

1 **Individual Behavioral and Physiological Responses During Different**
2 **Experimental Situations – Consistency over Time and Effects of**
3 **Context**

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12

13 **Abstract**

14 In a number of species, consistent behavioral differences between individuals have been
15 described in standardized tests, *e.g.* novel object exploration, open field test. Different
16 behavioral expressions are reflective of different coping strategies of individuals in stressful
17 situations. A causal link between behavioral responses and the activation of the physiological
18 stress response is assumed but not thoroughly studied. Also, most standard paradigms
19 investigating individual behavioral differences, are framed in a fearful context, therefore the
20 present study aimed to add a test in a more positive context, the feeding context. We assessed
21 individual differences in physiological (heart rate, HR) and behavioral responses (presence or
22 absence of pawing, startle response, defecation, snorting) of twenty domestic horses (*Equus*
23 *caballus*) in two behavioral experiments, a novel object presentation and a pre-feeding
24 excitement test. Experiments were conducted twice, in summer and autumn. Both
25 experiments caused higher mean HR in the first ten seconds after stimulus presentation
26 compared to a control condition, but mean HR did not differ between the experimental
27 conditions. Interestingly, in the novel object experiment, horses displaying stress-related
28 behaviors during the experiments also showed a significantly higher HR increase compared
29 to horses which did not display any stress-related behaviors, reflecting a correlation between
30 behavioral and physiological responses to the novel object. On the contrary, in the pre-
31 feeding experiments, horses that showed fewer behavioral responses had a greater HR
32 increase, indicating the physiological response being due to emotional arousal and not
33 behavioral activity. Moreover, HR response to experimental situations varied significantly
34 between individuals, and although we found HR to be significantly repeatable across
35 experiments, repeatability indices were low. In conclusion, our findings show that horses'
36 behavioral and physiological responses differed between test situations and that high

37 emotional reactivity, shown via mean HR and HR increase, is not always displayed

38 behaviorally.

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40 Key words: individual variation, heart rate, novel object, pre-feeding excitement, domestic

41 horses, *Equus caballus*

42

43

44 **Introduction**

45 The perception of a potential threat to homeostasis, caused by extrinsic or intrinsic
46 stimuli (stressors) results in the activation of the physiological stress response in animals
47 (Chrousus and Gold 1992; Moberg 1985). Regardless of the intensity of the stressor,
48 individuals respond both behaviorally and physiologically, and these responses aim to
49 counteract the effects of the stimuli and to re-establish homeostasis (Chrousus and Gold
50 1992). Physiological activation in response to a stressor causes a fast release of
51 norepinephrine in the brain, triggering the activation of the sympatho-adreno-medullary
52 (SAM) axis, and the hypothalamic-pituitary-adrenal (HPA) axis (Goldstein 2010). As a
53 consequence, heart rate (HR) and glucocorticoid levels increase (Elia et al. 2010; McGreevy
54 et al. 2005).

55

56 The way an individual perceives a stressor can significantly influence its
57 responsiveness (Moberg 1985). Indeed, both endogenous and exogenous factors have been
58 shown to modulate an animal's sensitivity to a perceived threat, which results in individual
59 differences in responsiveness within the same species (Gosling 2001). For example, the
60 presence of constant environmental stressors can lead to a desensitization of the threat-
61 perception, or learned helplessness, which can cause a reduction of individual responses to a
62 stressor (Ellis et al. 2014). Furthermore, early differences in environmental stimuli, such as
63 differences in maternal investment, may cause individuals to follow diverse trajectories,
64 shaping their behavioral repertoire and physiological responsiveness in different manners
65 (Claessens et al. 2011; Stamps 2003). For example, Meany (2001) showed how rat pups
66 raised by low-licking mothers are more fearful and more sensitive to stress in adulthood
67 compared to pups reared by high-licking mothers. Moreover, animals can be bred to either
68 show high or low responsiveness to stressors, indicating that individual differences in stress-

69 responsiveness are heritable (Carere et al. 2003; Flaherty and Rowan 1989). Studies on great
70 tits (*Parus major*) have shown individual physiological stress responses to be related to
71 differences in exploration strategies (Carere and van Oers 2004) and heritable throughout
72 four generations (Drent et al. 2003). If individual differences in physiological and/or
73 behavioral responses are found to be relatively stable across time and contexts, they are
74 regarded as temperamental traits (Cockrem 2007; Goldsmith et al. 1987; Lansade et al. 2008;
75 Le Scolan et al. 1997).

