**Horse mouth behaviour related to selected kinematic variables representing horse-rider interaction: a pilot study**

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**Abstract**

The objective of this pilot study was to investigate the influence of rein contact and the movement of the rider's hand on the horse's behaviour, analysing data on horses ridden in two different head and neck positions. We hypothesized that the rider's hand movements and rein tension generate behavioural responses from the horse, and more so when ridden on the bit compared to free and unrestrained. Data were collected from seven dressage horses/riders in sitting trot on a high-speed treadmill. Kinematics were recorded using a 12-camera, infrared-based opto-electronic system. Behavioural recordings were made from video and three horses wore a rein tension meter. After stride split, data were standardised to 0-100% stride duration. Mixed models were used to analyse how the behaviours varied over the stride cycle; trial within horse was treated as a random effect, while percentage of stride, rein tension and kinematic variables mainly related to the rider's hand were entered as fixed effects. Behaviours discerned were lip movement, mouth movement, open mouth, ear position, head tilt and tail movement. Mouth movements were associated with the suspension phase of the trot and percentage of stride was highly significant (*P*<0.0001). Head and neck position was non-significant in the final models, while rein tension and the distance between the rider's hand and the horse's mouth affected the amount of mouth movements. The results from this preliminary study convey the large variations between horses and riders, as well as the complexity of the interaction.

*Keywords:* Equine behaviour; Dressage; Rider hand movement; Rein tension

## Introduction

While trotting, vertical and horizontal accelerations and decelerations of the horse's trunk occur at each diagonal stance (Byström et al., 2009). These natural forces lead to a variation in the pressures the rider applies on the horse's body. The rider is pressed against the saddle at deceleration (from the beginning of the stance phase to midstance) and pushed out of the saddle at acceleration (from midstance to the beginning of the next stance, including the suspension phase) (Byström et al., 2009). The rider's ability to adjust to the horse's movement affects the pressure signals applied and poor absorption may lead to the rider exerting undue force on the reins in an attempt to regain position (Heleski et al., 2009). Training horses generally involves using negative reinforcement, e.g. an applied pressure is released when the horse responds in the desired way, the timing of the release being the crucial element of learning (McGreevy and McLean, 2007). If variations in rein tension are made accidentally and interpreted by the horse as signals, it may result in confusion and poor learning (Saslow, 2002). The bit has further implications: oral behaviours are displayed as a response to bit pressure (Manfredi et al., 2010), excessive bit pressure causes discomfort (Manfredi et al., 2005), and scars in the mouth of the riding horse are common (Tell et al., 2008), but light rein cues and repeated release from bit pressure may lead to more wanted behaviour (Egenvall et al., 2012).

This study is part of a larger project studying the biomechanical effects of various head and neck positions (HNPs) on the movement of the horse's back and limbs (Weishaupt et al., 2006; Rhodin et al., 2009), as well as on motion patterns of the saddle and the rider's seat (Byström et al., 2009). Using data from the same experiment, the aim of this study was to investigate the influence of rein contact and movement of the rider's hand on the horse's behaviour. We hypothesized that riding the horse on the bit, compared to in the free, unrestrained position, would be more associated with behavioural displays from the horse and that the rider's hand movements and rein tension would be temporally correlated to the stride cycle.

## Material and methods

### *Material*

The study participants were Warmblood breed horses (1.70 ± 0.07 m) competing at Grand Prix level (*n*=6) and Intermediaire level (*n*=1) ridden by their usual riders (three males and four females, weight 78 ± 17 kg) with their own saddles and bridles, snaffle bits and English nosebands (some also wore flash nosebands). The nosebands were tightened to fit two fingers between the skin and the noseband ventral to the mandibula. The study had ethical approval from the Animal Health and Welfare Commission of the canton of Zürich (188/2005).

### *Study design*

The data collection was performed on a high-speed treadmill (Mustang 2200) with an integrated force measuring system (Weishaupt et al., 2002) sampling at 420/480 Hz. The horses and riders were fitted with reflective markers, 19 mm in diameter. These markers were placed on the horse's head (crista facialis; left/right), between the eye and the ear (eye; left/right) as well as on the withers (thoracic vertebra six, T6) and the lumbar vertebra three (L3) of the horse and, in addition, on the rider's hands (hand; left/right). The markers were set in relation to a global coordinate system, calibrated before measuring each of the horses by creating a stance file aligned with the treadmill. To register the position of the markers 12 infrared cameras (ProReflex, Qualysis) were used at a frame rate of 140/240 Hz for 12 s (*n*=2) or 15 s (*n*=5). Position was registered in millimetres along the X-, Y- and

Z-axes. The X-axis was horizontal and positive in the horse's direction of motion, the Y-axis horizontal and positive to the left, and the Z-axis vertical and positive upwards. The trials were captured on video (left side of the horse). Three of the horses wore a rein tension meter (Futek 2357 JR S-Beam mini load cell force sensor) between the bit and the reins, weighing 28 g, and a Computer Boards AD-converter was used to register the signal. The sampling rate was 140 Hz for 15 s. The rein tension meter was calibrated before measuring each individual horse by suspending known weights ranging from 0 to 3 kg (rein tension results are presented in Newton). All the equipment was synchronised by a hardware start trigger pulse. Data collection was performed at trot, with the head and neck of the horse in the free, unrestrained position with loose reins (HNP1, 1 trial/horse) and with the neck raised, poll high and bridge of the nose slightly in front of the vertical, on the bit, as in dressage competitions (HNP2, 3-5 trials/horse) with the HNPs performed in random order (Rhodin et al., 2009). For the initial experiment, the horses were ridden in a speed series in HNP2 for speed matching to other HNPs (Weishaupt et al., 2006). For this reason there are numerous trials per horse in HNP2.

