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Stress during foal training in New Zealand

Stress bij het trainen van veulens in Nieuw Zeeland

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WORD IN ADVANCE

The master thesis ‘Stress monitoring during foal training in New Zealand’ is a research in the framework of a collaboration with the foal education centre Foal NZ in New Zealand. Foal NZ is a world-renowned foal training centre that educates foals to become champion race horses.

In this context, research is done on the stress levels of the foals during their trainings. The purpose is to monitor stress in real-time and find measures to evaluate the progress of the foals in their training program. Here, the opportunity to use heart rate (HR) and activity (ACT) measurements to meet the intended purpose, is investigated.

Before diving into details about the research, I first want to thank some people without whom this thesis would not have been possible. First, I want to thank Leigh and Steve Wills of Foal NZ for the collaboration. Thank you, Leigh and Steve, for performing the measurements and collecting the data. By measuring the HR and ACT with great precision and following the protocol with such a punctuality, you created the high-quality data I could start from.

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ABSTRACT

As a first step in the development of real-time stress monitoring during foal training, modelling techniques are used to obtain off-line stress monitoring. Based on the model parameters of dynamic autoregressive models of the foal's HR, features are calculated and used to monitor stress. Some of the features enable the monitoring of stress, while others can be used to detect high levels of stress. One of the features defined in this research is able to detect high stress levels with on average an accuracy of almost 70 %.

The HR measurements are also used to calculate HR measures that can be used to evaluate the progress of the foals in their training program. In a training session, the easily calculated median, mean and minimum (min) HR are good representatives for the mean stress level of the foals during the training. Based on the mean HR, it is observed that – in an ideal training program – the foals experience significantly ($p = 0.00330$) less stress at the end of the initial training program with respect to the first training session, which is confirmed by the behaviour-based gold standard stress levels. The progress of the foal in the program, involving the decrease of the mean stress level, is observed when taking into account the full training, but also for every respective part of the training: HEADSTALL ON ($p = 0.00764$), ROPE ON ($p = 0.00260$), LIFITNG LEGS ($p = 0.00180$), TOURS ($p = 0.00190$), ROPE OFF ($p = 0.02830$), HEADSTALL OFF ($p = 0.02160$) and TOUCH HEAD ($p = 0.01789$). The occurrence and intensity of short moments of high stress levels are represented by the maximum (max) HR and are also significantly ($p = 0.02341$) lower in the last initial training session with respect to the first. Also, the dynamic range of the HR, represented by the standard deviation (std) of the measured HRs, decreases throughout the training program, with already in the sixth training session a significant ($p = 0.04525$) decrease with respect to the first training. Meanwhile, it is interesting to evaluate the stress within a training session. In the beginning of a training session when the headstall is put on, stress is induced and only decreases again significantly ($p = 0.00030$ for stress level 1 and $p = 0.01350$ for level 3) once the headstall is taken off again, which indicates the end of the training. Based on the mean HR, the significant ($p = 0.04640$) higher stress level in the beginning, in this case when the rope is put on, and the end of the training session when the rope is taken off again, is also observed. Thus, based on HR measurements, the stress levels of the foals can be monitored off-line using dynamic autoregressive models, while the progress of the foals can be evaluated both within a training session and as well as throughout the training program using HR measures.

SAMENVATTING

Met het oog op de ontwikkeling van real-time stress monitoring tijdens de training van veulens, is offline stress monitoring verwezenlijkt door gebruik te maken van modeleringstechnieken. Op basis van model parameters, geschat met behulp van dynamische auto-regressieve modellen, zijn features berekend die worden gebruikt om stress te monitoren. Afhankelijk van de definitie, kan de feature ofwel stress ofwel momenten van hoge stress detecteren. Een van de features die hoge stress detecteert, kan dit met een accuraatheid van gemiddeld bijna 70 %.

De hartslag wordt ook gebruikt om waarden te berekenen die gebruikt kunnen worden om de progressie van het veulen in hun trainingsprogramma op te volgen. Op basis van de mediaan, het gemiddelde en de minimum hartslag, wordt het gemiddelde stress level van de veulens in een training opgevolgd. De evolutie van de gemiddelde hartstag wijst, in een ideaal trainingsprogramma, op significant ($p = 0.00330$) minder stress in de laatste training van het initiële trainingsprogramma in vergelijking met de eerste. Deze trend wordt bevestigd door de goud standaard stress levels die gebaseerd zijn op gedrag. Progressie in het programma betekent dat de gemiddelde stress daalt. De progressie is niet alleen zichtbaar wanneer de gehele training in acht wordt genomen, maar ook in elk deel van de training: HEADSTALL ON ($p = 0.00764$), ROPE ON ($p = 0.00260$), LIFITNG LEGS ($p = 0.00180$), TOURS ($p = 0.00190$), ROPE OFF ($p = 0.02830$), HEADSTALL OFF ($p = 0.02160$) en TOUCH HEAD ($p = 0.01789$). Het voorkomen en de intensiteit van korte moment van hoge stress worden geëvalueerd met behulp van de maximale hartslag en is ook significant ($p = 0.02341$) lager in de laatste initiële training in vergelijking met de eerste. Het dynamische bereik van de hartslag, voorgesteld door de standaardafwijking van de gemeten hartslagen in een tijdsinterval, daalt ook over het trainingsprogramma met reeds een significant ($p = 0.04525$) verschil vanaf de zesde training. Daarnaast is het interessant om te kijken naar de evolutie van stress in de training zelf. In het begin van een trainingssessie, wanneer het hoofdstel wordt aangedaan, wordt stress geïnduceerd dat pas terug significant ($p = 0.00030$ voor stress level 1 en $p = 0.01350$ voor level 3) daalt op het einde van de training wanneer het hoofdstel terug wordt uitgedaan. Op basis van de gemiddelde hartslag is dezelfde significante ($p = 0.04640$) daling zichtbaar tussen het moment waarop het touw rond het veulen wordt gedaan en er terug wordt afgenomen. Dus op basis van hartslagmetingen kan het stress level van de veulens offline gemonitord worden door gebruik te maken van dynamische auto-regressieve modellen en de progressie van het veulen kan geëvalueerd worden zowel in een training, als ook in zijn trainingsprogramma, met behulp van eenvoudig waardes berekend op basis van de hartslagmetingen.

LIST OF ABBREVIATIONS AND SYMBOLS

%	percent
α	significance level of statistical tests
β-endo	beta-endorphin stress hormone
ΔHR	difference between HRs
ACC	acceleration
ACTH	adrenocorticotropic hormone
ACT	activity
ADH	anti-diuretic hormone
ARX	AutoRegressive model with eXogenous input
AvSE	Average Standard Error
a_i	autoregressive model parameter a_0, a_1, …
A_i	variable for sample i defined as the sum of A_{11}, A_{22} and A_{33}
A	agreement point with examples A_{11}, A_{22} and A_{33}
bpm	beats per minute
BP	Blood Pressure
BTB	Beat-To-Beat interval or the time between two successive R peaks
b_i	model parameter b_1, b_1, …
CITD	Complex, Individual, Time-variant and Dynamic
Cl^-	chloride ion
CNS	Central Nervous System
COV	Cardiac Output Volume
CRF	Corticotropin-Releasing Factor
CSV file	Comma Separated Values file
DARX	Dynamic AutoRegressive model with eXogenous input variables
DAR	Dynamic AutoRegressive model
DNA	Deoxyribose Nucleic Acid or genome
D	disagreement point with example D_{ij}

d	Cohen’s d
d	time delay
ECG	electrocardiogram
ELAN	labelling program
Ep	epinephrine hormone
ES	endocrine system
e_I	model error CONDITION I
e_{II}	model error CONDITION II
FU	follow-up training session
F	feature
GHs	Growth Hormones
g	g-forces [m/s^2]
HPAC	Hypothalamic-Pituitary-Adrenal Cortical axis
HRV	Heart Rate Variability
HR	Heart Rate
Hz	Hertz [s^{-1}]
IOR	Intra-Observer Reliability
IOV	Inter-Observer Variability
ISES	International Society for Equitation Science training principles
k	discrete moment in time
LAB	labeller
LEVEL 1	stress level 1
LEVEL 2	stress level 2
LEVEL 3	stress level 3
M3-BIORES	research group of the KU Leuven
max	maximum
min	minimum
MISO	Multiple Input Single Output model

MWU	Mann-Whitney U test
m	model order input polynomial(s)
m	meter
Na^+	sodium ion
NaN	Not a Number in Matlab
NE	norepinephrine hormone
NS	Nervous System
n	model order autoregressive polynomial
n	amount of test horses
N	total amount of sample points
p-value	probability value
PSN	Parasympathetic Nervous System
p	probability value
QRS complex	characteristic of an ECG cycle
RAW	raw unfiltered non-preprocessed data
RMSSD	Root Mean Square of Successive beat-to-beat intervals
RR-interval	time intervals between R peaks of consecutive heartbeats
RR	Respiration Rate
q^{-1}	backward shift operator
R^2	correlation coefficient
R peak	characteristic of an ECG cycle
SAM	adrenomedullary stress response
SDRR	standard deviation of the beat-to-beat interval
SISO	Single Input Single Output model
SNS	Sympathetic Nervous System
SRF	Somatotropin-Releasing Factor

std	standard deviation
S	semi-agreement points
s	second unit of time
T3	triiodothyronin hormones
T4	thyroxine hormones
TRAIN	foal training
TRF	Thyrotropin-Releasing Factor
TSH	Thyroid-Stimulating Hormone
T	foal training
u	input signal
x	x-direction
YIC	Youngs Identification Criterion
y	output signal
y	y-direction
z	z-direction

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INTRODUCTION

Foal NZ is world leader in thoroughbred foal training and behavioural science. They are dedicated to improving the performance of race horses by means of foal education and behavioural research. According to them, the road to racing success begins with establishing foundation behaviour early in the foal's life. Foal NZ offers a foal training program in which the foals learn the fundamentals of human interaction. They use gentle scientifically-proven training methods to train the foals with the least amount of stress to enable the horses to cope with the most amount of stress.

With the eye on improving welfare and performance, they provide the foals the very best start to their racing careers. In trying to make the world better for these horses, they want to deal with real equine industry issues. Due to the typical life of a racing horse, they are confronted with a lot of stressful situation, both during their training as well as their racing career. On the racing track, they observed that sometimes the horses are unable to overcome the high amounts of stress. They observed horses refusing before the race by staying in the staring box or declining in the middle of the race.

To tackle the problems in the industry, Foal NZ invests in scientific research to improve the thoroughbred's sensitivity for stress. Based on research, they continuously improve their training program with new findings. By providing the foals with a solid foundation to cope with stress, they want to make the competitive life easier for the race horses. For scientific research they collaborate with universities all over the world. Having excess to a lot of horses, Foal NZ is responsible for the data collection, while universities have excess to research tool and techniques to transfer the data into information. Meanwhile, Foal NZ contributes to the science by sharing their experience and observations.

The master thesis 'Stress monitoring during foal training in New Zealand' is the result of a collaboration of Foal NZ with the M3-BIORES research group of KU Leuven university. In this context, research is done on the stress levels of the foals during their trainings. The purpose is to investigate the opportunities to use HR and acceleration (ACC) measurements to monitor stress in real-time and find measures to evaluate the progress of the foals in their training program.

To illustrate the context of the research, in the beginning of the literature study, the reader can find a brief introduction about the thoroughbred horse, including the initial breeding, the physical characteristics and some background information about the equine industry and flat racing in New Zealand. In a second part, the training strategy of Foal NZ is discussed. In this part, the focus goes to the incorporation of the ISES training principles in the Foal NZ's training program, the advantages of the proximity of the mare during foal training and why they decided to begin with initial foal trainings at an age of three weeks old. This part end by emphasizing on the importance of performing foal trainings within an optimal stress level range and the possible consequences in case of too much stress, both on the learning efficiency during training as well as on the foal's welfare and health on the long term.

The third section starts with a definition for stress, followed by an illustration of the types of stressors that can induce a stress response. After an explanation on how a physiological stress response is initiated, an elaborated discussion on the physiological stress response itself is given, including the endocrine, neuro-endocrine and endocrine stress response. The section is closed with the trophotropic stress response or the de-stress response.

In the next section, it is clarified how the behavioural stress response is used as the gold standard and which behaviours can be linked with stress. The section continuous by illustrating the complexity, time-variance, individual differences and dynamic characteristics of a living organism.

The literature review ends by a discussion about the state-of-the-art technique to monitor stress, including some concerns about the technique. By proposing to use physiological variables instead, the transition is made towards the objectives of this master thesis, namely the investigation of the opportunities of using HR and ACC data in stress monitoring and in defining measures to evaluate the progress of foals in their training program.

LITERATURE REVIEW

In the thoroughbred industry, New Zealand is one of the most successful in the world [1]. Large New Zealand studs like Windsor Park Stud and Cambridge Stud are specialised breeders of the world-famous thoroughbred horse. They are producing thoroughbred foals with the eye on breeding the next champions in the highest competition levels. On a yearly basis, the foals of the previous year are demonstrated at the national yearling sales. Since industry requires the foals to be confident, calm and able to walk and stand on command at the age of one year old, the foals are trained to prepare them for selling.

In New Zealand, some companies, like Foal NZ, specialised themselves into the training of young foals. Foal NZ offers studs a foal training program, consisting of six to nine training sessions in which the foals get used to human contact, learn some basic commands and acquire coping strategies to handle their stress properly. A foal that is trained by Foal NZ will graduate form the program when it has acquired all predetermined skills [2].

Notice here the importance of offering the foals basic techniques to control their stress level. Throughout their life, from foal to race horse until their post-racing career, thoroughbred horses will be confronted with many different stressful situations and environments. To perform even in the most stressful situations, especially during their racing career, they need to be able to withstand a lot of stress. Being resilient to stress can only be achieved by having acquired a wide range of coping strategies. The for a horse available strategies to release stress are determined by early life experiences. If a particular coping behaviour was successful in lowering the stress level of the horse in an earlier moment of high stress levels, the horse will be inclined to perform the same behaviour later on. Considering the importance of early life experiences, it is essential that foals learn how to cope properly with stress at young age [2].

To achieve a constructive training, the stress level of the foal must stay within a range: a certain degree of mental arousal is needed for learning to occur [2], while too much stress will impair the learning process. When experiencing too much stress, the foal will have difficulties to focus on the training, since it will be preoccupied with trying to lower its stress. In case of high stress, if the foal is unable to lower its stress level, it might perform a fight-or-flight response, in which it will try to escape the stress inducing situation or try to fight the stress trigger. Notice that a systematic triggering of the fight-or-flight response during training, might result in the development of learned helplessness, a condition of an animal in which it has learned to believe that a situation is unchangeable or unescapable. By performing a fight-or-flight response the foal tries to escape from the situation and lower its stress level. In case of systematic triggering, the foal will have no time to release stress and will eventually learn that its response to stress is useless. It will learn that it has no control over the situation and accepts the feeling of helplessness. Consequently, it will generalise the acceptance of being helpless to other situations, resulting in a passive behaviour of undergoing situations. Then, in case of high stress levels, the horse will passively wait until the trigger fades, instead of actively trying to do something about it by performing coping behaviours or a fight-or-flight response [3], [4]. This behaviour is also referred to as passive coping behaviour [5], [6]. To prevent the development of learned helplessness, it is extremely important that the trainer offers the foal correct coping strategies in case of high stress levels during training. Also, the wider the range of obtained coping behaviours, the more options a horse has to control its stress. When the horse is triggered, it will less likely perform a fight-or-flight response, although the flight response can never be eliminated completely and may reappear spontaneously in extremely stressful situations [2].

Hence, to obtain optimal training efficiency, continuously keeping track of the stress level of the foal is extremely important. Currently, Foal NZ trainers are continuously observing the behaviour of the foals to determine their stress level. To improve their training program, Foal NZ can use complementary techniques to assist in monitoring the stress level of the foals during training in a more automated way [2].

THOROUGHBRED HORSE

The thoroughbred horse, also known as racing horse, is one of the most intriguing horse breeds in the world. It is known for its athletic posture, strength and strong spirit, making the thoroughbreds the undefeatable champions in horse racing. The breeding program of the thoroughbred horse owns a long history and was executed with an incredible precision, hence the name ‘thoroughbred’ horse or ‘thorough-bred’ horse, referring to the pure and careful breeding program.

INITIAL BREEDING

The oriental breeding of the thoroughbred horse brings us back to the Middle ages in England. At the end of the 17^{th} century, horse racing became a very popular sport in England. Driven by the passion for racing, the English royal family founded a breeding program with the eye on breeding faster and stronger horses [7]. They decided to start from a selection of their own best mares, meaning the oriental breeding mares of the thoroughbred breed were native English. The native English mare, developed in northern Europe before the 13th century [8], [9], were known for their strength, power and soundness. However, to breed a champion race horse, physical power was not enough. Therefore, convinced of the mental strength and temper of the Middle East horses, the oriental breeders decided to cross the native English mares with Middle East stallions [10].