76

77 Temperamental traits have been found in both vertebrate and invertebrate taxa (Carere
78 et al. 2003; Kralj-Fišer et al. 2010; Momozawa et al. 2003; Riechert and Hedrick 1993;
79 Verbeek et al. 1994; While et al. 2009; Øverli et al. 2006). Studies have shown that
80 individual differences along the shy-bold axis are linked with aggressiveness (Kralj-Fišer et
81 al. 2010), exploratory behavior (Sibbald et al. 2009), neophobia (Momozawa et al. 2003), and
82 physiological responsiveness, such as hormonal (Carere et al. 2003), and HR modulation
83 (Kralj-Fišer et al. 2010). Heart rate presents a valid indicator of emotional arousal, defined as
84 an internal state, which is triggered by specific extrinsic or intrinsic stimuli (Anderson and
85 Adolphs 2014). Arousal can range between the subject being calm – low arousal, and excited
86 – high arousal, as well as the experience being of positive or negative valence (Russell 1980).
87 Briefer et al. (2015) have studied the effect of emotional arousal and valence on the
88 physiological and behavioral response in goats, showing both, positive (feeding) as well as
89 negative (frustration, isolation) emotional context to cause a significant physiological
90 response compared to a control situation.

91

92 An evaluation of individual differences in emotional arousal in response to situations
93 of positive emotional valence or the relationship of activation of the physiological stress

94 response in situations of different emotional valence is still lacking. In the present study, we
95 aim at investigating individual differences in emotional arousal in response to two
96 experimental paradigms, a novel object exposure and a test of pre-feeding excitement. So far,
97 most studies investigating temperamental traits in non-human animals have tested those in
98 fearful contexts, *e.g.* novel object exploration or open field tasks (Dall and Griffith 2014;
99 Smith and Blumstein 2008). Thereby we aimed to investigate physiological and behavioral
100 responses in a fearful (novel object) and anticipatory (pre-feeding excitement) context.

101

102 In horses (*Equus caballus*), diverse factors influencing individual differences in
103 behavior and physiology have been identified in terms of experience, such as habituation
104 (Leiner and Fendt 2011), diet (Bulmer et al. 2015), handling (Visser et al. 2002), and
105 maternal behavior (Haupt and Hintz 1983). Similarly, breed has also been found to strongly
106 influence individual reactivity, suggesting a relationship between individual responsiveness
107 and the heritability of traits (Hausberger et al. 2004; Lloyd et al. 2008). Further studies on
108 equine temperament have focused on the assessment of horse responsiveness to different
109 stimuli such as diverse environmental conditions (McCall et al. 2006; Schmidt et al. 2010a;
110 Schmidt et al. 2010b), novel situations (Ellis et al. 2014; Fureix et al. 2009; Leiner and Fendt
111 2011; Visser et al. 2001; Visser et al. 2002) human interactions in terms of both handling
112 (Ellis et al. 2014; Fureix et al. 2009; König von Brostel et al. 2011) and riding (Visser et al.
113 2008) and have shown how an individual's response to threat – or 'fearfulness' – is stable
114 across time (Lansade et al. 2008; Visser et al. 2001; Visser et al. 2003). However, contrasting
115 results have been found according to the relationship between HR and behavioral parameters
116 (Christensen et al. 2005; Lansade et al. 2008; Momozawa et al. 2003). For instance, only
117 Momozawa et al. (2003) found behavioral correlations with HR in their fear-inducing

118 experiments; results which were not supported in subsequent research (Christensen et al.
119 2005; Lansade et al. 2008).

