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Abstract

Eye-temperature measured with infrared thermography have in previous studies showed significant correlation to competition results in harness racing. Studies also show a positive correlation between serotonin level in blood and eye-temperature which indicates that this method could serve as an indicator of stress and welfare in harness racing. The purpose of this study was to measure eye-temperature during competition with the help of infrared-thermography and analyse if it could explain the behavioural profiles of the horses. A factor analysis where 5 principal components were constructed from the behaviour measures explained 71% of the variance. Horses with higher scores in factor 1 are fearful, remember unpleasant events and horses with lower scores in factor 1 tend to not desire to win and tend to not learn the task of racing. Horses with higher scores in factor 2 recovers well and are cooperative. Horses with higher scores in factor 3 are calm, horses with higher scores in factor 4 have good appetite after competitions and are focused. Horses with higher scores in factor 5 are stubborn, fearful and tend to lose control. A univariate linear model was constructed to test the significance of different co-variables. Breed had a significant effect on factor 2 and sex had a significant effect on factor 5. The eye-temperature variation could not explain the behaviours of the horses. To find strong evidence for the impact of eye-temperature on behaviour, additional interrogated horses are required to verify what exactly eye temperature is a measurement of. Future applications of these very preliminary results might lead to faster breeding progress, with better competition result and better welfare among sport horses.

Sammanfattning

Ögontemperatur, mätt genom termografi har i tidigare studier visat signifikanta korrelationer till tävlingsresultat i travsport. Ögontemperaturen har även visats ha en positiv korrelation till serotoninhalten i blodet vilket skulle göra denna metod till ett bra hjälpmedel för att mäta stress och välfärd i samband med travtävlingar. Denna studie syftade därför till att genom insamling av egna data i form av mätningar av ögontemperatur och beteendeenkäter förklara vilka beteendeprofiler som finns hos travhästar samt ifall dessa kan förklaras genom variation i ögontemperatur under fysisk ansträngning. Genom faktoranalys kunde fem principiella komponenter konstrueras utifrån beteendeparametrarna vilka tillsammans beskrev 71% av variansen. Hästar med högre poäng i faktor 1 tenderar att komma ihåg obehagliga händelser och är rädda, de med lägre poäng vill inte vinna samt har svårt att lära sig nya saker. Hästar med högre poäng i faktor 2 har lätt att återhämta sig och är samarbetsvilliga. Hästar med högre poäng i faktor 3 är mindre nervösa och blir sällan spända. Hästar med högre poäng i faktor 4 har bra aptit efter tävlingar samt är koncentrerade. Hästar med högre poäng i faktor 5 har lätt att tappa självkontrollen, är envisa samt rädda. En linjär modell konstruerades för att hitta vilka variabler som hade effekt på beteendena. Ras hade en signifikant effekt på faktor 2 och kön på faktor 5. Variationen i ögontemperatur kunde inte förklara hästarnas beteenden. För att hitta bevis för att beteendet hos travhästar kan påverkas av ögontemperaturen behövs mer data och mer forskning för att verifiera exakt vad ögontemperatur är ett mått på. Framtida användningsområden från vidare forskning kan vara avel för ökad prestation och ökad välfärd hos sporthästar under tävlingssammanhang.

Introduction

In this study the Norwegian-Swedish coldblooded trotter and the Standardbred trotter serve as a model to investigate how the modern sport horse cope with stress during competition. According to the Swedish trotting association (Svensk Travsport 2019a), the horses should not experience too much stress during the race. Moreover, in the code of conduct of the International Equestrian federation (Fédération Equestre Internationale 2018), the horse's welfare during competition is of highest priority. Whether it is dressage, show-jumping or harness racing, the horse is exposed to a new environment and stimuli which may impact the allostatic load. The allostatic load is the extra physiological changes needed to maintain homeostasis, a high allostatic load requires extra energy and might lead to stress (McEwen & Wingfield 2010). Stressors cause changes primarily in behaviour, the autonomic nervous system, immune response and neuroendocrine system. The immediate response to these changes is known as the "fight or flight" or stress response (e.g. aggressive behaviour or "passive fear" combined with cortisol release), to make sure the metabolism, immune system and reproduction function is equipped for changes in the environment that might be life-threatening (Bartolomé & Cockram 2016). The authors explain that stress that is perceived as a threat activate the neuroendocrine- and immune response, also the parasympathetic nervous system and sympathetic nervous system are activated to maintain homeostasis. This induces serotonin release regulating immune response, metabolic- and reproductive functions which are affected during a longer period. The sympathetic nervous system will directly increase the concentration of free fatty acids, glycogen and blood flow to the muscles. These are all important steps to maintain homeostasis during physical exercise, the stress turns into distress when the "off switch" is not working. Then it will cause problems like loss of muscle strength and suppression of the immune response. As previous mentioned serotonin plays an important role in the response to stress and Valera *et al.* (2012) found significant correlations between salivary cortisol and eye-temperature (ET). The temperature around the eye-area gives a good indication of both stress and discomfort. Studies on humans show that stress and fearful situations activate the sympathetic nervous system which cause a temperature rise in areas around the eyes due to more blood that goes to superficial blood vessels in the skin (Pavlidis *et al.* 2000). The authors mean that this extra blood flow to the eye-area is a must for quick eye-movements to detect possible threats.

