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**Dually Investigated: the effect of training on the behaviour, discomfort and stress of horses in a pressure headcollar**

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## 1 **Abstract**

2 The Dually™ is a control headcollar designed to improve equine behaviour during  
3 handling challenges by applying greater pressure than a standard headcollar.  
4 Previous research indicated it did not improve compliance in naïve horses but did  
5 result in higher Horse Grimace Scale scores (HGS) indicative of discomfort.  
6 However, subjects had not been trained to step forward to release the pressure  
7 applied by the headcollar. The aim of the current study was to determine the effect of  
8 training on behaviour and physiology of horses wearing the Dually™ headcollar  
9 during handling challenges. To this end, subjects received three training sessions  
10 prior to completing two distinct novel handling tests, one wearing a Dually™ with a  
11 line attached to the pressure mechanism and one attached to the standard ring as a  
12 control. Behaviour was coded by hypothesis blind researchers: time to cross the  
13 obstacle and proactivity were recorded as indicators of compliance and the Horse  
14 Grimace Scale was used to measure discomfort caused by each configuration of the  
15 device. Infrared thermography of ocular temperature, heart rate variability (RMSSD  
16 and low/high frequency ratios (LF/HF)) and salivary cortisol were measured as  
17 indicators of stress and arousal. Data from the previous study on Naïve horses was  
18 also included to compare responses to the Dually in Naïve and Trained horses (Ijichi  
19 et al., 2018). Training resulted in a decrease in RMSSD ( $p = 0.002$ ) and an increase  
20 in LF/HF ( $p=0.012$ ), compared to rest, indicating arousal. As per the original study,  
21 horses did not complete the tests more quickly in the Dually, compared to control  
22 ( $p=0.698$ ). Further, trained horses tended to be more proactive in the Dually  
23 compared to Controls ( $p=0.066$ ) and significantly more so than Naïve horses  
24 ( $p=0.002$ ) suggesting that behaviour deteriorates as a result of early Dually training.

25 Yet, stress and HGS indicators were not higher in the Dually compared to Control  
26 during testing. Results indicate the Dually has a negative effect on behaviour but not  
27 on stress or discomfort during short handling challenges. Further research is  
28 warranted to determine the long-term effect of Dually experience on behaviour and  
29 welfare.

30 **Keywords:** heart rate variability; infrared thermography; salivary cortisol; horse  
31 grimace scale; proactivity; horse welfare

32

### 33 **1. Introduction**

34 The horse is a large prey animal for which domestication has dampened, but not  
35 extinguished, innate biological flight responses (Brubaker and Udell, 2016). These  
36 responses make it difficult to retain stimulus control at all times (McGreevy and  
37 McLean, 2007) as environmental stimuli often exert more control over the horse's  
38 behaviour than their human handler is able to. Williams and Ashby (1995) state 20%  
39 of accidents occur during handling and allude to horse behaviour being the primary  
40 cause. Similarly, Sandiford et al., (2013) reported 12% of patients admitted to a UK  
41 hospital with horse related injuries sustained them in non-ridden accidents.

42 Therefore, it is understandable that many owners seek solutions to reduce such risky  
43 behaviour during daily interactions, often by using devices which increase the  
44 salience of human cues in order to compete with environmental stimuli.

45 The Dually™ headcollar is a commercially available control headcollar which  
46 increases the pressure a handler can apply in order to maintain control of a horse. It  
47 has two settings: a standard ring under the chin and two side rings which operate an  
48 inbuilt pressure-release mechanism. When the lead-rope is attached to the side ring,  
49 if the horse pulls back or fails to walk forward when pressure is applied by the

50 handler, the inbuilt mechanism tightens, increasing the level of pressure exerted  
51 around the jaw and nose of the horse (Roberts, 1999). The patent for the Dually™  
52 states *“It is extremely effective for training the animal to lead, to stand still, to walk  
53 into a truck or trailer, to walk slowly through narrow passages, to walk over unfamiliar  
54 objects...”* (Roberts, 1999). However, research investigating bridles which apply  
55 pressure to similar sensitive facial structures highlights welfare concerns (Doherty et  
56 al., 2017; Fenner et al., 2016; McGreevy et al., 2012). Further, Ijichi et al., (2018)  
57 found the Dually™ did not improve compliance in naïve horses but did result in  
58 higher Horse Grimace Scale scores (HGS). However, subjects were naïve to the  
59 Dually™ and had not been trained in how to release the pressure applied by the  
60 headcollar. Therefore, the headcollar may still be valuable in modifying the behaviour  
61 of horses that are trained to step forward to release the pressure.