120

121 In the present study, we aim at gaining further understanding of the relationship
122 between individual differences in behavioral and physiological reactivity in horses. Heart rate
123 represents a standardized, objective and non-invasive measure to infer an individual's
124 emotional arousal level, readily available to inexperienced behavioral observers (Lansade et
125 al. 2008; Visser et al. 2003). We question whether physiological responses during a novel
126 object presentation and pre-feeding test are caused by behavioral changes or purely emotional
127 arousal, in the absence of behavioral activity. Furthermore, we ask whether behavioral and
128 physiological responses are stable across time and contexts. We expect that horses show a
129 greater physiological reaction to a fear-inducing situation such as being exposed to a novel
130 object, compared to the anticipatory pre-feeding exposure.

131 **Methods**

132 *Animals and housing*

133 The study was conducted at the equine yard of the College of West Anglia (United
134 Kingdom) between July and November 2017. The research was conducted on 20 horses
135 which were individually stabled in loose boxes. Five of the 20 horses were tested only in one
136 of the two experimental conditions, two solely for pre-feeding excitement and three only for
137 novel object test, due to their lack of availability during testing periods (Table 1). The sample
138 included 14 geldings (Age: mean 11.8 ± 3.8 years (yrs), range 6 - 18 yrs) and 6 mares (Age:
139 mean 11.8 ± 2.6 yrs, range 10-17 yrs) of diverse breeds, use, and training experiences (Table
140 1). The horses were fed twice a day: once in the morning (0800-0830) and once in the
141 afternoon (1500-1600). Water was available *ad libitum*, and feces were removed from the
142 stables after the horses were fed. In the late afternoon (1600-1700), some of the horses were
143 turned out in the paddock for the night.

144

145 *Experimental design*

146 We conducted two experimental tests – the pre-feeding excitement and the novel
147 object test, presented in random order, with one trial per horse and repeated in summer
148 (July/August) and in autumn (October/November). Behavior was recorded by video camera
149 (Canon Legira HF R56), and HR was recorded using a Polar V800 system belt, placed around
150 the chest of the horses. The belt consists of an electrode belt with a built-in transmitter,
151 connected via Bluetooth to a wristwatch (receiver). To optimize the contact between the belt
152 and the skin, both the coat of the horse in the interested area and the belt were wetted. The
153 receiver was placed at the stable entrance or inside the stable. Prior to each test, an
154 adjustment period of 5 minutes was allowed to let the horse habituate to the belt.

155 The pre-feeding excitement test was conducted during morning feeds (0800-0830)

156 and started with the horses being shown a bucket containing their individual mix of hard feed
157 on the floor outside the stable, while the other horses were fed. The horses' physiological and
158 behavioral responses were recorded for five minutes. Thereafter, the horses were fed by
159 placing the feeding bucket inside the box and their behavioral and physiological responses
160 were measured for the following ten minutes.

161 In the novel object test, the horses were exposed to two of three different objects in
162 their stable. The first object was formed of a main cylindrical hard body (approximately 30
163 cm in length) filled with gravel which was fixed to a soft foam rubber ball (about 15 cm in
164 diameter) and covered in fabric. The second object was formed of two cylindrical plastic
165 tubes fixed together to form an 'x'. Similar to the first object, the cylinders were
166 approximately 30cm in length, filled with gravel and covered with fabric. In addition, twelve
167 tennis balls of different colors and materials were pierced and attached to 4 strings (3 balls
168 per string) of approximately 50 cm in length. These were then tied to the main body of the
169 object and left hanging. The third object was an inflatable guitar of approximately one meter
170 in length. All objects were attached to a string, around four-meters-long, to allow their
171 retrieval from the stable.