*Behaviour and kinematics*

The horses' behaviour was studied frame by frame (25frames/s) in a GOM player (Gomlab, Gretech Corp.) by one reviewer. For each frame one or several behaviours were registered, described in Table 1. Because of a safety belt on the side of the treadmill, the horses' mouth sometimes ended up out of sight. In the main analysis mouth-out-of-sight frames were considered absent for mouth behaviour (the frames were not excluded). To validate within-reviewer agreement in the behavioural data, the same reviewer re-evaluated one randomly chosen film for each horse (2477 frames). The criteria for agreement were that the same behaviour had to be registered as present or absent in the equivalent frame.

Mouth-out-of-sight was not validated.

Data were transferred to Matlab (The Math Works Inc.) where strides were divided at left forelimb first contact and time-standardised to 101 data points (0-100% of stride duration). A virtual marker defining the position and movement of the horses' mouth (mouth) was created by calculating the distance and angle between the eye, crista facialis and the corner of the mouth in ImageJ (ImageJ 1.46k) using a picture of each horse's head, extracted from the video films, standing still without tension on the reins. The kinematic variables studied were the distances: T6-mouth, mouth-hand (left/right), T6-hand (left/right) and L3-hand (left/right) as well as the 'nose' angle of the horse's head defined by the horizontal plane, the eye and the mouth (Appendix 1).

*Statistical analysis*

Descriptive statistics are presented for behavioural variables, kinematic variables, and rein tension, both as overall averages (means ± SD, range of motion (ROM) ± SD) and for selected variables over the stride and related to HNP. Mouth movements were analysed further as they appeared frequently in both HNPs and in all horses (Table 1). Mixed models (SAS Institute Inc.) were created with the dependent variable mouth movement. The most normal transformation of mouth movements ($1/y^2$, 1/y, natural logarithm of y, square root of y or $y^2$) was chosen based on a mean close to the median, a 'small' standard deviation, and low values of skewness and kurtosis. Trial within horse was used as random variable and the covariance structure was set to compound symmetry (indicating the within-horse correlation to be identical through trials). Fixed effects variables were percentage of stride cycle (baseline 0% of the stride), HNP (baseline HNP1, kept as a forced variable), speed (continuous, forced), rein tension (three horses) and the kinematic variables. The kinematic variables were transformed subtracting the minimum value for each horse. Both left/right variables were kept if only one was significant. Rein tension and the kinematic variables were transformed to dummy variables (four categories of which the two middle were equidistant) and tested for linearity. The 'rein tension model' was created using percentage of stride, rein tension (left/right), the distance mouth-hand (left/right), speed and HNP, while the 'kinematic model' included all variables except rein tension. Full multivariable models were reduced to models containing only variables with a group *P*-value of <0.05 (for at least one of the left/right variables). Percentages of stride were deemed as significant when consecutive Wald *P*-values were <0.0001. Univariable models (or bivariable for left and right variables) were created and finally, as percentage of stride possibly was systematically associated with mouth-out-of-sight, a model with percentage of stride as the only fixed effect was run where these observations were set to be present instead of absent.

**Results**

In total the seven horses were seen in 36 trials; 29 in HNP2 and seven in HNP1. In HNP2 the speed varied from 2.7 m/s to 3.4 m/s and in HNP1 2.9 m/s to 3.3 m/s. Rein tension was registered in three horses, 3 and 13 trials in HNP1 and HNP2, respectively. The mouth was out of sight in 2% (*n*=54) of the frames for HNP1 and in 10% (*n*=1067) for HNP2. In the behavioural validation there was 95% agreement of whether the same behaviour was present or absent in the equivalent frame. Mouth movement had an agreement of 79% (*n*=1967/2477 frames), lip movement and ears to the sides 93% (2306 and 2315 of 2477 frames). Other listed behaviours agreed ≥95%.

*Behaviour*

Behaviours discerned were lip movement, mouth movement, open mouth, ear position, head tilt and tail movement. The overall mean and range of horse-means of each of the behaviours, per HNP, are found in Table 1. The mouth behaviours; mouth movement, lip movement and open mouth showed a temporal association to the suspension phase of the trot in HNP2 (Figs. 1-2). In HNP1 these behaviours had a more even distribution (data only shown for mouth movements, Fig. 1). The other behaviours (ear position, tail movement and head tilt) were not found to be related to HNP or temporally to the stride cycle and were therefore not further studied.

*Kinematic data*

Fig. 3 and Appendices 2-3 show the kinematic variables over the stride cycle, from which the minimum (standardised) value for each horse has been subtracted. Graphically, similar results were found for both HNPs except for the variation in distance mouth-hand, where the maximum distances were found during the suspension phase for HNP2, whilst in the first part of stance for HNP1 (Fig. 3). The first part of stance was also when the maximum distance T6-hand was found, while the maximum distance T6-mouth occurred around midstance (Appendix 2). Further, midstance was associated with a maximum nose angle (Appendix 3) and a maximum distance L3-hand (Appendix 2).

*Rein tension*

Fig. 4 demonstrates rein tension relative to the stride cycle. In HNP2 both reins showed peaks of tension at suspension and midstance with emphasis on the suspension phase, as well as higher tension for the right rein. In HNP1 peaks of rein tension occurred around midstance.

*Statistical models*

Square root transformation was deemed the best way to process mouth movement data. In the model with only percentage of stride as fixed effect, the ranges 11-39% and 57-84% were significantly different (*P*<0.0001) from percentage 0 (seven horses, 35 trials, 3535 observations). In the multivariable kinematic model all variables except speed and HNP were significant. Table 2 shows univariable and multivariable results, where the latter have also been transformed to the original scale, showing how much the behaviours would be expected to change compared to the baseline category. Percentages of stride from 12-37% and 60-76% had a significantly lower frequency of mouth movement (*P*<0.0001). Increasing the distance mouth-hand left increased the mouth movements most pronouncedly.

