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1 Horses could perceive riding differently according to the way they express poor welfare in the stable

2

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12

13 **Abstract**

14 This study investigated the relationships between four behavioural indicators of a compromised
15 welfare state in loose boxes (stereotypies, aggressive behaviours towards humans, withdrawn posture
16 reflecting unresponsiveness to the environment and alert posture indicating hypervigilance) and the
17 way horses perceived riding. This perception was inferred using a survey completed by the usual
18 riding instructor and during a standardised riding session (assessment of behaviours and postures,
19 Qualitative Behaviour Assessment (QBA) and characterisation of the horses' locomotion using an
20 inertial measurement unit). According to ear and tail positions and the QBA, stereotypic and the most
21 hypervigilant horses in loose boxes seemed to experience a more negative affective state during the
22 riding session compared to non-stereotypic and less hypervigilant animals ($p < 0.02$ in all cases).
23 Horses which were aggressive towards humans in loose boxes had higher scores regarding the
24 occurrence of discomfort and defensive behaviours on the survey than non-aggressive horses ($p =$
25 0.03). They also presented higher dorsoventral accelerations at canter during the riding session ($p =$
26 0.03), requiring the rider to increase his spinal movement ($p = 0.005$). These results suggest that
27 aggressive horses may be harder to ride than non-aggressive animals. The expression of

28 unresponsiveness to the environment in loose boxes was related to more reluctance to move forward,
29 as assessed in the survey ($p = 0.006$). This study suggests that a compromised welfare state in the
30 stable is related to horses having a more negative perception of riding. This perception could vary
31 according to the expression of poor welfare.

32

33 ***Keywords***

34 Animal welfare, Behaviour, Horse, Housing conditions, Kinematics

35

36 ***Highlights***

- 37 - When ridden, stereotypic and hypervigilant horses showed negative affective signs.
- 38 - Aggressive horses may be more difficult to ride than non-aggressive animals.
- 39 - Unresponsive horses were more frequently reluctant to move forward.
- 40 - Poor welfare in boxes could be related to a more negative perception of riding.

41

42 ***1. Introduction***

43 Animal welfare is a multidimensional concept which includes the interaction between physical,
44 physiological and affective components [1]. As in other farm animals [2,3], the study of behavioural
45 indicators can be used to detect alterations in the welfare state of horses [4]. At least four main
46 behavioural indicators have been identified as allowing to infer the experience of negative internal
47 states in horses living in loose boxes: stereotypies [5–7], aggressive behaviours towards humans [8,9],
48 withdrawn posture [10] reflecting unresponsiveness to the environment [11,12] and alert posture [13]
49 indicating hypervigilance ([14]; see [15] for more details on the internal states likely associated with
50 these indicators).

51 Nowadays, a large number of horses are involved in riding activities [16], although being ridden can
52 be perceived as aversive [17] and could sometimes exceed the mental and physical capacities of

53 animals [18]. Numerous factors related to riding have been associated with a negative perception of
54 this practice by horses. These factors concern the equipment, the health of feet and shoeing, the use of
55 artificial aids such as spurs [19], the position, skills and technique of the rider [20,21] and certain
56 controversial riding practices such as hyperflexion of the neck [22–27]. Another key factor could be
57 the overall welfare state of the horse in the living environment. However, as stated by Hall and
58 Heleski [22], the direct relationship between a compromised welfare state in the stable and the horse’s
59 affective state when ridden has received little attention. A few studies have shown the effects of
60 housing conditions (e.g., boxes versus pasture) and animal management (e.g., playing music in the
61 stable) on the behaviour and physiological stress responses of horses during riding activities [28–30].
62 These results suggest that the welfare state of horses in their living environment and when ridden are
63 related, but this requires further investigation by evaluating the animals in both contexts.

64 Three kinds of indicators could enable the affective state of horses to be inferred while being ridden
65 [31]. The first concerns behavioural and postural indicators. To date, the majority of such indicators
66 reflect negative affective and/or physical states (e.g., bucking, bolting, raised tail carriage,
67 asymmetrical and backward ears positions; see [31] for a recent review), whereas positive indicators
68 remain scarce (e.g., snorts at walk [32]). The second is a Qualitative Behaviour Assessment (QBA),
69 which could be a useful tool to assess the affective state of horses in riding situations. It consists of a
70 “whole-animal” approach based on the assessment of the overall behavioural expression of the subject
71 using descriptors such as “relaxed” or “frightened” [33]. Due to its integrative nature, it is difficult to
72 define descriptors precisely using specific behavioural indicators [34], but a growing number of
73 studies support the validation of this tool by correlating descriptors with observable behaviours [35],
74 relevant physiological measures and health parameters [33,36]. To our knowledge, only one study has
75 used a QBA in horses during riding situations [37], and further validations are still required in this
76 context [22,38]. However, it constitutes a supplementary tool to be used in conjunction with
77 behavioural and postural indicators, particularly as it allows positive affective states to be assessed
78 [39]. The third involves the study of locomotion characteristics which may provide insights into
79 affective states (for a recent review in humans and non-human animals, see [40]). For example,

80 anxiety in mice can be expressed through a stretch-attend posture when walking [41]. In humans, it is
81 possible to differentiate states such as anger, depression or anxiety through gait patterns by studying
82 accelerations and velocities of different parts of the body (e.g., velocity of chest movements; [42–44]).
83 Accelerations of some parts of the horse’s body have been studied using inertial measurement units
84 consisting of accelerometers located close to the animal’s centre of gravity (i.e., sternum; [45]). To
85 date, the assessment of the locomotion in horses has mainly been studied to improve sport
86 performance and detect pathologies, but with regard to both human and animal literature, such
87 parameters could also be influenced by specific affective states and thus could constitute relevant
88 indicators. Moreover, the existence of two-way biomechanical interactions between the horse and the
89 rider is well described [46–48]. It could thus be hypothesized that the affective state of horses could
90 affect the movements of the rider through specific locomotion patterns, and potentially induce long-
91 lasting health issues.

92 The aim of this study was to investigate the relationships between four behavioural expressions of a
93 compromised welfare state in loose boxes using stereotypies, aggressive behaviours towards humans
94 and withdrawn and alert postures and the affective state of horses while being ridden, by recording the
95 three aforementioned kinds of indicators. For each horse, a survey was first completed by the usual
96 riding instructor of horses to obtain an integrative view of their behaviour during different riding
97 sessions and over time. Then, a standardised riding session was carried out with an expert rider, during
98 which the affective state of horses was inferred through recording behavioural and postural indicators,
99 a QBA assessment, and the 3-dimensional accelerations of the horse’s trunk characterising overall
100 locomotion, using an inertial measurement unit [49]. The movements of the rider’s spine were also
101 measured with two additional inertial sensors. We hypothesised that the expression of the four
102 behavioural indicators reflecting a compromised welfare state in loose boxes would be related to
103 behavioural and postural indicators of negative affective states (e.g., fear or anxiety-related behaviours
104 in the survey, bucking or asymmetrical ears during the riding session) and negative descriptors in the
105 QBA (e.g., “alarmed”) when ridden. As observed in humans, we expected to record different
106 accelerations of the horse’s trunk related to behavioural indicators of a compromised welfare state

107 expressed in loose boxes and different movements of the rider's spine in response to the horse's
108 locomotion pattern. As this study is the first to investigate the links between locomotion and welfare in
109 horses, no precise hypothesis could be formulated as to the direction of variations in acceleration.