172 The object assigned to each individual was randomized for each season as well as the
173 order of the horses tested. To avoid the horses seeing the object before testing, the objects
174 were covered from sight when carried around the yard. The novel object tests took place
175 between the hours of 0900 and 1300 and between 1500 and 1800 when the yard was quiet,
176 and the horses were fed. The testing procedure was based on the one used by Dai et al. (2015)
177 and Górecka-Bruzda et al. (2011) and adapted for the present experiment. A novel object was
178 placed over the box entrance, with the cord hanging over the stable door to keep the object at
179 the height of approximately one meter. The object was kept in this position for the following

180 five minutes and was then dropped to the floor (the objects filled with gravel created a
181 muffled noise). The horse reaction was recorded for the following five minutes. Thereafter,
182 the object was removed from the stable, whilst behavioral monitoring and HR measurement
183 continued for another 15 minutes.

184 *Ethical statement*

185 All applied methods were non-invasive, and the experimental procedure was
186 approved by Anglia Ruskin University's Departmental Research Ethics Panel.

187 *Data processing*

188 Raw HR data were purged with a moving average filter to remove biologically
189 implausible outlier values. The following HR variables were calculated: (1) mean HR in
190 beats per minute (bpm) for the ten seconds preceding and following the food presentation, as
191 well as preceding (control) and following the presentation, drop and removal of the object;
192 (2) HR increase in bpm following the food presentation and novel object presentation, drop,
193 and removal, calculated as difference between maximum value and three seconds average HR
194 before the presentation of the stimuli.

195 Behavioral responses of the horses were analyzed from videos using SOLOMON
196 Coder v. beta 17.03.22 (András Péter, www.solomoncoder.com). The behavior of the
197 individuals was analyzed for the five minutes prior to the presentation of the hard feed for the
198 pre-feeding excitement. For the novel object task, the five minutes following the presentation
199 and drop of the object and the two minutes following its removal were analyzed. Behavior
200 was recorded as continuous variables, *e.g.*, feeding as duration of behavior in s per
201 observation period, or frequency of behavior per observation period, *e.g.*, snorting. The
202 classification of the individual in high and low behavioral respondents was based on the

203 frequency and duration of vocalizations, pawing behavior, startle response and defecation
204 that the individuals performed during the experimental settings (Table 2).

205

206 *Statistical Analysis*

207 All data were analyzed using R version 3.4.3 (R Core Team, 2017; RStudio Team
208 2016). Generalized linear mixed models (GLMM) were conducted with the additional
209 packages ‘glmmADMB’ (Skaug et al. 2016). For the comparison of HR, two generalized
210 linear models were calculated. Experiment (pre-feeding excitement versus novel object), test
211 period and behavioral category (high versus low respondents) were considered as fixed
212 factors in both models, together with the interaction between behavioral category and
213 experimental condition. For the purpose of the analysis, the different conditions of each
214 experiment were individually included in the dataset, resulting in horses having multiple
215 values per each experimental condition. The response variable was assigned to the ten second
216 average HR (GLMM1) and the HR increase (GLMM2). Tukey test for multiple comparisons
217 was chosen to gain further understanding of the effect of the categories of the fixed factors in
218 the models. Specifically, the ‘multcomp’ (Hothorn et al. 2008) package was used to conduct
219 the post hoc analysis. Moreover, we used a likelihood ratio test to compare models fit
220 according to presence or absence of the individual random effect. Also, individual
221 repeatability of the physiological and behavioral measurements was assessed using the ‘rptR’
222 package (Stoffel et al. 2017). In particular, we assessed the repeatability of the ten second
223 average HR and HR increase with 1000 permutations for the physiological reactivity data
224 collected for the control, novel object and pre-feeding conditions. The significance level was
225 set at $\alpha = 0.05$.

226 **Results**

227 *Seasonal variation and behavioral categorization*

228 Horses had a significantly higher mean HR during summer compared to autumn
229 (GLMM1: $z = 2.713$, $p = 0.007$), but HR increase was not significantly different between the
230 seasons (GLMM2: $z = -0.94$, $p = 0.348$). Out of the 20 horses tested, only four individuals
231 were consistently categorized as high behavioral responders and one individual as low
232 behavioral responder, in both experiments (novel object, pre-feeding excitement) and seasons
233 (summer and autumn; see Table 1 for details).

