing area, horses were fitted with a Polar Equine V800 heart rate monitor by K.G. (Polar Electro Oy, Kempele, Finland). The elasticated surcingle was attached to the girth area, which had been moistened with water to aid conductivity. After confirming that HR was being detected, subjects were given a minimum of 5 minutes to habituate to the monitor. This was deemed sufficient as all subjects had previously worn girths and/or lunging rollers. During habituation, subjects were outside of the indoor testing arena and could not see the novel objects.

117 Test order and handler order was randomised and horse order was pseudo-randomised,

depending on the availability of subjects. Each handler was blind to the subject's behaviour with the alternate handler. Additionally, owners were expressly forbidden from discussing the likely behaviour of the subject. Double-blinding was possible as the test arena had solid doors and a research assistant remained outside at all times to prevent the second handler from attempting to see into the arena. Subjects entered the arena with the first handler and proceeded to a designated area for eye temperature measurement. This was marked by two parallel jump poles in the same position and direction within the enclosed area. This was to reduce the potentially
confounding effects of direct sunlight and environmental factors on IRT readings (Church et al.,
2014). The research assistant (K.S.) stood at a marked point approximately 1m and 90 degrees
from each eye (Travain et al., 2015; Yarnell et al., 2013). Images were taken using a FLIR E4
thermal imaging camera (FLIR Systems, USA.). The handler then led the subject towards Test
A or B as randomly allocated.

130 Upon successful completion of the task, or termination at 3 minutes, the subject was led back to 131 the designated area for post-test eye temperature readings. Recordings were taken as per pre-132 test procedures. Horses that completed the task in less than 3 minutes were then held within the 133 arena for the remainder of the available crossing time. This ensured the second handler could 134 not deduce the subject's behaviour during the preceding task, as all horses remained in the arena for a similar amount of time. Upon leaving the test arena, the subject had a minimum of 5 135 minutes to recover, before re-entering with the second handler. The procedure was then 136 repeated verbatim. 137

138 2.4 Analysis

139 2.4.1 Behaviour

140 Crossing time began when the first fore-limb bore weight after the ground pole 2m in front of the obstacle. Crossing time ended when the last hind-limb bore weight on the tarpaulin for Test A 141 142 (ljichi et al., 2013), or when the tail of the subject had passed through the frame for Test B 143 (Squibb et al., 2018). Horses that did not complete the test were recorded a Crossing Time of 144 180 seconds. Proactivity (outlined below) was calculated as per ljichi et al. (2013). Refusal behaviour was defined as any behaviour which did not contribute to crossing the object. This 145 included moving backwards, sideways, forwards but away from the object, rearing or remaining 146 147 stationary. Refusal that lasted for 10 seconds or more was analysed to determine how proactive 148 that refusal was (Tarpaulin: N = 13, Streamers: N = 36). Proactive refusal was defined as any

refusal behaviour that involved movement thus excluding stationary refusal. 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). Twelve subjects exhibited refusal behaviour for both tests, allowing a comparison between handlers.

154 2.4.2 Infrared Thermography

IRT was analysed using FLIR Tools software (ver. 5.9.16284.1001) post-hoc. This was to 155 reduce any stress inducing effects of prolonged IRT recordings (Travain et al., 2015) required to 156 157 record accurate readings from a small area. Eve temperature recordings were the maximum 158 temperature within the palpebral fissure from the lateral commissure to the lacrimal caruncle 159 (Yarnell et al., 2013). A mean of the left and right eyes was calculated for each subject, pre and 160 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 161 score indicates a hotter right eye, whilst a negative score indicates a hotter left eye. This may 162 163 provide an indicator of ipsilateral hemispheric dominance (Lush and Ijichi, 2018) and lateralised processing of stimuli (De Boyer Des Roches et al., 2008). 164

165 2.4.3 Heart Rate

166 Heart rate readings were taken from the point of the first IRT reading to the second IRT reading, for each test. Heart rate analysis was carried out using Kubios HRV (ver. 2.2, Biomedical Signal 167 168 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 169 170 (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 171 interval was more than 30% different from the preceding interval, it is deemed to be an incorrect 172 173 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. Heart rate readings for both tests were recorded for 26 subjects,
allowing a comparison between handlers.

