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**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”  
4 an attachment figure provides, which manifests in better coping and increased exploration  
5 during potential threat. However, there is limited research exploring the existence or benefits of  
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, improving horse behaviour and physiological stress responses during novel  
9 handling tests. Horses completed two different handling tests, one with their owner and the  
10 other with an unfamiliar experimental handler (n = 46). Test and handler order was randomised  
11 and handlers were double blind to the performance of the horse with the alternate handler. Time  
12 taken to complete the tests and proactive behaviour were measured as indicators of  
13 performance and compliance. Core temperature, discrepancy in eye temperature, heart rate  
14 and heart rate variability were recorded to assess stress responses. If horses experience a  
15 “safe base” effect in the vicinity of their owner, they would be expected to show lower stress  
16 responses and greater behavioural compliance, compared to being handled by a stranger.  
17 However, there was no difference in behaviour or any physiological stress response between  
18 the handlers. This indicates that a calm, competent, but unknown handler is equally effective to  
19 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  
21 between human and horse is not the most salient factor in equine well-being or compliance  
22 during training and handling. These findings have implications for veterinary and clinical  
23 behaviour counselling, where novel human handlers must modify behaviour under potentially  
24 stressful circumstances.

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

27

## 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  
31 infants and their caregivers in humans (Cassidy, 1999) and mammalian species (Newberry and  
32 Swanson, 2008). It is theorised that appropriate bonds aid in survival because vulnerable  
33 offspring keep close to their mothers in such species. Since domestic animals depend on  
34 human caregivers to a certain extent, some level of attachment-type bond may exist. A fully  
35 developed relationship bond is characterised by proximity seeking, secure base, safe haven and  
36 separation distress (Cassidy, 1999). Secure base refers to reduced stress under perceived  
37 threat and increased exploration in the presence of the attachment figure (Mikulincer and  
38 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  
43 bonds are thought to be integral to the success of partnerships during challenging and highly  
44 pressurised situations (Fallis, 2013). However, due to this perceived importance, and the fact  
45 that many human carers feel strong bonds towards their animal companions, it may be that  
46 reciprocal bonds are incorrectly perceived. Species that are highly dependent upon their care-  
47 giver, such as dogs, may be presumed to have more opportunities to bond. Indeed, separation  
48 anxiety is a relatively commonly recognised phenomenon in dogs isolated from their owners  
49 (Riemer et al., 2016) and the safe base effect has been observed in dogs (INSERT MIKLOSI).

50 Horses do not live as inter-dependently with their carers, yet studies indicate that horses can  
51 discern the difference between familiar and unfamiliar humans and that this elicits different  
52 cognitive responses (Proops and McComb, 2012). Therefore, it is possible that such bonds do  
53 form in a species that does not live in such close proximity with their carers, though this has not  
54 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 novelty, depending on whether they are with their owner  
59 or a stranger. To this end, horses completed two novel handling tests, one with their owner and  
60 the other with an unknown experimental handler. Time taken to complete the task and  
61 proactivity during refusal were measured as indicators of compliance and performance. Heart  
62 rate, heart rate variability, core temperature and the discrepancy between eye temperatures  
63 were measured as physiological indicators of stress and affective states. If an owner provides a  
64 safe base as the result of a human-horse bond (Cassidy, 1999), horses would be expected to  
65 take less time to complete the tasks, show less potentially dangerous proactive behaviour and  
66 have lower physiological indicators of stress, compared to when handled by an unfamiliar  
67 person.

68

## 69 2. METHOD

70 The current experiment was conducted within an indoor arena at Hartpury College Equestrian  
71 Centre, Gloucestershire (UK) in October 2016. Subjects were liveries at this facility which  
72 allowed testing to occur in a home arena, reducing the effects of environmental novelty (Wolff et  
73 al., 1997). Forty-six horses of mixed breeds and genders (26 geldings and 20 mares) took part.  
74 Age ranged from 3 – 20 years (mean =  $9.33 \pm 4.20$ ). All subjects had completed at least

75 preliminary work under saddle. Subjects were housed and managed as per owner preferences  
76 on a large livery yard. In general, subjects were provided forage three times a day with hard-  
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*