In the rein tension model percentage of stride (14-32% and 62-79% lowered the frequency of mouth movement) and left rein tension were significant, with increasing rein tension increasing the mouth movement (three horses, 16 trials, 1616 observations, Table 3). Rein tension was not linearly related to the dependent variable. Comparing the categories for the left rein tension, all categories (>2-≤10 N, >10-≤18 N, >18 N) increased the frequency of mouth movement compared to baseline ≤2 N. The results from the multivariable model are partially different from the graphical (univariable) presentation.

Speed was not significant in any model, while HNP was significant (*P*<0.0498) in the univariable kinematic model. The sensitivity analysis of setting mouth-out-of-sight registrations as present for mouth movements, instead of absent, did not show any differences regarding the conclusion relative to percentage of stride (data not shown).

## Discussion

The most prominent finding was that mouth movements appeared significantly more often in the suspension phase of the trot in HNP2 (Fig. 1), as did lip movements and open mouth (Fig. 2), compared to midstance. Controlling for other variables in the model, HNP did not affect mouth movements in the final model. Then again, from the horse's point of view the difference between the HNPs in terms of interaction with the rider might have been quite small due to the nature of the experiment. During the 12 s/15 s data collection on the treadmill, the horses were already in the correct speed and head carriage and rider influence was likely limited. Further, HNP was completely associated with each trial and hence had a low statistical power. The effect of HNP on mouth behaviour therefore merits further investigation.

Rein tension peaking around midstance when horses received no (or minimal) rein influence from the rider (HNP1) is similar to earlier findings (Clayton et al., 2011). The rein tension data for HNP2 was more complicated to interpret. Unexpectedly, the left rein tension, and not the right, increased the amount of mouth movements, while the right rein actually decreased the amount of mouth movements. This is contradictory since the right rein showed a more pronounced association to the suspension phase (Fig. 3). The large variation between the three riders, in magnitude, frequency of spikes as well as left and right hand synchronisation (data not shown), could explain these complex results. The considerable differences between the left and right rein tension are interesting from an equestrian perspective as laterality/handedness in both riders and horses is very typical, while straightness is considered one of the cornerstones for progression in training.

The correlation between mouth movements and the suspension phase is puzzling, especially in the light of the inconclusive results from the rein tension data. However, the distance mouth-hand (left/right) increased and decreased simultaneously with the mouth movements in HNP2 (Fig. 3), likely related to the rider being pushed out of the saddle during the suspension phase, as found by Byström et al. (2009) and suggests that the hand acting on the mouth creates the mouth movements. Further comparing to Byström et al. (2009), in the vertical and sagittal plane, the distance L3-hand peaked before the distance L3-rider seat (data not shown), which might indicate that the hand is more synchronised with the mouth than the seat, as the distance L3-hand (left/right) was largest at midstance when the distance mouth-hand was shortest. This separation of the hand from the seat is one of the hallmarks of an independent seat, but the ideal synchronisation with the mouth and how to achieve it is yet to be elucidated. We suspect that a sub-optimal seat may affect hand movements in a way less controlled by the rider (Engell et al., unpublished results). What the registered mouth movements in this study indicate from a behavioural point of view also needs further scrutiny. The vast literature on riding in general agrees that some mouth behaviours are desired by the rider. Manfredi et al. (2010) suggests that desirable mouth behaviour is mouthing the bit, referring to when the horse is displaying mandibular and/or tongue movement without separating the incisors by more than 1 cm, which resembles the mouth movements recorded in this study.

Other interesting findings were that mouth movements decreased almost linearly with an increasing distance T6-mouth, i.e. an elongated neck, and that compared to baseline, mouth movements increased with an increasing nose angle. The latter may suggest that poll bending influences mouth movements. Then again, the horse can hold its head perpendicular in both HNP1 and HNP2, while it is the height of the horse's head and neck that determines the degree of poll bending. It would thus have been useful to also study the poll angle (mouth-atlas-T6).

Only few horses participated in this study and whether results can be used for extrapolation to wider horse populations is uncertain. Frame by frame analysis had the advantage of behaviour being synchronized with all data. However, as cause-effect relationships in the horse-rider interaction cannot be expected to be precisely synchronised in time, other approaches that could have been used to find associations between kinematic and behavioural data may have involved time-shifting the data or other techniques to match the time series. A weakness of this study is that inter-observer reliability was neglected. In addition, the same data collection taking place over ground, instead of on a treadmill, may yield somewhat different results (Buchner et al., 1994).

Horse and rider interaction is complex, involving multiple parameters affecting the outcome, as seen from the modeling where by principle only significant variables remain and almost all selected variables did so. A certain non-signal variation in rein tension is likely unavoidable. The question is when the demonstrated variation becomes a sign of horse or rider instability, interfering with the communication or comfort for horse and rider (Heleski et al., 2009).

**Conclusions**

By combining recordings of the horse's behaviour with kinematic data representing the rider's hand movement, we attempted to find variables affecting horse-rider interactions. Findings were that the horse displayed mouth movements mainly during the suspension phase of the trot and it is suggested that the rider's hand movements create this behaviour. The rein tension data was complicated to interpret, but it can be concluded that rein tension differs immensely between horses and riders and we suggest that assessing rein tension in relation to the stride cycle is as important as having a large number of study objects. The results confirm the complexity of horse-rider interactions and the large variations between horses and riders. Nevertheless, considering this study as a pilot, including limited ability for extrapolation and mainly emphasizing the results with the lowest p-values, we believe that combining ethological studies with biomechanical measurements has considerable benefits when studying horse-rider interaction.