178 2.5 Statistical Analysis

179 Statistical analysis was carried out using R (R Development Core Team, 2017). Data normality

180 was tested using Shapiro-Wilks, which indicated that data was not normally distributed.

181 Therefore, non-parametric tests were used throughout. Wilcoxon Signed-Rank tests were used

to detect potential differences in crossing time, proactivity, heart rate, heart rate variability, core

temperature and discrepancy between eye temperature between familiar and unfamiliar

184 handlers.

185 2.6 Ethics

186 Owners provided informed consent for each subject via the completion of a participant

information form. All data provided was 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, the current study was authorised by the

190 Hartpury College Ethics Committee. The authors read and abided by this journals policy on

191 animal ethics.

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193 3. RESULTS

194 There was no statistically significant difference in behaviour or any indicator of stress,

depending on whether horses were handled by a familiar or unfamiliar person (Table 1).

196

197 **Table 1.** There were no significant differences in behaviour or physiological indicators of stress

Variable	n =	Handler	Median	IQR	v =	p =
Crossing Time (secs)	46	F	20.04	4.41 - 61.57	415	0.354
		UF	63.82	5.19 - 146.8		
Proactivity (%)	12	F	24.1	4.52 - 47.73	58	0.151
		UF	17.17	7.05 - 33.26		
Pre-test IRT ^o C	46	F	33.13	32.46 - 33.69	412	0.236
		UF	33.33	32.54 - 34.09		
Post- test IRT °C	46	F	33.15	32.54 - 33.49	440	0.388
		UF	33.08	32.3 - 33.69		
Pre-test Discrepancy ^o C	46	F	0.1	-0.3 - 0.7	454	0.832
		UF	0.218	-0.4 - 0.6		
Post-test Discrepancy ^o C	46	F	0.268	-0.2 - 0.5	411	0.373
		UF	0.1	-0.4 - 0.3		
Heart Rate	26	F	63.98	51.67 - 83.1	126	0.333
		UF	64.22	55.85 - 81.55		
Heart Rate Variability	26	F	98.79	70.71 - 143.3	163	1
		UF	98.92	80.31 - 122.9		

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201 4. DISCUSSION

The aim of the current study was to ascertain whether a safe base effect of bonding could be observed in horses during mildly stressful handling procedures. Forty-six horses completed two novel handling tests with a familiar and unfamiliar handler. Time taken to complete the tests, proactive behaviour and physiological indicators of stress were measured. Results of the current experiment do not support the existence of a "safe base" effect of bonding in human-horse interactions (Cassidy, 1999).

208 Stress responses of subjects did not differ depending on whether they were handled by their

209 owner or the unfamiliar handler. There was no difference in core eye temperature, the

discrepancy in temperature between eyes, heart rate or heart rate variability. Owners care for,

and train, their horses daily and, as such, are the most likely sources of human attachment.

212 During the unfamiliar handler procedure, horses were separated from their owners and presented with a potential threat, without a "safe base". However, this does not appear to cause 213 214 stress in horses, indicating that neither safe base (Cassidy, 1999) nor separation distress 215 (Mikulincer and Shaver, 2003) features of bond were salient here. Time taken to complete the 216 handling tests also did not differ dependent on whether the horse was handled by their owner or 217 an unfamiliar experimental handler. In addition, there was no difference in potentially dangerous 218 proactive behaviour shown by subjects between the two handlers. This indicates horses do not 219 respond differently under situations where bonding is not possible and are not distressed at 220 being separated from their owners, even during challenging scenarios. This has implication for 221 industries such as veterinary medicine, clinical behavioural counselling and horse racing where humans influence the behaviour of horses they have not interacted with previously. 222