Previous research on Spanish trotting horses confirmed a relationship between eye-temperature and performance which indicates a breakpoint where stress turns in to distress and affects competition results negatively (Negro *et al.* 2018). Long term damages of stress are immunosuppression, weight loss, stomach ulcer and stereotypic behaviours (Mills & Nankervis 2013). To evaluate welfare and stress-level at competitions this study will use a non-invasive method to measure ET with help of infrared-thermography. The purpose of this study is also to analyse behavioural profiles of the horses at the competition via a survey to find out if ET can explain the horses' behaviour.

Question formulations:

- Are there any specific behavioural profiles in racing horses?
- Can ET variation from exercise explain behaviour profiles among trotting horses?

Literature review

Infrared-thermography as a tool for measuring eye-temperature

The infrared-thermography camera has been used in several studies. In the study of Valera *et al.* (2012) ET was measured during show-jumping competitions. In total, samples of 16 horses were analysed. Results showed that ET before and just after the competition differed significantly. The study also revealed a significant correlation of 0.72 between salivary cortisol and ET intervals (five minutes after the competition to three hours after the competition). The same method has been used by Bartolomé *et al.* (2013) with data from 173 horses that competed in show-jumping. Results showed that correlation between performance and ET-intervals was negative, thereby low ET was correlated to better result in show-jumping competitions.

Another study made by McGreevy *et al.* (2012) measured the effect of different nosebands (double-bridle, regular noseband and without noseband). The study showed significant results with higher ET among the horses wearing double-bridle or a regular noseband. Measurements assessed with infrared-thermography (IRT) have also been done during lunging with Pessoa as a training aid or method for horses to help them use their back-muscles (Designed by show-jumping rider Nelson Pessoa) (Hall *et al.* 2011). The result of wearing the Pessoa during lunging contributed to higher ET compared to lunging without using the Pessoa and the author's conclusion correspond to previous mentioned studies, IRT is a helpful non-invasive tool to measure stress and welfare.

Behavioural tests in sport horses and genetics

Personality tests for horses have previously been used to confirm reliable methods to describe correlations between behaviour traits and rated personality (Lloyd *et al.* 2007). A survey including 20 behaviour traits such as excitability, anxiousness and tension was given to trainers or owners of 44 horses in the UK who rated each trait from one to seven depending on how often the horse expressed the particular trait. The trainers' or owners' scores were compared with the jury's scores, and the results showed that owners and the jury agreed on the score of 71.2% of the horses with the conclusion that questionnaires could be a reliable method to evaluate horse behaviour. Furthermore, the results from surveys like this can be used in breeding organisations to select for different traits (König von Borstel, 2013). The authors conclusion is that heritability (h^2) for behavioural traits varies in studies from 0 to 0.4, with significant and higher h^2 obtained from test stations. Most of the breeding associations include behavioural traits in their breeding objectives, and temperament seems to be most frequent trait to determine if the horse could perform in the given sport (König von Borstel, 2013).

In the breed standard description of the Swedish Standardbreds and Coldblooded trotters the horses should be easy to handle and have a great willingness to cooperate (Svensk Travsport 2019b). This can also be described as tractability. This specific trait has a genetic association in Thoroughbred horses to serotonin receptor 1a (HTR1A) which regulate anxiousness (Hori *et al.* 2015). The authors used a behavioural survey that the caretakers of Thoroughbred fillies and colts at a training centre in Japan answered. The replies from the survey were associated with two variants of the HTR1A gene (c.709G>A and c.771G>C). The behavioural survey had a scale of three points (1=easy, 2=somewhat difficult and 3= difficult) to describe how the horses react to different stimuli (e.g. first grooming, driving or riding on 800m track). Horses carrying an A allele at c.709G>A showed lower tractability. Five principal components were constructed and reflected different kinds of tractability. The genotype HTR1A:c.709G>A had significant impact on four of these components among fillies and only one (reaction to starting gates and trailers) among colts, reflecting a sex effect.