**Conflict of interest statement**

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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**Appendix A. Supplementary material**

Supplementary data associated with this article can be found in the online version, at doi:….

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**Table 1**

**Ethogram; percentages of the time each of the behaviours was displayed**

| Ethogram | Description | HNP | Mean | Range | SD | Range | Min | Range | Max | Range | Median | Range |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Upper lip movement | Upper lip is drawn upwards or outwards, teeth visible | 1 | 1 | (0, 3) | 1 | (0, 4) | 0 | (0, 0) | 3 | (0, 17) | 0 | (0, 2) |
| | | 2 | 6 | (0, 16) | 7 | (0, 10) | 0 | (0, 8) | 16 | (0, 26) | 2 | (0, 17) |
| Lower lip movement | Lower lip is drawn downwards, teeth visible | 1 | 2 | (0, 12) | 4 | (0, 9) | 0 | (0, 0) | 12 | (0, 26) | 0 | (0, 12) |
| | | 2 | 2 | (0, 9) | 3 | (0, 9) | 0 | (0, 5) | 9 | (0, 21) | 1 | (0, 8) |
| Mouth movement | Slight opening of the mouth or slight lip movement | 1 | 14 | (1, 36) | 13 | (1, 13) | 1 | (0, 18) | 36 | (6, 67) | 8 | (0, 4) |
| | | 2 | 23 | (10, 44) | 13 | (4, 11) | 10 | (4, 34) | 44 | (16, 52) | 19 | (6, 44) |
| Gaping | Space is visible between upper and lower jaw | 1 | 1 | (0, 4) | 2 | (0, 3) | 0 | (0, 0) | 4 | (0, 12) | 0 | (0, 5) |
| | | 2 | 2 | (0, 7) | 3 | (0, 6) | 0 | (0, 4) | 7 | (0, 12) | 2 | (0, 6) |
| Ears pressed back | Ears pressed back and downward | 1 | 0 | (0, 2) | 1 | (0, 3) | 0 | (0, 0) | 2 | (0, 6) | 0 | (0, 0) |
| | | 2 | 0 | (0, 0) | 0 | (0, 0) | 0 | (0, 0) | 0 | (0, 1) | 0 | (0, 0) |
| Ears back | Ears angled backwards | 1 | 25 | (0, 100) | 43 | (0, 4) | 0 | (0, 100) | 100 | (0, 100) | 0 | (0, 100) |
| | | 2 | 28 | (0, 100) | 36 | (0, 47) | 0 | (0, 100) | 100 | (0, 100) | 14 | (0, 100) |
| Ears forward | Ears angled forward | 1 | 51 | (0, 100) | 48 | (0, 8) | 0 | (0, 100) | 100 | (0, 100) | 46 | (0, 100) |
| | | 2 | 59 | (0, 100) | 34 | (0, 45) | 0 | (0, 100) | 100 | (0, 100) | 58 | (0, 100) |
| Ears to the sides | Ears angled to the sides | 1 | 23 | (0, 90) | 35 | (0, 6) | 0 | (0, 88) | 90 | (0, 94) | 0 | (0, 88) |
| | | 2 | 13 | (0, 28) | 10 | (0, 33) | 0 | (0, 12) | 28 | (0, 76) | 14 | (0, 18) |
| Tail movement | Rotating, or lateral or vertical movement of the tail | 1 | 1 | (0, 4) | 2 | (0, 3) | 0 | (0, 0) | 4 | (0, 6) | 0 | (0, 6) |
| | | 2 | 2 | (0, 16) | 6 | (0, 12) | 0 | (0, 0) | 16 | (0, 25) | 0 | (0, 19) |
| Head tilt | The head is held oblique | 1 | 1 | (0, 4) | 2 | (0, 4) | 0 | (0, 0) | 4 | (0, 12) | 0 | (0, 3) |
| | | 2 | 1 | (0, 3) | 1 | (0, 4) | 0 | (0, 0) | 3 | (0, 8) | 0 | (0, 2) |
| Head shake | Throwing the head upward, downward or from side to side | 1 | 0 | (0, 0) | 0 | (0, 0) | 0 | (0, 0) | 0 | (0, 0) | 0 | (0, 0) |
| | | 2 | 0 | (0, 0) | 0 | (0, 0) | 0 | (0, 0) | 0 | (0, 0) | 0 | (0, 0) |
| Out of sight | Mouth hidden behind a vertical belt next to treadmill | 1 | 2 | (0, 8) | 3 | (0, 0) | 0 | (0, 0) | 8 | (0, 0) | 1 | (0, 0) |
| | | 2 | 11 | (1, 30) | 11 | (1, 15) | 1 | (0, 13) | 30 | (2, 40) | 7 | (1, 38) |

The ethogram presents the percentages of the time each of the behaviours were seen during the standardised stride cycle in the free position (head and neck position 1, HNP1) and on the bit (HNP2). The table describes the mean of individual mean values of the entire group and the ranges for the individual horses between brackets. The data are collected from seven horses in 29 trials for HNP2 and seven trials for HNP1.

