Horses Could Perceive Riding Differently Depending on the Way They Express Poor Welfare in the Stable

Alice Ruet, Sophie Biau, Cécile Arnould, Patrick Galloux, Alexandra Destrez, Eléna Pycik, Laetitia Boichot, Léa Lansade

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Alice Ruet [a,*], Sophie Biau [b], Cécile Arnould [a], Patrick Galloux [b], Alexandra Destrez [c], Elena Pycik [b], Laetitia Boichot [b] and Léa Lansade [a]

[a] *UMR 0085 INRAE, PRC, CNRS, IFCE, Université de Tours, 37380 Nouzilly, France*
[b] *I.F.C.E. Ecole National d'Equitation, Terrefort, BP 207, 49411, Saumur Cedex, France*

[c] *AgroSup Dijon, Université de Bourgogne Franche-Comté, INRAE, CNRS, UMR6265 CSGA – Centre des Sciences du Goût et de l'Alimentation, Dijon F-21000, France*

* Corresponding author: alice.ruet@sfr.fr

Abstract

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indicators of a compromised welfare state in loose boxes (stereotypies, aggressive behaviours towards humans, withdrawn posture reflecting unresponsiveness to the environment and alert posture indicating hypervigilance) and the way horses perceived riding.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

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

Keywords

Animal welfare, Behaviour, Horse, Housing conditions, Kinematics

Highlights

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

1. Introduction

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

Nowadays, a large number of horses are involved in riding activities [16], although being ridden can be perceived as aversive [17] and could sometimes exceed the mental and physical capacities of animals [18]. Numerous factors related to riding have been associated with a negative perception of this practice by horses. These factors concern the equipment, the health of feet and shoeing, the use of artificial aids such as spurs [19], the position, skills and technique of the rider [20,21] and certain controversial riding practices such as hyperflexion of the neck [22–27]. Another key factor could be the overall welfare state of the horse in the living environment. However, as stated by Hall and Heleski [22], the direct relationship between a compromised welfare state in the stable and the horse's affective state when ridden has received little attention. A few studies have shown the effects of housing conditions (e.g., boxes versus pasture) and animal management (e.g., playing music in the stable) on the behaviour and physiological stress responses of horses during riding activities [28–30]. These results suggest that the welfare state of horses in their living environment and when ridden are related, but this requires further investigation by evaluating the animals in both contexts.

Three kinds of indicators could enable the affective state of horses to be inferred while being ridden [31]. The first concerns behavioural and postural indicators. To date, the majority of such indicators reflect negative affective and/or physical states (e.g., bucking, bolting, raised tail carriage, asymmetrical and backward ears positions; see [31] for a recent review), whereas positive indicators remain scarce (e.g., snorts at walk [32]). The second is a Qualitative Behaviour Assessment (QBA), which could be a useful tool to assess the affective state of horses in riding situations. It consists of a "whole-animal" approach based on the assessment of the overall behavioural expression of the subject using descriptors such as "relaxed" or "frightened" [33]. Due to its integrative nature, it is difficult to define descriptors precisely using specific behavioural indicators [34], but a growing number of studies support the validation of this tool by correlating descriptors with observable behaviours [35], relevant physiological measures and health parameters [33,36]. To our knowledge, only one study has used a QBA in horses during riding situations [37], and further validations are still required in this context [22,38]. However, it constitutes a supplementary tool to be used in conjunction with behavioural and postural indicators, particularly as it allows positive affective states to be assessed [39]. The third involves the study of locomotion characteristics which may provide insights into affective states (for a recent review in humans and non-human animals, see [40]). For example, anxiety in mice can be expressed through a stretch-attend posture when walking [41]. In humans, it is possible to differentiate states such as anger, depression or anxiety through gait patterns by studying accelerations and velocities of different parts of the body (e.g., velocity of chest movements; [42–44]). Accelerations of some parts of the horse's body have been studied using inertial measurement units consisting of accelerometers located close to the animal's centre of gravity (i.e., sternum; [45]). To date, the assessment of the locomotion in horses has mainly been studied to improve sport performance and detect pathologies, but with regard to both human and animal literature, such parameters could also be influenced by specific affective states and thus could constitute relevant indicators. Moreover, the existence of two-way biomechanical interactions between the horse and the rider is well described [46–48]. It could thus be hypothesized that the affective state of horses could affect the movements of the rider through specific locomotion patterns, and potentially induce long-lasting health issues.