234

235 *Physiological and behavioral responses to experimental situations*

236 Average HR of the horses was significantly higher during the novel object experiment
237 compared to the control period (Tukey: $z = 5.205$, $p < 0.001$; Figure 1A, Figure 2) and tended
238 to be higher during the pre-feeding excitement compared to the control period (Tukey: $z =$
239 2.197 , $p = 0.0662$; Figure 1A, Figure 2). Heart rate between novel object and pre-feeding
240 excitement was not significantly different (Tukey: mean: $z = -1.887$, $p = 0.133$; Figure 1A,
241 Figure 2), despite horses showing a significantly lower HR increase in the pre-feeding
242 condition (GLMM2: $z = -3.59$, $p < 0.001$; Figure 1B). We found a significant interaction
243 between behavior and experiment affecting HR. Mean HR during the novel object
244 experiment was significantly higher in the group of horses showing a high behavioral
245 response compared to horses showing a low behavioral response (GLMM1: $z = -3.139$, $p =$
246 0.002 ; Figure 1A). This pattern reversed regarding HR increase in the pre-feeding
247 experiment, with the horses showing a low behavioral response having a higher HR increased
248 compared to individuals with a high behavioral response (GLMM2: $z = 3.56$, $p < 0.001$;
249 Figure 1B). Finally, the models including individual identity as random factor had a
250 significantly better fit compared to the models without the random effect (ANOVA: mean

251 HR: Deviance = 14.632, df = 1, p = 0.001; HR increase: Deviance = 7.984, df = 1, p = 0.005),
252 indicating that the random effect also significantly variance in the data.

253

254 *Repeatability*

255 Individual horses' HR was significantly repeatable across experiments. However, the
256 repeatability indices were low for both parameters tested, with the highest repeatability score
257 recorded for the HR increase ($R = 0.265$, CI 95% [0.07, 0.442], $p < 0.001$); followed by mean
258 HR ($R = 0.201$, CI 95% [0.06, 0.343], $p < 0.001$).

259

260

261 **Discussion**

262 In the present study, we investigated individual behavioral and physiological
263 responses of horses during two experimental procedures, a novel object experiment and a
264 pre-feeding excitement test. Surprisingly, we found rather little behavioral consistency across
265 tests and only a limited number of individuals responded similarly across contexts and
266 seasons, which would have been expected when behavioral responses in experimental tests
267 are indicative of temperamental traits. In contrast to behavior, heart rate (HR) was
268 significantly repeatable across experiments, although it has to be pointed out that the
269 repeatability indices were low. This indicates that physiological responses to experiments
270 were consistent over time and contexts, as expected for temperamental traits. Repeatable
271 individual variation in behavior, *i.e.* personality has been very much in focus of scientific
272 research in recent years and was described in a vast variety of species, including horses
273 (Grajfoner et al. 2010; Momozawa et al. 2005), however most of these studies are focusing
274 on behavior rather than underlying physiological processes. For example, Grajfoner et al.
275 (2010) compared individual horse behavioral ratings between high and low performers,
276 showing how the combination of multiple traits, such as a horse being “nice”, “patient”,
277 “easy to handle”, shape the perceived personality of horses. On the contrary, only a few
278 studies investigated individual differences in physiological responses and their relationships
279 with behavioral reactivity in non-human animals (Briefer et al. 2015; Ellis et al. 2014).