**Table 2**

**Univariable and multivariable 'kinematic' models**

| | | | Univariable model | | | | Multivariable model | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | | N | Estimate | SE | *P*-value | Group *P*-value | Estimate | SE | *P*-value | Group *P*-value | Back-transformed value |
| T6-mouth (mm) | >90 | 804 | -0.18 | 0.013 | <0.0001 | <0.0001 | -0.14 | 0.019 | <0.0001 | <0.0001 | -0.02 |
| | >60<=90 | 748 | -0.10 | 0.009 | <0.0001 | | -0.07 | 0.012 | <0.0001 | | -0.005 |
| | >30<=60 | 1078 | -0.02 | 0.008 | 0.0018 | | -0.01 | 0.009 | 0.13 | | -0.0002 |
| | <=30 (BL) | 905 | 0 | | | | 0 | | | | 0 |
| L3-hand left (mm) | >75 | 704 | -0.25 | 0.011 | <0.0001 | <0.0001 | 0.02 | 0.019 | 0.41 | <0.0001 | 0.0003 |
| | >50<=75 | 947 | -0.17 | 0.009 | <0.0001 | | -0.01 | 0.014 | 0.41 | | -0.0001 |
| | >25<=50 | 1092 | -0.06 | 0.008 | <0.0001 | | 0.02 | 0.010 | 0.02 | | 0.0006 |
| | <=25 (BL) | 792 | 0 | | | | 0 | | | | 0 |
| L3-hand right (mm) | >75 | 772 | -0.23 | 0.010 | <0.0001 | <0.0001 | 0.0004 | 0.020 | 0.98 | <0.0001 | 0 |
| | >50<=75 | 913 | -0.17 | 0.009 | <0.0001 | | -0.02 | 0.015 | 0.23 | | -0.0003 |
| | >25<=50 | 1052 | -0.05 | 0.008 | <0.0001 | | 0.02 | 0.011 | 0.12 | | 0.0003 |
| | <=25 (BL) | 798 | 0 | | | | 0 | | | | 0 |
| T6-hand left (mm) | >60 | 785 | -0.14 | 0.018 | <0.0001 | <0.0001 | -0.03 | 0.019 | 0.07 | 0.16 | -0.001 |
| | >40<=60 | 875 | -0.08 | 0.013 | <0.0001 | | -0.01 | 0.012 | 0.61 | | -0.00004 |
| | >20<=40 | 1127 | -0.05 | 0.010 | <0.0001 | | -0.01 | 0.009 | 0.49 | | -0.00004 |
| | <=20 (BL) | 748 | 0 | | | | 0 | | | | 0 |
| T6-hand right (mm) | >60 | 893 | 0.02 | 0.015 | 0.16 | <0.0001 | -0.09 | 0.016 | <0.0001 | <0.0001 | -0.01 |
| | >40<=60 | 800 | 0.05 | 0.012 | 0.0002 | | -0.02 | 0.012 | 0.04 | | -0.001 |
| | >20<=40 | 1076 | 0.05 | 0.010 | <0.0001 | | -0.002 | 0.009 | 0.79 | | 0.00001 |
| | <=20 (BL) | 766 | 0 | | | | 0 | | | | 0 |
| Mouth-hand left (mm) | >75 | 875 | 0.01 | 0.016 | 0.36 | <0.0001 | 0.02 | 0.023 | 0.35 | 0.0003 | 0.0004 |
| | >50<=75 | 577 | 0.06 | 0.014 | <0.0001 | | 0.05 | 0.016 | 0.0005 | | 0.003 |
| | >25<=50 | 919 | 0.03 | 0.011 | 0.01 | | 0.03 | 0.010 | 0.0007 | | 0.001 |
| | <=25 (BL) | 1164 | 0 | | | | 0 | | | | 0 |
| Mouth-hand right (mm) | >75 | 890 | 0.08 | 0.015 | <0.0001 | <0.0001 | 0.003 | 0.022 | 0.88 | <0.0001 | 0.00001 |
| | >50<=75 | 842 | 0.06 | 0.014 | <0.0001 | | 0.01 | 0.017 | 0.72 | | 0.00004 |
| | >25<=50 | 767 | 0.08 | 0.011 | <0.0001 | | 0.04 | 0.011 | <0.0001 | | 0.002 |
| | <=25 (BL) | 1036 | 0 | | | | 0 | | | | 0 |
| Nose angle (degrees) | >18 | 1203 | -0.01 | 0.020 | 0.55 | 0.01 | 0.01 | 0.024 | 0.62 | 0.01 | 0.0001 |
| | >12<=18 | 750 | 0.04 | 0.020 | 0.04 | | 0.05 | 0.020 | 0.01 | | 0.002 |
| | >6<=12 | 601 | 0.01 | 0.014 | 0.49 | | 0.03 | 0.013 | 0.03 | | 0.0008 |
| | <=6 (BL) | 981 | 0 | | | | 0 | | | | 0 |
| Speed (m/s) | linear | | 0.002 | 0.002 | 0.14 | 0.14 | 0.001 | 0.001 | 0.61 | 0.61 | 0.0000005 |
| HNP | HNP2 | 2828 | 0.04 | 0.020 | 0.05 | 0.05 | 0.03 | 0.038 | 0.50 | 0.50 | 0.0006 |
| | HNP1 (BL) | 707 | 0 | | | | 0 | | | | 0 |

'Univariable' (with left and right variables where existent) and multivariable (with all variables; intercept; 0.31 (SE 0.428)) mixed models for the kinematic variables with the dependent variable mouth movement (square root transformed). Data were collected from seven horses in 35 trials with 101 data points for each standardised trial (*n*=3535). Trial within horses is incorporated as a random effect and head, neck position (HNP) and speed are forced. The kinematic variables were transformed subtracting the minimum value for each horse. Stride index has a group *P*<0.0001 in the multivariable model (see text for further details). (BL-baseline, HNP1-free head and neck position, HNP2-on the bit, L3-lumbar vertebra three, Nose angle-the angle: horizontal plane-eye-mouth, T6-thoracic vertebra six).