The aim of this study was to investigate the relationships between four behavioural expressions of a compromised welfare state in loose boxes using stereotypies, aggressive behaviours towards humans and withdrawn and alert postures and the affective state of horses while being ridden, by recording the three aforementioned kinds of indicators. For each horse, a survey was first completed by the usual riding instructor of horses to obtain an integrative view of their behaviour during different riding sessions and over time. Then, a standardised riding session was carried out with an expert rider, during which the affective state of horses was inferred through recording behavioural and postural indicators, a QBA assessment, and the 3-dimensional accelerations of the horse's trunk characterising overall locomotion, using an inertial measurement unit [49]. The movements of the rider's spine were also measured with two additional inertial sensors. We hypothesised that the expression of the four behavioural indicators reflecting a compromised welfare state in loose boxes would be related to behavioural and postural indicators of negative affective states (e.g., fear or anxiety-related behaviours in the survey, bucking or asymmetrical ears during the riding session) and negative descriptors in the QBA (e.g., "alarmed") when ridden. As observed in humans, we expected to record different accelerations of the horse's trunk related to behavioural indicators of a compromised welfare state expressed in loose boxes and different movements of the rider's spine in response to the horse's locomotion pattern. As this study is the first to investigate the links between locomotion and welfare in horses, no precise hypothesis could be formulated as to the direction of variations in acceleration.

2. Materials and methods

2.1. Animals, housing and management conditions

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

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

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

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

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

The percentages of scans during which each of the four behavioural indicators was observed were calculated according to the total number of scans recorded for each horse. As stereotypies and aggressive behaviours towards humans were expressed by less than 35 % of the horses (Table 1) and showed little variability, these two indicators were subsequently considered as two binary variables for each horse (the indicator was expressed at least once by the horse or was not expressed at all). Withdrawn and alert postures were retained as continuous variables and expressed as the percentages of scans of each indicator for each horse. Mean percentages of the expression of these two postures are presented in Table 1.

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

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

2.4. *Standardised riding session with an expert rider*

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

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

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

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

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

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

$$SAC = (1 - \left(\frac{rmsST}{rmsL5}\right)) * 100$$

in which rmsST is the magnitude of anteroposterior accelerations at the rider's sternum (ST) and rmsL5 is the magnitude of anteroposterior accelerations at the rider's fifth lumbar vertebra (L5). This coefficient describes the ability to reduce acceleration from the rider's L5 to the rider's ST (Table 3). The higher the SAC coefficient, the higher the acceleration attenuated by the rider's spine.

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

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

This second part of the riding session, lasting 5.3 ± 0.2 minutes, immediately followed the first part. This part consisted of a series of circles, gait transitions between walk and trot, lines in extended trot and canter and leg-yielding in both directions (Table S1). The rider was instructed to ride the horses uniformly, with as few constraints as possible, to limit the impact of the rider's technique on the horse's affective state.

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

The QBA was performed using thirteen descriptors adapted from the AWIN Horse protocol [53] by the same experimenter for all the horses (Table 5), who also assessed the behavioural and postural indicators during the riding session. The experimenter was trained to perform the QBA assessment (PhD in ethology). The latter consisted of observing the complete riding session on video recordings and then making a mark on a visual analogue scale from 0 to 100 (one scale per descriptor). A score of 0 indicated that the descriptor was not observed at all, and a score of 100 reflected that the descriptor was present during the whole riding session.

2.5. Statistical analyses

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

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

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

The multiple regression models used were:

$$y_i = \beta_0 + \beta stereotypies_i + \beta aggressiveness_i + \beta withdrawn\ posture_i + \beta alert\ posture_i + \varepsilon_i$$

in which y is the outcome variable (e.g., the occurrence of tail swishing per minute during the riding session), β_0 is the intercept, $\beta stereotypies$, $\beta aggressiveness$, $\beta withdrawn\ posture$ and $\beta alert\ posture$ are the fixed-effect parameters (predictors) and ε is the residual term. A log transformation was applied for the following outcome variables: head shaking / tossing, abnormal mouth behaviours, tail swishing, the SAC at trot and canter and the anteroposterior acceleration at canter. A square-root transformation was carried out on the raised tail carriage variable to approximate more accurately a normal distribution. As the usual discipline of each horse could influence the horse's locomotion [56] and the behavioural indicators of affective states [57,58], the confounding effect of this parameter was controlled for all the outcomes by quantifying changes in the values and significances of the coefficients of the fixed-effect parameters when the discipline was included in the models or when this variable was excluded. When a change of at least 10 % of the values was observed, the discipline was considered as a confounding factor and was therefore retained in the final model [59]. Tukey post-hoc tests (glht function, *multcomp* R library) were performed to investigate further the effects of significant parameters.