110

111 **2. Materials and methods**

112 *2.1. Animals, housing and management conditions*

113 This study was performed in a riding school (France) and included 43 clinically healthy horses (30
114 geldings, 13 mares) aged 12.8 ± 0.4 (mean \pm SEM) years. All the animals were Warmblood horses
115 (Anglo-Arab; N = 9, French Saddlebred; N = 27, Belgian Warmblood; N = 4, German Warmblood; N
116 = 3). They were housed in loose boxes of approximately 9 m², cleaned six mornings out of seven, on
117 straw (N = 36), wood shavings (N = 2) or pellets (N = 5) bedding. Horses were fed with two rations of
118 hay (4.5 kg per meal) and three of pellets of varying quantities per day, according to their body
119 condition. Water was provided *ad libitum* by automatic drinkers with pressure valves. All horses had
120 visual contact with conspecifics from the opening in the door of their loose boxes. They were released
121 for approximately 1 hour per week into individual sand paddocks for free exercise. The horses were
122 ridden in three different disciplines (dressage; N = 11, jumping; N = 12 or eventing; N = 20) during
123 5.7 ± 0.1 hours per week by future professional riders who were preparing for a riding instructor
124 diploma.

125 *2.2. Assessment of four behavioural indicators of a compromised welfare state in loose boxes*

126 The assessment of the four behavioural indicators reflecting a compromised welfare state was carried
127 out in the 6.5 ± 0.9 weeks (mean \pm SEM) preceding the riding session using a scan sampling method
128 [50]. This method was chosen to maximize the likelihood of detecting stereotypies and aggressive
129 behaviours towards humans, as well as to quantify the expression of withdrawn and alert postures over
130 time. It has already been successfully used to assess the effects of factors related to the architecture of
131 the loose box and animal management, the enrichment of the living environment and the weaning
132 method on the expression of these behavioural indicators [15,51,52].

133 Horses were observed during ten 90-minute sessions (two sessions between 09:00 and 10:30; 10:30
134 and 12:00; 12:00 and 13:30; 13:30 and 15:00; and 15:00 and 16:30) that were randomly distributed
135 over 9.7 ± 0.1 days. A maximum of two different observation sessions per day were performed, and
136 twelve scans per horse were recorded per session (7 minutes between two scans of the same horse).
137 The average final number of scans per horse was 90.8 ± 2.5 . Variations resulted from the absence of
138 the horse or the presence of the caretaker in the loose box at the time of the observation.

139 An experienced observer in equine ethology conducted the observations by walking slowly and
140 silently through the central corridor of the stables. The observer looked at the horses for 5 seconds and
141 recorded whether the animal expressed one of the four behavioural indicators of a compromised
142 welfare state [15]. The duration of a scan was extended by a few seconds (5 seconds instead of one) to
143 clearly differentiate the withdrawn posture, mainly characterized by the opening and fixity of the eyes
144 [10], from the standing resting posture, in which the eyelids blink and gradually become droopy. The
145 descriptions of the four behavioural indicators are presented in Table 1. Additional stereotypies were
146 taken into account in the assessment but were not observed in the sample: wind sucking, box walking,
147 compulsive licking or biting the environment, teeth rubbing and others repetitive head movements
148 such as bobbing. A human-animal relationship test (approach-contact test) was also conducted to
149 assess aggressive behaviours towards humans (see details of the test in [53]), but the results did not
150 discriminate sufficiently the horses, as nearly 90 % of them did not express the behavioural indicator
151 of interest. Therefore, the results of this test could not be analysed statistically.

152 The percentages of scans during which each of the four behavioural indicators was observed were
153 calculated according to the total number of scans recorded for each horse. As stereotypies and
154 aggressive behaviours towards humans were expressed by less than 35 % of the horses (Table 1) and
155 showed little variability, these two indicators were subsequently considered as two binary variables for
156 each horse (the indicator was expressed at least once by the horse or was not expressed at all).

157 Withdrawn and alert postures were retained as continuous variables and expressed as the percentages
158 of scans of each indicator for each horse. Mean percentages of the expression of these two postures are
159 presented in Table 1.

160 *2.3. Behavioural survey completed by the usual riding instructor*

161 A behavioural survey was carried out with the riding instructor in charge of the horses, to obtain an
162 integrative view of each animal's behaviour in different riding contexts and over time. The survey
163 consisted of three questions, formulated to be easily understandable to a field professional while being
164 based on scientific literature. A likert-type scale from 0 (the behaviour is never expressed in riding
165 situations) to 3 (the behaviour is very frequently expressed in riding situations) was used to assess the
166 level of expression of each behaviour [54]. The survey questions and descriptive statistics are
167 presented in Table 2.

168 *2.4. Standardised riding session with an expert rider*

169 Thirty of the 43 horses were randomly selected for the standardised riding session. They were all
170 tested once between January and March 2019. The standardised riding session took place between
171 08:00 and 09:00, before the horses' usual riders arrived, in an indoor arena of approximately 20 * 50
172 metres located at the riding school and known to horses. No other horses were present in the test arena.
173 Each horse was ridden by the same expert rider who was totally blind to their welfare state in loose
174 boxes. The same equipment was used for all the horses: a fitted snaffle bridle with a loose noseband
175 (two fingers could be inserted between the noseband and the nose) and a single-jointed bit, a
176 saddlecloth along with a jumping saddle and two tendon boots on the forelegs. The rider had a whip
177 but did not wear spurs. The horses were ridden on a daily basis with identical equipment and were
178 used to the use of a whip. After being led to the arena, the rider warmed up the horses at walk and trot,
179 in both directions staying on the track around the arena (except for changes of direction through the
180 diagonal), for 7.5 ± 0.4 (mean \pm SEM) minutes before starting the standardised riding session. The
181 average duration of the riding session was 7.9 ± 0.2 minutes, and included two parts: first, the
182 assessment of the horse's locomotion and the movements of the rider's spine, and then the assessment
183 of the horse's behavioural and postural indicators and the QBA (see Table S1 in Supplementary
184 materials for the description of the riding session).

185 2.4.1. Assessment of the horse's locomotion and the movements of the rider's spine during the
186 riding session

187 The data recorded on 24 out of the 30 horse-rider dyads could be analysed. Three inertial measurement
188 units (IMUs; APDM, USA) were located on the dyad: one on the horse's sternum, one on the rider's
189 fifth lumbar vertebra (L5) and one on the rider's sternum (ST; Figure 1). These positions were chosen
190 because they were close to both horse' and rider's centre of gravity, and because the attachment of the
191 IMU to the girth ensured its stability [55].