62 The aim of the current study was to determine the effect of training on behaviour and  
63 physiology of horses wearing the Dually™ headcollar during handling challenges. To  
64 this end, subjects received three training sessions prior to completing two novel  
65 handling tests, one wearing a Dually™ with a line attached to the pressure  
66 mechanism and one attached to the standard ring as a control. Time to cross the  
67 obstacle and proactivity were blind scored as indicators of compliance (Ijichi et al.,  
68 2013). The Horse Grimace Scale was scored by an observer blind to the  
69 experimental study design (Dalla Costa et al., 2014). Ocular temperature measured  
70 by infrared thermography (IRT) (Yarnell et al., 2013), heart rate variability (HRV) (von  
71 Borell et al., 2007) and salivary cortisol (Hughes et al., 2010) were measured as  
72 indicators of stress and arousal. Data from the previous study on naïve horses (Ijichi  
73 et al., 2018) was also included to compare the responses of trained and naïve  
74 horses. Results were compared between Control and Dually™ in Trained horses and

75 between Naïve and Trained horses. It was predicted that Dually™ Training would  
76 result in improved compliance, and reduced arousal and HGS scores compared to  
77 Trained Control and Naïve Dually™ horses.

78

## 79 **2. Method**

80 A sample of 16 resident Nottingham Trent University horses (10 geldings and 6  
81 mares) aged between 4 and 22 years (mean = 13 years  $\pm$  4.85) participated in the  
82 study. Subjects were housed and managed as per normal protocol. In general,  
83 horses were provided with forage three times a day, hard feed dependent on  
84 workload and nutritional requirements and had access to fresh water at all times. At  
85 the time of testing, subjects were housed individually or with a companion during the  
86 day and turned out at night. The study took place in an enclosed outdoor research  
87 arena at Brackenhurst campus between 14<sup>th</sup> and 17<sup>th</sup> May 2019. Horses were paired  
88 according to companion preference and both were present in their allocated pair in  
89 the arena during training and testing to prevent isolation stress. All horses were  
90 handled by the same experimental handler for all training and testing sessions (CI).

91

### 92 *2.1 Training Protocol*

93 Subjects underwent three 10-minute training sessions wearing a correctly fitted  
94 Dually™ headcollar (Roberts, 1999) with the lead-rope attached to the left side ring.  
95 All three training sessions were carried out on the same day over a 1-hour period for  
96 each pair, alternating 10-minute training sessions with 10 minutes of rest. Pair order  
97 was pseudo-randomised to account for subject availability. A training chute 2m x  
98 12m was marked along the short side of the arena using standard jump poles laid

99 end-to-end along the ground. This area was filmed using a Canon Legria HFR606  
100 camcorder.

101 The handler held the lead-rope approximately 2 inches from the side ring and  
102 maintained a light contact. Horses were led to the training chute and given a cue  
103 every four strides by applying pressure to the lead-rope. Pressure increased until the  
104 desired response was offered and then immediately released. No vocal or other  
105 tactile stimuli were used. Once at the end of the chute, the handler released the  
106 contact, scratched the horse on the withers and offered verbal praise in a soft tone.  
107 They allowed the horse to lower their head if they chose and walk at their preferred  
108 speed as they guided them in an arc around to the start of the training chute. Once  
109 at the start of the chute this process was repeated until the 10-minute training  
110 session was complete, whereupon the horse was led to the rest area. This training  
111 protocol resulted in a high number of trials (Table 1) with inter-trial intervals of  
112 approximately 5 seconds, but regular short breaks of approximately 30 seconds  
113 every three-four trials and larger 10-minute breaks between sessions to consolidate  
114 learning and minimise arousal. After completing three training sessions, subjects  
115 were returned to their stables. All subjects were able to stop, step forward,  
116 accelerate, decelerate and back-up two steps at the end of the training day (Table  
117 1). Subjects had a rest day following training with testing on the subsequent day.

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123 Table 1. Targeted responses and number of trials per session and in total.

	<b>Task</b>	<b>Number of Trials</b>
<b>Training Session 1</b>	Stop & step forward	Mean = 61 ( $\pm$ 13)
<b>Training Session 2</b>	Accelerate & decelerate	Mean = 38 ( $\pm$ 9)
<b>Training Session 3</b>	Stop, step forward, accelerate, decelerate, back-up two steps	Mean = 58 ( $\pm$ 12)
	<b>Total</b>	<b>Mean = 157 (<math>\pm</math>22)</b>

124

## 125 *2.2 Testing Protocol*

### 126 *2.2.1 Novel Handling Tests*

127 For the novel handling test, subjects were asked to cross two distinct obstacles (Test  
128 A & B) to avoid habituation from the first attempt. Subjects completed one test with a  
129 lunge-line attached to the side ring (Dually™) and one attached to the under-chin  
130 ring (Control) as per Ijichi et al. (2018). Test and treatment order were randomised in  
131 a counterbalanced design. Test A consisted of a 2.5m x 3m yellow tarpaulin secured  
132 to the ground by tent pegs; a piece of red carpet was placed on top of the tarpaulin  
133 allowing for a trim of approximately 0.75m of tarpaulin to be visible. Test B consisted  
134 of a green camouflage tarpaulin secured to the ground with individual tent pegs with  
135 a piece of pale blue carpet placed on top of the tarpaulin to leave a trim visible as per  
136 Test A.