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

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

2.6. Ethics statement

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

2.7. Graphic design

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

3. Results

3.1. Behavioural survey

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

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

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

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

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

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

3.2. Standardised riding session

Among the behavioural and postural indicators reflecting affective states during the riding session, no horse reared or produced snorts at walk, and only two horses bucked and bolted. Thus, these behaviours could not be statistically analysed. However, it is interesting to note that the horse that bucked twice also expressed both stereotypies and aggressive behaviours towards humans in its loose box, and the horse which bolted once expressed the highest level of alert postures in its loose box (3.8 % of scans).

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

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

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

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

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

Relationships between the expression of alert posture in loose boxes and the riding session. The expression of alert posture in loose boxes was significantly related to asymmetric ear position (F = 5.92, $p = 0.02$; N = 30) and tended to be related to forward ear position (F = 3.59, $p = 0.07$; N = 30) during the riding session: the more alert postures the horses expressed, the more asymmetric ($\beta = 3.81$; Figure 5.a) and forward ($\beta = 4.58$) the ear positions were. The expression of alert postures was also significantly related to the second axis of the PCA performed on the QBA descriptors ($F = 6.04$, $p = 0.02$, $N = 29$). This axis explained 18.9 % of the total variance and was mainly represented by "alarmed" for the positive scores, as opposed to "annoyed" and "pushy" for the negative scores. Thus, the more alert postures the horses expressed, the more they were judged as "alarmed" during the riding session ($\beta = 63.3$; Figure 5.b).

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

4. Discussion

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

Stereotypic horses in loose boxes seemed to experience a more negative affective state during the riding session, compared to non-stereotypic horses. Indeed, they more often expressed raised tail carriage, which has been described as an indicator of stress or fear in several conditions, in both non-ridden and ridden horses [13,38,60–64]. This may be in line with previous studies showing that stereotypic horses (crib-biters) were more stress sensitive and presented a higher cortisol response following an ACTH challenge test [65] and a larger increase in heart rate and locomotor behaviours after a sudden event [66] compared to non-crib-biters horses. The results of the current study suggest that stereotypic horses in general, and not only crib-biters, would present a higher sensitivity to stress. The riding session could have been perceived as a stressful event for stereotypic horses, probably in part because no other horses were present in the arena and because they were ridden by an unknown rider. However, this result needs to be confirmed, for example by adding physiological measures such as cortisol measurements, since only one behavioural indicator of negative affective states was highlighted. The results of this study suggest similar conclusions regarding the affective state when ridden for horses expressing hypervigilance in loose boxes. The increase in hypervigilance was related to asymmetrical and forward ear positions being expressed more. In horses, it has been demonstrated that asymmetrical ears could reflect a negative affective state during grooming by humans [67] and ears pointing forward towards external stimuli would indicate attention to the environment [68]. Moreover, the more horses expressed hypervigilance, the higher their scores on the PCA axis of "alarm" resulting from the QBA analysis, which supports the experience of a negative affective state when ridden (the "alarmed" descriptor is described as "tense, apprehensive, on guard against a threat"). These results are illustrated anecdotally by the most hypervigilant horse in loose boxes that bolted once during the riding session, i.e., demonstrating a flight response to a stressful event. It is likely that more horses could have expressed such extreme behaviours but that the expert rider prevented their expression.

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

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

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

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

5. Conclusion

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

Declarations of interest

The authors have no conflicts of interest to report.

Author contributions

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 experiments; A.R., S.B., C.A., E.P. and L.L. analysed and interpreted the data; A.R., S.B., C.A. and L.L. wrote the paper; all the authors reviewed the manuscript.