**Table 3**
**Univariable and multivariable ‘rein tension’ models**

| | | | Univariable model | | | | Multivariable model | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | Estimate | SE | *P*-value | Group *P*-value | Estimate | SE | *P*-value | Group *P*-value | Back-transformed value |
| Left rein tension (N) | >18 | 191 | -0.02 | 0.023 | 0.31 | <0.0001 | 0.05 | 0.023 | 0.02 | 0.01 | 0.003 |
| | >10<=18 | 352 | -0.02 | 0.021 | 0.44 | | 0.04 | 0.020 | 0.04 | | 0.002 |
| | >2<=10 | 574 | 0.05 | 0.016 | 0.003 | | 0.05 | 0.015 | 0.001 | | 0.002 |
| | <=2 (BL) | 499 | 0 | | | | 0 | | | | 0 |
| Right rein tension (N) | >18 | 210 | 0.02 | 0.021 | 0.28 | <0.0001 | -0.04 | 0.021 | 0.05 | 0.04 | -0.002 |
| | >10<=18 | 308 | -0.04 | 0.020 | 0.07 | | -0.02 | 0.020 | 0.23 | | -0.0006 |
| | >2<=10 | 668 | -0.09 | 0.014 | <0.0001 | | -0.04 | 0.015 | 0.01 | | -0.001 |
| | <=2 (BL) | 430 | 0 | | | | 0 | | | | 0 |
| Distance mouth-hand left (mm) | >75 | 343 | -0.16 | 0.032 | <0.0001 | <0.0001 | -0.05 | 0.035 | 0.13 | <0.0001 | -0.003 |
| | >50<=75 | 290 | 0.02 | 0.022 | 0.28 | | 0.04 | 0.023 | 0.10 | | 0.001 |
| | >25<=50 | 478 | 0.01 | 0.017 | 0.77 | | 0.05 | 0.017 | 0.006 | | 0.002 |
| | <=25 (BL) | 505 | 0 | | | | 0 | | | | 0 |
| Distance mouth-hand right (mm) | >75 | 449 | 0.20 | 0.028 | <0.0001 | <0.0001 | 0.08 | 0.031 | 0.007 | 0.008 | 0.007 |
| | >50<=75 | 433 | 0.11 | 0.025 | <0.0001 | | 0.06 | 0.025 | 0.02 | | 0.003 |
| | >25<=50 | 290 | 0.11 | 0.020 | <0.0001 | | 0.06 | 0.019 | 0.002 | | 0.004 |
| | <=25 (BL) | 444 | 0 | | | | 0 | | | | 0 |
| Speed | linear | | -0.0001 | 0.003 | 0.96 | 0.96 | -0.001 | 0.003 | 0.68 | 0.68 | -0.000001 |
| HNP | HNP2 | 1313 | 0.06 | 0.126 | 0.65 | 0.65 | -0.007 | 0.129 | 0.96 | 0.96 | -0.00005 |
| | HNP1 (BL) | 303 | 0 | | | | 0 | | | | 0 |

‘Univariable’ (with left and right variables where existent) and multivariable (with all variables; intercept; 0.81 (SE 0.896)) mixed models for the rein tension model with the dependent variable mouth movement (square root transformed). Data were collected from three horses in 16 trials with 101 data points for each standardised trial (*n*=1616). Trial within horses is incorporated as a random effect and head, neck position (HNP) and speed are forced. The kinematic variables were transformed subtracting the minimum value for each horse. Stride index has a group *P*<0.0001 in the multivariable model (see text for further details). (BL-baseline, HNP1-free head and neck position, HNP2-on the bit).

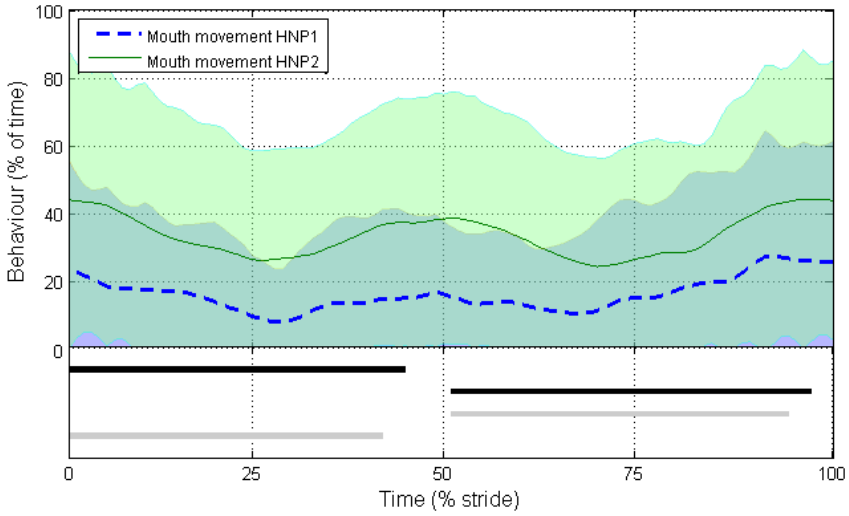



**Fig. 1.** The mean percentage of mouth movements (±SD, blue line and filled blue area HNP1 (head and neck position 1), green line and filled green area HNP2, SD values are truncated at zero) standardised to 0-100% stride cycle in the free position (HNP1) and on the bit (HNP2). Data were collected from seven horses during 29 trials for HNP2 and seven trials for HNP1. Stance bars (top to bottom; left fore, right fore, left hind and right hind) demonstrate the stride cycle.