Materials and methods

Collection of data for the study took place at Bjerke Racetrack in Oslo-Norway the 30th of April 2019 from the time the first horse arrived, to the end of last race, approximately from 3 pm to 10 pm. In total, 33 randomly selected horses were evaluated belonging to the breeds Norwegian-Swedish Coldblooded trotter (NSCT) and Standardbred trotter (SB).

ET measures with IRT were collected with a FLIR I6 camera as shown in Figure 1. In total three pictures/measurements of each horse were taken; first photo before the race, second photo immediately after the race, and third photo approximately one hour after the race. Position of the camera was one meter 90° from the eye. Pictures were only taken of the left eye. Out of the 33 horses only 26 had fully completed results that could be used in this study. All 26 horses contributed to the first and second photo, while third photo results from 17 of them were analysed. The remaining nine horses went home before the minimum time required (1h) and therefore they lack information about third ET. Out of the 26 horses there were 12 mares, 5 geldings and 9 stallions. The breed distribution was 20 SBs and 6 NSCT. At the same time as the photo was taken, air-temperature and humidity were measured with a hygrometer to obtain corrected results from the IRT-camera.

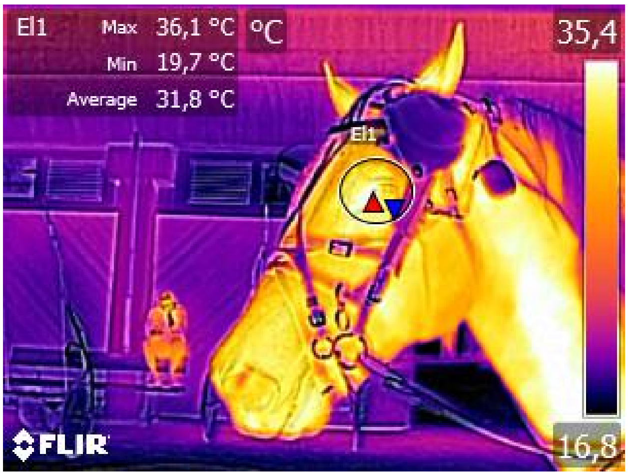

Figure 1. Infrared thermography-photo of the left eye taken after the race collected with FLIR I6 camera, maximum temperature (36,1°) is found in the red triangle and is shown in degrees Celsius in the upper left corner.

A survey was distributed to the owners or trainers of the horses containing questions about how they experienced the horse's behaviour at competitions on a scale from one to seven as seen in Appendix 1. The survey included 12 questions with alternative answers in the scale from 1-7 depending on how often the specific behaviour was observed in racing competitions. 1=Never, 2=Rarely, 3=Occasionally, 4=Sometimes, 5=Often, 6=Usually, 7=Always. The following behaviours and descriptions were used: **nervousness**: tends to become nervous or anxious, **excitability**: tends to get excited or agitated easily, **fearfulness**: tends to be afraid easily,

concentration: tends to be focused and unaffected by the environment, **learning**: tends to learn the task of racing/competing quickly, **memory**: tends to memorize/remember unpleasant events, **cooperation**: tends to be cooperative, have good attitude, **will to win**: tends to desire to win/run fast, **stubbornness**: tends to be obstinate once it resists a command, **self-control:** tends to panic, escape and lose control, **recovery**: tends to relax quickly, and **appetite:** tends to have poor appetite between races.

Results from the behavioural survey were analysed using the software Statistica v13. Both ET and behaviour were compared to a normal distribution. A factor analysis (FA) of the behavioural traits was first used, following a linear model to test for the influence of age, sex, breed, ET and time on behaviour. Sex was included as fixed effect with three classes: mare, gelding and stallion. Age, ET and time was used as linear covariates. Breed was included as a fixed effect with 2 classes (SB or NSCT). The following linear model was used to test significance of the effect for each factor:

$$y = \mu + Age + Sex + Breed + ETpre + \Delta ET + \Delta Time + Residual$$

y is the factor score (factor 1 to 5), μ is a common intercept, $ETpre$ is the ET before the competition, ΔET is the difference in ET from the second photo (after competition) to the first photo. $\Delta Time$ is the time increment from when the race started to the time of the second photo. Results are considered significant if $p \leq 0.05$.