137 The start of each test was marked by a single horizontal pole placed on the ground  
138 2m in front of the obstacle. The handler walked the horse toward the obstacle and  
139 asked the horse to cross by applying pressure to the headcollar with no additional  
140 pressure, verbal commands or further encouragement, as per the training sessions.  
141 Pressure was applied if the horse stopped, moved sideways or away from the



142 obstacle and was immediately released when the horse took a step toward the  
143 obstacle in accordance with learning theory (McGreevy and McLean, 2007).

#### 144 *2.2.2 Behaviour Analysis*

145 The area covering the pole and the tarpaulin was filmed using Canon Legria HFR606  
146 for retrospective analysis of behaviour by a hypothesis blind researcher (AB).

147 Crossing time for each test began when the subject's front hoof crossed over the  
148 pole and bore weight on the ground. Time stopped when the last rear hoof bore

149 weight on the tarpaulin. Horses engage their rear legs first when transforming into  
150 faster gaits. Therefore, horses that showed a flight response on the tarpaulin were

151 not given faster crossing times. For the attempt to be classed as a successful

152 crossing, all four hooves must have been placed onto the tarpaulin. Incomplete

153 crossings resulted in the horse being returned to make another attempt. A time limit

154 of 3 minutes was allotted for each attempt as previous research indicated that

155 subjects which had not completed the test within this time were unlikely to do so

156 (Ijichi et al., 2013). Once the 3-minute threshold had been reached the test was

157 ended. A crossing time of 180 seconds was given to any horse reaching this time

158 limit.

159 Refusal behaviour was defined as any behaviour which did not contribute to crossing  
160 the object (Ijichi et al., 2013). This included moving backwards, sideways, forwards

161 but away from the tarpaulin, rearing or remaining stationary. Refusal that lasted for

162 10 seconds or more was analysed to determine how proactive that refusal was. Nine

163 horses refused both tests for 10 seconds or more, providing data for paired tests.

164 Proactive refusal was defined as any refusal behaviour that involved movement.

165 Proactive refusal was then recorded as the percent of total refusal time for any

166 individual which showed refusal behaviour (which included remaining stationary) and  
167 reported as “proactive behaviour”. A higher value indicated a greater amount of  
168 proactive behaviour (Ijichi et al., 2013).

### 169 *2.2.3 Salivary Cortisol*

170 Saliva samples were taken from subjects immediately prior to each Training and  
171 Testing session and again 10 minutes after to allow any cortisol changes to reach  
172 the saliva (Yarnell et al., 2013). Baseline salivary cortisol measures were not taken in  
173 the stable at the same time as heart rate variability as cortisol fluctuates with diurnal  
174 rhythms (Hoffis et al., 1970). Therefore, changes from baseline may be the result of  
175 confounding factors, rather than experimental conditions per se. Saliva samples  
176 were taken with an Equisal swab gently moved over the tongue and lips of the  
177 subject (Ijichi et al., 2019). These swabs are specifically designed for use in horses  
178 and are routinely used to test for tapeworm. Subjects were familiar with similar  
179 sampling as they are regularly wormed, tested for worms and have saliva taken for  
180 cortisol analysis for other studies. Samples were placed in a cooler box with ice  
181 packs before being transferred to the laboratory freezer within 2 hours of collection.

182 A competitive ELISA (Cortisol ELISA, IBL International, Hamburg, Germany)  
183 developed for quantitative analysis of free cortisol in human saliva was used. The  
184 assay was performed according to manufacturer instructions. Saliva samples were  
185 thawed and centrifuged at 500 rpm at room temperature for 3 min using Hereaus  
186 Fresco 17 centrifuge (ThermoScientific, West Sussex, United Kingdom). The plate  
187 was shaken for 5 min using an orbital shaker (Flow Laboratories DSG Titertek,  
188 Pforzheim, Germany). The plate was washed 4 times with 1X wash buffer by gently  
189 squirting the buffer into each well with a squirt bottle. Optical density was measured

190 by a Multiscan EX (Thermo Labsystems, Vantaa, Finland). The results were  
191 calculated using four-parameter-logistic as recommended by the manufacturer. To  
192 determine the effect of training, the average of the three sessions was calculated.  
193 The change in salivary cortisol from pre-test to post-test A and B were used to  
194 determine the difference between Dually and Control, to account for diurnal  
195 fluctuations in cortisol (Hoffis et al., 1970).