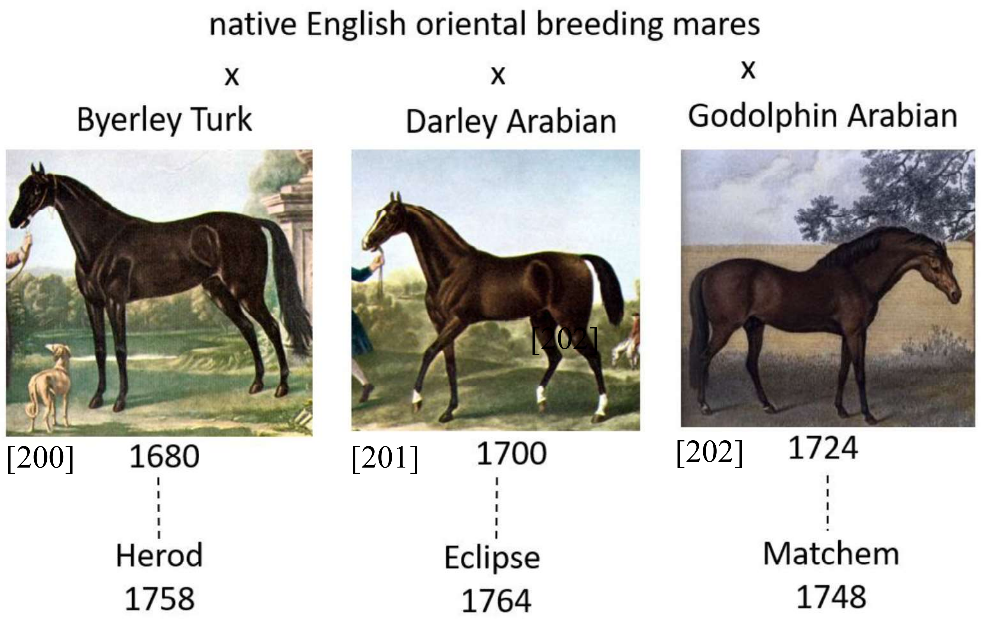


Figure 1 Initial breeding of the thoroughbred horse

According to historical writings, 160 Middle East stallions were involved in the oriental breeding, but three stallions Byerley Turk (1680), Darley Arabian (1700) and Godolphin Arabian (1724) are considered to form the male foundation in the oriental breeding [7], [11]. The other stallions had a rather small influence but some of them made noteworthy contributions to the breed. For example, Brownlow Turk is thought to be responsible for the gray coat colour in some of the thoroughbreds [10].

The male blood lines of the three foundation sires are still preserved today. In the end of the 18th century, Matchem (1748) preserved the male line of Godolphin Arabian [11] by being the only descendant, while Herod (1758), a great-great-grandson of Byerley Turk, preserved the male line of Byerley Turk [10]. Finally, great-great-grandson Eclipse (1764) continued the male blood line of Darley Arabian [12],[10]. The male bloodline of the three descendants of the oriental breeding stallions, Matchem, Herod and Byerley Turk, represent thoroughbred sire blood lines up until today. The oriental breeding of the thoroughbred breed is visualised in Figure 1. The reconstruction of the oriental breeding was done based on historical writings and in 1791, they compiled the information in a first general Stud Book [1]. The Stud Book, still existing today, contains basic information about every thoroughbred horse: a physical description, the genetic background, racing results and information about its further breeding [13]. The Stud Book makes is possible to trace the background of all thoroughbred horses through many generations, up until their foundations in England in the 17th century [1].

CHARACTERISTICS

By combining the strength of the native English mares with the spirit and tall, slim, athletic posture of the Middle East stallions, the ultimate race horse was born. A thoroughbred has typically high withers, strong hindquarters, a short back and long lean legs, making them agile and fast [14], [15]. Beside these physical characteristics, illustrated in Figure 2, it is mainly their strong spirit that makes the thoroughbred horse an excellent racing horse. It is because of their bold temper, that the thoroughbred horse is referred to as a hot-blooded breed [13].

Figure 2 Thoroughbred characteristics [203]

NEW ZEALAND

In the 19th century, the thoroughbred breed was introduced in New Zealand. Ever since, the race horse industry in New Zealand has been growing. In the beginning of the 20th century, some New Zealand breeders started to invest in a strong broodmare population and some world class stallions, which resulted in the production of some of the most famous racing horses of all time, like the mare Desert Gold (1912), sire Phar Lap (1926), sire Sir Tristram (1971) and his son Zabeel (1986) and the famous mare Sunline (1995). With decennia of experience in breeding thoroughbreds, it does not sound surprising that the New Zealand equine industry is still market leader in the world today. For example, New Zealand is still home to some of the most famous thoroughbred breeders in the world like Waikato Stud, Windsor Park Stud and Cambridge Stud and owns some great champion stallions. For example, Windsor Park Stud is home to Turn Me Loose, Vanbrugh, ShamExpress, Rip Van Winkle, Mongolion Khan and Falkirk [16], while Cambridge Studs owns Tavistock, Almanzor, Gurgundy and Highly Recommended [17].

FLAT RACING

Most of the New Zealand thoroughbreds are racing in competitions called flat races. Whereas horse racing was the driving force for the breeding of thoroughbreds, it is not surprising that horse racing and thoroughbred breeding share a common history. At least since 1174, flat races were organised at fairs and markets at Smithfield in London [18]. At that time, a flat race consisted of a four-mile race [18], whereas nowadays, different distances exist: from sprints over 1000 to 1500 meters, to a mile of 1600 meters and middle distances between 1800 and 2400 meters, until longer races that can go over 2400 meters, called staying races. Depending on the horse’s breeding, the horse performs best on a particular distance. However, a truly high champion race horse can perform over a wide range of distances. As the thoroughbred horse, initially bred with the eye on creating the fastest racing horse, is still the most successful today [13], almost exclusively thoroughbreds are competing in the flat races.

POST RACING CAREER

The racing career of a horse starts from an age of two years old and last maximally until the age of four or five years old. Supposing they accomplished good results at the racing track, earning honour, race horses are retired to stud to serve as breeding stallion or broodmare after their racing career. Otherwise, they have a chance to attain a second career as sport or leisure horse. For example, the famous Zabeel was retired to stud in the Cambridge Stud in New Zealand, after a racing career of four years.

FOAL NZ TRAINING STRATEGY

Foal NZ, highly investing in scientific research about equine behaviour, is continuously improving their training strategy, illustrated in Figure 3. In this context, Foal NZ has already successfully implemented the theory of the International Society for Equitation Science (ISES) training principles in their training strategy. For example, the eighth ISES principle is about training persistence of the requested response, meaning that once a cue is given, the foal will need to perform the desired response until it receives a cue to stop. After giving the cue to start walking, Foal NZ stimulates the foal to keep on walking forward by letting the mare walk before the foal. The foal will naturally synchronise its movement with the mare, resulting in the desired foal behaviour [2]. After discovering the advantage of using the mare during foal training, they gave the mare a central role in the training sessions. Foal NZ also optimised the timing of the sessions based on scientific research on the biological development of horses: they synchronised their training with critical learning phases in the life of a foal. Foal NZ has already proven efficiency, since some of the foals, trained by Foal NZ, became multiple winners, like for example the great champions So You Think, Jimmy Choux and Military Move.

ISES TRAINING PRINCIPLES

The ISES training principles are based on horse learning strategies and consist of the following ten independent principles: 1) take into account the horse's ethology and cognition, 2) use learning theory appropriately, 3) train easy-to-discriminate cues, 4) shape responses or movements, 5) elicit responses one at a time, 6) train only one response per signal, 7) form consistent habits, 8) train persistency of responses, 9) avoid and disassociate flight responses and 10) demonstrate minimal levels of arousal [19].

The training strategy is based on understanding equine ethology, or animal behaviour, cognition and mental state. Hereby, they take into account not only the abilities of the equine brain, but also its limitations. The small brain size of a horse causes a limited ability to learn new tasks. To avoid potential confusion in the horse's brain, it is extremely important that horses are experiencing consistency in learning and training throughout their life. The foal needs to be held in a consistent way: right hand in solid contact with the withers and left hand holding the leading rope under the halter. The signals, used as stimulus to provoke a desired response, must be as clear as possible and distinguishable from each other. If the horse is not able to discriminate the different signals or the same signal is used for two or more responses, confusion will arise and cause unnecessary stress for the horse. Also, the signals must be distinguishable in time. If multiple stimuli are given at the same time, the horse will have difficulty to understand the individual signals and will most likely be unable to respond to any of the stimuli. To ensure consistency in a horse's training throughout their career, it is important that the stimuli used in the initial training are transferred to the next trainer or handler [19].

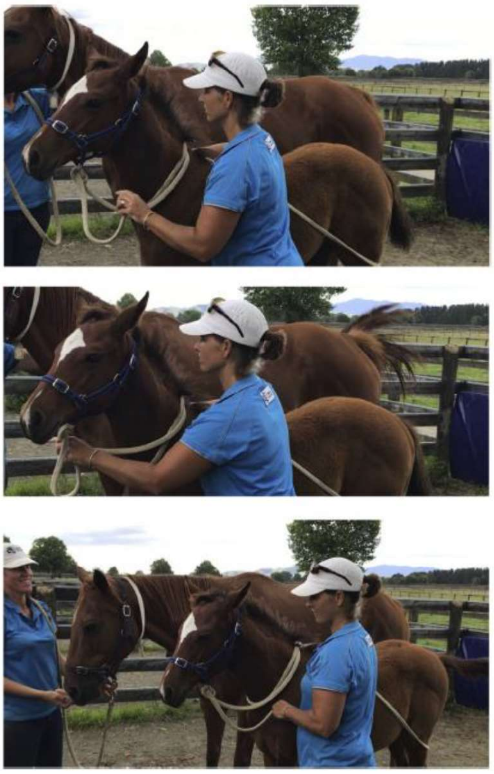


Figure 3 Foal NZ foal training [204]

The foals are trained using operant conditioning. An operant behaviour is a voluntary behaviour that is initially spontaneous, rather than a response to a prior stimulus, and whose effect – positive or negative – may reinforce or inhibit the repetition of the behaviour respectively. During operant conditioning, an operant behaviour will be conditionally linked by a certain stimulus, making stimulus control over the foal possible. Then, when a conditioned stimulus is given, the foal will respond with the desired operant behaviour [20]. To enhance the likelihood of occurrence of the operant behaviour during conditioning, training is combined with rewarding or positive reinforcements. To understand the learning process of operant conditioning in combination with rewarding, the example of learning how to be led is explained in detail. Walking forward is an operant natural behaviour of a horse. During conditioning, the stimulus, applying a gentle pressure on the hindquarters and simultaneous pulling on the leading rope, is linked with walking forward [2].

In equine training, the goal is to achieve a persistent response to a short stimuli. To avoid conditioning by continuous stimuli, the stimulus – pressure on the hindquarters and on the leading rope – is immediately removed after the onset of the desired behaviour. Once the stimulus is given, the horse needs to maintain the response until a stop stimulus is given. To enhance the likelihood of showing the desired behaviour and decrease the chance of showing a non-desired behaviour, the onset of the desired response is followed by a rewarding action [2].

Another important aspect of the ISES training principles includes the process of shaping responses or movements. Throughout the training process, shaping means the reinforcement of successive behaviours progressively approximating the desired response [21]. To ensure progress towards the desired response, trainers need to be careful with rewarding approximations of the desired behaviour [2].

Finally, the key to succes for this training strategy is a good timing of the given cue and the onset of the operant behaviour. The cue to start walking forward must be synchronised with the foal's operant behaviour of walking forward. Knowing that the foal has the natural urge to follow its mother, the mare is used to initiate the foal's movement. A precise timing of the cue, with respect to the occurrence of the operant behaviour, is required to obtain the desired result of the conditioning. Only then, the given cue will be associated with the desired response in the brain of the foal [2].

Foal NZ has observed that training young thoroughbred foals by means of a consistent training based on the ten ISES training principles, results in improved welfare for the foals both during training sessions as well as in their further life as racing horse. During foal training the foals also learn how to cope with stress. By learning this at young age, they will be able to withstand large amounts of stress later on, which will improve their performance during race trainings and racing career. The better they can cope with stress, the higher the chance to become a champion race horse [2].

MARE PROXIMITY

After the successful implementation of the ISES training principles in their training program, Foal NZ obtained confidence in the profit of using the mare during training. Conducting the foal's training sessions in the proximity of the mare is advantageous because of many reasons.

First of all, at young age, foals are still very attached to their mother, forming the major part of the foal's social and physical environment. By performing the training sessions in close proximity of the mare, the foal remains in its natural environment. Hereby, avoiding abrubt changes in their direct environment certainly lowers the foal's stress level. By performing most parts of the training with the foal between the mare and the trainer, the foal can easily seek comfort with its mother whenever it is experiencing stress [2].

Secondly, as discussed before, the mare is used to facilitate the operant training strategy of Foal NZ. To encourage the foal to start walking, they simply let the mare walk in front of the foal and the foal will instinctively follow its mother [2]. Thirdly, at the age of three weeks old, the foal is at the beginning of its socialisation phase, meaning it is starting to develop its own senses and emotions. Henry suggested that, at this age, the foals easily take over behaviours and emotions from their mother. Therefore, involving a previously positively handled mare in the foal's training can help the transfer of positive emotions with respect to humans of the mare towards her foal [22]. Consequently, the foals will show less avoidance and will more easily interact with humans. They will show less flight or flight responses and will more easily adapt to new environments and situations. Since a confident foal that experiences less stress, learns more easily, Foal NZ will encourage foal-mare interactions during training [2].

While Foal NZ takes advantage out of the interaction between the foal and mare during the training sessions, they also take into account the possible drawbacks. While a calm and confident mare can be used to lower the stress level of the foal [22], a mare experiencing stress, might, for instance, unnecessarily cause stress in the foal. Therefore, it is extremely important, the mare's stress level is kept as low as possible during foal training [19]. Notice also that the foal-mare interation works in two directions. Due to concern over her foal, the mare's stress level might increase as response to a stressed foal, which will consequently trigger the foal even more. Foal NZ trainers will try to keep the mare as calm as possible by, for example, stroking their head and neck or rubbing their back. If, however, the mare is experiencing stress, they will interrupt the interaction between the foal and mare to avoid the mare to increase the stress level of the foal. For example, Foal NZ trainers prevent further communication by distracting the mare by providing a salt block to lick or some small food chunks to eat [2].

In this way, Foal NZ tries to create a low-stress training environment for the foal, enabling a more efficient learning process and less fight-or-flight responses [2]. The strong bond between foal and mare changes however over time. According to Henry, the strong bond persists at least until the age of one year old, enabling the possibility to influence the foal's emotions using the mare for at least one year [22]. Because of the above-mentioned reasons, the foal training sessions of Foal NZ are performed in the near proximity of the mare.

INITIAL TRAINING

Based on research of Brubaker and Udell on the biological development of horses [23], the training program of Foal NZ is further optimised by synchronizing the training program with critical biological development periods in the life of a foal, for example the socialisation phase [2]. In New Zealand, foals are born in the period from August until the beginning of December. After birth, foals run with their mothers in a herd in vast paddocks, giving the foal time to gain strength, form a strong bond with its mother and socialise with other horses [24]. At the age of three weeks old, the socialisation phase sets in, in which the foals start to develop senses and emotions and form new bonds with other horses. Starting foal training at this age, has some important advantages [25].

First, during the socialisation phase the foal is easily forming new relationships. This enables the trainer to build a mutual trustworthy bond with the foals. A trusting foal will intuitively follow the instructions of the trainer, which facilitates training. Second, in this socialisation period, the foals start exploring their environment and begin to learn from outside stimuli or influences. The foals are able to learn and memorise the given cues and desired responses learned during training [26]. In addition, at the age of three weeks old, the foal's movement and coordination is sufficiently refined. In a particular part of the training, the trainer learns the foal how to lift its legs, by elevating the foal's legs one by one, while the foal needs to hold balance on the three remaining legs. At three weeks old, the foal's balance is sufficiently developed to complete this part of training [2].

Finally, with its emotions and thus its stress response not yet fully developed, the foal will not directly respond with a stress response to an unknown stimulus. Instead, the foal will mainly rely on its mother to determine how to respond in a particular situation. This again illustrates the importance of the presence of the mare during foal training and the nessecity to keep the mare's stress levels as low as possible. Therefore, starting the initial foal training at the age of three weeks old, is standard procedure for Foal NZ [23].

FOAL EDUCATION PROGRAM

The education program, as schematically illustrated in Figure 4, starts with the initial training program, starting at the age of three weeks old. In a period of two or three weeks, the foal is completing seven to nine training sessions, depending on the learning capacity of the foal. During the sessions, foals are getting used to human contact and become more comfortable being approached and touched. They adapt to wearing a halter, learn to distinguish different cues and respond correctly according to the given cue. Ideally, the program is completed before the age of eight weeks old. A follow-up (FU) training is done at the age of five months old to check the effectiveness and profit of the initial training program. In between training sessions, the foals can spend time with the herd in a vast grazing, which stimulates the further physical and mental development of the foal. Before every training session, the foal and mare are isolated from the herd in a small fenced part of the grazing [24].

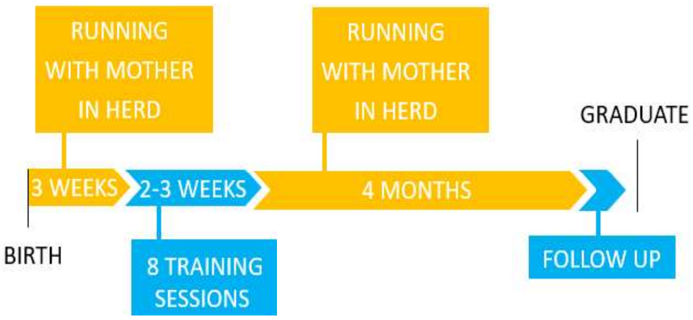


Figure 4 Training strategy of Foal NZ

TRAINING SESSION

At the start of the first training session, the foal is approached by a trainer and subsequently touched for the first time. The trainer will gently touch the foal's back and neck before gradually going toward the head. This is particularly important for the foal to gain trust in the trainer and become comfortable being touched [2]. Once the foal is comfortable, the headstall is put on, followed by a rope tied to the headstall and wrapped around the foal behind its hindquarters, as illustrated in Figure 3.