Acknowledgements

This project was funded by the IFCE and the “Fonds Eperon”. This funding source has no role in the study design, data collection and analyses or in the preparation and submission of the manuscript. The authors are very grateful to the staff of the French Horse and Riding Institute and INRAE (Nouzilly, France), and especially to Marc-André Morin. We would like to thank the Springer Nature Author Services team and Sue Edrich from Interconnect for correcting the English manuscript.

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Table captions

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 ± SEM; [Min - Max]). N = 43.

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.

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

Table 4. Descriptions of the behavioural and postural indicators reflecting affective states assessed during the riding session (mean ± 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.

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

Figure captions

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

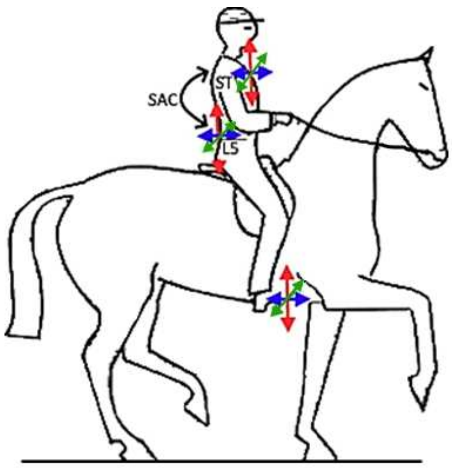
Figure 2. Scores assigned to the questions "Does the horse show reluctance to move forward" in the behavioural survey completed by the usual riding instructor, according to the percentages of scans of withdrawn postures in loose boxes. A score of 0 corresponds to "the behaviour is never expressed in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

Figure 3. Mean percentages of scans (± SEM) with raised tail carriage during the riding session for stereotypic and non-stereotypic horses ($N_{Stereotypic} = 7$; $N_{Non\text{-}stereotypic} = 22$; F-tests results from multiple regression models with the percentages of scans with raised tail carriage as the outcome variable). ** $p \leq 0.01$.

Figure 4. Mean values of the dorsoventral accelerations of the horse (a) and the mean shock absorption coefficients of the rider (b) at canter (± SEM) during a riding session according to the expression of aggressive behaviours towards humans in loose boxes ($N_{Non\text{-}aggressive} = 13$; $N_{Aggressive} = 11$; F-test results from multiple regression models with dorsoventral accelerations and shock absorption coefficients as the outcome variables). * $p \leq 0.05$; ** $p \leq 0.01$.

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

Figures



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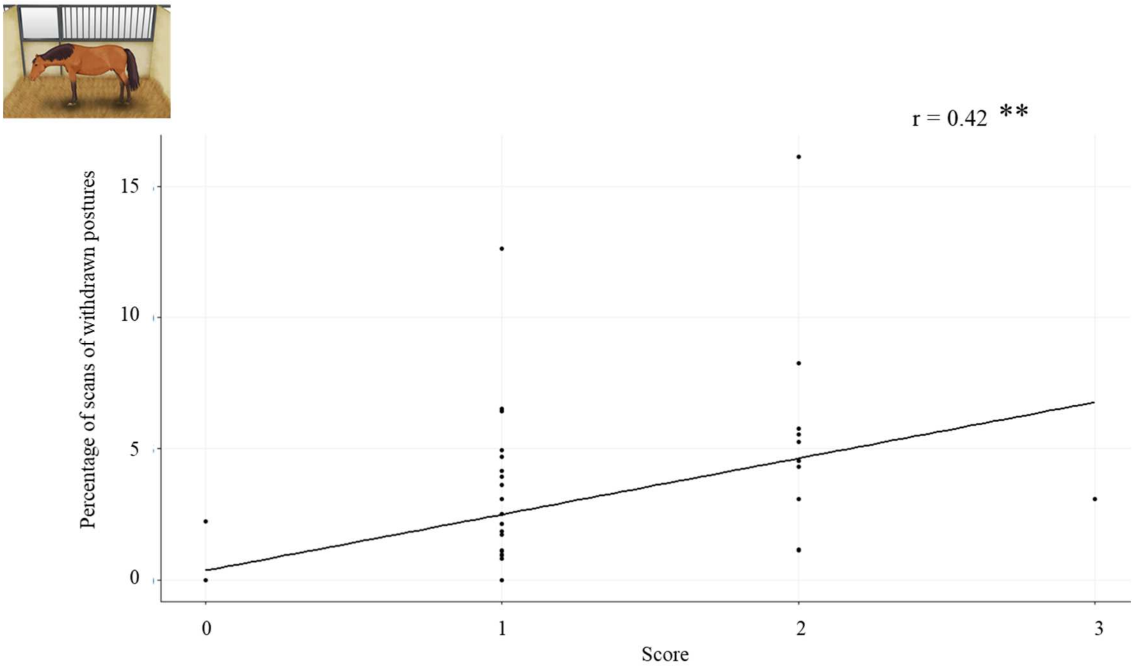

