**COW, LION AND HORSES – methods + statistical results**

Methods (brief description)

Before the tests, the horses were given the pellets in a container to check whether they will approach the experimenter with the food. All horses approached the experimenter.

The tested horse was equipped with HR monitor (heart area and the areas from the withers down to the sternum were moisten with the ECG gelly; the belt with electrodes was put around the chest, with the receiver in form of a wrist watch). Then, the monitor was started and the experimenter(s) went out of the box (horse alone in its box, Time point1; TP1). After 10 minutes, the experimenter opened the box door and stood motionless with the container half-full of pellets for 3 minutes (TP2), then shoot the box door. After 1 minute interval, the experimenter opened the box door and stood motionless with the container half-full of pellets and stimulus feces for 3 minutes (TP3) After 1 minute interval, the experimenter opened the box door and stood motionless with the container half-full of pellets for 3 minutes (TP4) then shoot the box door. Then, the horse was alone in its box for next 10 minutes (TP1).

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| --- | --- | --- | --- | --- |
| Timepoint | Procedure | Start-Stop (minute) | Duration  (minutes) | comments |
|  | HR monitor instalation |  |  |  |
| TP1 | none | 1-10 | 10 | Not analysed for behaviour |
| TP 2 | Presentation of food | 10-13 | 3 | Analysed |
|  | interval | 13-14 | 1 | Not analysed for behaviour |
| TP 3 | Presentation of food+feces | 14-17 | 3 | Analysed |
|  | interval | 17-18 | 1 | Not analysed for behaviour |
| TP 4 | Presentation of food | 18-21 | 3 | Analysed |
| TP 5 | none | 21-31 | 10 | Not analysed for behaviour |

Behavioural indices of the alertness

They are the following:

Approach\_lat latency to approach the container in s (indicator of relax, appetite)

Sniff\_dur duration of sniffing the container in s (attention, exploration, anxiety)

SniffPC sniffing the container - per cent of time point time (180s) when the head was visible

Chew\_dur duration of chewing the food in s

ChewPC chewing per cent

Ears\_forw duration of ears pointed forward in s (attention, exploration, anxiety)

EarsPC ears forward per cent

Withdr\_dur duration of time the horse withdraws from experimenter (disgust, anxiety)

WithdrPC withdrawal per cent

Interact\_dur duration of interaction with food container (touching, tossing the container, pushing its with the lip and nose)

InteractPC interaction per cent

Blows\_occur total number of audible blows of the air expulsed by the nostrils when sniffing the content of the container (to enhance the air flow above the feces)

Durations were transformed into the per cent of the time the head of the horse was visible. Since not all behavioural variables converged (SAS was unable to produce the results with GLIMMIX), so I decided to run non-parametric tests.

The factors (independent variable) were:

* time points (TP1 – time 0 to 10min: pre-test HR basal recording; TP2 – 3min: food presentation1; TP3 – 3 min: stimulus presentation; TP4 – 3min, food presentation2; TP5 – post-test basal 10min-HR recording). Actually, only TP2, TP3 and T4 (all 3-min periods) were analysed.
* stimulus (cow feces, lion feces)
* sex (gelding, mare)
* age (below 10yo, above 10yo)

|  |  |  |
| --- | --- | --- |
|  | test statistics (CMH) | P-value |
| **Time point** |  |  |
| Approach\_lat | 5.41 | 0.07 |
| SniffPC | 25.0 | **<0.01** |
| ChewPC | 20.0 | **<0.01** |
| EarsPC | 14.4 | **<0.01** |
| WithdrPC | 10.3 | **<0.01** |
| InteractPC | 3.91 | 0.14 |
| Blows\_occur | 21.0 | **<0.01** |
|  |  |  |
| **Stimulus** (only time point 3) | test statistics (Z) | P-value |
| Approach\_lat | -0.76 | 0.48 |
| SniffPC | 0.53 | 0.60 |
| ChewPC | -0.31 | 0.76 |
| EarsPC | 2.01 | 0.06 |
| WithdrPC | -0.76 | 0.46 |
| InteractPC | -2.49 | **0.02** |
| Blows\_occur | -0.48 | 0.63 |

**Time point** affected (AGB comments):

* sniffPC (increased when the stimulus was presented) practically only then the horses investigated the content of the container, which means this was something strange for them. When offered the food during TP4, they shortly sniff the content to reassure whether it is a normal food
* chewPC (decreased) it is obvious that the horses hadn’t eat the feces, so the chewing dropped when the feces were presented. Occassional chewing resulted from chewing the content of the mouth still present after TP1 or a hay grasped during 1-min interval between TP2 and TP3.
* earsPC (increased) the horses show the attention to the stimulus, pointing the ears in direction to the stimulus reflect the interest in the “strange food”.
* withdrawPC (increased) the horses discouraged by an (aversive?) smell switched their activity on eating the straw or hay from the litter, or just stood indifferently. Although it was not significant, lion feces seemed more aversive.
* blows\_occur (increased) the audible blows were short expulsions of the air by the nostrils to provoke the scent molecules to move; it seem to be helpful for scent analysis. Notably, it was not needed in the case of food.