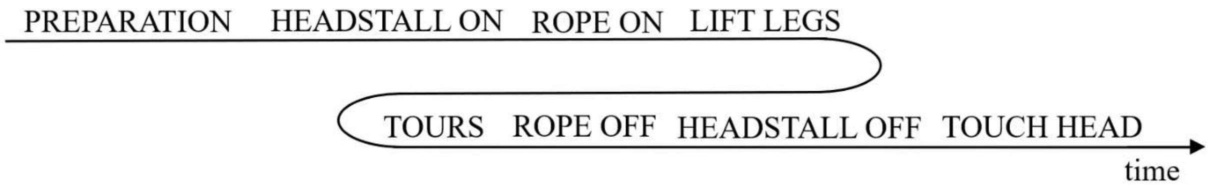


Figure 5 Training steps of a standard training session

The purpose of the rope is for the foal to get used to the feeling of something touching its back, making it more easy for the foal to accept a saddle or rider later on. Subsequently, the trainer will learn the foal to present its feet by lifting the legs of the foal one by one. Meanwhile, the foal must keep its balance on the three remaining legs. The cue to present its feet is given by touching the base of the leg. To demonstrate the foal the desired behaviour, the trainer slowely moves the foal’s foot upwards.In the next part of the training, the foal will learn how to be led. Here, it is important that the trainer holds the foal in the standard position: left hand holding the lead under the halter and right hand in solid contact with the withers, as illustrated in Figure 3. Starting from this standard position, the trainer can give different cues, for example to start or stop walking, to the foal, by applying pressure on different parts of the foal’s body or releasing pressure [2].

In the first three to four sessions, the training is limited to one way of leading, in which the trainer walks next to the foal and the cue to start walking is given by applying a short pressure pulse on the whiters of the foal. Only when the foal is familiar with this first way of leading, the trainer will start with a second way, in which the trainer is walking in front of the foal and the cue is given by applying a short forward pull on the rope of the halte. The foal is asked to start walking and follow the trainer in front of him. At the end of the training session, the rope and halter are taken off, followed by the trainer touching the head and ears of the foal and some positive reinforcements [24].

Each training session, visually represented in Figure 5, will contain the previously mentioned parts, represented as: HEADSTALL ON, ROPE ON, LIFTING LEGS, TOURS, ROPE OFF, HEADSTALL OFF and TOUCH HEAD. Usually, the step are performed in this order, but might sometimes be replaced in time due to practical considerations. Sometimes steps are repeated multiple times during the same training: in case the foal didn’t react well on a particular part, the part is repeated later on in the training until the foal makes some approvement [24].

GOAL

The goal of the foal training program is twofold. On one side, the foal is learning how to be led properly, while on the other side, it is taught how to cope with stress. Leading the foal can be achieved by the foal associating given cues with a certain desired repsonse, for example start or stop walking. While being able to lead the foal is recuired for the foal presentation at the national yearling sales, being able to properly handle their stress is important for the general welfare and health of the foal. Already at a young age, foals develop coping strategies, e.i. natural behaviours that offer comfort and satisfaction and lower their stress level. In highly stressful situations, it can be crucial for a race horse to be able to lower its stress to an agreeable level. The wider the horse's range of coping strategies, the easier it can handle stress in all kinds of situations. Race horses with well developed coping stategies are calmer and more confident, perform better and have higher chance of making it to the racing track. Thus, during foal training, Foal NZ focus also on the development of natural coping behaviours [24].

DURATION

A training session lasts between seven to fifteen minutes. Especially in the the beginning of the program, trainings are maximally seven to eight minutes long. Horses have a limited brain size, restricting their concentration span to maximally twenty minutes. Performing longer sessions would be inefficient. Also, the time between consecutive sessions is important. A horse needs enough time to process the information received during training, which is why foals never receive two trainings in one day or more than three consecutive days of training sessions [2].

GRADUATION

After on average eight initial training sessions and one FU session, the foals are confident and able to respond correctly to different stimuli. They are comfortable wearing a halter with a rope wrapped around their body and can present their feet when asked. They walk forward and stand still on command. If the foal meets the above-mentioned requirements, the foal graduates from the Foal NZ training program and are ready to start further training programs. Typically, at the age of one year old, they move to the next training facility to start a race training program, in which they are prepared to become a champion race horse [24].

STRESS DURING TRAINING

Although too much stress during foal training must be avoided, it is inevitable that the foals experience stress during training. Especially at the beginning of the first training session, the abrupt change of environment typically induces a lot of stress. Subsequently, the learning process will induce stress as well. Learning to associate unknown stimuli with desired behaviours, causes confusion and stress. Once the foal knows which behaviour is desired when a certain stimulus is given, the foal will experience less confusion and thus less stress [2].

The duration and efficiency of a foal's learning process depends on its learning capacity and is thus different for every foal. While some foals learn rather quickly, others encounter more difficulties in differentiating between the cues. Nevertheless a foal performs well on one part of the training, it can experience difficulties on another part. Depending on the progress in a particular part of the training, the foal will be triggered accordingly in that particular part, inducing a corresponding increase in its stress level [2].

ACUTE STRESS RESPONSE

Excessive stress levels during training not only impair the learning efficiency during the training, but also involve welfare and health issues on the long term. At moments of high stress levels, while the available coping mechanisms of the foal are insufficient to lower its stress to normal levels, every stimulus can potentially elicit an acute stress response. In an acute stress response, the foal will no longer try to cope with its stress using regular coping techniques, but will try to eliminate the stressor. In this case, two options remain: the foal can either fight the stressor or flee to avoid it. Therefore, the acute stress response is also known as the fight-or-flight response [28]. In the context of foal training, fighting the stressor might involve the foal trying to bite or hit the trainer. If, otherwise, the foal prefers the flight, it will try to run towards its mother to seek shelter or towards the other side of the training area [24].

OPTIMAL STRESS LEVEL

With the eye on obtaining maximal efficiency during the learning process of the foal, an optimal learning environment must be created during training. Valenchon recognized in 2013 that high stress levels have a negative impact on the learning ability of the foals [27]. Therefore, it is important to avoid the development of too much stress. At the same time, some level of arousal is needed to keep attention on the training: no arousal means no concentration and no motivation to learn. Hence, regarding to stress, an optimal range exists in which learning efficiency is maximalised [19].

COPING TECHNIQUES

The optimal learning environment is a relatively low-stress enviroment in which they avoid triggers that might induce too much stress. Nonetheless, if trainers recognise initial signs of an emerging fight-or-flight response, they pause the training and try to avoid it, for example, by blocking escape routes for the foal. At this critical point of high stress, Foal NZ trainers teach the foals apropriate coping techniques that help them reduce their high stress level to an acceptable level. For example, the trainers encourage the foals to lick or chew. The licking behaviour can be provoked by offering the foal a salt block, while chewing is elicited by putting their finger into the mouth of the foal. Licking and chewing creates physical relaxation of the face and jaws, which results in mental relaxation and thus decrease in stress level [2].

CONDITIONED FIGHT-OR-FLIGHT RESPONSE

Repeatedly tiggering the fight-or-flight response during training, involves the risk of developing a preference of the foal for a fight or fligh response to release stress above regular coping behaviours [2]. If the stimuli given during training repeatedly trigger a fight-or-flight repsonse, the stimuli can also become associated with a fight-or-flight instead of the desired behaviour. To avoid this undesired conditioning and encourage the displacement of the desired behaviour, Foal NZ trainers use positive reinforcements when the foal performs the desired behaviour, while avoiding high stress levels as much as possible [29]. If, however, the foal gets used to escape highly stressful situations by fighting or fleeing, instead of using apropriate coping behaviours, a conditioned fight-or-flight response is developed, which is accompanied with a deteriorated welfare for the horse and health risks for both the horse and its handlers [30].

CHRONIC STRESS

During the initial foal trainings, the foals have their first experiences with highly stressfull situations. These moments are critical in the development of coping strategies. The strategy they use then to lower their stress determines how they will cope with stress later on in life. If initially, licking and chewing adequately lowered their stress level, they will be inclined to reuse these strategies in other stressful situations. However, if the foal is triggered too often to perform a fight-or-flight response, it will prefer to fight-or-flight instead of using appropriate coping strategies. Considering the importance of early experiences in the development of coping strategies, investing time in teaching the foals apropriate strategies in an early stage of life is incredibly important. A horse lacking the ability to cope properly with stress obtains a high risk to develop chronic stress [2]. Although the New Zealand racing industry inclines to make the life of the thoroughbreds as comfortable as possible, the life of a racing horse is far from natural. Racing horses are frequently confronted with highly stressful situations that induce acute stress responses [24]. During an acute stress repsonse, adaptive mechanisms in the horse's body induce a physicological stress respone, which results in an increased HR, blood pressure (BP) and stress hormone levels [31]. Normally, after an acute stress response, the HR, BP and stress hormone levels automatically decrease to normal levels but horses can also actively lower their stress level by performing coping behaviours, like licking or chewing.

Unfortunately, coping mechanisms can only provide a limited stress relief [31]. In case of persisted stimulation, the horse will no longer be able to lower its stress level, which results in sustained high stress hormone levels and increased HR and BP [32]. A frequent overactivation of the stress response causes the development of chronic stress [31].

Chronic stress is characterised by a constant high level of corticosteroid stress hormones, making it possible to diagnose chronic stress by testing the stress hormone levels in the blood. However, chronic stress will also cause behavioural changes, making it possible to observe chronic stress by looking at the general appearance and behaviour of the horse as well. Horses that are suffering from chronic stress look apathic and show loss of interest in their environment. They demonstrate a despaired withdrawn posture: standing with a stretched neck on similar height of their back with no ear or eye movement. They show lower reactivity to tactile stimuli but suffer from high levels of anxiety: the smallest trigger can evoke an overemotional reaction in which their depressed appearance quickly change to an intense stress response.Desperate to relieve their excess of stress, they also display modified displacement behaviours, involving repetitive movements or destructive behaviours [33]. For example, some chronically stressed horses start biting their own chest. However this behaviour is highly destructive for the horse, biting their chest gives them short moments of stress relieve, which encourages them to continue with this destructive behaviour. Another typical example of a modified displacement behaviour is the rhythmical shaking of the head [31].

The effect of chronic stress can last for days, weeks, month or even years. Once a horse suffers from chronic stress, it is hard to cure. Besides modified behaviours, chronic stress is also associated with bad health related outcomes, like a permanent high BP, suppressed immune system, growth inhibition, increased risk for heart diseases and damaged muscle tissue [32]. Moreover, it is also associated with an impaired digestion process and poor nutrient absorption, leading to vitamin and mineral deficiencies. While high stress levels also cause longer healing times when injured and lower fertility, it also damages the hippocampus which impairs the memory [31].

Lacking appropriate coping behaviours thus has a severe impact on the physical and mental health of a horse. A horse unable to cope properly with stress, will experience a reduced general welfare and health, as well as demonstrate a lower performance [2].

PHYSIOLOGICAL STRESS RESPONSE

Before being able to monitor stress during foal training, it is necessary to give an undisputable definition for stress first. Next, two types of stressors that can induce stress are identified, followed by a discussion about the initiation and coordination of the physiological stress response.

DEFENITION FOR STRESS

Although the term stress is widely used, there is discussion about an explicit definition. A lot of researchers have tried to give a general definition for stress, but pioneers in defining stress are Walter Cannon and Hans Selye. Although Walter Cannon, an expert in physiology, used the term stress for the first time in medicine [34], it was Hans Selye who was the first to use the term 'stress' to describe the physiological and/or psychological state of unpleasantness in living organisms. In the early 20th century, he saw the similarity between mechanical stress in materials and the unpleasant feeling – caused by stress – in living organisms and decided to deduce the term 'stress' to describe the unpleasant or uncomfortable feeling [34], [35].

Eventually, Cannon defined the stress response as a mechanism of the body to adapt and deal with stress inducing situations or triggers. If, however, the body is unable to adapt properly, stress results in an acute stress response, also known as a fight-or-flight response, in which the stress inducing situation is avoided instead of adapting to the situation [34], [36], [37]. Peterson, on the other hand, described stress as the non-specific response of an organism to any stressor that endangers the integrity of the organism. Reinhardt, on his turn, considers stress as a state of threatened homeostasis, by either a physiological or psychological stressor, that results in the activation of a complex repertoire of physiological and psychological responses [38]. According to Dewil, expert in animal welfare, stress is a state of an organism that appears with a sudden decrease in predictability and/or possibility to influence relevant changes in the environment [39]. The definition of stress thus depends on the expertise of the researcher or the performed experiments. Here, stress is defined as a combination of the definitions by Cannon, Peterson, Reinhardt and Dewil:

> Stress is an uncomfortable tensed state, after being triggered by either a physical or psychological stressor, that induces a stress response with associated physiological changes.

DIFFERENT TYPES OF STRESSORS

In the definition of stress, it is mentioned that stress is either triggered by a physical or psychological stressor. To fully understand stress and the stress response, Selye expanded the research of Cannon by investigating in more detail the triggers that can cause a stress response, called stressors. According to Selye, stressors are triggers that endanger the integrity of an organism and unbalance the homeostatic equilibrium [34], [40].

The homeostatic equilibrium refers to the steady state of the internal conditions of the body at an optimal level. Optimal internal conditions include for example a constant body temperature, pH around seven and vital concentrations of minerals and essential compounds. These optimal conditions are important to ensure a proper functionality of all systems, organs and pathways. Maintaining homeostasis is thus important for the regulation and well-functioning of the entire body, and therefore for survival. The homeostatic balance is a dynamic equilibrium, regulated by a complex network of self-regulating processes and pathways, coordinated by the autonomic nervous system. This means that despite changes in the conditions, the body will adapt to maintain the internal body conditions [41].

Changes in the conditions can refer to environmental changes or psychological changes. Environmental changes can be sensed by one or more senses, like sight, tactile sense or olfactory sense, and are therefore called physical stressors. Think, for example, about high temperatures, drought or touch. Psychological changes refer to the development of non-favourable thoughts or fear, induced by a possible threat. It can also refer to uncertainty or a decreased predictability about changes in the environment or the lack of having an influence on the changes. In this case, the changes in the conditions are referred to as psychological stressors [41].

Selye found that independently of the nature of the stressor, physical or psychological, the induced unbalance in the homeostatic equilibrium results in the same physiological stress response, involving the same physiological changes with the purpose of restoring the homeostatic balance. The nature of the trigger that originally induced the homeostatic unbalance has no influence on this process. Selye called this principle the general adaptation syndrome of living organisms [34], [40].

INITIATION OF THE STRESS RESPONSE

After the sensation of a physical stressor by organ-specific receptors, the stimulus is transferred into a neurological signal. The receptor information is then sent as an efferent signal through the neurological system to the brain [42] [43] [44]. In the sensory cortex of the brain, the received sensory information of all sense organs is combined and further processed. Here, the nature of the stressor is defined, but further processing, involving other parts of the brain, is needed before the stressor can be interpreted [45].

For the interpretation of the sensory information, the limbic system and the hippocampus are involved. The limbic system is considered as the central coordination centre of emotions and mental state of an organism, while the hippocampus, which is also a part of the limbic system, plays an central role in the formation and storage of memories and later consolidation of information [46], [47], [48]. The evaluation of the threat level of the perceived physical stressor is done by comparing it with earlier experiences and the associated memories and emotions. If the stressor is interpreted as a threat for the organism, the stressor will provoke a stress response [45].

A psychological stressor, on the other hand, cannot be perceived by sensory organs and thus only involves the interpretation of information in the hippocampus and limbic system. The thought of a possible threat or danger is enough to provoke a stress response [45].

If a stressor is intense enough to induce a stress response, neurons in the posterior part of the hypothalamus, a small but important part of the limbic system [41], are activated [49] and the stress response is initiated. From here on the stress response can be divided into three separate responses: the neurological response, the neuro-endocrine response and the endocrine response. In the neurological stress response, a neurological signal is sent from the activated posterior part of the hypothalamus, to the brain stem and then to the involved organs. Whereas in the neurological response the signal transduction is exclusively done by means of neurological signals sent through the nervous system (NS), in the neuro-endocrine and the endocrine stress response, the endocrine system (ES) is also involved. In the neuro-endocrine stress response, the signal transduction is partly neurological and partly endocrine: an activating neurological signal is sent from the hypothalamus towards the adrenal medullary where it stimulates the production of stress hormones. In the endocrine stress response, on the other hand, the entire signal transduction is endocrine [45].

PHYSIOLOGICAL STRESS RESPONSE

Although the stress response is a complex system of physiological processes, involving the NS and the ES, the hypothalamus, is responsible for the initiation [50] and coordination of the stress response [41], [51], [52], [53]. To understand the mechanism of the stress response, basic knowledge about the physiology of the NS is beneficial. Therefore, a more detailed overview on the physiology of a horse is given in Appendix I.

The neurological, neuro-endocrine and endocrine stress response are occurring next to each other and have complementary functionalities. Since, in general, the signal transduction through the nervous system is faster than in the ES, the effect of the neurological stress response can be observed first, followed by the effects of the neuro-endocrine and endocrine response [45].

NEUROLOGICAL STRESS RESPONSE

In the neurological stress response, the activated hypothalamus sends a signal towards the brain stem, where it activates the sympathetic autonomous nervous system (SNS) [54]. Activating the SNS has a general stimulating effect and will bring the body in a more alert state. It will almost immediately increase the HR, BP, cardiac output volume (COV) and respiration rate (RR), allowing an increased energy and oxygen flow. By regulating vessel constriction, the blood flow is regulated to increase the blood flow towards the brain, the skeletal muscles, some important glands and critical organs, like for example the liver. At the same time, it will decrease the blood flow towards less critical organs, like the digestive system, internal organs and the skin. The activation of the critical organs and excreting glands provides the blood flow with more directly-available glucose, providing extra energy for the brain and skeletal muscles. The body is in a more alert state and the muscles have more energy available which allows faster and stronger contraction [55].

Although the neurological stress response enables an almost immediate reaction to a stressor, the general activating and stimulating effect of the SNS doesn't last long [50]. The activating neurological signal is sent, stimulates the organs and disappears. A more persistent stimulation is offered by the neuro-endocrine stress response [45].