*Figure 2. Scores assigned to the questions "Does the horse show reluctance to move forward" in the behavioural survey completed by the usual riding instructor, according to the percentages of scans of withdrawn postures in loose boxes. A score of 0 corresponds to "the behaviour is never expressed in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

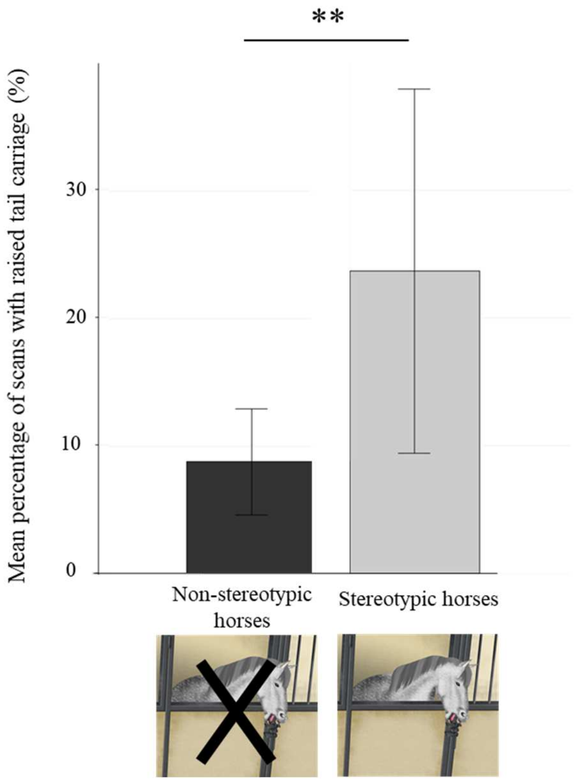

*Figure 3. Mean percentages of scans (± SEM) with raised tail carriage during the riding session for stereotypic and non-stereotypic horses ($N_{Stereotypic}$ = 7; $N_{Non\text{-}stereotypic}$ = 22; F-tests results from multiple regression models with the percentages of scans with raised tail carriage as the outcome variable). ** $p \leq 0.01$.*