196

#### 197 *2.2.4 Infrared Thermography*

198 A FLIR E4 thermal imaging camera (FLIR Systems, USA.) was used to record eye  
199 temperature (°C). IRT images were taken immediately before and after each Training  
200 and Testing session. Baseline IRT was not taken in the stable at the same time as  
201 heart rate variability as this fluctuates with environmental conditions (Church et al.,  
202 2014). Therefore, changes from baseline may be the result of confounding factors,  
203 rather than experimental conditions per se. After pre-session saliva samples were  
204 collected, horses were led to the measurement chute. This consisted of two jump  
205 poles laid parallel 1m apart. A small cavaletti block at one end marked where the  
206 horses head should be once stationary. Two cavaletti were positioned 1m away from  
207 this central marker 90° to the left and right to mark where the IRT camera should be  
208 positioned for the left and right eye. This kept the horse straight and in the same  
209 direction for all images and standardised the optimal camera angle and distance as  
210 the angle of measurement significantly affects temperature readings (Ijichi et al,  
211 Resubmitted).

212 Images were analysed using FLIR Tools software (ver. 5.9.16284.1001) to obtain a  
213 measurement for each eye. All images were analysed by the same two researchers  
214 (C.I. & H.W.). Eye temperature recordings were the maximum temperature within the

215 palpebral fissure from the lateral commissure to the lacrimal caruncle (Yarnell et al.,  
216 2013). A mean of the left and right eyes was calculated for each subject, pre and  
217 post-test, for each training session and test. The average temperature change was  
218 calculated to determine the effects of training. The change in average temperature  
219 from pre-test to post-test was used to account for individual differences and  
220 fluctuations in core temperature due to changing environmental conditions.

221

### 222 *2.2.5 Heart Rate Variability*

223 Heart rate variability was recorded with a Polar Equine V800 portable heart rate  
224 monitor for baseline and all Training and Testing sessions (Polar Electro Oy,  
225 Kempele, Finland). The surcingle was fitted to each subject after the first saliva  
226 collection at the start of Training and Testing days and remained on until the subject  
227 had completed data collection for the day. The girth area of each subject was wetted  
228 to ensure contact and enhance electrical conductivity. Electrodes were positioned in  
229 the region of the upper left thorax and the ventral midline (Yarnell et al., 2013). The  
230 receiving watch was looped onto the surcingle to ensure it remained within  
231 connectivity boundaries at all times.

232 Baseline heart rate variability was recorded to determine changes as a result of  
233 training and testing. To mitigate any potential impact of anticipatory stress, baseline  
234 heart rate and heart rate variability parameters were recorded after a period of  
235 wearing the heart rate monitor undisturbed in the home stable. Data was collected  
236 between 10.30am and 3.30pm between 11<sup>th</sup> – 14<sup>th</sup> February 2019. Horses were  
237 loosely tethered in their home environment with a headcollar and leadrope and fitted  
238 with a Polar Equine V800 Science heart rate monitor before being released. RR  
239 interval data was recorded continuously for 35 minutes while the horses were left

240 undisturbed in their home environment. Potential environmental disturbances were  
241 recorded by an observer. Thereafter, horses were caught and tethered again, the  
242 recording stopped and the heart rate monitor removed. If no environmental  
243 disturbance was observed during the recording, mean heart rate and heart rate  
244 variability readings were extracted from the section of the recording between 25 and  
245 30 minutes. If an environmental disturbance was observed that visibly affected heart  
246 rate (n=2: neighbouring horse removed), readings were taken from the 5 minutes  
247 immediately preceding that disturbance.

248 For Training and Testing, subjects were allowed 5 minutes to habituate to the  
249 surcingle, deemed to be sufficient as all subjects have previously worn these heart  
250 monitors on several occasions. Heart rate recording commenced when the horse left  
251 the measurement chute to begin testing and ceased when the horse re-entered the  
252 measurement chute post-test after the last training or testing session of the day.

253 Kubios software (version 3.0.2 Biomedical Signal Analysis and Medical Imaging  
254 Group, Department of Applied Physics, University of Eastern Finland, Kuopio,  
255 Finland) was used to analyse heart rate data and determine HRV. Artefact correction  
256 was set to custom level 0.03, removing RR intervals varying more than 30% from the  
257 previous interval. Trend components were adjusted using the concept of smoothness  
258 priors set at 500ms, to avoid the effect of outlying intervals (Ille et al., 2014).

259 Frequency Domain analysis was set at  $>0.01 - \leq 0.07$  for Low Frequency (LF) and  $>$   
260  $0.07 - \leq 0.5$  for High Frequency (HF) (Stucke et al., 2015). The full recording from  
261 leaving the IRT measurement chute to returning after completing each training or  
262 test session was selected for analysis. RMSSD values were used as these reflect  
263 high frequency beat-to-beat variations indicative of vagal activity (Stucke et al.,  
264 2015). In addition, Frequency Domain Analysis (FDA) was conducted using a fast

265 Fourier transformation which were expressed as ratios for enhanced comparability  
266 (Stucke et al., 2015). The ratio of Low to High Frequency (LF/HF) reflects both  
267 parasympathetic and sympathetic tone as well as cardiac sympatho-vagal balance.  
268 The average RMSSD and LF/HF for the three training sessions was calculated to  
269 determine the effects of training.