NEURO-ENDOCRINE STRESS RESPONSE

In the neuro-endocrine stress response, visualised in Figure 6, the activated neurons in the posterior part of the hypothalamus send a neurological signal through the spinal cord towards the adrenal medulla [49]. The adrenal medulla is activated and starts to excrete two catecholamine stress hormones norepinephrine (NE) and epinephrine (Ep), which is also known as noradrenaline or simply adrenaline. In the adrenal medulla, mainly Ep is excreted [52], with approximately 80 percent of all medullary catecholamines [56]. But Ep is almost exclusively produced in the adrenal medulla, while NE is also excreted by adrenergic neurons of the central nervous system (CNS) and in the innervations of the SNS to the critical organs. Because of the hormone excretion in the adrenal medulla, the neuro-endocrine stress response is also called the adrenomedullary stress response (SAM) [53].

The produced catecholamines are then transported through the blood circulatory system towards target organs, where Ep binds on the β-adrenoreceptors and enhances the sympathetic activating and stimulating effect, similarly to the neurological stress response. Therefore, Ep stimulates the HR, BP, COV and RR to increase. Ep also regulates the dilation and constriction of particular blood vessels, so it can increase the blood flow towards the brain, critical organs and skeletal muscles, while minimizing the blood flow towards less critical organs [57], [58], [59], [60]. NE, on the other hand, binds to the α-adrenoreceptors of the viscera, but has a negligible stimulating effect. The Ep hormone also stimulates the liver to make and release more glucose and cholesterol, providing cells in the heart, muscles and brain with more energy [31]. By making more energy available, the body prepares itself for a possible emerging fight-or-flight response [59]. The neuro-endocrine stress response can thus be seen as an elongation of the non-long-lasting effect of the neurological stress response. As long as the concentration of Ep in the blood is high, the stimulating effect remains [45].

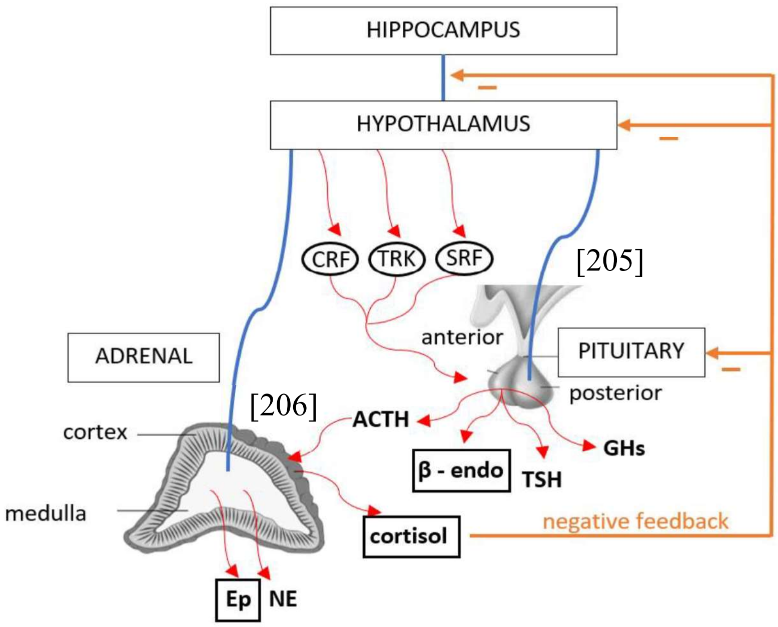


Figure 6 Neuro-endocrine and endocrine stress response

ENDOCRINE STRESS RESPONSE

So far, the hypothalamus acted as a central coordination centre for the neurological and neuro-endocrine stress response, sending only neurological signals. However, before going into more detail about the endocrine stress response, the unique characteristics of the hypothalamus are illustrated. The hypothalamus contains specific secretory neuronal cells that excrete signalling factors in the endocrine stress response, which gives the hypothalamus the unique characteristic of combining neurological and endocrine signal transduction [45].

In the endocrine stress response, also visualised in Figure 6, the hypothalamus coordinates the production of hormones in the anterior and posterior pituitary gland, located just underneath the hypothalamus, while specific secretory cells in the hypothalamus itself produce the stress hormone beta-endorphin (β-endo). β-endo has no stimulating effect but is a stress regulating hormone, known to reduce stress and maintain homeostasis, while it is also associated with thrill, hunger, pain and reward cognition [61]. The secretory cells in the hypothalamus also produce the following signalling factors: corticotropin-releasing factor (CRF), somatotropin-releasing factor (SRF) and the thyrotropin-releasing factor (TRF) [62]. After being released, the TRF, CRF and SRF releasing factors are recognized by specific receptors in the anterior pituitary and stimulate the production of the thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH) and growth hormones (GHs), respectively [63], [45].

The TSH is transported through the blood stream and when recognised by receptors in the thyroid, the TSH stimulates the production of two thyroid hormones: triiodothyronine (T3) and thyroxine (T4). T3 and T4 are known to increase the metabolism, HR and BP during the stress response, while in normal conditions, they are essential for a normal growth and development [42] [64] [65].

The ACTH is also transported through the blood stream but is recognised by receptors of the adrenal cortex, where it stimulates the endocrine glands to produce the stress hormones cortisol, corticosterone, aldosterone and deoxycorticosterone [53], [57], [60], [63], [65]. Cortisol in particular plays a central role in the regulation of the stress response, which will be discussed in the next section. Because, in the endocrine stress response, the hypothalamus stimulates the anterior pituitary to produce ACTH, which on his turn activates the adrenal cortex to excrete cortisol, the endocrine stress response is also called the hypothalamic-pituitary-adrenal cortical (HPAC) response [45], [53].

Aldosterone and deoxycorticosterone, on the other hand, have a totally different function than cortisol. They regulate fluid retention in the body during the stress response by regulating the absorption of sodium (Na^+) and chloride (Cl^-) in different parts of the body [57], [63], [53], [65], [60].

Concerning the function of the GHs produced in the anterior pituitary gland, no scientific consensus exists. According to Selye, the GHs stimulate the release of cortisol, corticosterone, aldosterone and deoxycorticosterone in the adrenal cortex [66]. Another hypothesis, defended by While Yuwiler, states that the GHs facilitates the release of stored fats, increasing the concentration of free fatty acids, next to the increased glucose concentration in the blood [65].

In contrast to the anterior pituitary, no releasing factors are involved in the activation of the posterior pituitary. The posterior pituitary is stimulated by a neural signal descending from the hypothalamus towards the posterior pituitary, which is why the posterior pituitary is also called the neuro-pituitary. After stimulation the neuro-pituitary produces the oxytocin hormone and vasopressin hormone, with the vasopressin hormone also known as the anti-diuretic hormone (ADH) with a positive effect on the re-absorption of water in the kidneys, ensuring water retention during the stress response [67], [64].

REGULATION

In the previous section, an overview of the stress response is given, including all released signalling factors and hormones. However, three primary stress hormones are known to mainly control the stress response: Ep, cortisol and β-endo. Ep is released in adrenal medulla in the neuro-endocrine response and is mainly responsible for the stimulating effect of the stress response. It induces the typical physiological changes: increased HR, BP, COV and RR [45].

Cortisol and β-endo, on the other hand, have a regulatory function. In non-stressful situations, the cortisol level demonstrates a typical circadian pattern with high cortisol levels in the beginning of the day, while the level gradually decreasing towards the end of the day. The cortisol level is highly regulated by three negative feedback loops on different levels of the cortisol production, see Figure 6. The negative feedback loops prevent perturbations from the typical day-night pattern of the blood cortisol level. A first feedback loop acts upon the neural input from the hippocampus to the hypothalamus, while a second loop acts directly on the hypothalamus. These two feedback loops counteract the release of the CRF and thus indirectly the release of more cortisol. The third feedback loop acts upon the pituitary gland, inhibiting the production of ACTH and thus also indirectly the production of more cortisol.

In non-stressful situations, the cortisol feedback loops prevent perturbations from the circadian pattern. In case of stress, however, extra cortisol is produced in the endocrine stress response and stimulates the sympathetically mediated release of glucose and fat, while the negative feedback loops will inhibit the production of more cortisol. The cortisol produced in the endocrine stress response can be seen as a perturbation from the normal circadian pattern. By inhibiting the overproduction of cortisol, the negative feedback loops also inhibit the overproduction of other stress hormones. Therefore, the cortisol level, in combination with the intrinsic negative feedback loops, prevent an overexpression of the stress response, making cortisol responsible for the regulation of the stress response. By preventing an overactivation, cortisol restrains stress hormones to cause permanent damage to the body. The other regulation stress hormone β-endo is responsible for the reduction of stress after a stress response and encourages the resume and conservation of the homeostatic balance [45].

TROPHOTROPIC RESPONSE

After a stress response, the increased HR, BP, COV, RR and stress hormone levels decrease again in the trophotropic response or de-stress response. In the trophotropic stress response, the parasympathetic nervous system (PNS) is activated which has an opposite effect on the critical organs with respect to the stimulating SNS. The organism releases stress and restores the initial homeostatic balance [68], [53], [42], [69].

The trophotropic response is initiated by activating PNS neurons in the anterior part of the hypothalamus. After activation, a neurological signal is sent through the PNS towards the target organs [54], where it restores the normal activity of the heart muscle, smooth muscles, intestines and glands, resulting in the recovery of normal regular HRs, BPs, COVs and RRs. The normal body functions that were temporally inhibited during the stress response, like an effective digestion, defaecation and urination, are restarted and the resting homeostatic balance is restored [55].

BEHAVIOURAL STRESS RESPONSE AS GOLD STANDARD

The gold standard for the detection of stress is the behavioural stress response, including horse-specific stress-related behaviours.

BEHAVIOURAL STRESS RESPONSE

All animals demonstrate species-specific behaviours i.e. a collection of different behaviours that are genetically determined and stereotype for a certain animal. The behaviours are mainly programmed in the DNA but depend also on previous experiences and the animal's life conditions and environment. A horse experiencing stress, displays other behaviours than in a non-stressful situation. The horse will, on one side, show evidence of an uncomfortable or tensed feeling, and on the other side, perform coping behaviours to release stress [31].

ETHOGRAM

These typical signs and behaviours are listed in the stress-related ethogram, attached in Appendix II. The ethogram forms a complete list of all possible signs and behaviours related to stress, but a horse in stress doesn't necessarily demonstrate all the signs. Horses are individually different: every horse has its own way of expressing stress and it can moreover differ from day to day. The behaviours associated with stress in the ethogram are not exclusively demonstrated in stressful situation [70]. Some of the behaviours also occur in non-stressful situations and mean, in that case, something totally different. Since an ethogram forms a list of species-specific behaviours of an animal in a specific environment, lots of ethograms can be found in literature. Horse-specific ethograms can be found for free horses [71], [72], [73], [74], [75], stabled horses [76], [77] and horses being ridden [73], [78], [79], [80], [81], [82].

Since, in this experiment, stress is monitored during foal training, in which the foals and mares can be considered as being free, the stress-related ethogram used here is based on the horse-specific ethograms for free horses. The ethogram is further optimized by adding additional behaviours, retained from the online learning tool of the cooperation 'Groene kennis' [83] and an article of the famous dressage rider and horse training coach Appleton [70]. The final ethogram used in this experiment can be found in Appendix II.

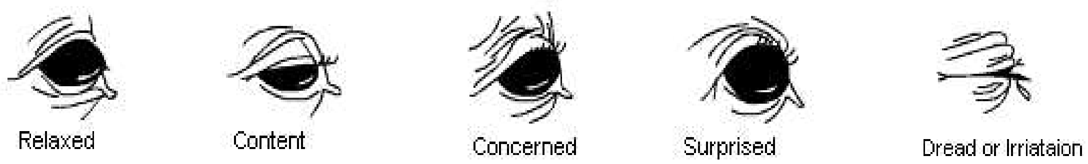


Figure 7 Facial expressions to clarify the descriptive stress-related ethogram for the eyes [207]

The ethogram consists of two parts. In the first part, signs of a tensed or uncomfortable feeling are listed. To keep a clear overview over the abundance of sings, this part is further divided into subparts. The first subpart contains a list of facial expressions that can be linked with stress and is further divided in categories: eyes, ears, mouth, tongue and nose. In the second subpart, signs regarding to different body parts are given: head and neck, legs, tail and general posture. In the third subpart, the movement is analysed, including the gait, velocity changes and sudden movements. In the fourth subpart, changes of focus are linked with stress, while the last subpart focusses on sounds related to stress. The second part of the ethogram contains the typical coping behaviours used by horses to release stress.

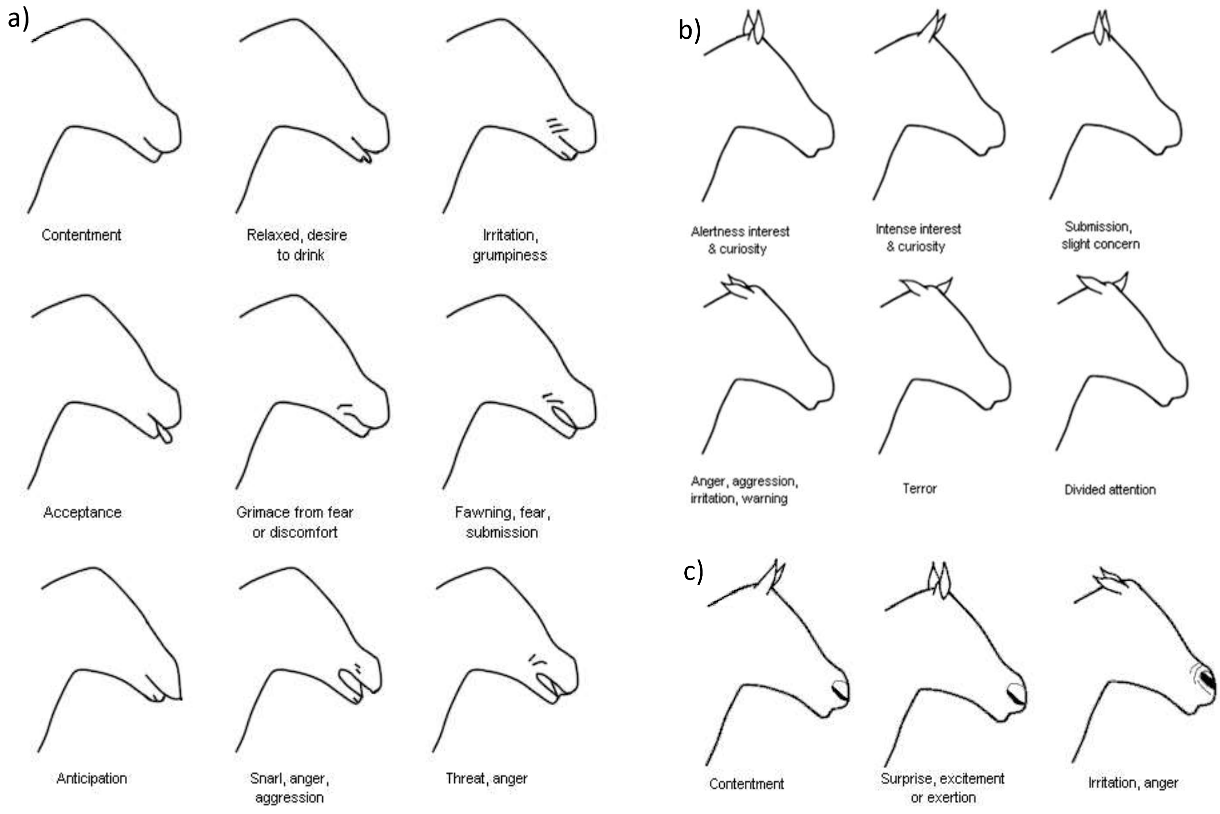


Figure 8 Facial expressions to clarify the descriptive stress-related ethogram, with a) mouth expressions, b) ear position and c) nostril in combination with ear position [207]

In the ethogram, all stress-related signs and behaviours are linked to a particular stress level, indicated as stress level 1, 2 or 3. A horse, being in stress level 1, is not experiencing any stress. The horse is relaxed and shows no stress-related signs or coping behaviours. If, however, the horse is triggered by a stressor, the stress response causes the horse to be tensed. The horse is more alert and has more attention for changes in its environment, resulting in the typical signs indicated in the ethogram under stress level 2. The more the horse is triggered, the higher the stress level of the horse will be. At a certain point, the horse will show signs of fear and behaviours indicating attempts to avoid the stressor. At this point, the horse is shifted to a stress level indicated as stress level 3. If the horse is not immediately able to avoid the stressor or release its stress, a fight-or-flight response is initiated. The horse will either fight the stressor or run away from it as a desperate last attempt to avoid the stressor. After experiencing high levels of stress, the horse will try to release the excess of stress by performing coping behaviours, indicated in the last part of the ethogram. A horse performing coping behaviours is trying to lower its stress from stress level 3 to stress level 1 and is therefore categorised as being in stress level 2.

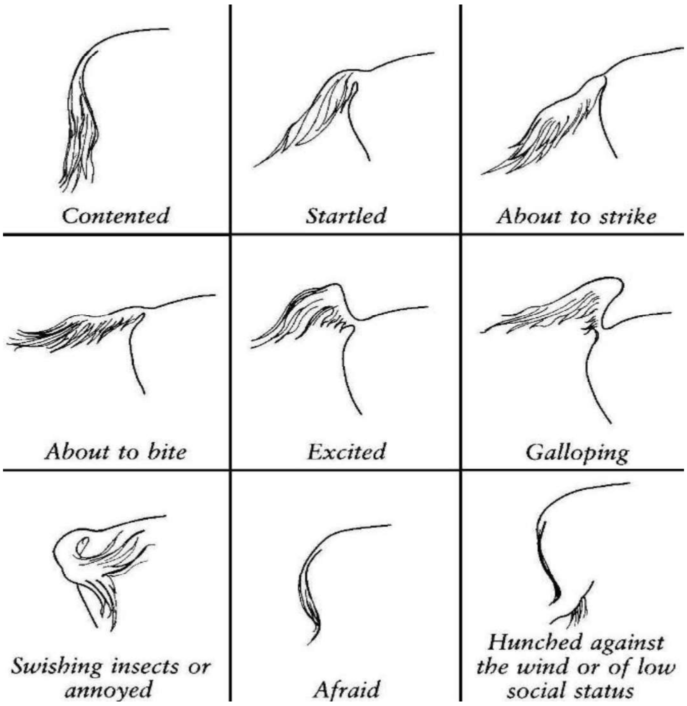


Figure 9 Tail position to clarify the descriptive stress-related ethogram [208]

To clarify the descriptive stress-related ethogram, figures about the discussed behaviours are provided. Figure 7 and 8 show facial expressions and give the associated emotions. Figure 8 includes expressions of the mouth, ears and nostrils, while Figure 7 includes expressions of the eyes. Figure 9, on the other hand, contains examples of some tail positions and their associate emotions.