Results

Descriptive statistics

The first IRT- photo was taken when the horses arrived to the stable at the racecourse, results of ET are shown in Table 1. The mean-value of the first photo was 35.18 ˚C, mean value for the ET on the second photo was 36.97 ˚C and the mean value for the third photo was 35.89 ˚C. All IRT- photos followed assumptions of normal distribution.

Table 1. Medians, means and standard deviation (SD) for eye-temperature (ET) pre- and post-test, ET change 1 (Difference between first and second photo), ET at rest and ET change 2 (Difference between second and third photo) in Degrees Celsius

ET	N	Median	Mean	SD
ET Pre	26	35.05	35.18	0.72
ET Post	26	36.85	36.97	1.08
ET Change 1	26	1.8	1.79	1.16
ET Rest	17	35.8	35.89	1.07
ET Change 2	17	-1.3	-1.39	1.42

From the behavioural survey results from medians, means and standard deviation (SD) are shown in Table 2. Results did not follow the assumption of normal distribution. The median for nervousness was 2.5 meaning the horses rarely or occasionally tended to get nervous at competitions. The median for excitability was 3 meaning they occasionally tended to get excited at competitions. The median for fearfulness was 2 meaning they rarely tended to get afraid easily, the median for concentration was 6 meaning they are usually focused and unaffected by the environment. The median for learning was 6 meaning they learn the task of racing or competing quickly. The median for memory was 2 meaning they rarely remembered unpleasant events. The median for cooperation was 6 meaning they are usually cooperative. The median for will to win was 6 meaning they usually are willing to win. The median for stubbornness was 2 meaning they rarely get obstinate once they resist a command. The median for self-control was 1 meaning they never tends to panic, escape or loose control. The median for recovery was 6 meaning they usually tends to recover quickly. The median for appetite was 1 meaning they never tends to lose appetite between races.

Table 2. Medians, means and Standard deviation (SD) for scores in each trait in the 26 fully completed behavioural surveys

Trait	Median	Mean	SD
Nervousness	2.5	2.8	1.5
Excitability	3	3.4	1.4
Fearfulness	2	2.1	1
Concentration	6	5.3	1.6
Learning	6	6.3	0.8
Memory	2	3.2	2.1
Cooperation	6	6.3	0.8
Will to win	6	5.8	1.5
Stubbornness	2	2.5	1.8
Self-control	1	1.4	0.6
Recovery	6	5.7	1.5
Appetite	1	1.7	1.3

Principal components and Factor analysis

The behavioural profiles (observed correlated variables) among the trotting horses were explained using principal component analysis and factorial analysis. In total there were five principal components constructed that together explained 71% of the variance. Factor 1 explained 23% of the variance, factor 2 explained 15 % of the variance, factor 3 explained 12% of the variance, factor 4 explained 11% of the variance and factor 5 explained 10% of the variance. Positive values from the factor analysis correspond to higher scores on the behavioural questionnaire (Maximum:7=Always) and negative values from the factor analysis correspond to lower scores from the questionnaire (Minimum:1=Never). As seen in Figure 2, each trait is either on the positive or negative side of each factor. Traits on the same side, below -0.4 or above 0.4 are those who most likely have a relationship or interact with each other. Depending on how strongly correlated each trait/behaviour is to the factor they will have different loadings. The loading is the weight of each trait converted from points in the survey to a scale from - 1 to 1 depending on its weight. In factor 1, fearfulness and memory load on the positive side and will to win and learning load on the negative side. In factor 2, cooperation and recovery load on the positive side. In factor 3, nervousness and excitability are on the negative side and load together. In factor 4, concentration and appetite are on the positive side and load together. In factor 5, stubbornness, self-control and fearfulness are positive and load in the same direction.