#### 270 *2.2.6 Horse Grimace Scale*

271 During testing, images were taken of each subject with a Panasonic camera (Model,  
272 DMC-FZ72, Japan). The photographer (H.W.) used a zoom lens to take detailed  
273 images of the subject's face from a distance of approximately 3m. Images were  
274 included in analysis if the lunge line formed a straight line from the handler's hand to  
275 the ring of the headcollar, indicating that pressure was being applied to the  
276 headcollar in that instance. Therefore, subjects who completed the task without  
277 hesitation did not provide images for analysis, as no pressure was required to  
278 indicate they should walk forward. Crossing time also influenced the number of  
279 images available for each subject. Images that were clearly in focus were  
280 preferentially selected. A total of 256 photographs (Control: subjects with images =  
281 12, mean images per subject = 8.67; Dually: subjects with images = 12, mean  
282 images per subject = 10) were then analysed against the Horse Grimace Scale  
283 (Dalla Costa et al., 2014) by a researcher blind to the research hypothesis (FD).  
284 Where an area of the face (facial action unit) was obscured it was not scored. The  
285 mean score for each Facial Action Unit from all images was calculated and then  
286 totalled to give the HGS score for each subject in each treatment.

287

288

289

### 290 *2.2.7 Retrospective Analysis*

291 To determine a potential effect of training on behaviour and physiology in horses  
292 wearing a Dually™ headcollar, previously collected data from 20 naïve horses who  
293 had not been trained in a Dually™ headcollar was also included (Ijichi et al., 2018).  
294 These subjects underwent the same testing procedure over novel objects, full details  
295 of which are reported by Ijichi et al (2018). Eye temperatures, crossing times and  
296 proactive behaviour were available for these subjects, but not HRV or salivary  
297 cortisol. Images of the subject's faces were re-analysed by the same researcher  
298 (FD) using the method stated in 2.2.6 in order to provide comparable data. A total of  
299 150 images was available for analysis (Control: subjects with images= 13, mean  
300 images per subject = 6.5; Dually: subjects with images = 12, mean images per  
301 subject = 7.5). The behaviour, HGS and physiology of Trained and Naïve horses was  
302 then compared.

303

### 304 *2.3 Ethics*

305 The yard manager provided informed consent for all subjects via the completion of a  
306 participant information form. Both researchers and the manager had the right to  
307 withdraw a subject at any time, for any reason, until the point of data analysis. Prior  
308 to commencement, the current study was authorised by the Nottingham Trent  
309 University Ethics Committee.

310

### 311 *2.4 Statistical Analysis*

312 Statistical analysis was carried out using R (R Development Core Team, 2017).  
313 Shapiro-Wilks tests were used to test the distribution of the residuals between paired

314 variables. Differences between baseline or pre-training and post-training physiology,  
315 pre and post-testing, and between Control and Dually™ treatments were  
316 investigated using either Paired T-tests or Wilcoxon tests as appropriate for  
317 normality. Shapiro-Wilks tests were used to test the distribution of variables and  
318 Levene Tests were used to test homogeneity of variance for independent tests of  
319 difference. Differences between Naïve and Trained horses were tested using  
320 Independent T-tests or Mann Whitney U-tests as appropriate for normality and  
321 homogeneity of variance. Tests of difference between Trained and Naïve were only  
322 conducted if there was no difference in Control. Otherwise, differences observed  
323 may have been due to different samples. Post-hoc effect sizes were then calculated  
324 as per Field et al. (2012).

325

### 326 **3. Results**

#### 327 *3.1 Effect of Training on physiology*

328 RMSSD was significantly lower on average during training, compared to baseline  
329 (Paired T-test:  $T = -3.98$ ,  $N = 12$ ,  $P = 0.002$ ,  $D = 0.754$ ). LF/HF was significantly  
330 higher on average during training, compared to baseline (Wilcoxon:  $V = 78$ ,  $N = 14$ ,  
331  $P = 0.021$ ,  $D = -0.541$ ). No other indicators of stress were significantly different  
332 between rest and training (Table 2).

333



334

335 Table 2. Differences in physiology as a result of training. Paired T-Tests (PTT) and Wilcoxon  
336 tests (W) are used as appropriate for normality.

