

**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, 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
11 randomised and handlers were double blind to the performance of the horse with the alternate
12 handler. Time taken to complete the tests and proactive behaviour were measured as indicators
13 of 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 There was no difference in behaviour or any physiological stress response between the
18 handlers. This indicates that a calm, competent, but unknown handler may be equally effective
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
21 between human and horse may not be the most salient factor in coping or compliance during
22 training and handling. These findings have implications for veterinary and clinical behaviour
23 counselling, where novel human handlers must modify behaviour under potentially stressful
24 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 both within humans (Cassidy, 1999) and other mammalian species
32 (Newberry and Swanson, 2008). It is theorised that appropriate bonds aid in survival because
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.
35 A fully developed relationship bond is characterised by proximity seeking, secure base, safe
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
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, the safe
48 base effect has been observed in dogs (Gácsi et al., 2013), whilst separation anxiety is a
49 relatively commonly recognised phenomenon in this species when isolated from their owners

50 (Riemer et al., 2016) Horses do not live as inter-dependently with their carers, yet studies
51 indicate that horses can discern the difference between familiar and unfamiliar humans and that
52 this elicits different cognitive responses (Proops and McComb, 2012). Therefore, it is possible
53 that such bonds do form in a species that does not live in such close proximity with their carers,
54 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
62 performance. Heart rate, heart rate variability, core temperature and the discrepancy between
63 eye temperatures were measured as physiological indicators of stress and affective states. If an
64 owner provides a safe base as the result of a human-horse bond (Cassidy, 1999), horses would
65 be expected to take less time to complete the tasks, show less potentially dangerous proactive
66 behaviour and have lower physiological indicators of stress, compared to when handled by an
67 unfamiliar 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 ± 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 subject walked over to mark the start of the
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 (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 subject's behaviour with
119 the alternate handler. Additionally, 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 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
135 arena for a similar amount of time. Upon leaving the test arena, the subject had a minimum of 5
136 minutes to recover, before re-entering with the second handler. The procedure was then
137 repeated verbatim.

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
141 obstacle. Crossing time ended when the last hind-limb bore weight on the tarpaulin for Test A
142 (Ijichi 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 Ijichi et al. (2013). Refusal
145 behaviour was defined as any behaviour which did not contribute to crossing the object. This
146 included moving backwards, sideways, forwards but away from the object, rearing or remaining
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

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

154 *2.4.2 Infrared Thermography*

155 IRT was analysed using FLIR Tools software (ver. 5.9.16284.1001) post-hoc. This was to
156 reduce any stress inducing effects of prolonged IRT recordings (Travain et al., 2015) required to
157 record accurate readings from a small area. Eye 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
161 eye to indicate the discrepancy between both eyes, pre and post-test, for each test. A positive
162 score indicates a hotter right eye, whilst a negative score indicates a hotter left eye. This may
163 provide an indicator of ipsilateral hemispheric dominance (Lush and Ijichi, 2018) and lateralised
164 processing of stimuli (De Boyer Des Roches et al., 2008).

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,
167 for each test. Heart rate analysis was carried out using Kubios HRV (ver. 2.2, Biomedical Signal
168 Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern
169 Finland, Kuopio, Finland.). Kubios settings were adjusted in line with previous equine studies
170 (Ille et al., 2014). Specifically, artefact correction was set to custom level 0.3, thus removing RR
171 levels varying by more than 30% from the previous interval. This means that if a single RR
172 interval was more than 30% different from the preceding interval, it is deemed to be an incorrect
173 reading. Trend components were adjusted using the concept of smoothness priors set at

174 500ms, to avoid the effect of outlying intervals. The STD RR value, being the standard deviation
175 of RR intervals, was used as the HRV figure to reflect both short-term and long-term variation
176 with the series of RR intervals. Heart rate readings for both tests were recorded for 26 subjects,
177 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
182 to detect potential differences in crossing time, proactivity, heart rate, heart rate variability, core
183 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
187 information form. All data provided was held in accordance with the Data Protection Act (1998).
188 Both researchers and owners had the right to withdraw a subject at any time, for any reason,
189 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.

192

193 3. RESULTS

194 There was no statistically significant difference in behaviour or any indicator of stress,
195 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
 198 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		

199

200

201 **4. DISCUSSION**

202 The aim of the current study was to ascertain whether a safe base effect of bonding could be
 203 observed in horses during mildly stressful handling procedures. Forty-six horses completed two
 204 novel handling tests with a familiar and unfamiliar handler. Time taken to complete the tests,
 205 proactive behaviour and physiological indicators of stress were measured. Results of the current
 206 experiment do not support the existence of a “safe base” effect of bonding in human-horse
 207 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
 210 discrepancy in temperature between eyes, heart rate or heart rate variability. Owners care for,
 211 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
213 presented with a potential threat, without a “safe base”. However, this does not appear to cause
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
222 humans influence the behaviour of horses they have not interacted with previously.

223 Horses are prey animals that utilise flight to improve adaptive fitness and show consistent fear
224 responses (Lansade et al., 2008). Significant risk in horse sports and management is
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
228 that bonding has benefits for resolving issues that result from these factors (Roberts, 1997).
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

237 between horses and their owners. They do suggest that certain features seen in fully developed
238 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
241 used during training was not quantified or controlled for. The type of reinforcement is known to
242 affect subsequent reactions to humans (Sankey et al., 2010) and may therefore have
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
245 longer than previous studies assessing the effects of familiarity (Marsbøll and Christensen,
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
249 unlikely, despite even the best intentions, that owners in real-world scenarios can avoid any
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
253 between horses and dogs cannot fully be accounted for by these limitations. One key difference
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.

258

259 5. CONCLUSIONS

260 In the current study, the presence of a subject's owner did not affect behavioural or
261 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
270 that the sample tested here had not successfully developed bonds. Second, it is also possible
271 that other features of attachment are present in human-horse interactions but that a safe base
272 effect is not one of them. Future research into this subject is needed to explore these
273 possibilities.

274

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280

281 7. AUTHORSHIP STATEMENT

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

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