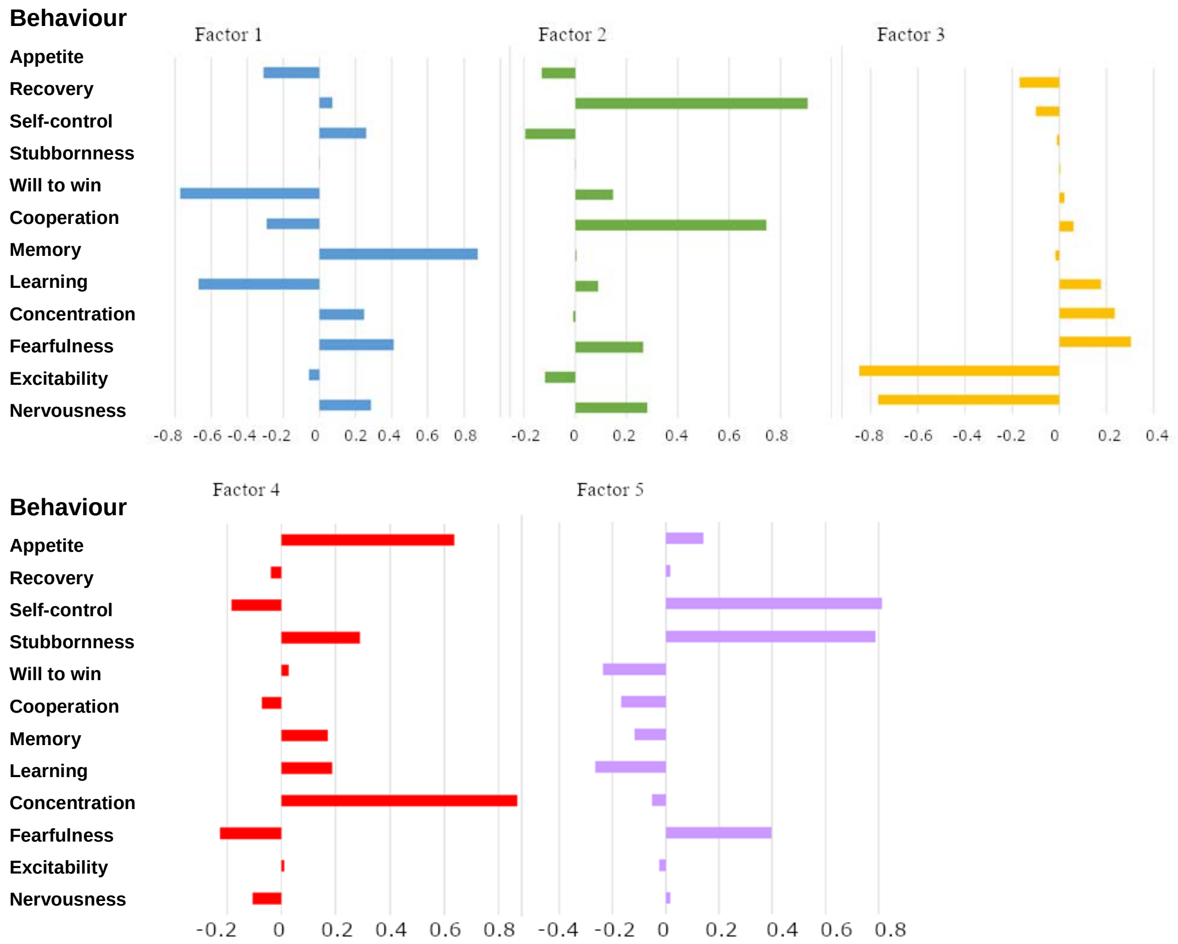

Figure 2. Factor 1-5, behavioural traits loading on either positive or negative side, absolute values over 0.4 create the factors.

Linear model and LS (Least Square) means

Results from LS-means and p-values are shown in Figure 3 and Table 3. From the univariate analysis of factor 1 none of the effects had significant impact on the factor. From the analysis of factor 2, breed had a significant effect on the factor (p=0.048) as seen in Figure 3. The mean-value of the NSCT horses in factor 2 was positive and close to 1 and the mean-value for the SBs in factor 2 was negative close to -0.3. In factor 2 cooperation and recovery loaded in the same positive direction and NSCT horses had higher scores than SBs. This means in general, that NSCT got higher scores that correspond to better recovery and cooperation in comparison with SB. From the univariate analysis of factor 3 none of the effects had any significant impact on the factor neither on factor 4. For the test of significance of factor 5, the sex had a significant effect on the factor (p=0.013) as seen in Figure 3. Geldings were on the positive side, mares and stallions on the negative side. In factor 5 self-control and stubbornness loaded in the same positive direction and the sex had a significant effect on the factor meaning that geldings had higher scores and tends to lose self-control and obstinate once it resists a command more often than mares and stallions.