280

281 We described context dependent links between behavioral and physiological
282 response. The two conducted experiments differed regarding their context, with the novel
283 object experiment being considered as a fear-inducing situation (Dalmau et al. 2009; Lansade
284 et al. 2008; Leiner and Fendt 2011), whereas the pre-feeding excitement test presents an
285 anticipatory context (Mahnhardt et al. 2014). In the novel object experiment, a classic

286 temperament test in non-human animals' personality research (Carter et al. 2013), individuals
287 who were behaviorally active also showed a higher average HR. This is especially relevant as
288 the presentation of a novel object can be seen as a fear-inducing situation (Leiner and Fendt
289 2011). The results of the present study confirm that increased physical activity resulting from
290 the performance of stress-related behaviors (*e.g.*, startle response) correlates with higher
291 mean HR compared to individuals not responding behaviourally in the experiment. It is
292 difficult to conclude whether the physiological activation is caused by increased emotional
293 arousal, *i.e.* the perception of fear or the increased physical activity, *e.g.* locomotion (von
294 Borell et al. 2007; Visser et al. 2002).

295

296 A different pattern became apparent during the pre-feeding excitement task. Here,
297 individuals lacking a behavioral response during the experiment showed a higher HR
298 increase. This indicates that emotional arousal, but not physical activity, accounted for the
299 increase in HR. Horses that were classified as highly behaviorally responsive during the pre-
300 feeding experiments had an undetectable increase in HR, reflecting that physical activity may
301 have increased their HRs before the presentation of the feed. Once the feed was presented
302 their arousal level may not have changed in amplitude, as no significant HR increase was
303 recorded, but possibly in valence, from negative to positive. Effects of emotional arousal on
304 physiological responses have already been identified in other non-human animals. For
305 example, in the study by Wascher et al. (2008), immobile greylag geese (*Anser anser*)
306 watching aggressive interaction between conspecifics showed a significantly higher increase
307 in HR compared to geese watching non-social interactions. Moreover, an increase in
308 physiological reactivity resulting solely from emotional arousal was also identified in guide
309 dogs (Fallani et al. 2007).

310

311 Our findings show that HR in the control situations did not necessarily predict HR
312 during the experiments and horses differed in the strength of their responses, with some even
313 decreasing HR. Such variation, together with the repeatability of the physiological reactivity,
314 reflected how behavioral responses of horses do not necessarily predict physiological
315 reactions during a novel object and pre-feeding excitement test. This is in line with previous
316 studies on horses describing that individual classified as calmer had higher HR compared to
317 more excited individuals despite showing less behavioral signs of stress when tested for pre-
318 feeding reactivity (Ellis et al. 2014), as well as with other studies on cattle (*e.g.*, Christensen
319 et al. 2005; Jezierski et al. 1999; Lansade et al. 2008; Welp et al. 2004).

320

321 Classical models regarding individual differences in behavior and physiology, assume
322 them to be associated with each other to form different coping styles (Koolhaas et al. 1999).
323 However, evidence for independent modulation of the hypothalamic-pituitary-adrenal axis
324 (Boulton et al. 2015; Dosmann et al. 2015; Ferrari et al. 2013), the sympatho-adreno-
325 medullary axis (Qu et al. 2018), and behavioral traits have been recently accumulating. Our
326 study provides further evidence that HR and behavior might be regulated independently.

327

328 To conclude, our study illustrates the importance of accounting for context when
329 studying individual differences in non-human animals, and we suggest that the inclusion of
330 tests in non-fear related contexts is desirable. Further, we point at the importance of studying
331 interconnectedness and independencies between physiological and behavioral reactions.

332

333 **Acknowledgements**

334 We would like to thank Elizabeth Ragg and Nikki Bentley from the College of West Anglia
335 for granting us access to their facilities and horses to conduct our experiments. Furthermore,

336 we would like to thank all members of staff for helping us to facilitate our research on site.

337 Finally, we would also like to thank Irene Susini for discussion and comments on the

338 manuscript.