192 This first part of the riding session lasted 2.8 ± 0.1 (mean \pm SEM) minutes. Fast gaits such as trot and
193 canter were preferred to walk to maximize the chances of observing the impact of a compromised
194 welfare state assessed in loose boxes on the horse's locomotion and the movements of the rider's
195 spine, due to the greater physical effort this required of the horses. During rising trot, the measures
196 were carried out for a total of twelve strides per horse (six strides per straight line of the arena) in one
197 direction (left rein) and twelve strides in the other direction (right rein). The same protocol was used at
198 canter (Table S1). For each gait, acceleration values of the two directions were averaged.

199 *Horse locomotion.* The magnitude of the anteroposterior (in blue on Figure 1), mediolateral (in green
200 on Figure 1) and dorsoventral (in red on Figure 1) accelerations were calculated using the root mean
201 square (rms) of the signal provided by the IMUs located on the horse's sternum (Table 3).

202 *Movements of the rider's spine.* To quantify the rider's ability to adapt to the horse's locomotion, i.e.,
203 to attenuate accelerations from the horse's trunk through the spine [56], a shock absorption coefficient
204 (SAC) was calculated as:

205
$$SAC = \left(1 - \frac{rms_{ST}}{rms_{L5}}\right) * 100$$

206 in which rmsST is the magnitude of anteroposterior accelerations at the rider's sternum (ST) and
207 rmsL5 is the magnitude of anteroposterior accelerations at the rider's fifth lumbar vertebra (L5). This
208 coefficient describes the ability to reduce acceleration from the rider's L5 to the rider's ST (Table 3).

209 The higher the SAC coefficient, the higher the acceleration attenuated by the rider's spine.

210 2.4.2. Assessment of behavioural and postural indicators and QBA during the riding session

211 The riding session was recorded using a Sony HDR-CX450 camera held by the experimenter, and the
212 rider wore a camera (Cambox ISIS®) fixed to his helmet to observe more precisely the position of the
213 horse's ears. All measurements were carried out on the video recordings using Boris software (version
214 7.8.2, Torino, Italy, 2019). Due to a camera dysfunction, the video-recording could not be analysed for
215 one horse. For this animal, only the analysis of ear positions could be carried out from the rider's
216 camera.

217 This second part of the riding session, lasting 5.3 ± 0.2 minutes, immediately followed the first part.

218 This part consisted of a series of circles, gait transitions between walk and trot, lines in extended trot
219 and canter and leg-yielding in both directions (Table S1). The rider was instructed to ride the horses
220 uniformly, with as few constraints as possible, to limit the impact of the rider's technique on the
221 horse's affective state.

222 Eleven behavioural and postural indicators reflecting affective states were taken into account: snorts at
223 walk, rearing, bucking, bolting, head shaking/tossing, abnormal mouth behaviours (wide opening and
224 teeth grinding), tail swishing, raised tail carriage, forward, backward and asymmetric ear position
225 (Table 4). The occurrences of snorts at walk, rearing, bucking, bolting, head shaking/tossing, abnormal
226 mouth behaviours and tail swishing were recorded *ad libitum* and then calculated as the number of
227 occurrence per minute of the riding session. Raised tail carriage and ear positions were recorded using
228 scan sampling (one scan per second throughout the riding session) and the percentage of scans with
229 these indicators was then calculated based on the total number of scans recorded. The rider's voice
230 could influence ear positions, therefore this indicator was considered as missing data when the
231 position changed immediately following the rider's vocal stimulation.

232 The QBA was performed using thirteen descriptors adapted from the AWIN Horse protocol [53] by
233 the same experimenter for all the horses (Table 5), who also assessed the behavioural and postural
234 indicators during the riding session. The experimenter was trained to perform the QBA assessment
235 (PhD in ethology). The latter consisted of observing the complete riding session on video recordings

236 and then making a mark on a visual analogue scale from 0 to 100 (one scale per descriptor). A score of
237 0 indicated that the descriptor was not observed at all, and a score of 100 reflected that the descriptor
238 was present during the whole riding session.

239 2.5. Statistical analyses

240 Scores of the behavioural survey were not normally distributed and were therefore analysed with non
241 parametric tests. Wilcoxon rank-sum tests with continuity correction (*wilcox.test* function, *stats* R
242 library) were used to compare the scores attributed to each question between stereotypic versus non-
243 stereotypic horses, as well as aggressive versus non-aggressive horses. Polyserial correlations were
244 calculated between the scores of each question and the percentage of scans of the withdrawn and alert
245 postures (*polyserial* function, *polycor* R library).

246 Multiple regression models (LMs; *lm* function, *stats* R library) were used to test the effects of the four
247 behavioural indicators of a compromised welfare state assessed in loose boxes on each of the variables
248 recorded during the riding session: each behavioural and postural indicator, the QBA profile, horse
249 locomotion and the SAC of the rider at trot and canter.

250 Residuals were checked graphically for a normal distribution and homoscedasticity. F-tests from type-
251 II ANOVAs along with *p*-values (*p*) were calculated using the *Anova* function of the *car* R library.

252 The multiple regression models used were:

$$253 y_i = \beta_0 + \beta_{stereotypies_i} + \beta_{aggressiveness_i} + \beta_{withdrawn\ posture_i} + \beta_{alert\ posture_i} + \varepsilon_i$$

254 in which *y* is the outcome variable (e.g., the occurrence of tail swishing per minute during the riding
255 session), β_0 is the intercept, $\beta_{stereotypies}$, $\beta_{aggressiveness}$, $\beta_{withdrawn\ posture}$ and $\beta_{alert\ posture}$ are
256 the fixed-effect parameters (predictors) and ε is the residual term. A log transformation was applied for
257 the following outcome variables: head shaking / tossing, abnormal mouth behaviours, tail swishing,
258 the SAC at trot and canter and the anteroposterior acceleration at canter. A square-root transformation
259 was carried out on the raised tail carriage variable to approximate more accurately a normal
260 distribution. As the usual discipline of each horse could influence the horse's locomotion [56] and the

261 behavioural indicators of affective states [57,58], the confounding effect of this parameter was
262 controlled for all the outcomes by quantifying changes in the values and significances of the
263 coefficients of the fixed-effect parameters when the discipline was included in the models or when this
264 variable was excluded. When a change of at least 10 % of the values was observed, the discipline was
265 considered as a confounding factor and was therefore retained in the final model [59]. Tukey post-hoc
266 tests (glht function, *multcomp* R library) were performed to investigate further the effects of
267 significant parameters.

268 The thirteen multivariate QBA descriptors were first reduced using a spearman Principal Component
269 Analysis (PCA) without rotation. Two descriptors were excluded because all horses presented null
270 values (i.e., “Happy” and “Looking for contact”). Two principal components were extracted and
271 accounted for 57.1 % of the total variance. Only variables with loadings $\geq |0.40|$ were interpreted (the
272 loadings of the 11 QBA descriptors are presented in Supplementary materials; Table S2). The
273 individual scores on the two selected axis were then tested as outcome variables in the multiple
274 regression models.