No effect of Time point was found for:

* approach\_lat, but it can be seen that some horses delayed their approach (one had not approach at all).
* interactPC, but some horses tossed the container, pushed it with their lips/nose, especially when the cow feces were visibly “disturbing” the normal food. In the pilot study, one of horses ate the cow feces, with no previous olfactory investigation.

The analyses were made with CMH (Friedman) test.

Pairwise comparisons were made with Sign exact test (SAS alternative for Wilcoxon matched-pair signed rank test). Descriptive statistics below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Median [Q1; Q3] Min-Max | | | |
| **Time point** | T2 (pre-stimulus) | T3 (stimulus) | | T4 (post-stimulus) |
| Approach\_lat | 1 [1; 6] 1-41 | 1 [1; 1] 1-3 | | 1 [1; 1] 1-181 |
| SniffPC | 0 [0; 0] 0-2.03 **A** | 24.0 [20.8; 45.9] 8.3-100 **B** | | 4.1 [0; 7.8] 0-7.8 **A** |
| ChewPC | 97.8 [95.2; 98.8] 87.8-100 **A** | 0 [0; 0] 0-79.4 **B** | | 96.7 [92.8; 100] 0-100 **A** |
| EarsPC | 4.46 [1.34; 19.4] 0-84.9 **A** | 38 [19.6; 74.2] 6.11-100 **B** | | 3.76 [0.56; 17.2] 0-38.9 **A** |
| WithdrPC | 0 [0; 0] 0-0 **A** | 1.39 [0; 65.3] 0-95 **B** | | 0 [0; 0] 0-21.1 |
| InteractPC | 0 [0; 0] 0-0 | 0 [0; 7.57] 0-75.6 | | 0 [0; 0] 0-27.8 |
| Blows\_occur | 0 [0; 0] 0-0 **A** | 4.5 [0; 15] 0-30 **B** | | 0 [0; 0] 0-0 **A** |
|  |  |  | |  |
| **Stimulus** (only time point 3) | cow | | lion | |
| Approach\_lat | 1 [1; 1] 1-3 | | 1 [1; 1] 1-1 | |
| SniffPC | 33.3 [26.1; 42.2] 8.30-69.4 | | 34.6 [25.6; 85.9] 20.6-100 | |
| ChewPC | 0 [0; 0] 0-79.4 | | 0 [0; 0] 0-9.3 | |
| EarsPC | 32.3 [17.4; 39.8] 6.11-77.2 | | 71.1 [20.8; 100] 20.6-100 | |
| WithdrPC | 0 [0; 2.78] 0-75.6 | | 40.6 [0; 86.1] 0-95 | |
| InteractPC | 6.67 [0-8.51] 0-75.6 **a** | | 0 [0; 0] 0-0 **b** | |
| Blows\_occur | 6 [1.0; 12.0] 0-30.0 | | 4 [0; 24] 0-25 | |

**Stimulus** affected only the duration of interaction with the container, cow’s feces provoking more pushes with lip/nose and no touch of container with lion’s feces (Wilcoxon two-sample test, SAS equivalent to Mann-Whitney U test).

**Age\_class** and **sex** did not affected any of the behaviour (Wilcoxon two-sample test).

Cardiac indices of the alertness (HR and HRV)

HR and HRV (RMSSD) were log transformed and the homoscedasticity of standard deviations were tested with Barlett’s test which proved normal distribution of transformed data for HRV.

Model used: HRlog/ HRVlog=Timepoint + Stimulus + Age\_class + Sex + ID (random) + e

GLIMMIX procedure with lognormal distribution assumed for HR and HRV was used. GLIMMIX (general linear mixed model) is the analysis taking into account the variance connected with particular animal (id) as a random effect as the comparisons of behaviour and HR on particular time points of the study involved the repeated measures on the same animal. The distribution of the variable can be individually adjusted in the model.

**Time point and Stimulus** had no effect on HR and HRV while **age** affected the HR (F=4.91. P=0.03). younger horses presenting higher HR (LSM: 1.28; 38.6 [36.4; 44.6] 33.2-61.8 bpm) than older horses (LSM: 1.26; 35.4 [34.4 [34.7; 37.4] 32.4-41.5 bpm).

The **sex** affected HRV (F=4.85. 0.03); mares showing higher HRV (LSM: 1.50; 77.6 [60.2; 126.0] 43.1-377.2 ms) that geldings (LSM: 1.42; 60.5 [47.9; 85.0] 35.0-115.8 ms).

Both effects were not related to presented stimuli, so they could be incidental or just physiological.