CITD SYSTEM

Every living organism is a CITD system, with CITD standing for Complex, Individual, Time-variant and Dynamic. This unique combination of characteristics discriminates biological systems from physical, mechanical and electronical systems [84].

COMPLEX

Although some physical and electronical systems can be very complex, imagine the complexity of pathways in living organisms. In the previous part, for example, the description of the stress response to small perturbations in the homeostatic balance illustrates the complexity of the stress response on organism level. Imagine the complexity of mechanisms on cellular or even molecular level [84]. To illustrate the complexity, an overview of metabolic pathways is given in Figure 11.

INDIVIDUAL

Due to the complexity, all living organisms are intrinsically different. That is why all organisms react individually different to the same stimulus. To illustrate this, a simple example is given, in which the HR response to an exercise of two test persons is observed. One test person is fit, while the other test person is unfit. In the experiment, the test persons are performing the same running exercise, while the HR response is measured. In Figure 10, the course of the HR of the two subjects is plotted: in blue, the HR of the fit test person and in red of the unfit person. At the onset of the running exercise, indicated with the first arrow, the HR of the unfit person clearly rises faster than the fit person, while it also attains a higher HR during the exercise. At the end of the exercise, indicated with the second arrow, the unfit person demonstrates a longer recovery period than the fit person. Although the two test persons are preforming the same exercise, their HR response is different due to individual differences [84].

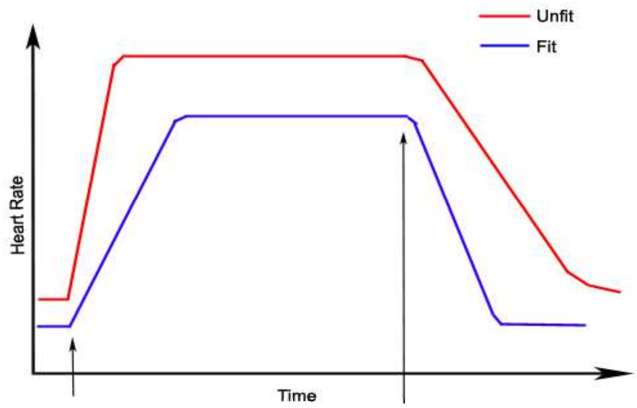


Figure 10 HR response [209]

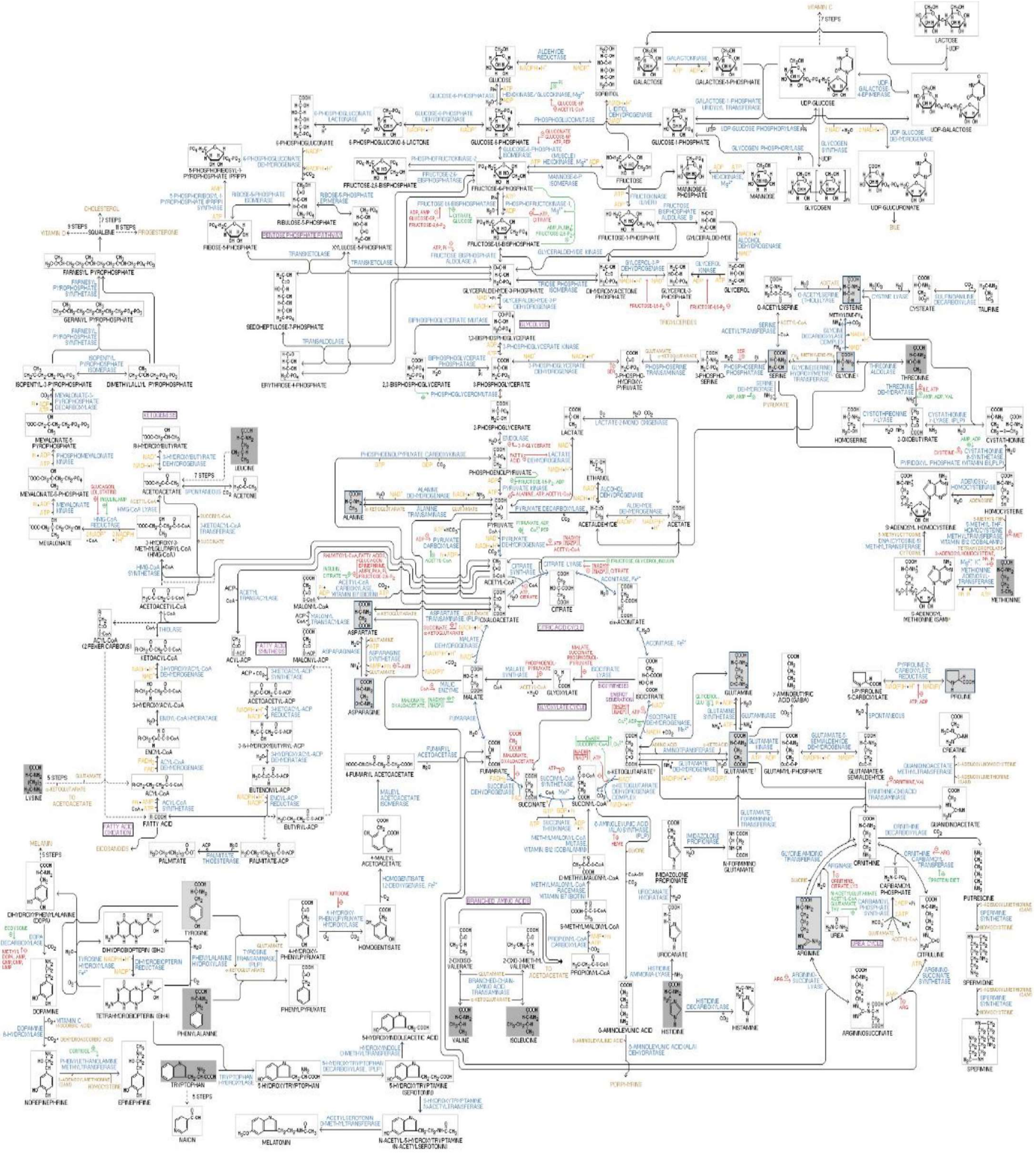


Figure 11 Metabolic pathways illustrating the complexity of living organisms [210]

TIME-VARIANT

Next to differences between individuals, variations within an individual exist as well. An organism is time-variant, meaning varying in time. For a living organism it is impossible to demonstrate the same physiological response twice. Even when the main external input is exactly the same, too many other variables, sometimes unknown or unmeasurable, have an effect on the observed response. To illustrate time-variance within an individual, another running experiment is done. The same test person is doing the same exercise at three different time instants, while the HR is measured. In Figure 12, the HR of the runner is plotted in time. Even though the exercise remained the same, the HR response is different every time [84].

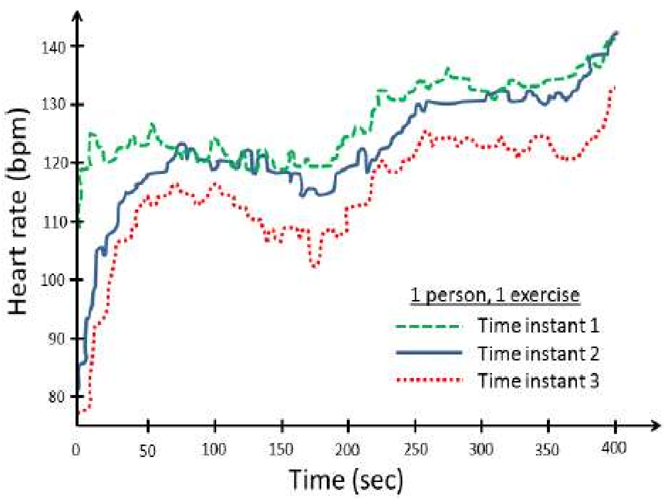


Figure 12 Illustration of time variance in a living organism [211]

DYNAMIC

The fourth unique characteristic of a living organism is the dynamic response. If a change in the internal or external environment of an organism results in the disturbance of a steady state condition, the organism will need to adapt to obtain a new steady state condition and the transition from one steady state to another, is dynamic. In Figure 13, the transition from one steady state to another is represented by a step input, in blue, while different possible dynamic responses are represented in red, green and purple. As illustrated in Figure 13, the dynamic response can be of different orders. However, in a living organism, dynamic responses are preferably first order, due to energy efficiency [84].

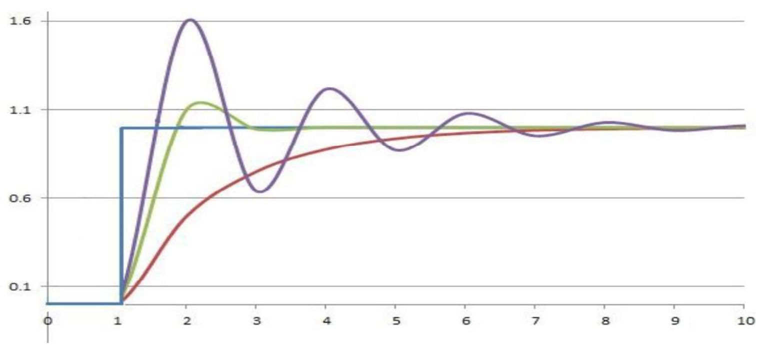


Figure 13 Dynamic response of different orders [212]

Although physical, mechanical and electronical systems can be very complex, or dynamic responses, it is the combination of the CITD characteristics Complex, Individual, Time-variant and Dynamic, that makes living organisms unique [84].

STRESS MONITORING

Because a horse is a CITD systems, finding objective measures to monitor stress becomes more difficult. The variables that change according to the stress level include stress hormone levels and physiological changes related to stress. In principle, if you can define the correlation between the variable and the stress level, the variable can be used to monitor stress. However, due to the horse's complexity, individual differences and time-variance, it is important to compare the measured variable with a correct reference.

STATE-OF-THE-ART

The state-of-the-art stress monitoring technique is measuring the hormone levels in the blood. Stress hormones, like ACTH, Ep and cortisol can be used, but cortisol is the one that is most commonly used [85], [86], [87], [88], [89].

OPPORTUNITIES

To be able to interpret the measured cortisol level, it is important to define all variables that might have an influence on the cortisol level. For example, earlier studies on horses have demonstrated that the level of cortisol not only increases in stressful situations like transportation [90] or restraint [91], but also after exercise [92]. While doing measurements, one can either avoid other affecting variables or identify them and take them into account. Cortisol is a stress hormone that is produced in the adrenal cortex in the stress response and after release transported through the blood stream. By taking blood samples, the cortisol concentration can be measured and used to determine the stress level of the horse [85], [86], [87], [88], [89].

DISADVANTAGES

Taking blood samples is, however, an invasive procedure. It will cause extra stress and consequently directly influence the cortisol level. As an alternative, the cortisol level can also be measured in the saliva. Nonprotein-bound cortisol rapidly diffuses from the blood into the saliva, which means the salivary cortisol level is directly related to the cortisol level in the blood [93]. Therefore, salivary cortisol concentration forms a good alternative. On the other hand, taking saliva samples remains semi-invasive. By taking salivary samples, the horse is still interrupted which might induce additional stress [94].

Another drawback of using cortisol concentration as to monitor stress is the discrete characteristic of blood samples. If the samples can only be taken at discrete points in time, a lot of dynamic information is lost. Besides, even though cortisol levels are widely used as a stress indicator, there is some disagreement regarding the interpretation of the data. Some authors consider small changes in the cortisol level as an indicator of stress, whereas others doubt the relevance of changes that do not exceed diurnal variations [95], [96]. Examples of a normal daily cortisol cycle, an inconsistent cycle and a horse lacking any circadian pattern, are illustrated in Figure 14. Note here that every horse is a CITD system and consequently, has its own horse-specific daily pattern, while the horse-specific pattern of a particular horse also changes from day to day [84]. Therefore, absolute cortisol measures obtain no information, but must be compared to the horse-specific daily pattern. Before deviations from the normal cycle can be investigated, the individual circadian pattern must be determined, which is a time consuming and still invasive procedure.

To overcome this problem, some researchers used the change in cortisol levels instead of the deviation from the normal pattern as an indicator for stress. Others, however, doubt the relevance, because the measured change in cortisol levels are small compared to the daily variation. Secondly, stress is not the only factor to induce deviations from the normal diurnal cycle. For example, several other psychosocial and psychiatric factors, like chronic stress, have an influence on the pattern of the diurnal cycle, as well as on the average daily level and on the flexibility of the cortisol level to change. Or, for example, unexpected cortisol levels can also result from medical issues like an impaired functioning of the ES or adrenal cortex glands [97].

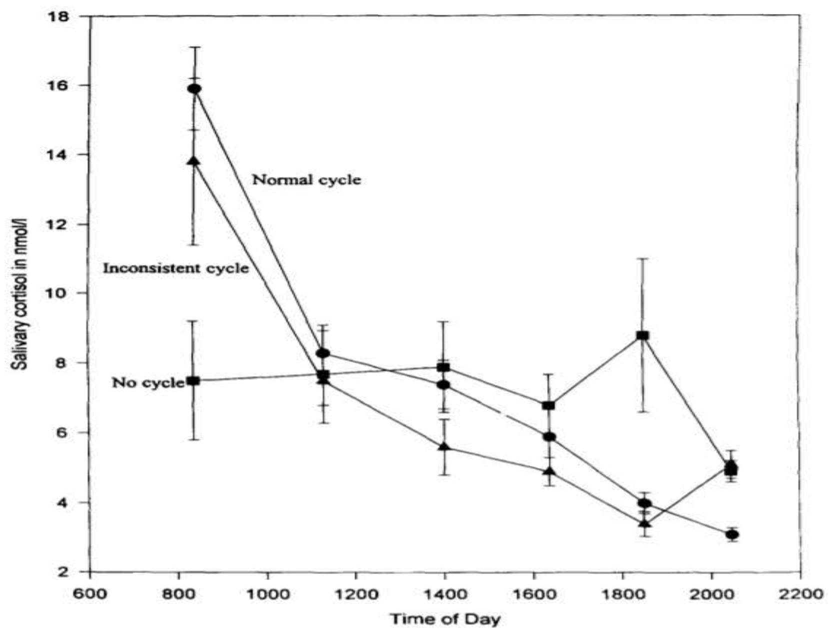


Figure 14 Example of diurnal cortisol level cycles. A normal diurnal cycle is compared with two impaired cycles: inconsistent and no cycle [213]

Because taking blood samples is an invasive technique, the cortisol levels can only be determined on discrete points in time, the horse-specific diurnal cortisol cycle, the multiple factors that might affect the cortisol level and the lack of consensus on the interpretation of the measurements, encourages the search for other techniques to objectively monitor stress.

PHYSIOLOGICAL PARAMETERS

As earlier indicated, stress is also associated with some physiological changes, like an increased HR, BP, COV and RR. These physiological variables can, beside stress hormone levels, be used as direct indicators of stress. Since they can easily be measured using less invasive or even non-invasive techniques, they might be preferential with respect to measuring hormone levels.

Since the HR is known to be strongly correlated with stress [69], and measuring HR is non-invasive, HR measurements might be the best alternative for cortisol measurements. Both the HR as well as the heart rate variability (HRV) can be used as a measure for stress. Whereas the HR represents the amount of heart beats per minute, the HRV reflects the short-term fluctuations in the HR [10]. Increased stress levels cause the HR to increase and the HRV to decrease.

The HR is simply represented by beats per minute [bpm], while the HRV can be represented using several features. For example, the standard deviation of the beat-to-beat interval (SDRR), the root mean square of successive beat-to-beat intervals (RMSSD) or the beat-to-beat interval (BTB), being the time between two successive R peaks of the QRS complex [98].

The advantage of HR measurements is that it can be done continuously. Continuous data does not only contain more information than discrete samples, it also enables real-time measurements. If the HR can be monitored in real-time, the stress levels of the foals can be monitored in real-time as well [85], [86], [89], [99]. Real-time stress monitoring enables the trainers to evaluate the effectiveness of the training on spot and enable them to adjust the training according to the foal’s current stress level. This advantage of real-time stress monitoring might improve the training efficiency significantly.

OBJECTIVE

In the context of a collaboration with the New Zealand foal training centre Foal NZ, the goal is to monitor stress during their foal trainings. In the literature review about the training program and strategy of Foal NZ it is indicated they are focussing on performing trainings in a low-stress environment. The goal of this research is to investigate the opportunities of using HR measurements to develop real-time stress monitoring, on one side, and on the other side, evaluate the foal's progress in their training program.

PROGRESS IN TRAINING PROGRAM

Based on the HR measurements, the goal is to investigate whether measures such as mean, median, and minimal HR can be used to represent the mean stress levels and whether the max HR can be used as a measure for short moments of high stress levels. To investigate how constant the stress level is throughout the training, it is tested whether the std of the measured HRs can be used as a measure.

By calculating the measures for the full training and for the respective parts of the training, it is investigated whether these measures can be used to evaluate the progress of the foals throughout their training program. To elaborate a positive progress, a decreasing mean stress level, less short moments of high stress and a constant stress level throughout the training session are required.

REAL-TIME STRESS MONITORING

Another goal is to undertake a first step in the development of real-time stress monitoring by performing off-line stress monitoring based on the HR measurements. The aim is to investigate whether the HR can be modelled and whether the model parameters can be used to monitor stress off-line.