Horses are prey animals that utilise flight to improve adaptive fitness and show consistent fear 223 responses (Lansade et al., 2008). Significant risk in horse sports and management is 224 225 acknowledged due to the combination of a large flight animal being routinely subjected to 226 potentially stressful procedures (Thompson et al., 2015). Some anecdotally based training 227 practices, which are often described as either "natural" or "sympathetic" horsemanship, claim that bonding has benefits for resolving issues that result from these factors (Roberts, 1997). 228 229 They attribute reduced flight responses and improved compliance as the result of "trust", or 230 "respect" for a leadership figure. The current experiment contradicts this and instead supports 231 previous research undermining the legitimacy of such claims (Hawson et al., 2010; McLean and 232 McGreevy, 2010). For example, it has been shown that horses will follow an unknown person 233 after "join-up" with a different individual (Krueger, 2007), or will even follow an inanimate object 234 (Henshall et al., 2012), within a round pen. In addition, the changes to behaviour resulting from 235 techniques such as round-pen interactions do not persist outside of this specialised context 236 (Krueger, 2007). Taken together, these results do not conclusively reject the possibility of bonds between horses and their owners. They do suggest that certain features seen in fully developed
attachments may not be meaningfully applied to human-horse interactions.

239 In the current study, the length of the relationship, the dynamic between caregiver and horse, 240 the hours spent together each day and whether positive or negative reinforcement was primarily used during training was not quantified or controlled for. The type of reinforcement is known to 241 affect subsequent reactions to humans (Sankey et al., 2010) and may therefore have 242 243 confounded the current study. In addition, it is assumed that bonds take time to develop and the 244 length of the relationship between horses and owners was not controlled for here, though it was longer than previous studies assessing the effects of familiarity (Marsbøll and Christensen, 245 246 2015). The current findings contradict those of Marsbøll and Christensen (2015), as their study 247 noted positive effects of familiarity on handling tests. However, the subjects of that study were 248 unusual in having only positive interaction with the familiar handler in a shorted time period. It is unlikely, despite even the best intentions, that owners in real-world scenarios can avoid any 249 250 negative interactions with their horses. Despite this, a safe-base effect has been observed in 251 human-dog relationships in which neither the length of the relationship nor the predominant 252 training method was controlled for (Gácsi et al., 2013). This suggests that the differences between horses and dogs cannot fully be accounted for by these limitations. One key difference 253 254 between Gácsi et al., (2013) and the current experiment, is that subjects in the former were 255 compared with and without any handler. In the current study, all horses were handled by the 256 same stranger and the particular attributes of this individual are likely to affect how horses 257 responded.

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259 5. CONCLUSIONS

In the current study, the presence of a subject's owner did not affect behavioural or
physiological indicators of stress in horses during handling tests. Results indicate that, in

262 general, horses can be handled just as effectively without prior experience of the handler. These 263 findings suggest that competent handling is more salient than bond in influencing horse 264 behaviour during handling. This has implications for industries such as veterinary practice, 265 behaviour consultations and racing, where humans must quickly and effectively modify the 266 behaviour of horses under potentially stressful circumstances. This experiment suggests that, in 267 general, the presence of the horse's owner did not confer a safe-base effect. This does not 268 conclusively reject the concept of bonds between horses and owners however. First, such 269 bonds may be influenced by the amount and type of interaction between the dyad. It is possible that the sample tested here had not successfully developed bonds. Second, it is also possible 270 that other features of attachment are present in human-horse interactions but that a safe base 271 effect is not one of them. Future research into this subject is needed to explore these 272 273 possibilities.

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281 7. AUTHORSHIP STATEMENT

The idea for this paper was conceived by Carrie Ijichi; the study was designed by Carrie Ijichi; the study was performed by Carrie Ijichi, Kym Griffin, Keith Squibb and Rebecca Favier; the data was analysed by Carrie Ijichi; the paper was written by Carrie Ijichi and edited by Kym Griffin, Keith Squibb and Rebecca Favier. 286

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