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

### 91 *2.2 Handling Tests*

92 Tests required subjects to navigate novel objects in response to leadrope pressure, which is an  
93 aid used to indicate that the horse should step forward (McGreevy and McLean, 2007). Each  
94 test was sufficiently different to prevent habituation, which might alter behaviour between the  
95 first and the second test. Task A consisted of a 2.5m x 3m blue tarpaulin secured to the surface  
96 of the indoor holding arena by 20 individual tent pegs (Ijichi et al., 2013). To complete this test,  
97 the subject walked over the tarpaulin. Test B consisted of a frame that was 2.5m high and 1.6m  
98 wide, from which hung 2m long coloured plastic streamers (Squibb et al., 2018). To complete

99 this test, the subject walked through the frame, causing the streamers to touch the face and  
100 body 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  
103 pole was placed 2m in front of each test, which the subjected walked over to mark the start of  
104 the 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 (Ijichi et al., 2013). Tests were recorded on  
108 video for post-hoc analysis.

### 109 *2.3 Experimental Design*

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

117 Test order and handler order was randomised and horse order was pseudo-randomised,  
118 depending on the availability of subjects. Each handler was blind to the behaviour of the subject  
119 with the alternate handler and owners were expressly forbidden from discussing the likely  
120 behaviour of the subject. Double-blinding was possible as the test arena had solid doors and a  
121 research assistant remained outside at all times to prevent the second handler from attempting  
122 to see into the arena. Subjects entered the arena with the first handler and proceeded to a  
123 designated area for eye temperature measurement. This was marked by two parallel jump poles

124 in the same position and direction within the enclosed area. This was to reduce the potentially  
125 confounding effects of direct sunlight and environmental factors on IRT readings (Church et al.,  
126 2014). The research assistant (K.S.) stood at a marked point approximately 1m and 90 degrees  
127 from each eye (Travain et al., 2015; Yarnell et al., 2013). Images were taken using a FLIR E4  
128 thermal imaging camera (FLIR Systems, USA.). The handler then led the subject towards Test  
129 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 for the  
133 remainder of the available crossing time. This ensured the second handler could not deduce the  
134 subject's behaviour during the first task as all horses remained in the arena for a similar amount  
135 of time. Upon leaving the test arena, the subject had a minimum of 5 minutes to recover, before  
136 re-entering with the second handler. The procedure was then repeated verbatim.

## 137 *2.4 Analysis*

### 138 *2.4.1 Behaviour*

139 Crossing time began when the first fore-limb bore weight after the ground pole 2m in front of the  
140 obstacle. Crossing time ended when the last hind-limb bore weight on the tarpaulin for Test A  
141 (Ijichi et al., 2013), or when the tail of the subject had passed through the frame for Test B  
142 (Squibb et al., 2018). Horses that did not complete the test were recorded a Crossing Time of  
143 180 seconds. Proactivity (outlined below) was calculated as per Ijichi et al. (2013). Refusal  
144 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 stationary. Refusal that lasted for 10 seconds or more was analysed to determine how proactive  
147 that refusal was (Tarpaulin: N = 13, Streamers: N = 36). Proactive refusal was defined as any  
148 refusal behaviour that involved movement thus excluding stationary refusal. Proactive refusal



149 was then recorded as the percent of total refusal time for any individual which showed refusal  
150 behaviour (which included remaining stationary). A higher value indicated a greater amount of  
151 proactive behaviour (Ijichi et al., 2013). Twelve subjects exhibited refusal behaviour for both  
152 tests, allowing a comparison between handlers.

#### 153 *2.4.2 Infrared Thermography*

154 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 record accurate readings from a small area. Eye temperature recordings were the maximum  
157 temperature within the palpebral fissure from the lateral commissure to the lacrimal caruncle  
158 (Yarnell et al., 2013). A mean of the left and right eyes was calculated for each subject, pre and  
159 post-test, for each test. In addition, the temperature of the left eye was subtracted from the right  
160 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 provide an indicator of ipsilateral hemispheric dominance (Lush and Ijichi, 2018) and lateralised  
163 processing of stimuli (De Boyer Des Roches et al., 2008).