Variable	Treatment	Mean/ Median	SD/ IQR	Test	V/T	P	Effect Size	N
<b>IRT Change (°C)</b>	Pre-Training	35.890	0.912	PTT	0.79	0.441	0.207	15
	Post-Training	36.089	0.517					
<b>RMSSD (ms)</b>	Baseline	103.640	43.899	PTT	-3.98	<b>0.002</b>	<b>0.754</b>	12
	Training	49.145	16.206					
<b>LF/HF</b>	Baseline	0.873	0.597	W	78.00	<b>0.021</b>	<b>-0.541</b>	14
	Training	1.178	0.730					
<b>Cortisol (µg/dL)</b>	Pre-Training	0.605	0.458	W	39.00	0.144	-0.365	16
	Post-Training	0.478	0.584					

337

### 338 3.2 Effect of Testing on physiology

339 RMSSD was significantly lower after testing for both Dually™ (Paired T-test: T =  
340 3.23, N = 12, P = 0.007, D = 0.667) and Control (Wilcoxon: V = 102, N = 12, P <  
341 0.001, D = 0.989). There was a tendency for LF/HF to increase after both Dually™  
342 (Paired T-test: T = -1.81, N = 14, P = 0.094, D = 0.448) and Control (Wilcoxon: V =  
343 23, N = 14, P = 0.067, D = -0.916). No other variables differed following Testing  
344 (Table 3).

345

346

347 Table 3. Differences in physiology as a result of Testing. Paired T-Tests (PTT) and Wilcoxon  
 348 tests (W) are used as appropriate for normality.

Variable	Treatment	Mean/Median	SD/IQR	Test	V/T	P	Effect Size	N
IRT (°C)	Pre-Dually	35.743	±0.924	PTT	0.30	0.765	0.078	16
	Post-Dually	35.681	±1.053					
	Pre-Control	35.600	±0.800	PTT	0.34	0.741	0.087	
	Post-Control	35.544	±0.753					
RMSSD (ms)	Baseline	103.644	±43.900	PTT	3.23	<b>0.007</b>	<b>0.667</b>	12
	Post-Dually	48.343	±26.640					
	Baseline	87.430	65.230	W	102.00	<b>&lt;0.001</b>	<b>-0.989</b>	
	Post-Control	49.567	24.027					
LF/HF	Baseline	0.873	±1.021	PTT	-1.81	<b>0.094</b>	<b>0.448</b>	14
	Post-Dually	2.542	±2.706					
	Baseline	0.558	0.597	W	23.00	<b>0.068</b>	<b>-0.916</b>	
	Post-Control	1.455	1.527					
Cortisol (µg/dL)	Pre-Dually	0.327	0.663	W	57.00	0.587	-0.136	16
	Post-Dually	0.276	0.258					
	Pre-Control	0.327	0.663	W	46.00	0.274	-0.273	
	Post-Control	0.29	0.3275					

349

350

351 *3.3 Differences between Treatment and Control*

352 Proactive behaviour had a tendency to be significantly higher in the Dually™,  
 353 compared to the Control (Paired T-Test: T = 2.214, N = 9, P = 0.066, D = 0.6). No  
 354 other differences were observed between Treatment and Control (Table 4).

355

356

357

358 Table 4. Differences in behaviour and physiology between Dually and Control in Trained  
 359 horses. Paired T-Tests (PTT) and Wilcoxon tests (W) are used as appropriate for normality.

Variable	Treatment	Mean/ Median	SD/IQR	Test	V/T	P	Effect Size	N
<b>HGS</b>	Dually	1.99	±0.75	PTT	-1.22	0.247	0.345	12
	Control	1.7	±0.93					
<b>IRT Change (°C)</b>	Dually	-0.056	±0.668	PTT	0.023	0.982	0.008	16
	Control	-0.063	±0.821					
<b>RMSSD (ms)</b>	Dually	49.567	±24.027	PTT	0.206	0.840	0.053	16
	Control	48.343	±26.639					
<b>LF/HF</b>	Dually	1.913	1.952	W	81	0.528	-0.158	16
	Control	1.455	1.527					
<b>Cortisol Change (µg/dL)</b>	Dually	-0.001	0.299	W	69	0.980	-0.006	16
	Control	-0.002	0.299					
<b>Crossing Time (secs)</b>	Dually	23.300	57.500	W	76	0.698	-0.097	16
	Control	20.700	47.750					
<b>% Proactivity</b>	Dually	53.290	±26.124	PTT	2.124	<b>0.066</b>	<b>0.600</b>	9
	Control	30.170	±36.772					