MATERIAL AND METHODS

The measurements for the experiments were performed during foal trainings by Foal NZ, starting in the beginning of October 2017 until the beginning of November of 2017, with the approval of the ethical commission added in Appendix VII. In this period, the foals were around the age of three weeks old and performing their initial training program. After four months, the measurements were repeated during the FU training in the end of February 2018.

DATA COLLECTION

TEST HORSES

The measurements were done on seventeen foals, whereof eight fillies, which are female foals, and eleven colts, or male foals, and are listed in Table 1. The foals were all born in the period from mid-September 2017 until the beginning of October of 2017. Most foals were trained in the proximity of their own mother, but two foals were fostered by another mare, indicated in bold in the Table 1. The mares are between five and seventeen years old and have different experiences with respect to nurturing foals. One of the mares is nurturing for the first time, while others already nurtured eight foals before 2017. Because of their young age, the foals haven't gotten a name yet. Therefore, the name of the mare, or foster mare in the case of Celtic Lass and High Light, is used to refer the foal as well.

Table 1 Test mares and foals

MARE	BIRTH	FOAL	BIRTH	BROODMARE	SIRE
Black Beauty	22/11/2005	filly	23/09/2017	Black Beauty	Charm Spirit
Celtic Lass	**12/11/2011**	**colt**	**19/09/2017**	**Krysia**	**Ocean Park**
Chaperone	30/10/2007	colt	27/09/2017	Chaperone	Charm Spirit
Chita Rivera	26/10/2002	filly	03/10/2017	Chita Rivera	Mongolian Khan
Code Black	09/10/2004	colt	23/09/2017	Code Black	Charm Spirit
Conchita	22/11/2009	colt	25/09/2017	Conchita	Mongolian Khan
Dumont	24/08/2011	colt	19/09/2017	Dumont	Rip Van Winkle
Go South	24/10/2007	filly	27/09/2017	Go South	Shamexpress
Hardassah	19/10/2011	filly	20/09/2017	Hardassah	Shamexpress
High Light	**10/12/2010**	**colt**	**04/10/2017**	**Crafty Mistress**	**Rip Van Winkle**
Permanent	29/10/2013	colt	14/09/2017	Permanent	Mongolian Khan
Reigning Dynasty	19/08/2011	filly	18/09/2017	Reigning Dynasty	Charm Spirit
Stitchentyne	18/11/2009	filly	25/09/2017	Stitchentyne	Atlante
Supreme	10/10/2010	filly	15/09/2017	Supreme	Showcasing
Time For A Kip	26/09/2014	colt	13/09/2017	Time For A Kip	Shamexpress
Turkey Lowe	02/10/2012	colt	11/09/2017	Turkey Lowe	Per Incanto
Winsome Kash	29/11/2002	filly	05/10/2017	Winsome Kash	Mongolian Khan

PROTOCOL

The experiment consists of HR and ACC measurements during the initial foal trainings of the above-mentioned test horses. Every training consists of the same steps, as already mentioned in the introduction, but sometime the order of the steps is changed, or in other trainings, some steps are repeated more than once during the same session. For completeness, the training steps are represented again in Figure 15.

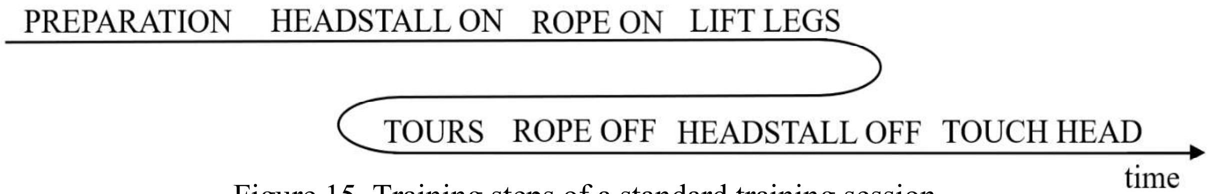


Figure 15 Training steps of a standard training session

The same three trainers are involved in all trainings: one trainer is responsible for the actual training of the foal, performing all the training steps of Figure 15. The second trainer is leading the mare, while the third trainer is responsible for the measurements and filming the training.

In Table 2, the amount of training sessions per test horse is given, as well as whether the measurements were repeated during its FU training, indicated as FU. In total, measurements were done on 107 training sessions, from whom 98 initial training sessions and nine FU sessions.

Table 2 Overview of trainings of testing foals

MARE	TRAINs		MARE	TRAINs		MARE	TRAINs	
Black Beauty	6	FU	Dumont	7		Stitchentyne	5	FU
Celtic Lass	7	FU	Go South	7		Supreme	7	FU
Chaperone	6	FU	Hardassah	3		Time For A Kip	5	
Chita Rivera	5		High Light	1		Turkey Lowe	6	
Code Black	7	FU	Permanent	6		Winsome Kash	5	FU
Conchita	7	FU	Reigning Dynasty	8	FU			

The training program can be divided into two phases, as illustrated in Figure 16. As mentioned in the introduction, Foal NZ teaches the foals two ways of leading: one while the trainer is walking next to the foal and one while the trainer is walking in front of the foal. In the first three training sessions, or PHASE I, the trainers limit themselves to the first way of leading. In the fourth training, TRAIN4, they introduce the second way of leading, signifying the start of PHASE II. In the FU session, yet another way of leading is introduced. In the first way of leading, the cue is given by a short pressure on the withers, while in the second way, a short

Figure 16 Training program consisting of PHASE I and PHASE II

pull on the rope is used. After the foal's first step forward, the cue to start walking is removed. In the third way of leading, the cue to start and keep on walking is also given by a pulling on the leading rope, but in the FU, however, the cue is maintained instead of released after the foal's first step, inducing again additional stress in the FU session.

PHYSIOLOGICAL MEASUREMENTS

PREPERATION

Before the start of a training session, the measurement equipment is put on, starting with the two HR sensors: one for the foal and one for the mare. The HR sensor is placed on the left-hand side of the body just behind the front leg, around the area of the horse's heart, as illustrated in Figure 17. To improve conduction, the skin is wetted before the HR sensor is put in place. Next, a surcingle is placed on top of the HR sensor, again, both on the foal and mare. A surcingle is a component of a horse harness or saddle fastened around the horse's girth. Normally, it is used to stabilize the weight of a rider, but in this experiment, a pocket on the surcingle is used to hold the ACC measurement equipment. Once the accelerometer is placed in the pouch of the surcingle, as illustrated on Figure 17, the measurements can start.

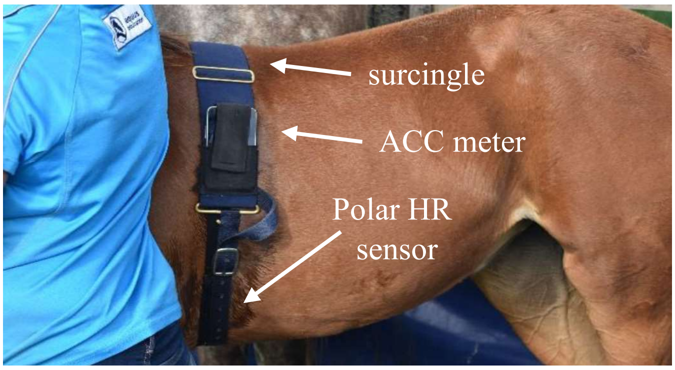


Figure 17 Foal with Polar HR sensor and surcingle

MEASUREMETNS

During every training, the HR and tri-axial ACC of both the foal and the mare are measured. The ACC data are collected using the build in accelerometer of a Samsung J1-6 SM-J120ZN smartphone, placed in the pouch of the surcingle. The ACC data are recorded using the Vieyra Physics Toolbox Accelerometer App Version 1.4.6. and measured in g-forces [m/s^2] at a sample frequency of 100 Hz. The HR is measured using Polar HR sensors, attached with a large Polar Equine Belt 80004 for the mare and with a small Polar HR Monitor Strap for the foal. The HR is measured in beats per minute [bpm] at a sample frequency of 1 Hz and automatically transmitted to the Polar Beat App Version 2.4.5 on the Samsung smartphone via a Bluetooth connection. The training is filmed using a Samsung Galaxy Note 3 Camera and recorded with a sample frequency of 30 frames per second. After completing the training, the video recording is ended and the smartphones are removed from the surcingle pockets. The HR recordings in the Polar Beat App are stopped and the data are transferred to an online Polar Flow Identity Account, on the Polar website. To avoid exchange of data between the foal and mare, both the foal and mare have their own Flow Identity Account. Also, the recordings of the ACC data are stopped. To transfer the ACC data to a computer, the smartphones are plugged in and the data of the Vieyra Physics Toolbox Accelerometer App are transferred manually to a folder on the computer.

SYNCHRONISATION

To facilitate the synchronisation of the HR and ACC recordings of both the foal and mare, all recordings are transferred to an Excel file. The HR recordings of the foal and mare are downloaded as a CSV file from their online Polar Flow Identity account and converted to a excel file, while the ACC data are manually transferred to an Excel file.

To avoid confusion afterwards, the Excel files are saved using a name with a conventional structure: the name of the mare, followed by the date of recording and 'Foal' or 'Mare' for the foal or mare respectively and ended by 'HR' or 'ACC' for the HR or ACC data respectively. For example, the Excel file of the mare's ACC recordings of Hardassah on October 12th 2018 is saved as 'Hardassah 20181012 Mare ACC'. All recordings of one training session, including the HR and ACC data of the foal and mare, are then simply synchronized by aligning the data with respect to the timelines, included in all data recordings.

Once the HR and ACC measurements are synchronised, the last step of the synchronisation procedure consists of synchronising the measurements with the videos. Therefore, a synchronisation point is determined, recurring in both the measurements and the videos. The synchronisation point is determined as the starting time of the foal ACC recordings, i.e. the moment the foal's ACC recording session is started in the Vieyra Physics App on the smartphone. To identify the start of the ACC recordings in the videos, the verbal cue "Foal accelerometer data starting NOW", is combined with a visible cue, a hand gesture at the same time of the verbal cue "NOW". Note that, for this last step in the synchronisation procedure, it is important that, at the beginning of every training session, the video is started before the ACC and HR measurement. Once the video is trimmed until just after the visible cue, deleting all video recordings prior to the "NOW", the video is synchronised with the start of the ACC recordings of the foal, and thus with the ACC and HR measurements of both foal and mare. The overall synchronisation procedure allows an accuracy of smaller or equal to one second.

GOLD STANDARD BEHAVIOUR

For all training sessions, for all horses, the gold standard stress levels were obtained by looking at the behaviour of the foals during training.

LABELLING OF BEHAVIOUR

Gold standard stress levels were obtained by labelling, or the analysis of audio-visual recordings. Using the labelling program ELAN, human observers examined the occurrence of behaviours included in the horse-specific stress-related ethogram in Appendix II. Based on the observed behaviours and the stress level linked to these behaviours in the ethogram, a stress label is indicated: stress level 1, stress level 2 or stress level 3.

To make a correct assessment of the stress level, it is important not to focus on certain body parts but look holistically to the horse. Only by considering all body parts, including eyes, ears, mouth, nose, neck-head position, shoulders, back, tail and movement, a correct assessment of the stress level can be made, keeping in mind the CITD characteristics of the horse. Every horse has its own way of showing emotions and stress. Proper knowledge of the personality of the horse and its way of communicating helps in assessing its stress levels [84].

During the labelling, the recordings are watched continuously while stress levels are assigned against a time base. A label is formed by indicating the beginning and ending of each stress level. This labelling procedure is called continuous sampling [100].

The labelling of the 107 videos is done by seven labellers who have experience with horses, whereof five female and two male labellers. The videos were randomly assigned to the different labellers to avoid one labeller to be responsible for the labelling of all training sessions of a particular horse or for all first training sessions or all the second and so on. However, a labeller, assigned to a video, was responsible for the labelling of both the foal's stress levels as well as the mare's stress levels in that video.

Once the labelling of the stress levels in ELAN was completed, the labels were saved as a text files and further processed using Matlab. In Matlab, the ELAN labels are converted into a vector containing the numbers 1,2 and 3 to indicate the stress level 1, 2 and 3 respectively, with a sample frequency of 100 Hz. This vector serves as the gold standard for the stress levels.

TRAINING PARTS

Next to the labelling of the stress levels of the foal and the mare, two other labelling procedures are performed in the ELAN labelling program: one extra for the foal and one extra for the mare. The same continuous sampling approach is used as for the stress level labelling.

FOAL

For the foal, the beginning and ending of the different parts of the training sessions are indicated, as well as the moments that the foal is walking versus standing still. In analogy to the stress level labelling, after the labelling in the ELAN program, the labels are saved as text files and further processed in Matlab.

To be able to work in analogy with the processing of the stress level labels in Matlab, the different parts of the training sessions are indicated with numbers. By using the numbers that refer to the respective parts of the training, a training vector can be created with a sample frequency of 100 Hz. In the training vector, numbers 1 refer to the preparation part of the training (PREP TRAIN), 2 refers to the moment the headstall is put on (HEADSTALL ON), 3 to the rope that is put on (ROPE ON), 4 to the moment where the trainer lifts the legs of the foal (LIFTING LEGS), 5 to the moment the rope is taken off again (ROPE OFF) and 6 refers to the moment the headstall is taken off (HEADSTALL OFF), while number 7 is used to indicate moments on which the trainer is touching the head of the foal (TOUCH HEAD). The other part of the training in which the foal is walking tours in the training area next to or around the mare, is indicated as the part between ROPE ON and ROPE OFF and is called TOURS.

The labels denoting the activity, indicated as standing or walking, are processed similarly to the stress level labels. In the activity vector of 100 Hz number 1 refers to moments where the foal is walking, while 0 represents the moments where the foal is standing still.

MARE

The additional labelling for the mare includes the representation of the focus of the mare: is the mare focused on the foal or is she distracted by something else? The moments the mare is focussing on the foal are indicated with the number 1, while the moments the mare is distracted is indicated with 2. Also, for the mare's focus, a focus vector with a sample frequency of 100 Hz is rendered. For the mare, the activity vector, indicating when the mare is walking versus standing still, is created in the exact same way as it was done for the foal.

DATA ANALYSIS

After the data collection, the data analysis can start. The analysis is performed in Matlab and includes the pre-processing of the measured HR and ACC data, the labeller analysis, HR analysis, stress level analysis and modelling.

PRE-PROCESSING

CALIBRATION

The ACC data is measured in three directions using three separate ACC sensors, having their own baseline. The measurements are illustrated in Figure 18, with in blue and yellow the ACC in the x and y direction respectively, running in parallel with earth's surface and perpendicular with respect to each other. The ACC data in the z direction, namely perpendicular to the earth's surface, is indicated in red.

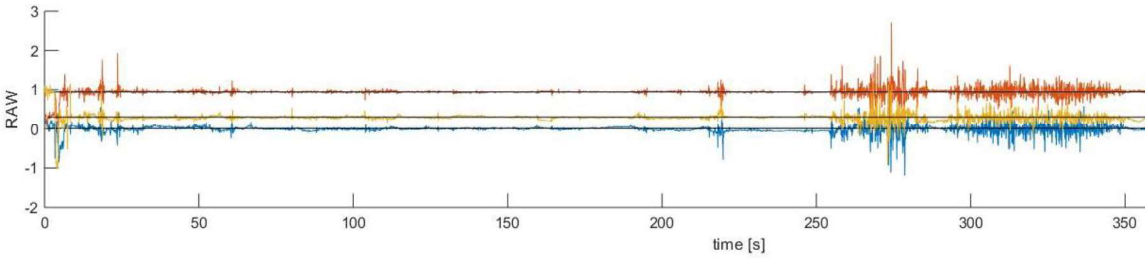


Figure 18 ACC measurements in the x,y and z-direction with respective baselines (black)

The baseline in the z direction is equal to 1.0000 g, representing earth's gravity, while the baselines in the x and y direction are calculated as 0.0145 g and 0.3034 g respectively. In Figure 18, the baselines are plotted on top of the ACC data. The off-line calibration of the ACC data then consisted of extracting the respective baselines from the ACC measurements in the x, y and z-direction.

OUTLIERS

Outliers appearing in the measurement can have different causes. For the HR measurements, loss of contact of the sensor with the skin is the main reason for the outliers to arise. Outliers are characterised by extremely high or low values. For example, HR values below 30 bpm and over 230 bpm are unrealistic and can be categorised as outliers. The outliers are detected by calculating the differential of the HR recording to represent the instantaneous changes in the HR. Where in time, the changes are higher than a manually determined threshold, the recordings are categorised as outliers and set to NaN. Afterwards, the NaN values are replaced using spline interpolation, using the Matlab function *interp1* and settings *'spline'*. At the end of every training session, the recording equipment is taken off, resulting in unmeaningful data at the end of the recordings lines. Since this part of the data part can also be categorises as outliers, the end of the recordings is deleted since the equipment is removed.

CALCUATION ACTIVITY

Based on the off-line calibrated ACC data, for convenience, here represented as x, y and z, the activity ACT is calculated using the following equation

$$\text{ACT} = \sum_{i=1}^{N} \sqrt{x_i^2 + y_i^2 + z_i^2}$$

with N = the amount of sample points. Since the ACC data is measured at 100 Hz, the obtained ACT vector has also a sample frequency of 100 Hz.

RESAMPLING

For some parts of the analysis, which will be discussed in the next session, a sample frequency of 1 Hz is required for the HR recordings, as well as the ACT. For others, a sample frequency of 100 Hz is desired. Therefore, the ACT data is resampled from 100 Hz to 1 Hz and the HR measurements, from 1 Hz to 100 Hz.

LABELLER ANALYSIS

The have an idea about the consistency of the labelling procedure, the intra-observer reliability (IOR) and the inter-observer variability (IOV) is calculated. The IOR is a measure for the consistency and reliability of a labeller, while the IOV is a measure for the constancy of a labellers with respect to each other. For the calculations of both measures, the gold standard vectors with sampling frequency of 100 Hz are used.