#### 164 *2.4.3 Heart Rate*

165 Heart rate readings were taken from the point of the first IRT reading to the second IRT reading,  
166 for each test. Heart rate analysis was carried out using Kubios HRV (ver. 2.2, Biomedical Signal  
167 Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern  
168 Finland, Kuopio, Finland.). Kubios settings were adjusted in line with previous equine studies  
169 (Ille et al., 2014). Specifically, artefact correction was set to custom level 0.3, thus removing RR  
170 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 reading. Trend components were adjusted using the concept of smoothness priors set at  
173 500ms, to avoid the effect of outlying intervals. The STD RR value, being the standard deviation

174 of RR intervals, was used as the HRV figure to reflect both short-term and long-term variation  
175 with the series of RR intervals. Heart rate readings for both tests were recorded for 26 subjects,  
176 allowing a comparison between handlers.

### 177 *2.5 Statistical Analysis*

178 Statistical analysis was carried out using R (R Development Core Team, 2017). Data normality  
179 was tested using Shapiro-Wilks, which indicated that data was not normally distributed.  
180 Therefore, non-parametric tests were used throughout. Wilcoxon Signed-Rank tests were used  
181 to detect potential differences in crossing time, proactivity, heart rate, heart rate variability, core  
182 temperature and discrepancy between eye temperature between familiar and unfamiliar  
183 handlers.

### 184 *2.6 Ethics*

185 Owners provided informed consent for each subject via the completion of a participant  
186 information form. All data provided was held in accordance with the Data Protection Act (1998).  
187 Both researchers and owners had the right to withdraw a subject at any time, for any reason,  
188 until the point of data analysis. Prior to commencement, the current study was authorised by the  
189 Hartpury College Ethics Committee. The authors read and abided by this journals policy on  
190 animal ethics.

191

## 192 3. RESULTS

193 There was no statistically significant difference in behaviour or any indicator of stress,  
194 depending on whether horses were handled by a familiar or unfamiliar person (Table 1).

195

196 **Table 1.** There were no significant differences in behaviour or physiological indicators of stress  
 197 between familiar (F) and unfamiliar (UF) handlers.

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 °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 °C	46	F	0.1	-0.3 - 0.7	454	0.832
		UF	0.218	-0.4 - 0.6		
Post-test Discrepancy °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		

198

199 **4. DISCUSSION**

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

206 Stress responses of subjects did not differ depending on whether they were handled by their  
 207 owner or the unfamiliar handler. There was no difference in core eye temperature, the  
 208 discrepancy in temperature between eyes, heart rate or heart rate variability. Owners care for,  
 209 and train, their horses daily and, as such, are the most likely sources of human attachment.

210 During the unfamiliar handler procedure, horses were separated from their owners and

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

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

235 between horses and their owners. They do suggest that certain features seen in fully developed  
236 attachments may not be meaningfully applied to human-horse interactions.

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

255

## 256 5. CONCLUSIONS

257 In the current study, the presence of a subject's owner did not affect behavioural or  
258 physiological indicators of stress in horses during handling tests. Results indicate that, in  
259 general, horses can be handled just as effectively without prior experience of the handler. These

260 findings suggest that competent handling is more salient than bond in influencing horse  
261 behaviour during handling. This has implications for industries such as veterinary practice,  
262 behaviour consultations and racing, where humans must quickly and effectively modify the  
263 behaviour of horses under potentially stressful circumstances. This experiment suggests that, in  
264 general, the presence of the horse's owner did not confer a safe-base effect. This does not  
265 conclusively reject the concept of bonds between horses and owners however. First, such  
266 bonds may be influenced by the amount and type of interaction between the dyad. It is possible  
267 that the sample tested here had not successfully developed bonds. Second, it is also possible  
268 that other features of attachment are present in human-horse interactions but that a safe base  
269 effect is not one of them. Future research into this subject is needed to explore these  
270 possibilities.

271

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277

## 278 7. AUTHORSHIP STATEMENT

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

283

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