360

### 361 3.4 Differences between Trained and Naïve Horses

362 There was no significant difference between Naïve and Trained Control HGS (T-  
 363 Test:  $T = 0.347$ ,  $N_1 = 13$ ,  $N_2 = 12$ ,  $P = 0.733$ ). There was also no difference in HGS  
 364 between Trained and Naïve horses when wearing the Dually (T-Test:  $T = 1.42$ ;  $N_1 =$   
 365  $12$ ,  $N_2 = 14$ ,  $P = 0.179$ ). Further, there was no difference in HGS between Dually  
 366 and Control in Naïve horses, when considering re-scored images (Mann Whitney:  $V$   
 367  $= 13$ ,  $N = 8$ ,  $P = 0.528$ ). When wearing the Dually™, Trained horses did not have  
 368 significantly lower IRT changes, compared to Naïve horses (T-Test:  $T = 0.448$ ,  $N_1 =$   
 369  $14$ ,  $N_2 = 16$ ,  $P = 0.251$ ). When wearing the Dually™, Trained horses did not cross  
 370 the obstacle significantly more quickly than Naïve horses (Mann Whitney:  $U = 188$ ,  
 371  $N_1 = 19$ ,  $N_2 = 16$ ,  $P = 0.239$ ). Trained horses did show significantly more proactive  
 372 behaviour than Naïve horses when wearing the Dually™ (T-Test:  $T = -3.904$ ,  $N_1 =$

373 13, N2 = 9, P = 0.002) and a strong effect was observed (D = 0.753). No difference  
 374 in proactivity was observed between Trained and Naïve horses in the Control (Mann  
 375 Whitney: U = 77, N1 = 14, N2 = 11, P = 1). No other variables differed between  
 376 Trained and Naïve horses (Table 5).

377

378 Table 5. Differences in behaviour and physiology between Trained and Naïve horses for  
 379 Dually and Control. Independent T-Tests (TT) and Mann Whitney U-Tests (MW) were  
 380 conducted as appropriate for normality.

Variable	Treatment	Mean/ Median	SD/IRQ	Test	U/T	P	Effect Size	N
<b>HGS</b>	Naïve Control	1.9	±1.9	TT	0.347	0.733	0.082	13
	Trained Control	1.7	±0.93					12
	Naïve Dually	2.96	±2.27	TT	1.42	0.179	0.366	12
	Trained Dually	1.99	0.75					14
<b>IRT Change</b>	Naïve Control	-0.443	±1.054	TT	1.181	0.251	0.439	14
	Trained Control	-0.056	±0.668					16
	Naïve Dually	-0.196	±0.814	TT	0.448	0.658	0.163	14
	Trained Dually	-0.063	±0.821					16
<b>Crossing Time</b>	Naïve Control	31	132.5	W	174	0.474	-0.119	19
	Trained Control	20.7	47.75					16
	Naïve Dually	40	128.5	W	188	0.239	-0.196	19
	Trained Dually	23.3	57.5					16
<b>% Pro- activity</b>	Naïve Control	17.15	15.32	W	77	1	0	14
	Trained Control	10.72	63.7					11
	Naïve Dually	15.65	±14.905	TT	-3.904	<b>0.002</b>	<b>0.753</b>	13
	Trained Dually	53.289	±26.124					9

381

#### 382 4. Discussion

383 The aim of the present study was to investigate how training horses to respond to  
 384 the pressure of the Dually™ headcollar affected compliance and stress in a novel  
 385 handling test. The impact of the Dually™ on stress physiology during training and  
 386 testing was also assessed. Following training, horses were asked to complete two  
 387 novel handling tests, once with the line attached to the side-ring and once with the

388 line attached to the standard under chin ring as a control. Results indicate the  
389 Dually™ may have a negative effect on compliance but does not cause welfare  
390 concerns in horses trained to respond to the pressure/release mechanism.

391 During the novel test, Trained horses in the Dually™ were not significantly quicker to  
392 cross the novel object than horses in the Control headcollar setting. Further, Trained  
393 horses did not cross more quickly than Naïve horses. The first Dually™ study also  
394 demonstrated no difference in crossing time between horses wearing the Dually™  
395 and those wearing a control headcollar (Ijichi et al., 2018). One of the limitations to  
396 the first study was that subjects had no prior training in the Dually™, therefore it  
397 could be expected that training would improve compliance. It is generally agreed that  
398 training horses to respond to handler signals via stimulus generated by pressure  
399 from a headcollar is an effective way to achieve compliance (McLean, 2005).

400 However, there was a tendency for Trained horses to be more proactive in the  
401 Dually™ than the Control and significantly more so than Naïve horses in the  
402 Dually™. No difference was seen for proactivity between Trained and Naïve horses  
403 for the Control setting, indicating that differences seen in the Dually cannot be  
404 explained by the different sample of horses. This suggests that training in fact  
405 increased resistance to the device, rather than improving it as the horse learns how  
406 to release the pressure. Taken together, this indicates that the Dually™ does not  
407 improve compliance during handling. It is not clear whether further training would  
408 extinguish or exacerbate this proactive response.