275 Statistics were carried out using R software (version 3.6.0, R Development Core Team, Vienna,
276 Austria, 2019) with a significance threshold at $p \leq 0.05$. Trends were considered for $p \leq 0.07$. Means \pm
277 SEMs or medians and 1st – 3^d quartiles are presented.

278 *2.6. Ethics statement*

279 The observation of the horses was approved by the Val de Loire ethics committee
280 (2019012211274697.V4 – 18939). The riding session included exercises commonly performed by the
281 horses studied. The duration and intensity of physical activity were monitored to avoid excessive
282 fatigue for the animals.

283 *2.7. Graphic design*

284 The images were drawn by Estel Blasi Palacios using Adobe Illustrator (version CS6, 16, San José,
285 USA).

286

287 **3. Results**

288 *3.1. Behavioural survey*

289 *Relationships between the expression of stereotypies in loose boxes and the survey.* Stereotypic horses
290 in loose boxes did not differ from non-stereotypic horses in any of the behaviours assessed in the
291 survey ($164 < W < 173$, $0.81 < p < 0.99$; Supplementary materials Table S3).

292 *Relationships between the expression of aggressive behaviours towards humans in loose boxes and*
293 *the survey.* Aggressive horses in loose boxes scored significantly higher regarding discomfort and
294 defensive behaviours in the survey compared to non-aggressive horses ($W = 127$, $p = 0.03$, $N = 43$).
295 They also tended to obtained a higher score regarding fear or anxiety-related behaviours compared to
296 non-aggressive horses ($W = 138$, $p = 0.06$).

297 Aggressive horses in loose boxes did not differ from non-aggressive horses regarding reluctance to
298 move forward assessed in the survey ($W = 206$, $p = 0.93$; Supplementary materials Table S3).

299 *Relationships between the expression of withdrawn posture in loose boxes and the survey.* The
300 expression of withdrawn posture in loose boxes was significantly positively correlated with reluctance
301 to move forward in the survey ($r = 0.42$, $p = 0.006$, $N = 43$; Figure 2).

302 The expression of withdrawn posture in loose boxes was not correlated to other behaviours in the
303 survey ($0.19 < r < 0.32$, $0.09 < p < 0.25$; Supplementary materials Table S3).

304 *Relationships between the expression of alert posture in loose boxes and the survey.* The expression of
305 alert posture in loose boxes was not correlated with any of the behaviours assessed in the survey (-0.13
306 $< r < 0.03$, $0.43 < p < 0.80$; Supplementary materials Table S3)

307 *3.2. Standardised riding session*

308 Among the behavioural and postural indicators reflecting affective states during the riding session, no
309 horse reared or produced snorts at walk, and only two horses bucked and bolted. Thus, these
310 behaviours could not be statistically analysed. However, it is interesting to note that the horse that

311 bucked twice also expressed both stereotypies and aggressive behaviours towards humans in its loose
312 box, and the horse which bolted once expressed the highest level of alert postures in its loose box (3.8
313 % of scans).

314 *Relationships between the expression of stereotypies in loose boxes and the riding session.* The
315 expression of stereotypies in loose boxes was significantly related to tail carriage during the riding
316 session ($F = 7.14, p = 0.01, N = 29$): stereotypic horses expressed significantly more raised tail
317 carriage than non-stereotypic horses ($Z = 2.67, p = 0.01$; Figure 3).

318 The expression of stereotypies in loose boxes was not related to any other indicators of affective states
319 or rider movements ($0.001 < F < 3.40, 0.08 < p < 0.98$; Supplementary materials Table S4).

320 *Relationships between the expression of aggressive behaviours towards humans in loose boxes and the*
321 *riding session.* The expression of aggressive behaviours towards humans in loose boxes was
322 significantly related to the horse's locomotion pattern at canter ($F = 5.93, p = 0.03, N = 24$):
323 aggressive horses showed significantly higher dorsoventral accelerations compared to those of non-
324 aggressive horses ($Z = 2.43, p = 0.03$; Figure 4.a). Aggressiveness was also significantly related to
325 rider movements ($F = 10.10, p = 0.005, N = 24$): the expression of aggressive behaviours towards
326 humans was significantly related to a higher rider shock absorption coefficient ($Z = 3.18, p = 0.005$;
327 Figure 4.b).

328 The expression of aggressive behaviours towards humans in loose boxes was not related to other
329 indicators of affective states or rider movements ($0.08 < F < 1.52, 0.23 < p < 0.93$; Supplementary
330 materials Table S4).

331 *Relationships between the expression of withdrawn posture in loose boxes and the riding session.* The
332 expression of withdrawn posture in loose boxes was not related to any indicators of affective states or
333 rider movements ($< 0.001 < F < 1.80, 0.19 < p < 0.99$; Supplementary materials Table S4).

334 *Relationships between the expression of alert posture in loose boxes and the riding session.* The
335 expression of alert posture in loose boxes was significantly related to asymmetric ear position ($F =$
336 $5.92, p = 0.02; N = 30$) and tended to be related to forward ear position ($F = 3.59, p = 0.07; N = 30$)

337 during the riding session: the more alert postures the horses expressed, the more asymmetric ($\beta = 3.81$;
338 Figure 5.a) and forward ($\beta = 4.58$) the ear positions were. The expression of alert postures was also
339 significantly related to the second axis of the PCA performed on the QBA descriptors ($F = 6.04$, $p =$
340 0.02 , $N = 29$). This axis explained 18.9 % of the total variance and was mainly represented by
341 “alarmed” for the positive scores, as opposed to “annoyed” and “pushy” for the negative scores. Thus,
342 the more alert postures the horses expressed, the more they were judged as “alarmed” during the riding
343 session ($\beta = 63.3$; Figure 5.b).

344 The expression of the alert posture was not related to other indicators of negative affective states or
345 rider movements ($0.02 < F < 3.59$, $0.10 < p < 0.88$; Supplementary materials Table S4).

346

347 **4. Discussion**

348 In accordance with our hypothesis, the four behavioural expressions of a compromised welfare state in
349 loose boxes appear to be related to the horse’s affective state and the movements of the rider’s spine
350 when ridden, in specific ways. The stereotypic and the most hypervigilant (alert posture) horses in
351 loose boxes showed more behavioural and postural indicators of negative affective states when ridden
352 by the expert rider compared to the non-stereotypic and less hypervigilant animals. Compared to non-
353 aggressive horses, aggressive horses towards humans in loose boxes obtained a higher score regarding
354 the expression of discomfort and defensive behaviours on the survey, and showed a specific
355 locomotion pattern at canter during the riding session, which impacted the movements of the rider’s
356 spine. Finally, the more unresponsive the horses were in loose boxes (withdrawn posture), the higher
357 the score for reluctance to move forward on the survey.