339 **Author Contribution statement**

340 A.S., D.V.H. & C.A.F.W. designed the experiments

341 A.S. & D.V.H. conducted the experiments

342 A.S. & C.A.F.W. analyzed the data

343 A.S., D.V.H. & C.A.F.W. wrote the paper

344

345 **Conflict of interest**

346 The authors declare that they have no competing interests.

347

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560 **Table 1.** Age in years, sex and breed of the 20 horses tested for this study. Mean heart rate
 561 and standard deviation during the different experimental situations as well as whether
 562 the horses were categorized as high (orange) or low (blue) behavioral responders.

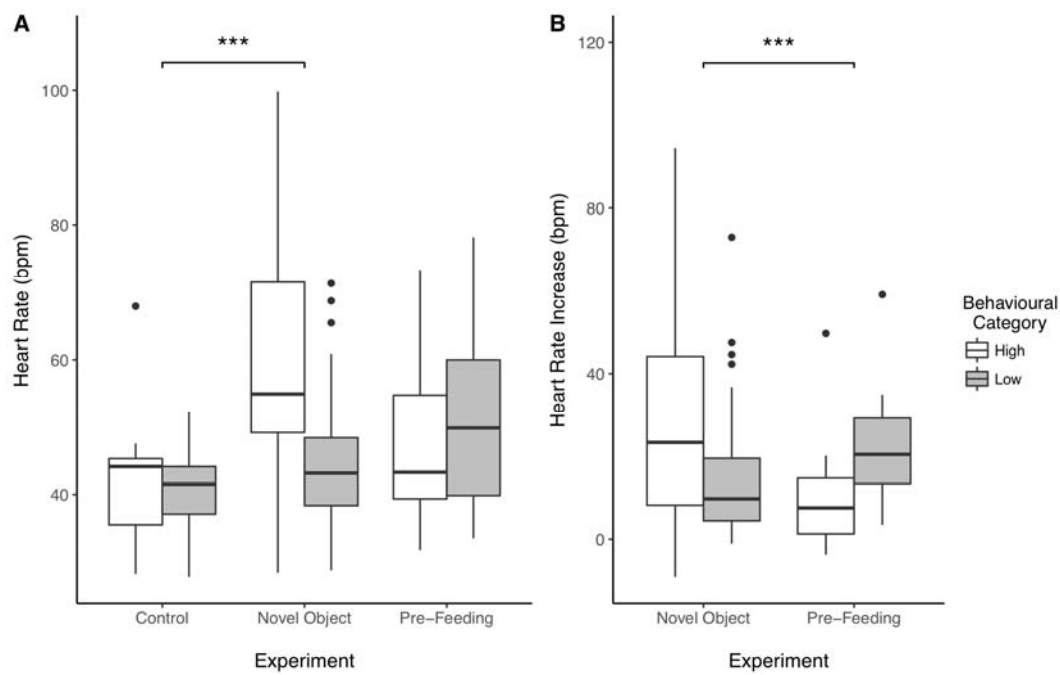
ID	Age	Sex	Breed	NO 1	NO2	PF1	PF2
1	13	Gelding	Cob	NA	NA	48.29 ± 9.89	57.14 ± 12.43
2	14	Gelding	Welsh Pony	51.47 ± 8.87	51.21 ± 5.66	67.27 ± 12.41	54.42 ± 8.01
3	17	Mare	Gypsy Cob	54.77 ± 9.71	45.16 ± 3.44	NA	NA
4	8	Gelding	Cob	44.54 ± 1.56	38.37 ± 0.40	41.08 ± 5.62	33.69 ± 2.48
5	14	Gelding	Cob	46.82 ± 2.69	54.67 ± 3.85	51.29 ± 9.89	55.47 ± 10.65
6	9	Gelding	KWPN	43.87 ± 6.93	45.93 ± 12.30	39.01 ± 2.80	39.95 ± 7.07
7	10	Mare	Welsh Crossbred	62.40 ± 12.40	43.16 ± 1.92	48.06 ± 5.75	65.76 ± 14.01
8	16	Gelding	Welsh Crossbred	71.47 ± 18.82	44.41 ± 2.40	68.63 ± 9.19	53.98 ± 13.29
9	13	Gelding	Cob	NA	NA	55.57 ± 5.06	50.73 ± 1.53
10	12	Gelding	KWPN	54.30 ± 5.87	40.24 ± 8.05	NA	NA
11	16	Gelding	Shire X Warmblood	83.26 ± 20.00	80.03 ± 29.02	60.78 ± 7.80	46.88 ± 6.05
12	11	Mare	Appaloosa X Cob	42.84 ± 1.54	41.92 ± 8.30	60.89 ± 3.36	48.30 ± 12.39
13	11	Gelding	Welsh Section A	62.73 ± 20.70	55.29 ± 25.91	NA	NA
14	12	Mare	Thoroughbred X Cob	43.93 ± 3.47	47.27 ± 4.77	61.14 ± 7.16	46.12 ± 3.40
15	18	Gelding	Thoroughbred	44.43 ± 8.47	36.94 ± 4.96	41.58 ± 1.78	33.59 ± 1.90
16	10	Mare	Cob	44.28 ± 12.98	66.93 ± 15.66	42.84 ± 4.98	44.89 ± 7.01
17	6	Gelding	KWPN	36.67 ± 2.59	31.41 ± 1.52	42.10 ± 4.30	38.41 ± 2.57
18	9	Gelding	Irish Sports Pony	69.63 ± 15.71	62.12 ± 8.10	64.30 ± 12.74	36.77 ± 3.04
19	11	Mare	Warmblood	40.25 ± 4.11	42.52 ± 6.27	50.69 ± 17.53	38.71 ± 4.06
20	6	Gelding	Cob	44.22 ± 9.89	39.63 ± 4.86	53.74 ± 8.18	37.48 ± 3.31