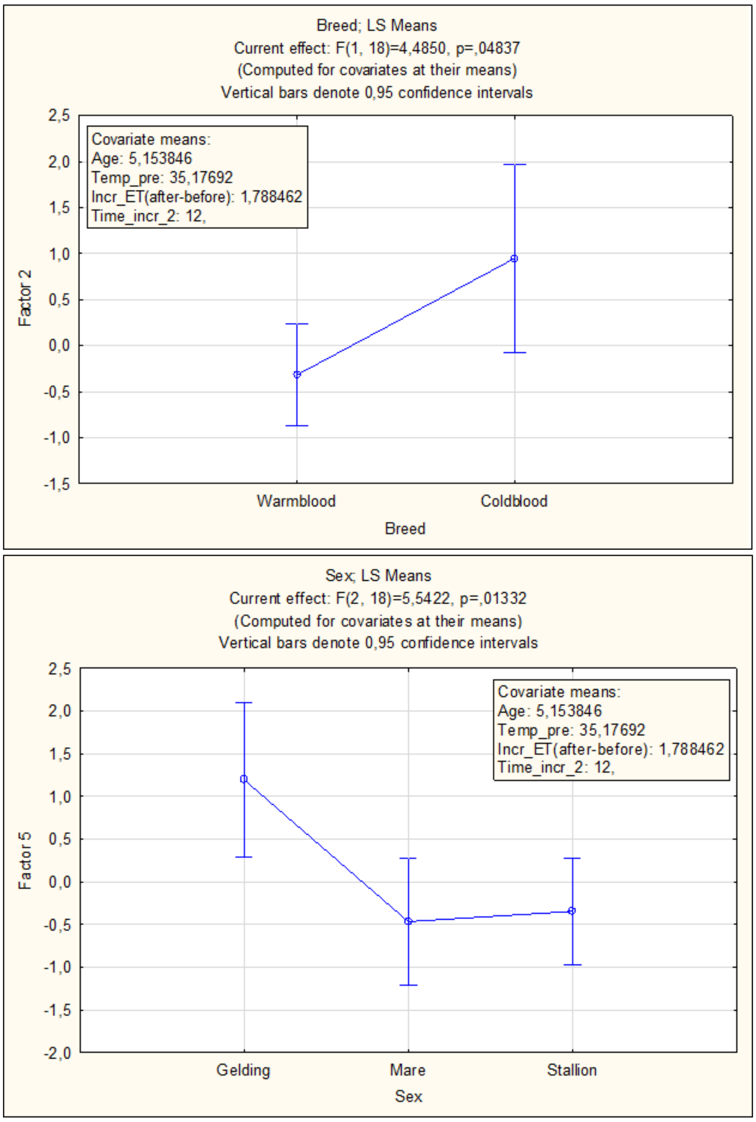

Figure 3. The y-axis explains mean factor scores and the x-axis breed in the left figure and sex in the right figure. Upper figure: Least Significant (LS) means for breed in factor 2, warmbloods: -0.3189, coldbloods: 0.9488. Lower figure: LS means for sex in factor 5, geldings: 1.1961, mares: -0.4659, stallions: -0.3457.

Table 3. p-values from univariate test of significance for each factor, significant effects marked in red

p-values							
	Intercept	**Age**	**Temp. Pre**	**ET change 1**	**Time Incr.**	**Sex**	**Breed**
FACTOR 1	0.34	0.69	0.34	0.18	0.71	0.58	0.33
FACTOR 2	0.41	0.41	0.37	0.49	0.59	0.43	0.05
FACTOR 3	0.91	0.84	0.88	0.24	0.73	0.25	0.97
FACTOR 4	0.58	0.52	0.58	0.42	0.30	0.30	0.30
FACTOR 5	0.57	0.14	0.53	0.69	0.71	0.01	0.46

Correlations

Correlations among behavioural traits and between behavioural traits and ET were analysed using Pearson's correlation coefficient. Results show that the following behavioural traits were significantly correlated to each other; nervousness positively correlated to excitability (0.42; p=0.031). Learning negatively correlated to memory (-0.42; p=0.031) and negative correlated to self-control (-0.45; p=0.022). Learning was positively correlated to will to win (0.55; p=0.03). Fearfulness was positively correlated to self-control (0.41; p=0.040). Cooperation was positively correlated to recovery (0.50; p=0.009). Will to win was negatively correlated to self-control (-0.41; p=0.040). Stubbornness was positively correlated to self-control (0.41; p=0.038). Eye-temperature of the first photo before the race and ET on the second photo after the race had no significant correlation to any of the behavioural traits in this study. One of the traits, will to win seems to have a significant positive correlation with increase in ET between first and second photo (0.50; p=0.039). Excitability had a significant positive correlation to the ET on the third photo (rest) (0.70; p=0.002) and a significant negative correlation to decrease in ET between the second and third IRT-photo (-0.50; p=0.040).