358 Stereotypic horses in loose boxes seemed to experience a more negative affective state during the
359 riding session, compared to non-stereotypic horses. Indeed, they more often expressed raised tail
360 carriage, which has been described as an indicator of stress or fear in several conditions, in both non-
361 ridden and ridden horses [13,38,60–64]. This may be in line with previous studies showing that
362 stereotypic horses (crib-biters) were more stress sensitive and presented a higher cortisol response

363 following an ACTH challenge test [65] and a larger increase in heart rate and locomotor behaviours
364 after a sudden event [66] compared to non-crib-biters horses. The results of the current study suggest
365 that stereotypic horses in general, and not only crib-biters, would present a higher sensitivity to stress.
366 The riding session could have been perceived as a stressful event for stereotypic horses, probably in
367 part because no other horses were present in the arena and because they were ridden by an unknown
368 rider. However, this result needs to be confirmed, for example by adding physiological measures such
369 as cortisol measurements, since only one behavioural indicator of negative affective states was
370 highlighted. The results of this study suggest similar conclusions regarding the affective state when
371 ridden for horses expressing hypervigilance in loose boxes. The increase in hypervigilance was related
372 to asymmetrical and forward ear positions being expressed more. In horses, it has been demonstrated
373 that asymmetrical ears could reflect a negative affective state during grooming by humans [67] and
374 ears pointing forward towards external stimuli would indicate attention to the environment [68].
375 Moreover, the more horses expressed hypervigilance, the higher their scores on the PCA axis of
376 “alarm” resulting from the QBA analysis, which supports the experience of a negative affective state
377 when ridden (the “alarmed” descriptor is described as “tense, apprehensive, on guard against a
378 threat”). These results are illustrated anecdotally by the most hypervigilant horse in loose boxes that
379 bolted once during the riding session, i.e., demonstrating a flight response to a stressful event. It is
380 likely that more horses could have expressed such extreme behaviours but that the expert rider
381 prevented their expression.

382 Aggressive horses towards humans in loose boxes presented higher scores for the occurrence of
383 discomfort and defensive behaviours and fear or anxiety-related behaviours on the survey compared to
384 non-aggressive horses. Anecdotally, it is interesting to notice that the only horse that bucked twice
385 during the riding session (a defensive behaviour), was also an aggressive horse in the stable. The
386 negative affective state of aggressive horses during the riding session was reflected through a specific
387 locomotion pattern, characterized by a higher dorsoventral acceleration at canter compared to non-
388 aggressive horses. Indeed, in humans, a high vertical velocity of chest movements is characteristic of
389 angry walkers [42,69], although it is impossible to directly transpose such an affective state to horses.

390 However, aggressiveness reflects a poor human-animal relationship, so it could be conceivable that
391 aggressive horses may also experience a negative affective state when ridden. This negative affective
392 state could stem from the experience of chronic back pain, as the expression of aggressiveness towards
393 humans in loose boxes has been linked to vertebral damage spread up to two thirds of the horses' spine
394 during a back examination [9]. In response to the higher dorsoventral accelerations of aggressive
395 horses' trunks, the expert rider adapted his movements by increasing absorption of the accelerations
396 with his spine. It is likely that, in the long term, the high recurrent absorption of the dorsoventral
397 accelerations transmitted by the locomotion of aggressive horses will affect the physical integrity of
398 the rider. In addition, although the expert rider was able to adjust his technique to the locomotion of
399 the horse [70,71], less experienced riders may not be able to do the same. In the latter case, the
400 increase in the horses' dorsoventral accelerations could lead them to being unsuitable for riding, due to
401 rider instability, and probability of falls. Such difficulties encountered by less experienced riders could
402 also lead to inappropriate behaviours towards horses, such as use of positive punishments, which
403 would reinforce the horses' negative perception of humans. These repeated aversive experiences when
404 ridden could lead horses to generalize to all riders [72,73], which could keep them in a compromised
405 welfare state as humans are omnipresent in domestic horses' lives. Overall, these results encourage
406 further studies to investigate the use of horses' locomotion, whether ridden or not, as an indicator of
407 negative or even positive affective states, to help refine animal welfare assessment.

408 Finally, the horses which were the more unresponsive in loose boxes were more reluctant to move
409 forward when ridden, as highlighted on the survey. This relationship could reflect a general state of
410 learned helplessness in these horses, which has been reported in this species [74]. This state could lead
411 to a decrease in responsiveness towards environmental stimulation, as well as rider aids, particularly
412 for horses in the current study that were ridden for training riders and which are thus potentially
413 exposed to more or less appropriate riding technics [20]. In the current study, this result was not
414 confirmed during the riding session, probably because the variables did not specifically assess this
415 aspect.

416 The overall results suggest that horses experiencing a compromised welfare state in loose boxes could
417 perceive riding more negatively. This perception is expressed differently according to the behavioural
418 expression of poor welfare in the stable (stereotypies, aggressiveness towards humans,
419 unresponsiveness to the environment or hypervigilance), suggesting that horses perception could vary.
420 Additional indicators could have been identified during the riding session to clarify the different
421 perceptions. Indeed, it is possible that the riding session, which was performed only once per horse,
422 was too short and not challenging enough to elicit stronger behavioural responses from the animals. It
423 would thus be interesting to continue monitoring horses with welfare concerns in various contexts
424 (e.g., in an unfamiliar environment, while performing more challenging exercises, with less
425 experienced riders) which could allow additional indicators of affective states to be detected in riding
426 situations. In terms of locomotion, other measures could also have been investigated. For instance, we
427 could have expected that horses who perceived the riding session as stressful (stereotypic and the most
428 hypervigilant horses) would have shown specific gait patterns. Indeed, in humans, fear is characterized
429 by small and rapid strides [42], two characteristics that could also be easily measured in horses. Horses
430 exhibiting unresponsiveness to the environment, which may reflect a depressive-like state [10], would
431 also present a specific locomotion. Indeed, humans suffering from depression show specific gait
432 patterns characterized by reduced speed and vertical head movements, and increased lateral upper
433 body movements [75]. This suggestion is particularly supported by the correlations observed between
434 this behavioural indicator of a compromised welfare state and reluctance to move forward when
435 ridden. Various tools could be used in horses to measure these characteristics, such as marker-based
436 motion capture systems that capture joint movements with high precision [49]. In terms of behaviour,
437 more subtle behavioural signs during the riding session, such as specific facial expressions, could also
438 be related to welfare indicators assessed in loose boxes, as stated by Hall and Heleski [22]. The use of
439 QBA appears interesting to capture the demeanour of horses in riding situations, but further validation
440 would be required before using this tool widely as a method to assess affective states when horses are
441 ridden, such as correlations with relevant behavioural and physiological indicators [22].

442

443 **5. Conclusion**

444 This study suggests that horses experiencing a compromised welfare state in their loose box could
445 perceive riding more negatively. This result was particularly highlighted for aggressive horses towards
446 humans, for which a convergence of results between the behavioural survey with the usual riding
447 instructor and the standardised riding session was observed. In addition, the way horses express a
448 negative perception of riding differed according to the behavioural expression of poor welfare in the
449 stable. These results could indicate that they feel different negative affective states in riding situations
450 and deserve further investigation. It seems therefore necessary to continue exploring the relationship
451 between the welfare state of horses in their living environment and in riding situations over a longer
452 term and in multiple contexts. As stated by the Fédération Equestre Internationale (FEI) Welfare Code
453 of conduct [76], the welfare state of horses involved in equestrian sports must be paramount at all
454 times.