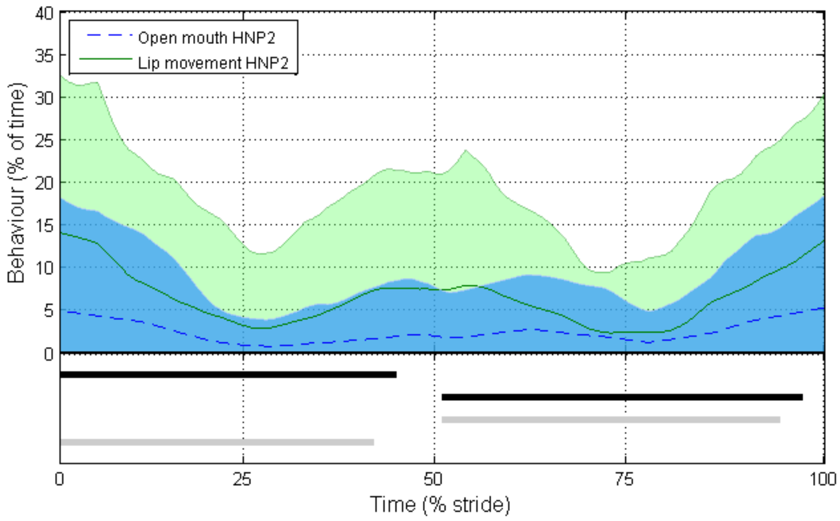



**Fig. 2.** The mean percentage of open mouth and lip movements (±SD, blue line and filled blue area open mouth, green line and filled green area lip movement, SD values are truncated at zero) when ridden on the bit (head and neck position 2, HNP2) , standardised over the stride cycle (0-100%). Data were collected from seven horses during 29 trials.

Stance bars (top to bottom; left fore, right fore, left hind and right hind) demonstrate the stride cycle.

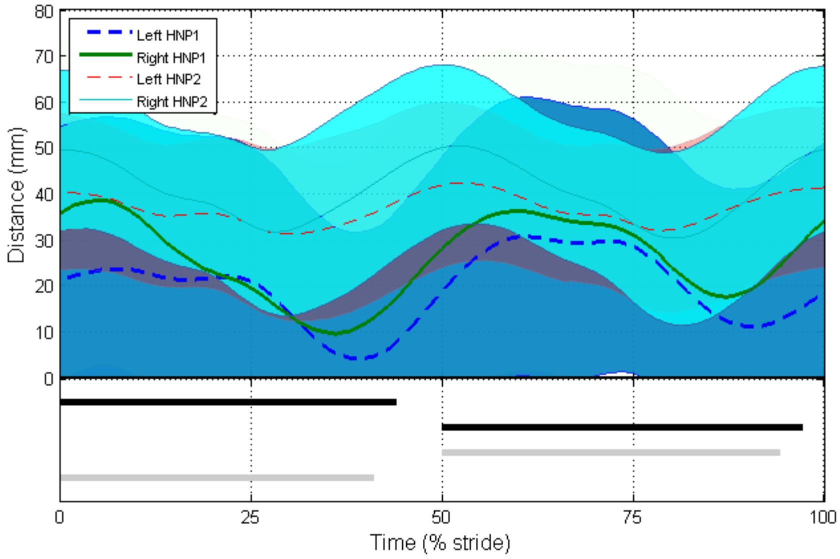



**Fig. 3.** The mean variation in distance between the horse's mouth and the rider's hands (left/right) (±SD, filled areas above and below lines belong to the lines of similar colour, SD values are truncated at zero) standardised over the stride cycle (0-100%) in the free position (head and neck position 1, HNP1) and on the bit (HNP2). Values have been transformed by subtracting the minimum value for each horse per HNP. Data were collected from seven horses during 29 trials in HNP2 and seven trials for HNP1. Stance bars (top to bottom; left fore, right fore, left hind and right hind) demonstrate the stride cycle.