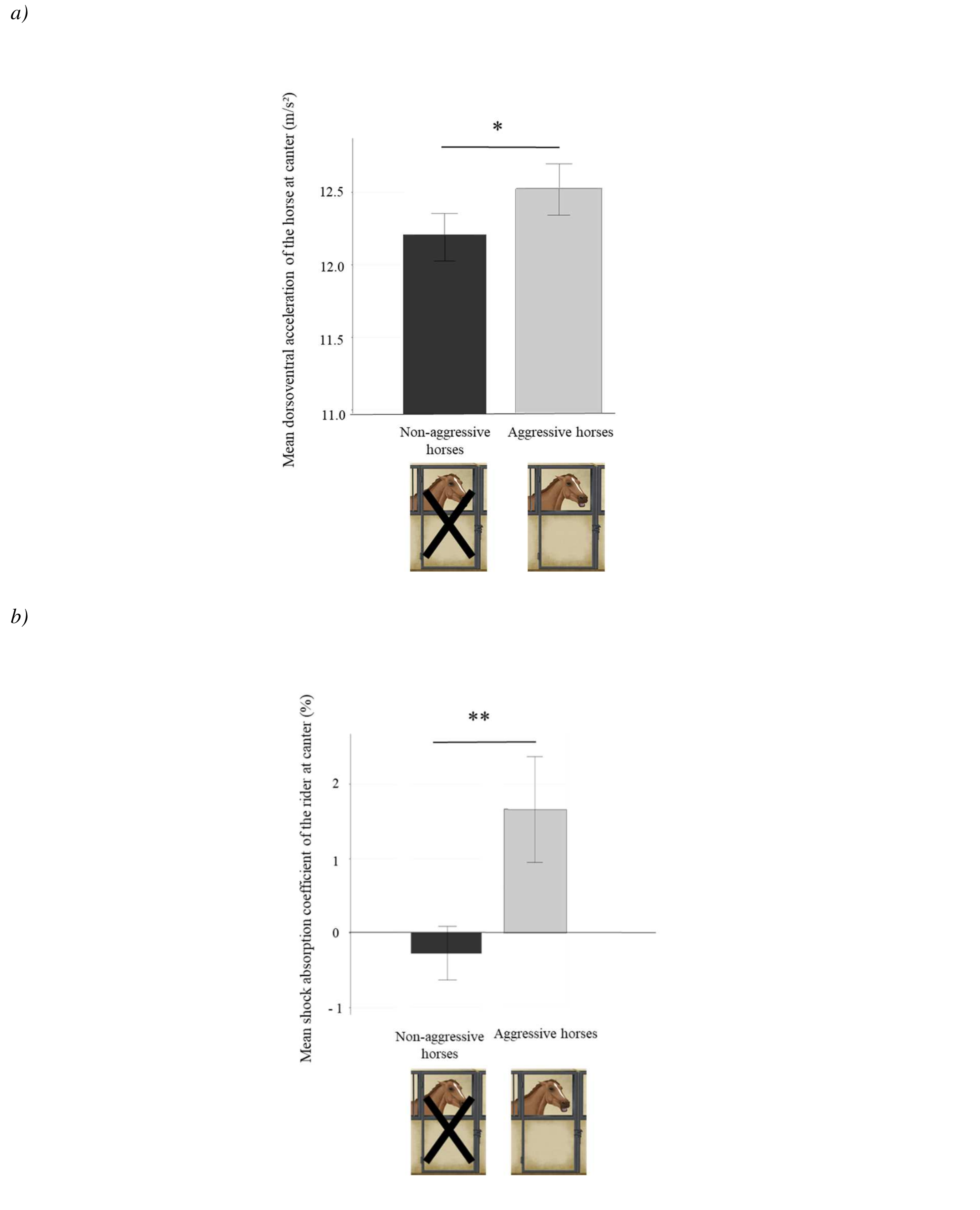

*Figure 4. Mean values of the dorsoventral accelerations of the horse (a) and the mean shock absorption coefficients of the rider (b) at canter (± SEM) during a riding session according to the expression of aggressive behaviours towards humans in loose boxes ($N_{Non\text{-}aggressive}$ = 13; $N_{Aggressive}$ = 11; F-test results from multiple regression models with dorsoventral accelerations and shock absorption coefficients as the outcome variables). * $p \leq 0.05$; ** $p \leq 0.01$.*