563 **Table 2.** Description of behavioral categories and number of individuals per category, per
564 season.

Condition	Value	Description	n	n
			<i>(summer)</i>	<i>(autumn)</i>
Pre-Feeding	Low	Absence or occasional vocalization (snort, neigh), no pawing behavior.	8	10
	High	Repetitive vocalization and/or pawing behavior.	9	7
Novel Object	Low	Total absence or occurrence of just one of the following behaviors: pawing at object, startle response, defecation, snorting.	9	14
	High	Presence of at least two of the following behaviors: pawing, startle response, defecation, snorting.	9	4

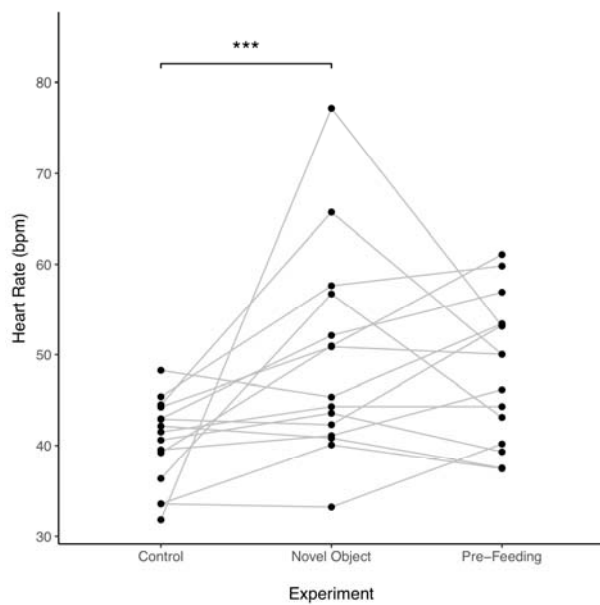
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567

568 **Figure 1.** Effect of behavioral categorization on the mean heart rate (HR) (A) and HR
569 increase (B) of the horses recorded during the study. Boxplots represent the median (black
570 bar), the interquartile range – IQR (boxes), maximum and minimum values excluding outliers
571 (whiskers) and outliers (black dots).



572

573 **Figure 2.** Individual average heart rate of horses across experiments. Points represent a value

574 per individual horse and the lines connect individual horses over different experiments.