INTRA-OBSERVER RELIABILITY

The IOR is a measure for how consistent a labeller is in assessing the stress labels. To enable the comparison between labels assessed by the same labeller, every labeller is asked to relabel two of its videos a few months after the initial labelling. The initial labels are then compared to the labels indicated during the relabelling by comparing every sample point.

Two approaches are used to calculate the IOR. In the first approach a sample point is considered as an agreement point (A) if the labeller has appointed the same stress level twice, while it is considered as a disagreement point (D) if the assessed labels don't agree. The agreement points A_{11}, A_{22} and A_{33} in Table 3 refer to agreement in stress level 1, 2 and 3, respectively.

The disagreement points D_{ij} represent the sample points on which the labeller indicated the point as being in stress level i during the initial labelling and as stress level j during the relabelling, with $i \neq j$.

Table 3 Intra-observer Approach 1

		relabel		
LEVEL		1	2	3
initial	1	A_{11}	D_{12}	D_{13}
	2	D_{21}	A_{22}	D_{23}
	3	D_{31}	D_{32}	A_{33}

Table 4 Intra-observer Approach 2

		relabel		
LEVEL		1	2	3
initial	1	A_{11}	S_{12}	D_{13}
	2	S_{21}	A_{22}	S_{23}
	3	D_{31}	S_{32}	A_{33}

For every sample point, the number 1 is assigned to one of the agreement of disagreement points. For example agreement point A_{11} is 1 if the labeller indicated level 1 during both labelling sessions, or A_{22} is 1 if the label of stress level 2 was indicated twice, and so on. If the labeller indicated, however, indicated stress level 2 during the first labelling and 3 during the relabelling 1 is assigned to D_{23}. Subsequently, for every sample point i, the variable

$$A_i = A_{11} + A_{22} + A_{33}$$

is calculated and for N = the amount of sample points, the intra-observer reliability can then be calculated as

$$\mathrm{IOR} = \frac{1}{N} \sum_{k=0}^{N} A_i \qquad [101]$$

In the second approach, besides the agreement and disagreement points a third kind of point is introduced, namely the semi-agreement points, indicated with an S, see Table 4. A sample point is indicated as a semi-agreement point, if the initial label only differs one stress level from the relabelled label. Because in the case of a semi-agreement point S_{ij}, the label i and label j still differ by one stress level, a semi-agreement point may only count for 0.5 instead of 1. Therefore, in the recalculation of IOR for the second approach, the A_i term is recalculated as

$$A_i = A_{11} + A_{22} + A_{33} + 0.5\,(S_{12} + S_{23} + S_{32} + S_{21})$$

while the formula for IOR can be reused.

INTER-OBSERVER VARIABILITY

The IOV is a measure that is used to compare the labellers with respect to each other. To make a comparison possible, two of the videos were labelled by all the labellers. For the IOV, the same two approaches are used as for the IOR calculations. Here, however, for every sample point, the stress levels denoted by labeller X, are compared to the level denoted by labeller Y.

STRESS LEVEL ANALYSIS

For the stress level analysis, the gold standard vectors are used to calculate the percentage of time the horse is in stress level 1,2 or 3 respectively. The calculations were also performed in Matlab using the functions *find* and *sum*. In analogy to the HR analysis, the analysis is repeated for the respective parts of the training.

HR ANALYSIS

In the HR analysis, features are calculated based on the HR recording and used to investigate the opportunity to use them to evaluate the progress of the foal in its training program. For both the foal and the mare, for every training session of every horse, the median and mean HR are calculated. The calculations were performed in Matlab using the functions *median* and *mean*. Also, the standard deviation (std) of the HRs measured during the training were calculated, as well as the min and the max HR, by using the Matlab functions *std*, *min* and *max*. To enable an analysis of the different parts of the training session, the median, mean, std, min and max HR were also computed for every part of the trainings respectively.

For a correct assessment of the significance of the differences between respective trainings a pairwise comparison is performed between the first training session TRAIN1 and the other sessions. To select the appropriate statistical approach, the data are first tested for homoscedasticity and normality using the Levene test and the Lilliefors test respectively with a significance level $\alpha = 0.05$. In case the data are both normal distribution and both groups have an equal variance, a two independent samples t-test with significance level $\alpha = 0.05$ is applied. If, however, normality is met, but both groups are characterized with unequal variances, a Welch's test with $\alpha = 0.05$ is used. In case the data are not normally distributed, but have equal variances, the comparison is done by means of the Mann-Whitney U test, with $\alpha = 0.05$. In case the data lack normality and homoscedasticity, both the Welch's test and the Mann-Whitney U test are applied. In this case, the trainings are only considered significantly different if both methods indicate a significant difference. For a final assessment of the significance of the difference between two trainings, the effect size is calculated using Cohen's d. In Table 5, the size of the effect is compared to the calculated d's. The bigger Cohen's d, the higher the effect size. To compare the differences between respective parts of a training session, for example compare HEADSTALL ON with LIFTING LEGS, the same procedure is applicable.

Table 5 Cohen's d and corresponding effect size

Cohen's d	Effect size
0.01	very small
0.20	small
0.50	medium
0.80	large
1.20	very large
2.00	huge

MODELLING

The final goal of this part of the research is to control the HR of the foals during their training sessions. Controlling HR is controlling a physiological parameter of a living system, or more generally, controlling output(s) of a biological process. The output of a biological system can be controlled by regulating the input(s) of the system with an actuator. This controlling principle is illustrated in the general scheme of Figure 19. Before control over the output(s) of a biological system can be established, an important first step is to identify the input(s) that have an influence on the output(s) of the system.

For this purpose, a mathematical model is estimated that represents the transfer of the input(s) into the output(s) of the biological system. Once the model of the biological system is known, it can be used to either monitor a status of the biological system or control the output by actuation the system with a particular input. To have control over a biological system, three essential steps are involved: 1) identify and measure the input(s) and output(s), 2) estimate a model that represents the biological system and eventually 3) manage the system, or in a nutshell measure, model, manage [84].

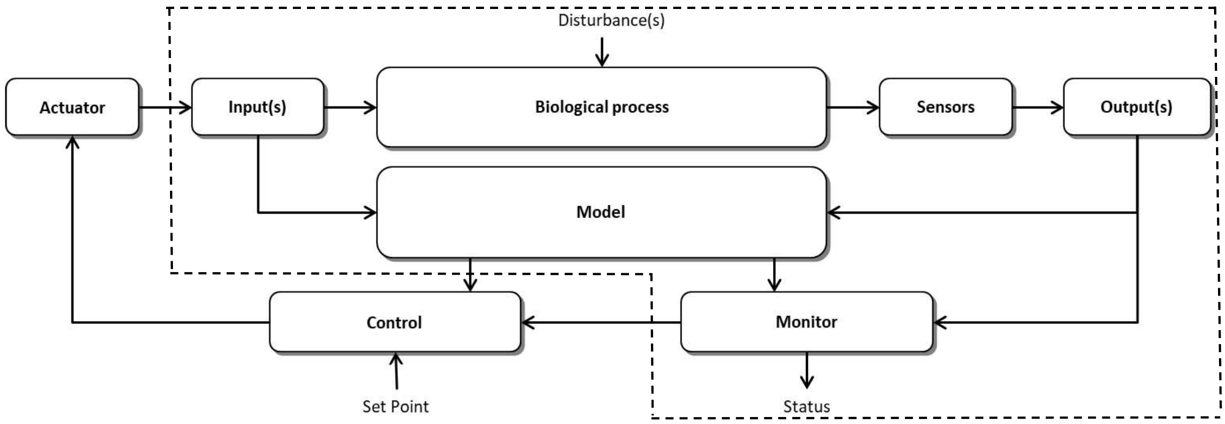


Figure 19 Measure, model, manage scheme [214]

BIOLOGICAL SYSTEM

By applying the approach of Figure 19 on this research, the foals are considered the biological system. The measured HR is considered the output of the biological system, and the ACT the input that has an influence on the output, while the psychological stressors are considered disturbance(s) for the biological system. The aim of the research is to estimate a model that represents the foal as a biological system that transfers the changes in ACT into the measured HR, while psychological stress disturbs the system. Additionally, the estimated model can be used to monitor the stress level of the foals as a status of the biological system. The parts of the scheme in Figure 19 relevant for this research are indicated with a dashed line [84].

Translating the general scheme to this research, results in the scheme of Figure 20. The foal is taking the place of the biological system, while the HR is considered as the output of the biological system and the ACT the main input that has an influence on the output. Since it is difficult to translate psychological stressors into numbers, the psychological stress is considered as a disturbing factor. A model, estimated using measurements of the inputs and the output, can be used to monitor the stress as a status of the biological system.

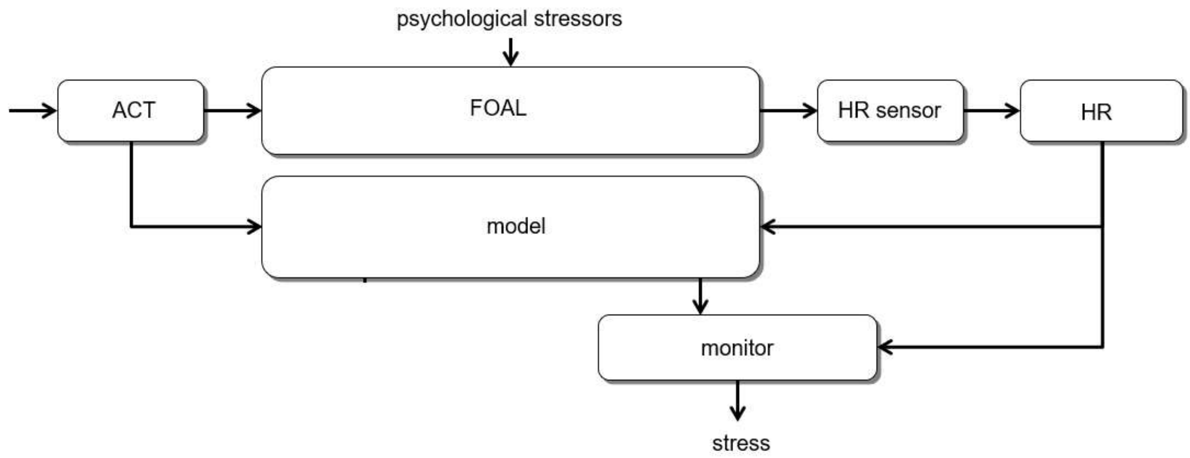


Figure 20 Measure, model, manage scheme applied on this research [214]

The estimated models are purely based on measurements of the inputs and output, and therefore referred to as data-based models. This type of models is also known as black box models because no prior knowledge of the biological system is taken into account. For the estimation of the biological system's model, two approaches are used: time-invariant models and time-variant models. Time-invariant models are defined as models with constant model parameters, meaning the parameters don't change in time. Contrarily, the model parameters of time-variant models may vary in time.

TIME-INVARIANT MODELS

In the time-invariant approach, the models are estimated using a Single Input Single Output (SISO) model, illustrated in Figure 21. Applying the SISO model of Figure 21 on this research results in the SISO model represented in Figure 22.

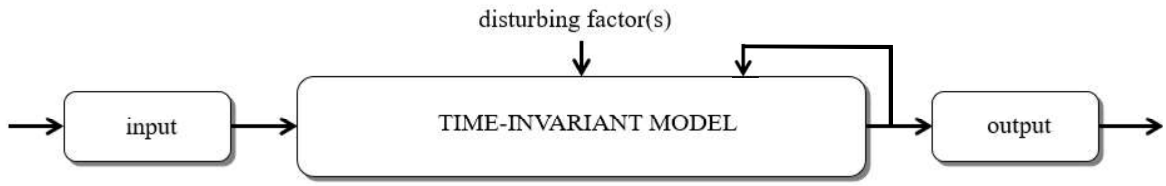


Figure 21 SISO time-invariant model

SISO MODELS

Since it is difficult to translate psychological stressors into numbers, the psychological stress is considered as a disturbing factor in the estimation of the SISO model representing the transfer of the ACT input into the HR output.

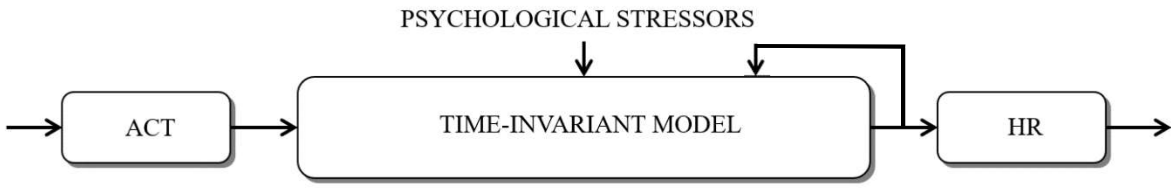
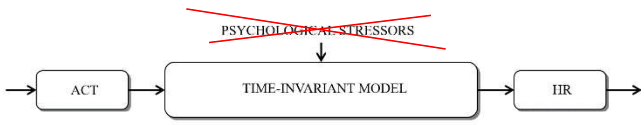


Figure 22 Applied SISO time-invariant model

The goal is to estimate the SISO time-invariant model that links the changes in HR to the variations in activity at a sample frequency of 100 Hz. To be able to capture only the changes in the HR that are the result of variations in the activity, the model must be estimated in a part of the training in which the foal is experiencing no stress, i.e. while the foal is in stress level 1. In general, based on the gold standard stress levels, two different conditions can be distinguished during a training session: CONDITION I and CONDITION II, illustrated in Figure 23 and Figure 24.

CONDITION I

stress level 1

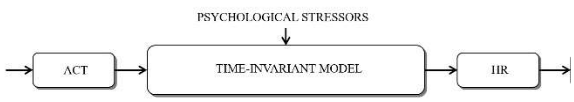


$$y(k) = \frac{b_0 + b_1 q^{-1} + \cdots + b_m q^{-m}}{1 + a_1 q^{-1} + \cdots + a_n q^{-n}} u(k-d) + e_I(k)$$

Figure 23 CONDITION I during training

CONDITION II

stress level 2 or 3



$$y(k) = \frac{b_0 + b_1 q^{-1} + \cdots + b_m q^{-m}}{1 + a_1 q^{-1} + \cdots + a_n q^{-n}} u(k-d) + e_{II}(k)$$

Figure 24 CONDITION II during training

In CONDITION I, the horse experiences no stress, so a SISO model estimated into CONDITION I is not disturbed by psychological stressors. Then, to enable the estimation of a SISO model, a step input is required, meaning an excitation step of the activity, followed by a steady state activity. The step input can be either going from no or low activity to a higher activity (step up) or going from a higher activity to a lower or no activity (step down).

To estimate the SISO model for CONDITION I, a part of the training session is selected in which both conditions are fulfilled, meaning a part in which the foal was labelled as stress free and exhibiting activity representing a step input. The estimated backward shift SISO transfer function model then represents the change in HR output y with respect to variations in the ACT input u exclusively, given by

$$y(k) = \frac{b_0 + b_1q^{-1} + \cdots + b_m q^{-m}}{1 + a_1q^{-1} + \cdots + a_nq^{-n}} u(k-d) + e_I(k)$$

with y(k) the output HR at time k; u(k) the input at time k; q^{-1} the backward shift operator; d the time delay of the model; e_I(k) the model error at time k.

The model is uniquely characterised by the model structure [n m d] with n number of a-parameters, m the number of b-parameters and d the time delay and the model parameters $a_1, a_2, \dots a_n$ and $b_0, b_1, b_2, \dots b_m$. For the identification of the model structure, the Matlab function *rivbjid* is used. 150 initial models are estimated with model structures going form first order until fifth order for both the a- and b-parameters and a delay between 0 and 5.

From the initial models the best model is selected based on the YIC value and R^2 as selection criterions. The R^2 is a measure for the goodness of fit and is calculated based on the fitting error. The YIC or Youngs Identification Criterion is another model identification criterion being a measure for how well the estimated model explains the data, but also takes into account over-parameterisation. The YIC is a logarithmic measure and therefore typically negative. The more negative the YIC value because the smaller the model error the more negative the YIC becomes, but the more parameters in the model, the higher the YIC. In general, the more model parameters estimated, the better the model can fit the output, the higher the R^2. Selecting the best model only based on the R^2 would favour higher order models, and therefore the YIC value is used as main identification criterion because the YIC value represents the trade-off between the amount of model parameters and the goodness of fit [102]. The best model is selected as the model with a low YIC value, being a large negative value, and a relatively high R^2. Once the model order is selected, the model is re-estimated using the Matlab function *rivbj*. The SISO models are estimated first by using the ACT, calculated based on the ACC measurements and secondly by using the labelled ACT.

For every horse, foal and mare, the SISO model in CONDITION I is estimated. The SISO models represent the relation between variations in the activity and the resulting changes in the HR. If we use this model to simulate the HR with the ACT input, the changes in the HR in the simulation are due to activity variations only. The difference between the measured HR and the simulated HR, indicated as ΔHR, is representative for the HR changes due to psychological stressors [103]. The principle is illustrated in Figure 25.

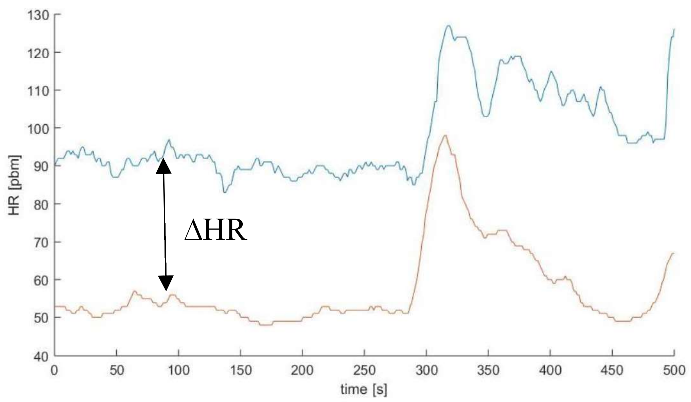


Figure 25 ΔHR as a feature for HR changes due to psychological stress with in blue the measured HR and in red the simulated HR using the SISO model

TIME-VARIANT MODELS

Time-variant models are, in analogy to time-invariant models, characterised by a model structure and model parameters. For time-variant models, however, the model parameters can change in time, also referred to as dynamic model parameters.