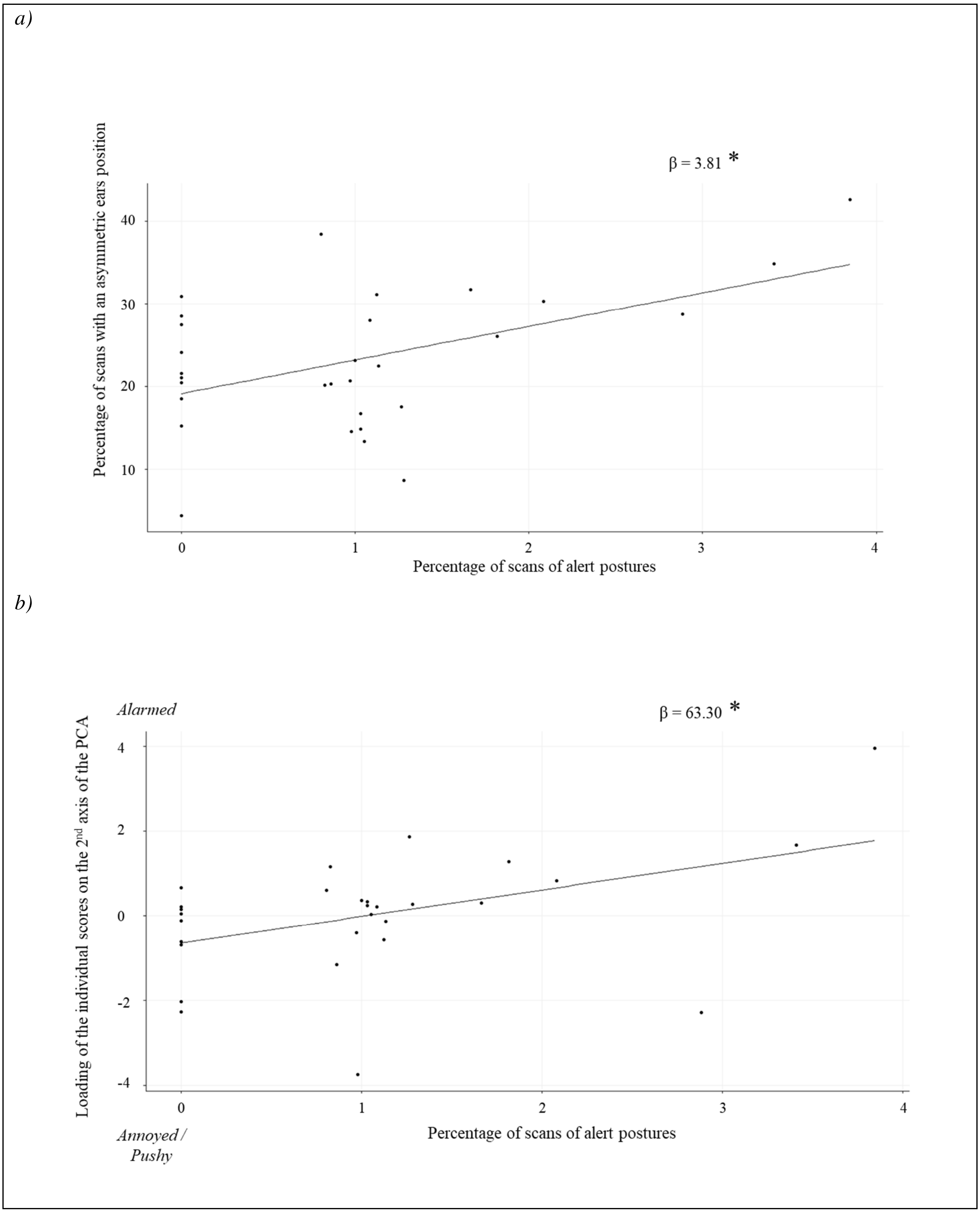

*Figure 5. Percentages of scans with asymmetric ear positions (a; N = 30) as well as individual scores on the second axis of the PCA (b; N = 29) performed on the QBA descriptors, according to the expression of alert postures in loose boxes. F-test results from multiple regression models with ear positions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

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 ± 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 ± 0.5 % [0 – 16.1 %]
Alert posture	Elevated neck and ears pricked forward, looking intensively at the environment, reflecting hypervigilance	1.1 ± 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 – 3d quartile]. N = 43.

Question	Median [1st – 3d 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 ± SEM; [Min – Max]). N = 24.

	Trot	**Canter**
Horse's locomotion	**Mean ± SEM [Min – Max]**	**Mean ± SEM [Min – Max]**
Anteroposterior acceleration (m/s²)	4.5 ± 0.1 [3.7 – 5.8]	4.4 ± 0.2 [3.6 – 6.7]
Mediolateral acceleration (m/s²)	3.6 ± 0.2 [2.07 – 6.02]	4.5 ± 0.2 [3.2 – 6.6]
Dorsoventral acceleration (m/s²)	12.4 ± 0.1 [10.9 – 14.3]	12.3 ± 0.1 [10.6 – 13.3]
Movement of the rider's spine	**Mean ± SEM [Min – Max]**	**Mean ± SEM [Min – Max]**
Shock absorption coefficient (SAC; %)	7.7 ± 0.5 [2.6 – 13.5]	0.6 ± 0.4 [(-2.3) – 5.2]

Table 4. Descriptions of the behavioural and postural indicators reflecting affective states assessed during the riding session (mean ± 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 ± 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 ± < 0.01 [0 – 0.25]
Bolting [a]	The horse runs off suddenly	< 0.01 ± < 0.01 [0 – 0.10]
Head shaking / tossing [a]	Fast lateral, circular or up-and-down movements of the head	1.2 ± 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 ± 0.3 [0 – 5.7]
Tail swishing [a]	Fast lateral, vertical or circular movements of the tail	2.5 ± 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 ± 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 ± 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 ± 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 ± 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 ± SEM; [Min – Max]). N = 29.

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