455

456 ***Declarations of interest***

457 The authors have no conflicts of interest to report.

458

459 ***Author contributions***

460 A.R., S.B., P.G. and L.L. designed the experiments; A.R., S.B., P.G., E.P. and L.B. performed the
461 experiments; A.R., S.B., C.A., E.P. and L.L. analysed and interpreted the data; A.R., S.B., C.A. and
462 L.L. wrote the paper; all the authors reviewed the manuscript.

463

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470

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682 **Table captions**

683 **Table 1.** Description of the four behavioural indicators of a compromised welfare state recorded using
684 scan sampling in the horses in loose boxes. Stereotypies and aggressive behaviours towards humans
685 were expressed by less than 35 % of the animals and were subsequently considered as binary variables
686 (the indicator was expressed at least once by the horse or was not expressed at all), while withdrawn
687 and alert postures were expressed as the percentages of scans of expression. Descriptive statistics are
688 presented (mean \pm SEM; [Min - Max]). N = 43.

689 **Table 2.** Behavioural survey consisting of three questions to the usual riding instructor of the horses,
690 scored from 0 (the behaviour is never expressed in riding situations) to 3 (the behaviour is very
691 frequently expressed in riding situations). Median; [1st quartile – 3^d quartile]. N = 43.

692 **Table 3.** Variables related to the horse's locomotion and the movements of the rider's spine (mean \pm
693 SEM; [Min – Max]). N = 24.

694 **Table 4.** Descriptions of the behavioural and postural indicators reflecting affective states assessed
695 during the riding session (mean \pm SEM; [Min – Max]). ^a variables measured in occurrence / minute. ^b
696 variables measured as a percentage of the total number of scans recorded. N = 29, except for the three
697 ear positions: N = 30.

698 **Table 5.** Qualitative Behaviour Assessment descriptors used on the horse during the riding session on
699 a scale of 0 to 100 (mean \pm SEM; [Min – Max]). N = 29.

700

701

702 **Figure captions**

703 **Figure 1.** The position of the three inertial measurement units used are represented on the horse's
704 sternum, the rider's fifth lumbar vertebra (L5) and the rider's sternum (ST). The coloured arrows
705 represent the three dimensions of accelerations measured (anteroposterior in blue, mediolateral in
706 green, dorsoventral in red). The shock absorption coefficient (SAC) represents the ability to reduce
707 accelerations via the rider's spine.

708 **Figure 2.** Scores assigned to the questions "Does the horse show reluctance to move forward" in the
709 behavioural survey completed by the usual riding instructor, according to the percentages of scans of
710 withdrawn postures in loose boxes. A score of 0 corresponds to "the behaviour is never expressed in
711 riding situations" and a score of 3 corresponds to "the behaviour is very frequently expressed in riding
712 situations". Polyserial correlations coefficients and regression lines are presented. ** $p \leq 0.01$.

713 **Figure 3.** Mean percentages of scans (\pm SEM) with raised tail carriage during the riding session for
714 stereotypic and non-stereotypic horses ($N_{\text{Stereotypic}} = 7$; $N_{\text{Non-stereotypic}} = 22$; F-tests results from multiple
715 regression models with the percentages of scans with raised tail carriage as the outcome variable). ** p
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717 **Figure 4.** Mean values of the dorsoventral accelerations of the horse (a) and the mean shock
718 absorption coefficients of the rider (b) at canter (\pm SEM) during a riding session according to the
719 expression of aggressive behaviours towards humans in loose boxes ($N_{\text{Non-aggressive}} = 13$; $N_{\text{Aggressive}} = 11$;
720 F-test results from multiple regression models with dorsoventral accelerations and shock absorption
721 coefficients as the outcome variables). * $p \leq 0.05$; ** $p \leq 0.01$.

722 **Figure 5.** Percentages of scans with asymmetric ear positions (a; $N = 30$) as well as individual scores
723 on the second axis of the PCA (b; $N = 29$) performed on the QBA descriptors, according to the
724 expression of alert postures in loose boxes. F-test results from multiple regression models with ear
725 positions and QBA scores as outcome variables. Regression lines are presented. * $p \leq 0.05$.

726

Figures

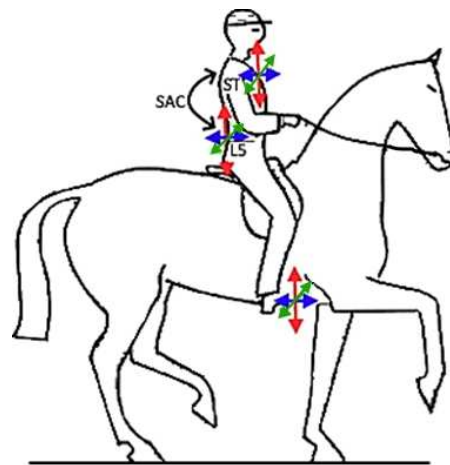


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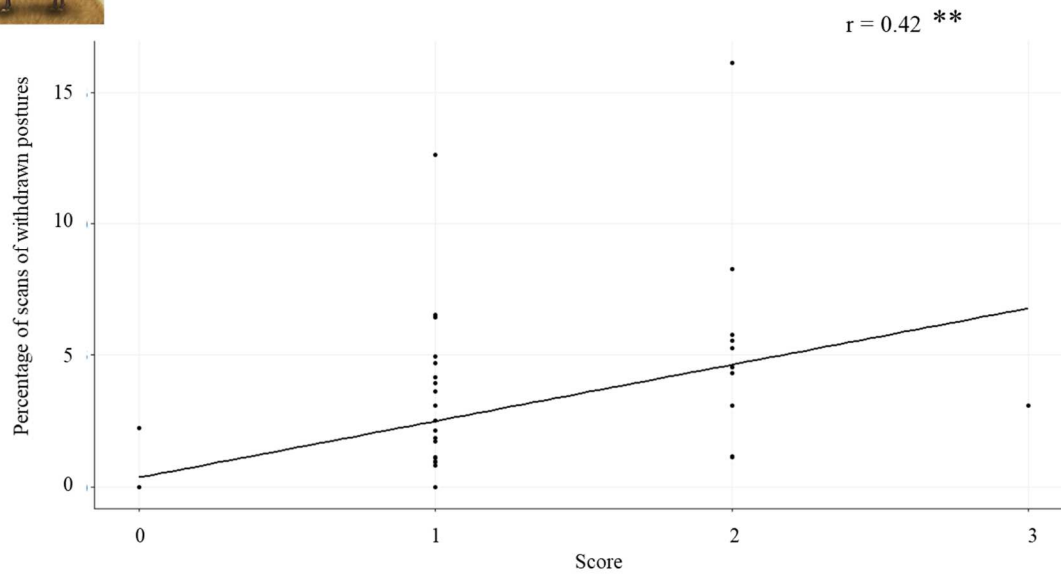