409 It may be that three training sessions were not sufficient to significantly alter the  
410 effect of the Dually™. However, subjects experienced an average of 157 ( $\pm 22$ )  
411 attempts in this time and during training all horses in the study were compliant and  
412 able to consistently offer the desired response. Another possibility is that the three-

413 minute handling challenge was not long enough for the effect of the Dually™ to be  
414 observed. This is contradicted by the fact that all but one horse crossed within this  
415 time. A counter explanation for the lack of effect of the Dually™ is that the handling  
416 tests were not aversive enough. However, most horses (60%) resisted crossing the  
417 obstacle in the current study. Further, LF/HF was elevated, whilst RMSSD  
418 decreased, indicating that the handling tests were inducing observable arousal. More  
419 aversive tests may not be considered ethically appropriate within the context of  
420 research. Finally, proponents of the device might explain this lack of improvement  
421 following training by noting that we did not perform “join-up” during training.  
422 However, multiple sources of evidence indicate this is not a useful training approach  
423 for building bond (Henshall et al., 2012) and does not generalise to other contexts  
424 (Krueger, 2007).

425 In the previous research, HGS scores were significantly higher in the Dually™  
426 compared to the control (Ijichi et al., 2018). However, the scorer was not blind to  
427 treatment, as these cannot easily be obscured from the photos without limiting how  
428 clearly the face can be observed. In the current experiment, a hypothesis blind rater  
429 was used to resolve this limitation. In the current study, there was no difference in  
430 HGS between Dually™ and Control in Trained horses. Whilst this might suggest that  
431 training reduces the discomfort caused by the Dually, there was no difference in  
432 HGS between Trained and Naïve subjects during Dually use. This indicates that it is  
433 not training per se that explains this finding. In fact, reanalysed HGS for Naïve  
434 horses did not show a significant difference between Dually and Control, challenging  
435 the finding of the original paper. This is likely to be the result of including all images  
436 (rather than a random sample) and calculating HGS by averaging each Facial Action  
437 Unit (FAU) and then totalling these (rather than using percentage to account for

438 missing FAU). Whilst HGS were still higher for Dually compared to Control this was  
439 no longer significant. An increased HGS score, although non significant, has been  
440 already described in horses experiencing fear (Dalla Costa et al 2016). Further  
441 research could be conducted to observe behaviour and HGS longitudinally in horses  
442 being tested in the Dually for the first time compared to after a period of training.

443 Although the Dually™ had a potentially negative effect on compliance, there was no  
444 effect of training on stress indicators. There was no difference in IRT, RMSSD,  
445 LF/HF or salivary cortisol between Dually™ and Control, suggesting the Dually™  
446 does not reduce welfare within a 3-minute handling challenge when compared to a  
447 standard headcollar. This does not contradict findings that the Dually caused greater  
448 proactivity, as proactive behaviour does not necessarily indicate higher arousal  
449 (Munsters et al., 2013; Squibb et al., 2018; Yarnell et al., 2013). Similar stress  
450 profiles between Dually and Control supports the observation in the original research  
451 which indicated there was no difference in IRT between Dually™ and Control in  
452 Naïve horses, despite higher HGS scores (Ijichi et al., 2018). Further, IRT did not  
453 differ between Trained and Naïve horses. However, it is worth considering that these  
454 indicators of arousal might alter if the testing lasted longer than 3 minutes. For  
455 example, studies investigating the effects of tight noseband, which apply pressure to  
456 the same anatomical structures, observed horses for 10 minutes (Fenner et al.,  
457 2016; McGreevy et al., 2012). It is important to know whether longer handling  
458 sessions more representative of typical behaviour modification sessions do result in  
459 stress. Indeed, average RMSSD significantly decreased whilst LF/HF significantly  
460 increased during Training compared to a stabled baseline. These HRV variables  
461 suggest that training in the Dually™ headcollar caused observable arousal (Stucke  
462 et al., 2015), though this was not seen in IRT or salivary cortisol changes. Further, it

463 is not clear whether the Dually™ caused more arousal than the same training in a  
464 standard headcollar, as Control training sessions were not conducted.

## 465 **5. Conclusion**

466 The findings of the current study indicate that the Dually™ does not improve  
467 compliance in trained horses as horses do not cross more quickly compared to a  
468 standard headcollar. In fact, potentially dangerous proactive behaviour was  
469 increased in the Dually™ and is exacerbated by training, rather than diminishing this  
470 response. It should be noted that the device does not appear to cause more stress  
471 or discomfort than standard headcollars in Trained horses, though the short testing  
472 time may not be sufficient to detect an effect of the headcollar on arousal. Therefore,  
473 while the efficacy of the device is questionable, it does not appear to cause poorer  
474 welfare and if owners perceive that it gives them more control this may justify its use.

475

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480

## 481 **Author Contributions**

482 The idea for this paper was conceived by Carrie Ijichi; the experiment was designed  
483 by Carrie Ijichi and Hayley Wild; data was collected by Carrie Ijichi, Hayley Wild,  
484 Heather Cameron-Whytock, Samuel White, Aurelie Jolivald, Sarah Hallam and  
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