Discussion

The purpose of this study was to find specific behaviour profiles among trotting horses and if the ET variation from exercise could explain these behaviours. All the measurements and data were collected using non-invasive methods such as behaviour survey and ET with an IRT-camera. The questionnaire was filled in by the trainer or the owner that was familiar with the horse. With the help of a factor analysis 5 factors were constructed which gave indications of how each trait interact with the other traits in the behavioural survey. The aim of a factor analysis is to find latent variables, which means by combining several behavioural traits one" general" factor can be constructed out of these.

Results for factor 1 showed that this factor describes horses that tend to remember unpleasant events, are fearful and tend to get afraid easily on competition sites. These horses did not tend to learn new things and did not have as high will to win as other horses. This factor explained the largest part of the variance in the different factors (23%) but neither age, nor sex, breed, ET pre-test, difference in ET pre and post-test or the time-difference between these photos had a significant effect in the linear model. This could either be because of the limited data material (too few horses that contributed to the result) or other effects that were not considered in the linear model.

Factor 2 describes horses that are cooperative and recovers easily after competitions because of high scores in the behavioural survey. None of the effects included in the linear model except breed had significant effect on factor 2. The NSCT horses had significantly higher scores than SB indicating better cooperation and faster recovery after races. It would be interesting to do some further research with more horses included and in relation to competition results if fast recovery after races could indicate better results due to less stress. In Spanish SB, Negro *et al.* (2018) found that stress could have a negative impact on competition results. This study used the same method as in Negro *et al.* (2018) to evaluate ET during harness racing but the authors did not evaluate the horses behaviours as in this study. Therefore, if more data from ET and behavioural surveys are collected to reach same number of horses as in the previous study (130 horses) results from ET might have a significant correlation with factor 2. A theory could be that animals receiving higher scores on cooperation and recovery also had a faster reduction in eye-temperature between the second photo (just after the race) and third photo (at rest).

Results from factor 3 showed that the horses in this factor are calmer and do not get excited easily on the competition sites. These two traits have previously been studied among Tennessee Walking horses where they also loaded in the same direction in one factor when analysed with FA (Staiger *et al.,* 2016). From the same study on the Tennessee Walking horses' other traits from the survey that also loaded in the same direction as nervousness and excitability in the same factor were; panic, skittishness (easily surprised), unpredictable, timidity (lack of courage) and watchful (about surroundings). The same method using a questionnaire for behavioural traits and a IRT-camera to measure ET have previously been used by Dai *et al.* (2015) to analyse reaction to novel objects in warmblood-, riding- and draft horses. The authors result from the FA also correspond to the result of the FA in this study, nervousness and excitability move in the same direction and high scores comes from horses that are fearful. Although these breeds are selected for different traits they are the same species and maybe these traits are a result of the horse's evolution as a pray, not selection for sport-horses.

Factor 4 describes horses that were more concentrated and had better appetite after competitions. In the study by (Staiger *et al.* 2016), they found concentration and cooperation loading on the same side and traits like nervousness and excitability loading on the other side in one of the factors called tractable. This factor could describe behavioural profiles of horses that are willing to cooperate and have better concentration, are less nervous and gets less excited on competitions.

Horses in factor 5 were more stubborn, tend to lose self-control and were more fearful. This also corresponds to Staiger *et al.* (2016) and the factor analysis where panic and stubbornness moved in the same direction in one of the factors. In the present study in factor 5, sex had a significant effect on the result where geldings received the highest scores in the questionnaire followed by stallions and mares ($p=0.013$). There was no linear model from the Tennessee Walking horse's behaviour to test significant effects for factors, but Tennessee Walker geldings mean factor values for the trait stubbornness in one factor also indicates that geldings in that factor tend to be more stubborn than mares and stallions (Staiger *et al.* 2016).