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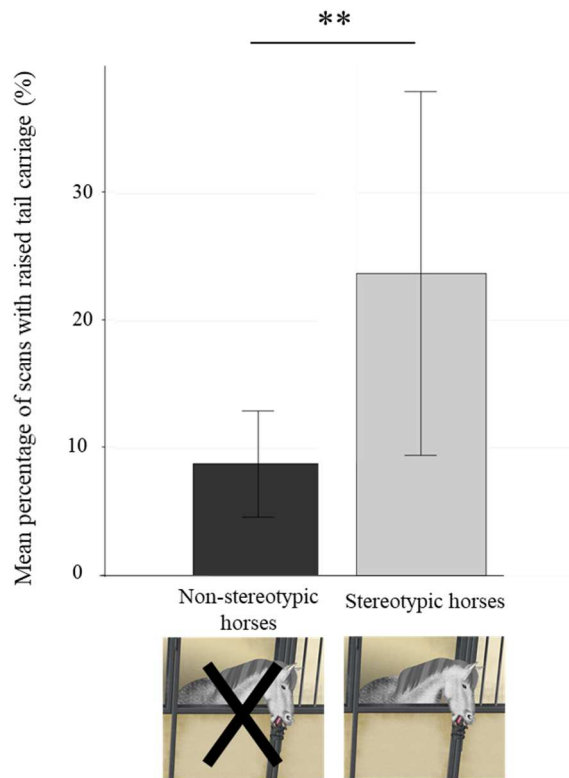
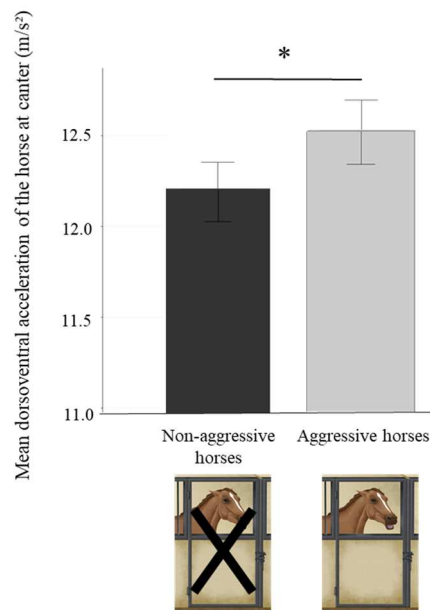


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a)



b)

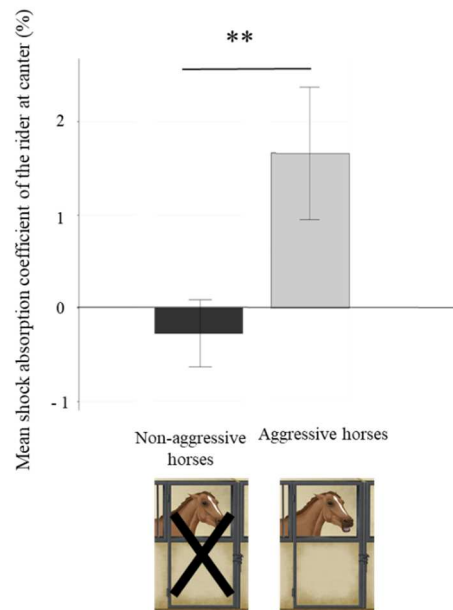


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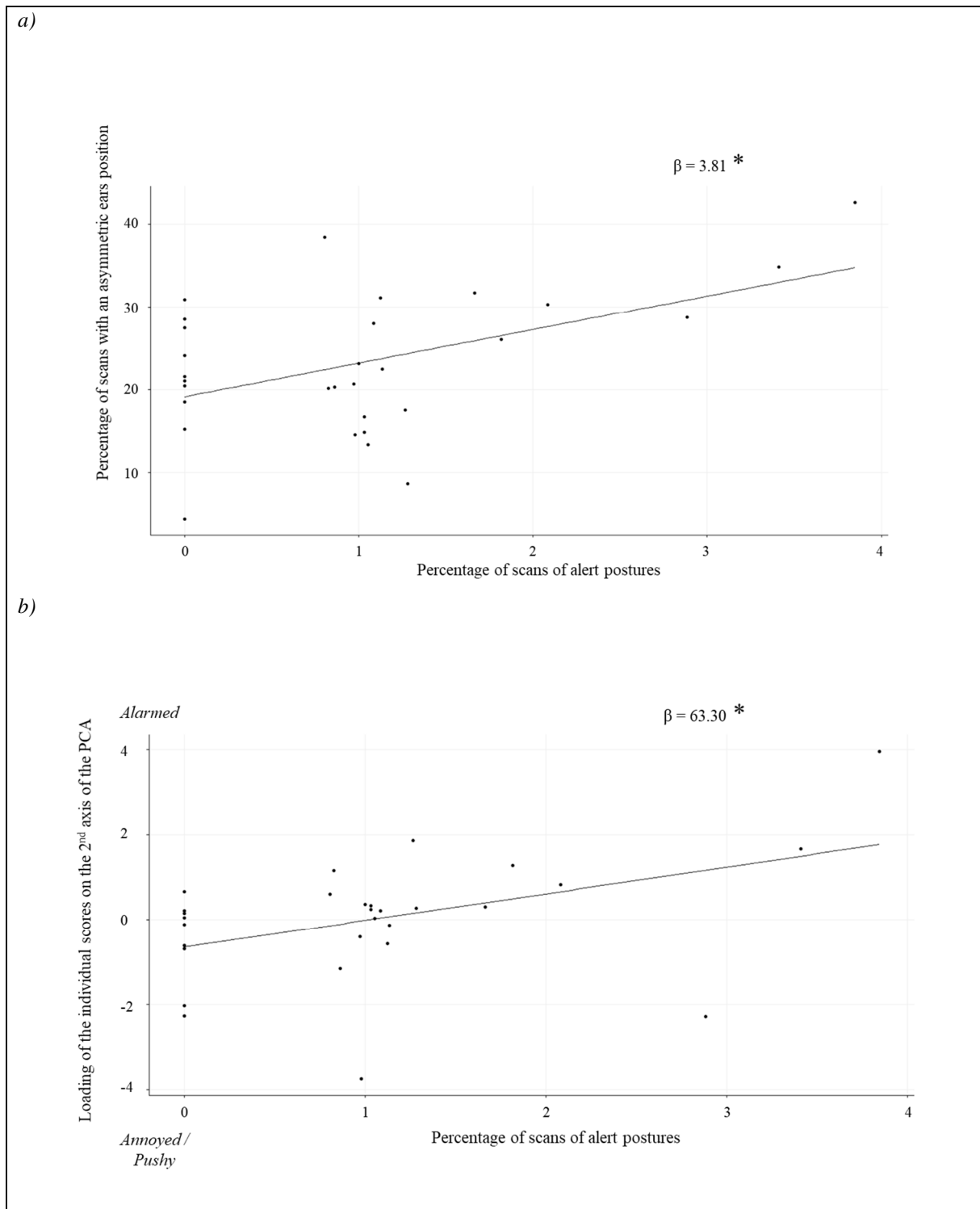


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Tables

Table 1. Description of the four behavioural indicators of a compromised welfare state recorded using scan sampling in the horses in loose boxes. Stereotypies and aggressive behaviours towards humans were expressed by less than 35 % of the animals and were subsequently considered as binary variables (the indicator was expressed at least once by the horse or was not expressed at all), while withdrawn and alert postures were expressed as the percentages of scans of expression. Descriptive statistics are presented (mean \pm SEM; [Min - Max]). N = 43.