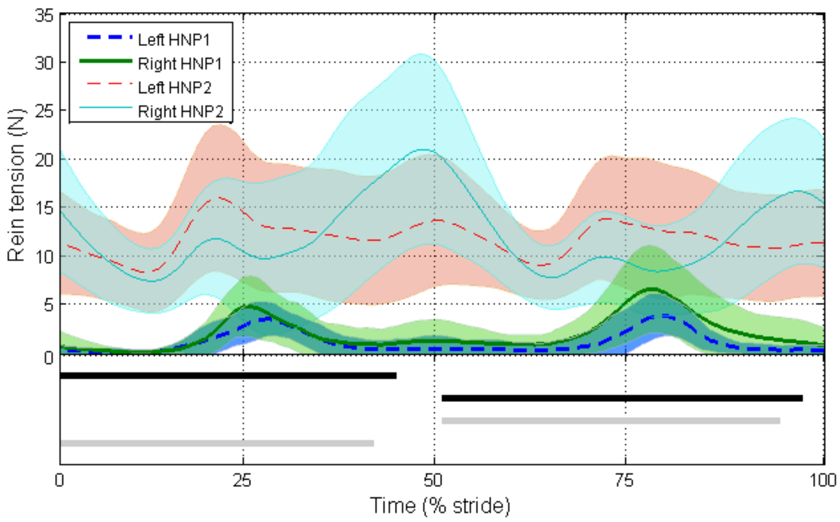
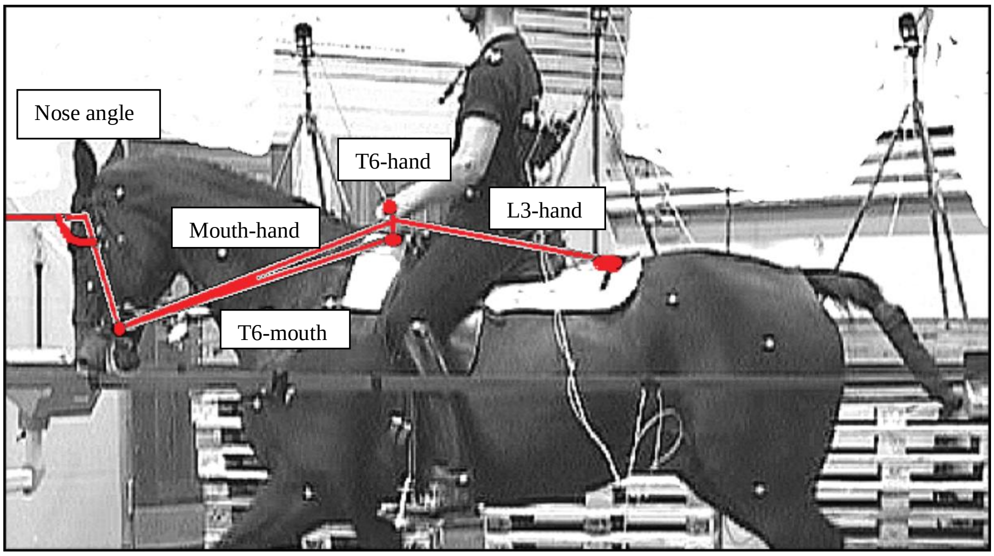
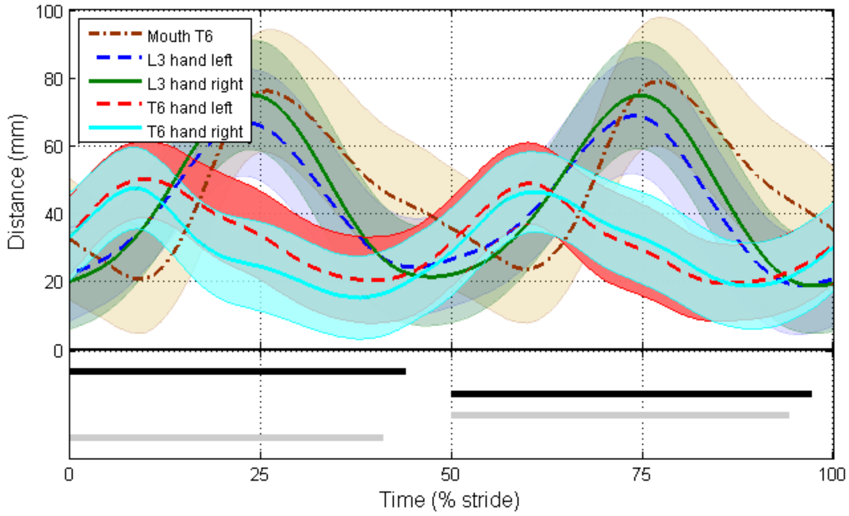


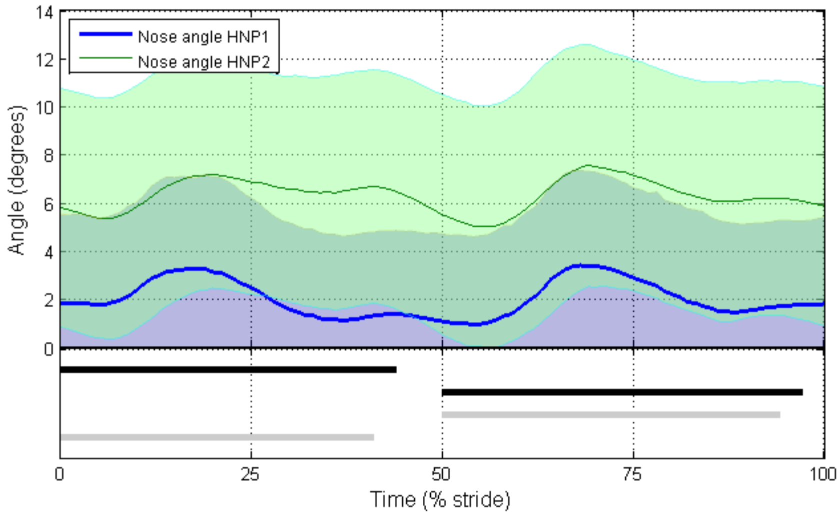

**Fig. 4.** The mean distribution of rein tension for the left and right rein (±SD, filled areas below and above lines belong to the lines of similar colour, SD values are truncated at zero) standardised over the stride cycle (0-100%) in the free position (head and neck position 1, HNP1) and on the bit (HNP2). Data were collected from three horses during 13 trials for HNP2 and three trials for HNP1. Stance bars (top to bottom; left fore, right fore, left hind and right hind) demonstrate the stride cycle.




**Appendix 1.** The kinematic variables studied. The red dots indicate the placement of the markers and the red lines between the dots indicate the distances studied. The angle between the horizontal plane, the eye and the mouth is shown on the horse's head. The picture has been retouched.




**Appendix 2.** The mean variation in distance between the horse's withers (T6) and the rider's hands (left/right), the horse's lumbar vertebra three (L3) and the rider's hands (left/right) and the horse's mouth and T6 (±SD, filled areas above and below lines belong to the lines of similar colour), standardised over the stride cycle (0-100%) when ridden on the bit (HNP2). Values have been transformed by subtracting the minimum value for each horse in HNP2. Data were collected from seven horses during 29 trials. Stance bars (top to bottom; left fore, right fore, left hind and right hind) demonstrate the stride cycle.




**Appendix 3.** The mean variation in angle in front of the horse's head between the horizontal plane, the eye and the mouth (±SD, filled areas above and below lines belong to the lines of similar colour, SD values are truncated at zero) standardised over the stride cycle (0-100%) when ridden in the free position (head and neck position 1, HNP1) and on

the bit (HNP2). Values have been transformed by subtracting the minimum value for each horse per HNP.  Data were collected from seven horses during 29 trials. Stance bars (top to bottom; left fore, right fore, left hind and right hind) demonstrate the stride cycle.