The only significant correlations found between behaviour traits and ET were excitability and will to win. Excitability was strongly positive correlated to ET at rest (third photo) which means the more excited the higher ET at rest. Excitability was also significantly correlated to ET change 2 (between second and third photo). The correlation was negative indicating that exited horses have less decrease in ET after the race, this could perhaps be a sign of bad recovery, stress and a welfare issue. In Negro *et al.* (2018) performance result of Spanish SB were compared to ET using the same method as in the present study. The result showed that better competition results were correlated to high ET just after the race but ET at rest was low. Results of ET change 2 and ET at rest in the present study was not corrected for time, which may impact the result. With more data, results of correlations between ET and behaviour would be more trustworthy and more significant correlations might be found, then ET measurements and behavioural patterns in trotters could serve as a basis in the selection for breeding. Factor analysis could be a suitable method combined with IRT, and in further research; competition result and genome-analysis to generate significant associations between these traits. This could be used to generate faster breeding progress, both in competition success and welfare.

The explanation for the large SD value from ET change 1 (Table 1) is that some horses did not have an increase but an decrease in ET between first and second photo, same in ET change 2 (Table 1) where some horses had a increase in ET instead of decrease. Due to different numbers of horses in second and third photo mean value considered in the SD for ET change 2 is based on the mean values of the 17 horses in the last photo, not the actual 26 horses that contributed with information to the second photo.

The method chosen for this study to evaluate behavioural profiles via a questionnaire and ET as an indirect measurement of stress and recovery have multiple times been used in other studies. As previously mentioned, questionnaires filled in by caretakers or trainers have been a successful tool to find behavioural profiles in different horse breeds. Staiger *et al.* (2016) mapped different behaviour profiles among Tennessee Walking horses, Dai *et al.* (2015) in different sport horses for reaction/fear to novel object and Hori *et al.* (2015) in young Thoroughbred horses for reaction to new stimuli. Advantages regarding this method is that results from the survey reflect behavioural profiles from a long time (at least as long as the trainer or owner has known the horse) not just at a specific time which would have been the case if a jury rated the behaviours seen at the competition. Disadvantages with the method in this study were that some people misunderstood some questions which may have impact on the result due to few completed surveys (26 of 33 distributed).

The IRT- camera also has advantages and disadvantages, one major advantage is that it is a non- invasive method and less stressful to the horse compared to e.g. blood-samples. The fact that IRT measures of ET is an indirect measurement of something else could be a disadvantage if the underlying factors are unknown. There are still evidence of that IRT- photos may have a correlation to cortisol-level (Valera *et al.* 2012), or with the serotonin and dopamine gene variants (Negro *et al.* 2019). With more studies on what exactly ET measurements are reflecting and more data from ET and behavioural surveys there is a huge potential to find results that can be implemented in breeding programs to improve athletic ability and welfare in trotting horses as well as other sport horses.

Conclusion

In conclusion, the FA gave indications of specific behaviour profiles among trotting horses. These factors could not be explained by ET but breed had an effect in factor 2 and sex in factor 5. The small sample size is believed to be the main reason to the non-significant results since previous studies confirm that IRT is a helpful tool to evaluate ET. With more data from behavioural surveys and ET there is a potential to use this information combined with e.g. competition results and genome-analysis to implement in the breeding programs. This could increase the welfare as well as competition results among trotting horses.

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Appendix 1
Please rate **how often you observe/d** the specific behaviour **in racing competitions**:

Trait	**Description**	**1** Never	**2** Rarely	**3** Occasionally	**4** Sometimes	**5** Often	**6** Usually	**7** Always
Nervousness	Tends/tended to become nervous							
Excitability	Tends/tended to get excited or agitated easily							
Fearfulness	Tends/tended to be afraid easily (e.g. novel environments)							
Concentration	Tends/tended to be focused and unaffected by the environment							
Learning	Tends/tended to learn the task of racing /competing quickly							
Memory	Tends/tended to memorize/remember unpleasant events							
Cooperation	Tends/tended to be cooperative, have good attitude (e.g. willing to work/no resistance)							
Will to win	Tends/tended to desire to win							
Stubbornness	Tends/tended to be obstinate once it resists a command							
Self-control	Tends/tended to panic, escape and lose control (e.g. impossible to handle or stop / damage itself)							
Recovery	Tends/tended to relax quickly							
Appetite	Tends/tended to have poor appetite between races							