Binary variables		
Behavioural indicator	Description	Percentage of horses expressing the indicator at least once
Stereotypies	Crib-biting, weaving, head nodding, lips repetitive movements (e.g., clapping of lips), tongue repetitive movements	23.2 %
Aggressive behaviours towards humans	Looking with ears pinned backward, approaching with mouth open, turning hindquarters, attempting to bite or kick (when someone walks in front of the loose box door)	32.5 %
Continuous variables		
Behavioural indicator	Description	Percentage of scans during which the indicator was recorded
Withdrawn posture	Neck horizontal at same level as the back, fixed stare, ears static and mainly oriented backward, reflecting unresponsiveness to the environment	3.0 \pm 0.5 % [0 – 16.1 %]
Alert posture	Elevated neck and ears pricked forward, looking intensively at the environment, reflecting hypervigilance	1.1 \pm 0.3 % [0 – 10.2 %]

Table 2. Behavioural survey consisting of three questions to the usual riding instructor of the horses, scored from 0 (the behaviour is never expressed in riding situations) to 3 (the behaviour is very frequently expressed in riding situations). Median; [1st quartile – 3^d quartile]. N = 43.

Question	Median [1 st – 3 ^d quartile]
Does the horse express fear or anxiety-related behaviours towards its environment? (The horse tries to bolt or jumps frequently, he looks intensely at elements of the environment, especially if they are new).	1 [0 – 1]
Does the horse express discomfort and defensive behaviours such as abrupt head movements, tail swishing, rearing or bucking?	1 [1 – 2]
Does the horse show reluctance to move forward and needs to be strongly stimulated by the rider, especially with artificial aids such as the whip or spurs?	1 [1 – 2]

Table 3. Variables related to the horse's locomotion and the movements of the rider's spine (mean \pm SEM; [Min – Max]). N = 24.

	Trot	Canter
Horse's locomotion	Mean \pm SEM [Min – Max]	Mean \pm SEM [Min – Max]
Anteroposterior acceleration (m/s ²)	4.5 \pm 0.1 [3.7 – 5.8]	4.4 \pm 0.2 [3.6 – 6.7]
Mediolateral acceleration (m/s ²)	3.6 \pm 0.2 [2.07 – 6.02]	4.5 \pm 0.2 [3.2 – 6.6]
Dorsoventral acceleration (m/s ²)	12.4 \pm 0.1 [10.9 – 14.3]	12.3 \pm 0.1 [10.6 – 13.3]
Movement of the rider's spine	Mean \pm SEM [Min – Max]	Mean \pm SEM [Min – Max]
Shock absorption coefficient (SAC; %)	7.7 \pm 0.5 [2.6 – 13.5]	0.6 \pm 0.4 [(-2.3) – 5.2]

Table 4. Descriptions of the behavioural and postural indicators reflecting affective states assessed during the riding session (mean \pm SEM; [Min – Max]). ^a variables measured in occurrence / minute. ^b variables measured as a percentage of the total number of scans recorded. N = 29, except for the three ear positions: N = 30.

Behavioural and postural indicators	Description	Mean \pm SEM [Min – Max]
Snort at walk ^a	More or less pulsed sound produced by nostrils vibrations while expulsing the air	0
Rearing ^a	The horse rises up on its rear limbs	0
Bucking ^a	The horse kicks with one or two rear limbs	< 0.01 \pm < 0.01 [0 – 0.25]
Bolting ^a	The horse runs off suddenly	< 0.01 \pm < 0.01 [0 – 0.10]
Head shaking / tossing ^a	Fast lateral, circular or up-and-down movements of the head	1.2 \pm 0.2 [0 – 3.2]
Abnormal mouth behaviours ^a	Wide opening of the mouth without chewing the bit for more than 3 seconds Teeth grinding	1.5 \pm 0.3 [0 – 5.7]
Tail swishing ^a	Fast lateral, vertical or circular movements of the tail	2.5 \pm 0.5 [0 – 9.2]
Raised tail carriage (%) ^b	The fleshy part of the tail is held horizontally, in line with the back, or above the croup, and shows minimal swinging movements with the horse's gait	12.3 \pm 4.6 [0 – 96.9]
Ears forward (%) ^b	Both ears are oriented forward. When recorded from behind, the inside of the auricle of both ears is completely invisible.	25.8 \pm 2.3 [4.9 – 56.3]
Ears backward (%) ^b	Both ears are oriented backward towards the rider. When recorded from behind, the inside of the auricle of both ears is visible	29.5 \pm 2.9 [6.9 – 65.9]
Ears asymmetric (%) ^b	One ear is pricked at the environment and the other is oriented backward towards the rider. When recorded from behind, only the inside of the auricle of one ear is visible, and the inside of the auricle of the other ear is completely invisible.	23.2 \pm 1.5 [4.4 – 42.6]

Table 5. Qualitative Behaviour Assessment descriptors used on the horse during the riding session on a scale of 0 to 100 (mean \pm SEM; [Min – Max]). N = 29.

Descriptor	Description	Mean \pm SEM [Min – Max]
Aggressive	Dominating, defensive aggression, ears pinned backward, tail swishing	17.2 \pm 4.8 [0 – 87.5]
Alarmed	Tense, apprehensive, jumpy, nervous, watchful, on guard against a possible threat	23.4 \pm 5.1 [0 – 97.5]
Annoyed	Irritated, bothered by something, upset	11.1 \pm 3.5 [0 – 72.5]
Apathetic	Having or showing little or no emotion, disinterest, unresponsive to the rider's aids	6.4 \pm 2.9 [0 – 80]
At ease	Calm, carefree, peaceful	54.6 \pm 5.4 [0 – 91.7]
Curious	Inquisitive, desire to investigate the environment	29.4 \pm 5.6 [0 – 88.3]
Friendly	Receptive to the rider's aids, kind, not hostile, confident	68.7 \pm 4.4 [11.6 – 95]
Fearful	Afraid, hesitant, timid, not confident	4.8 \pm 3.2 [0 – 92.5]
Happy	Feeling, showing or expressing joy, pleased, lively, playful, satisfied	0 \pm 0 [0 – 0]
Looking for contact	Actively looking for interaction, interested, close proximity, eager to approach	0 \pm 0 [0 – 0]
Relaxed	Not tense or rigid, easy-going, tranquil	45.3 \pm 5.2 [0 – 89.2]
Pushy	Assertive or forceful, dominant behaviour	1.7 \pm 0.6 [0 – 11.7]
Uneasy	Afflicted, uncomfortable, unsettled	48.2 \pm 6.4 [0 – 100]

