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Title	Dually investigated: The effect of a pressure headcollar on the behaviour, discomfort and stress of trained horses
Type	Article
URL	https://clock.uclan.ac.uk/id/eprint/47722/
DOI	doi10.1016/j.applanim.2020.105101
Date	2020
Citation	Ijichi, Carrie, Wild, Hayley, Dai, Francesca, Bordin, Alexandre, Cameron-Whytock, Heather, White, Samuel J, Yarnell, Kelly, Starbuck, Gareth, Jolivald, Aurelie et al (2020) Dually investigated: The effect of a pressure headcollar on the behaviour, discomfort and stress of trained horses. Applied Animal Behaviour Science, 232. ISSN 0168-1591
Creators	Ijichi, Carrie, Wild, Hayley, Dai, Francesca, Bordin, Alexandre, Cameron-Whytock, Heather, White, Samuel J, Yarnell, Kelly, Starbuck, Gareth, Jolivald, Aurelie, Birkbeck, Lauren, Hallam, Sarah and Dalla Costa, Emanuela

It is advisable to refer to the publisher's version if you intend to cite from the work.
doi10.1016/j.applanim.2020.105101

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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 14th and 17th 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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122

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

	Task	Number of Trials
Training Session 1	Stop & step forward	Mean = 61 (± 13)
Training Session 2	Accelerate & decelerate	Mean = 38 (± 9)
Training Session 3	Stop, step forward, accelerate, decelerate, back-up two steps	Mean = 58 (± 12)
Total		Mean = 157 (± 22)

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 11th – 14th 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

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

2.2.6 Horse Grimace Scale

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

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
IRT Change (°C)	Pre-Training	35.890	0.912	PTT	0.79	0.441	0.207	15
	Post-Training	36.089	0.517					
RMSSD (ms)	Baseline	103.640	43.899	PTT	-3.98	0.002	0.754	12
	Training	49.145	16.206					
LF/HF	Baseline	0.873	0.597	W	78.00	0.021	-0.541	14
	Training	1.178	0.730					
Cortisol (µg/dL)	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	0.007	0.667	12
	Post-Dually	48.343	±26.640					
	Baseline	87.430	65.230	W	102.00	<0.001	-0.989	
	Post-Control	49.567	24.027					
LF/HF	Baseline	0.873	±1.021	PTT	-1.81	0.094	0.448	14
	Post-Dually	2.542	±2.706					
	Baseline	0.558	0.597	W	23.00	0.068	-0.916	
	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
HGS	Dually	1.99	±0.75	PTT	-1.22	0.247	0.345	12
	Control	1.7	±0.93					
IRT Change (°C)	Dually	-0.056	±0.668	PTT	0.023	0.982	0.008	16
	Control	-0.063	±0.821					
RMSSD (ms)	Dually	49.567	±24.027	PTT	0.206	0.840	0.053	16
	Control	48.343	±26.639					
LF/HF	Dually	1.913	1.952	W	81	0.528	-0.158	16
	Control	1.455	1.527					
Cortisol Change (µg/dL)	Dually	-0.001	0.299	W	69	0.980	-0.006	16
	Control	-0.002	0.299					
Crossing Time (secs)	Dually	23.300	57.500	W	76	0.698	-0.097	16
	Control	20.700	47.750					
% Proactivity	Dually	53.290	±26.124	PTT	2.124	0.066	0.600	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, N1 = 13, N2 = 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; N1 =

365 12, N2 = 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, N1 =

369 14, N2 = 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 N1 = 19, N2 = 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, N1 =

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

377

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

Variable	Treatment	Mean/ Median	SD/IRQ	Test	U/T	P	Effect Size	N
HGS	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
IRT Change	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
Crossing Time	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
% Pro- activity	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	0.002	0.753	13
	Trained Dually	53.289	±26.124					9

381

382 4. Discussion

The aim of the present study was to investigate how training horses to respond to the pressure of the Dually™ headcollar affected compliance and stress in a novel handling test. The impact of the Dually™ on stress physiology during training and testing was also assessed. Following training, horses were asked to complete two 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 (± 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

476 **Acknowledgements**

477 We are indebted to Anna Gregory, Cath Hake and Jake Bromley-Fowles for
478 facilitating this research on the Brackenhurst yard. Salivary cortisol analysis was
479 supported by the European Regional Development Fund.

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
485 Lauren Birkbeck; analysis was done by Carrie Ijichi, Francesca Dai; Emanuela Dalla

486 Costa, Hayley Wild, Alex Bordin, Gareth Starbuck and Kelly Yarnell; statistical
487 analysis was done by Carrie Ijichi; the paper was written by Carrie Ijichi and drafted
488 by all authors. The authors of this manuscript have no conflict of interest to declare
489 and no funding bodies to acknowledge.

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