MISO MODELS

As already mentioned in the previous section, it is hard to translate psychological stressors into numbers, making it impossible to incorporate them into models. To find a measure that can be used to monitor stress, the following principle is applied.

First a dynamic autoregressive time-variant model with two ACT-related inputs is estimated, as illustrated in Figure 26. The ACT-related inputs represented as WALKING and STANDING. The WALKING vector is the activity vector of 100 Hz containing ones and zeros that was made based on the labelling procedure to determine the different parts of the training sessions resampled to 1 Hz. In WALKING the ones refer to the moments the foal is walking and the zeroes indicate the moments the foal is standing still. The STANDING input, on the other hand, contains ones when the foal is standing still and zeros when the foal is walking. Since the same sampling frequency is required for the input and output while modelling, the output HR of 1 Hz is used. Because the model is estimated using two input variables, the model is referred to as a Multiple Input Single Output or MISO model.

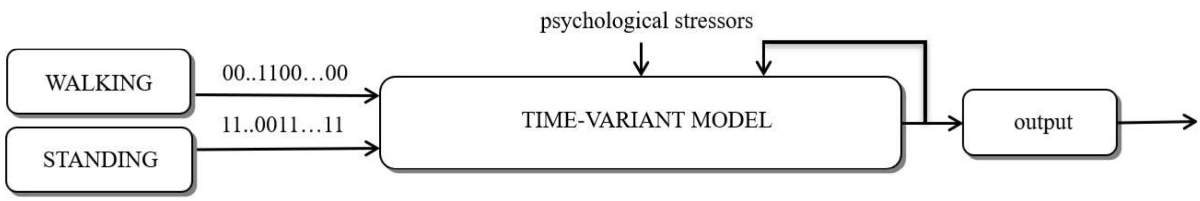


Figure 26 MISO time-variant model

The dynamic autoregressive time-variant model is estimated using the *darx* function in Matlab, with DARX standing for Dynamic AutoRegressive model with multi-eXogenous input variables. The general expression for the DARX model is given by

$$y(k) = \frac{B_1}{\mathrm{A}} u_1(k - d_1) + \frac{B_2}{\mathrm{A}} u_2(k - d_2) + \cdots + \frac{1}{\mathrm{A}} e(k)$$

with polynomials

$$\begin{aligned} \mathrm{A} &= 1 + a_1 q^{-1} + \cdots + a_n q^{-n} \\ B_{\mathrm{i}} &= b_{\mathrm{i}0} + b_{\mathrm{i}1} q^{-1} + \cdots + b_{im} q^{-\mathrm{i}m} \end{aligned}$$

with y(k) the output HR at time k; $u_1(k)$ the i$^{\mathrm{th}}$ input at time k; q^{-1} the backward shift operator; d_{i} the time delay for the i$^{\mathrm{th}}$ input; e(k) the model error at time k. For the estimation of the models, different model orders are tested for both the input and output polynomials.

AUTOREGRESSIVE MODELS

Another time-variant model that is estimated in the context of the modelling procedure is the time-variant autoregressive model, also known as Dynamic AutoRegressive or DAR models, illustrated in Figure 27. In an autoregressive model the output at time k is expressed as a function of previous outputs at time (k – 1), (k – 2), … (k – n) and the general expression is

$$y(k) = a_1\, y(k-1) + a_2\, y(k-2) + \cdots + a_n\, y_{\mathrm{n}}(k - \mathrm{n})$$

with a_1, a_2, … , a_{n} the time-variant model parameters.

For the estimation of the DAR models only the output HR vector is needed. Using the HR vector of 1 Hz, the time-variant model parameters are estimates. The first step is again the identification of the model structure by trying all possible combinations, going from first order until fifth order.

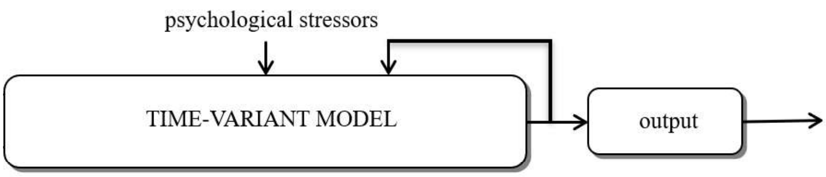


Figure 27 DAR time-variant model

MONITOR STRESS

Once a model is estimated, the model parameters can be used to monitor stress. While working on the long term towards real-time stress monitoring, the aim of this research is to use the model parameters and link them off-line to the gold standard stress levels. The general approach of the off-line monitoring procedure is visualised in Figure 28.

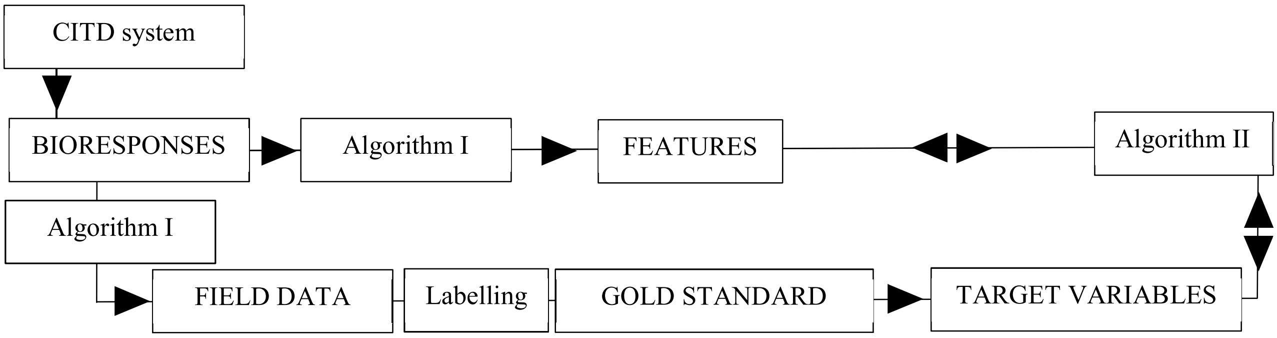


Figure 28 General approach of the data analysis [84]

In off-line monitoring, measured biological responses of a CITD system are used to calculate features, using Algorithm I. In the context of this research, the HR is modelled in Algorithm I and the model parameters are used to calculate features. To test whether these features can be used to monitor stress, they are compared with the gold standard stress levels in Algorithm II. The gold standard stress levels are referred to as target values in Figure 28 and are determined based on the field data, or in this research the video recordings of the training sessions. In the labelling procedure, the human observers investigated the occurrence of stress related-behaviours, the gold standard, and indicated directly the target variables: stress level 1, level 2 or level 3. In Algorithm II, the correlation between the calculated features and the gold standard target values determines whether the features can be used for monitoring [84].

Based on the findings of the modelling procedure and the analysis of the autoregressive model parameter a_1 in relation to the gold standard stress levels, four different approaches are used to calculated possible features that might be used for monitoring. The calculations of the features are based on the change of the a_1 parameter in time, with the overview given in Table 6. The calculated features are basically vectors containing zeros and ones, while F4 also contains twos.

Feature F1 is defined as one when the a_1 parameter is decreasing and as zero if the a_1 parameter is increasing. The ones of F1 represents the occurrence of stress, or the foal being in stress level 2 or level 3. The second feature F2 is defined as one when the a_1 parameter is decreasing and smaller than -1, while zero otherwise and is used to identify moment the foal is in stress level

3. Feature F3 is an adapted version of F2. For F3, stress level 3 is maintained for as long as the a_1 parameter is smaller than -1, although the a_1 parameter is not decreasing anymore. F3 is also used to indicate stress level 3. Feature F4 is, in contradiction to F1, F2 and F3, based on the derivation of the a_1 parameter with respect to -1. If the derivation is larger than threshold 1, feature F4 is one, and indicates stress level 2 in the training session and if the derivation is larger than threshold 2, F4 is two and indicates stress level 3.

Table 6 Possible features to monitor stress

	DESCRIPTION	STRESS LEVEL	
F1	a_1 decreases	level 2	level 3
F2	a_1 decreases and $a_1 < -1$		level 3
F3	F2 continued while $a_1 < -1$		level 3
F4	a_1 derivation > threshold 1	level 2	
	a_1 derivation > threshold 2		level 3

For all the training session of all horses, the four features F1, F2, F3 and F4 are calculated and compared to the gold standard stress levels, as illustrated in Figure 29.

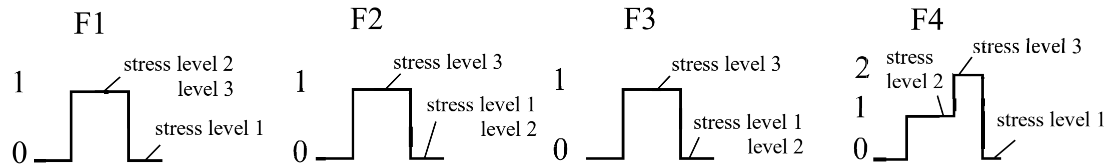


Figure 29 Comparison of F1, F2, F3 and F4 with gold standard stress levels

RESULTS AND DISCUSSION

LABELLER ANALYSIS

In the labeller analysis the IOR and IOV are calculated, with IOR a measure for the consistency and reliability of a labeller and IOV a measure for the constancy of the labellers with respect to each other. By looking at the IOR and IOV values of the labeller, the human observers can be compared with respect to each other.

INTRA-OBSERVER RELIABILITY

The IOR is a measure for how consistent a labeller is in assessing the stress labels. For the seven labellers, the calculated IOR values, based on both approaches, are given in Table 7. The IOR values of the first approach represent the exact comparison of the labels as denoted by the respective labellers. This means that, on average, the labellers were able exactly reproduce the same labels twice for 60.92 % of the labels. The IOR values of the second approach, take into account small deviations in the relabelling with respect the initial labelling. Labels that only vary one stress level from the initial labelling, are taken into account with a factor 0.50. For the second approach, the mean IOR is calculated as 79.23 %. This means that on average, (79.23 % - 60.92 %) x 2 = 36.62 % of the labels are denoted with an error of one stress level in the second labelling. This implies that, on average, only 100 % – (60.92 % + 36.62 %) = 100 % – 97.54 % = 2.46 % of the labels are relabelled with an error of two stress levels. The IOR values above the average are indicated in bold in Table 7. The same calculation is repeated for the respective labellers and the percentages of labels relabelled with an error of one stress level and two stress levels, are added in Table 7 in column ERROR 1 and ERROR 2, respectively.

Table 7 IORs for the two approaches

	Approach 1	Approach 2	ERROR 1	ERROR 2
LABELLER 1	**0.70556**	**0.84878**	**0.28640**	**0.00800**
LABELLER 2	**0.64917**	**0.81987**	**0.34140**	**0.00940**
LABELLER 3	**0.75607**	**0.87483**	**0.23750**	**0.00640**
LABELLER 4	0.54618	0.73381	0.37530	0.07860
LABELLER 5	0.25909	0.59933	0.68050	0.06040
LABELLER 6	**0.75784**	**0.87684**	**0.23800**	**0.00420**
LABELLER 7	0.59057	**0.79234**	**0.40350**	**0.00590**

By comparing the IORs of the respective labellers, LABELLER 1, 2, 3, 6 and 7 are explicitly more consistent than LABELLER 4 and 5, whit LABELLER 6 being the most reliable.

INTRA-OBSERVER VARIABILITY
In the IOV calculations, the labels of the respective labellers are compared with respect to each other. The IOVs based on the first approach are given in Table 8 and the IOVs according to the second approach in Table 9. The calculated IOVs based on the first approach, indicate the percentage of labels denoted as the same label by the respective labellers. On average, the labellers were able to indicate the same label for 57.09 % of the labels. The IOV values that are higher than the average value of 57.09 % are again indicated in bold in Table 8.

Table 8 IOVs according to the first approach

	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6
LAB 1						
LAB 2	0.46781					
LAB 3	0.43547	**0.87488**				
LAB 4	0.47476	**0.77640**	**0.79589**			
LAB 5	**0.69507**	0.45770	0.42169	0.45944		
LAB 6	**0.74593**	0.35344	0.31237	0.34808	**0.71077**	
LAB 7	**0.65694**	**0.57810**	0.55514	0.56629	**0.69580**	**0.60764**

Like for the IORs, in the second approach for the IOV calculations, errors of one stress level are taken into account with a factor 0.50. The IOVs calculated by using the second approach are on average 77.29 %. The IOVs higher than the average are indicated in bold in Table 9. Consequently, it can be calculated that the observers label on average (77.29 % - 57.09 %) x 2 = 40.40 % of the labels with an error of one stress level. This means, the observers only label with an error of two stress levels for 100 % – (57.09 % + 40.40 %) = 100 % – 97.49 % = 2.51 % of the labels.

Table 9 IOVs according to the second approach

	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6
LAB 1						
LAB 2	0.71421					
LAB 3	0.69875	**0.93744**				
LAB 4	0.72675	**0.88767**	**0.89758**			
LAB 5	**0.84198**	0.70899	0.69115	0.71902		
LAB 6	**0.86938**	0.64291	0.62499	0.65217	**0.84768**	
LAB 7	**0.82483**	0.77168	0.75831	0.76997	**0.84343**	**0.80166**

By looking at Table 8 and Table 9, it is conspicuous that LABELLER 1, 5, 6 and 7 are denoting the same labels, while the same is noticed for LABELLER 2, 3 and 4. However, LABELLER 2 and 7 are also reasonably consistent with respect to each other in assessing labels. The result of the IOVs and the IORs are similar, meaning the variations within the labellers in time is similar to the variation between the labellers.

STRESS LEVEL ANALYSIS

In the stress level analysis, the gold standard stress levels are analysed. The analysis involves the evolution of the foal's stress in time and over the respective parts of its training, looking for eventual differences between the respective parts. The stress level analysis ends with a short analysis on the mare's stress levels as well.

FOAL

To make progress in their training program, the foals needs to demonstrate improvement in three different aspects. First, a lower mean stress over the training is required. Secondly, progress involves the reduction of the occurrence and intensity of short moments of high stress levels. The last requirement has to do with the dynamic range of the HR within a training session. The lower the dynamic range of the HRs measured within a certain time interval, the more constant the stress level of the foal in that time interval. To ensure progress in the training program, improvement on the mean stress level, the moments of high stress levels and consistency of stress is required.

PROGRESS

The bar graph of Figure 30 represents the gold standard stress levels of the foals at group level, from the first training, until the FU session. The percentage of time the foal is in stress level 1, 2 or 3 is illustrated with a white, grey or black bar, respectively. At group level, the progress throughout the training program is less clear, although, on average, the percentage the foal is in stress level 3 is slightly lower in the second and in the sixth training.

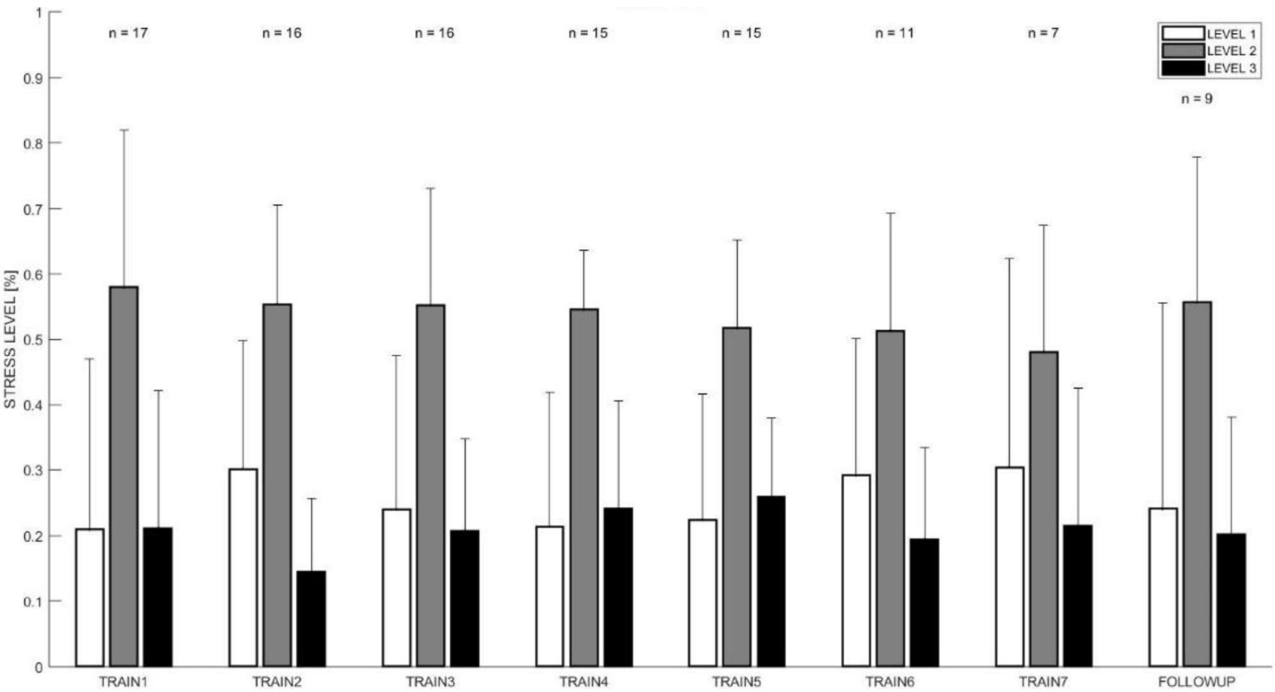


Figure 30 Evolution of gold standard stress levels of entire training at group level

Although at group level, the progress throughout the training program is less clear, the progress is visible by looking at the foals respectively. To illustrate the desired progress of a foal, the example of Conchita is given in Figure 31. In its first training session, the foal is 54.9 % of the time experiencing stress level 3. By looking at the evolution of this percentage in the first four sessions, referred to as PHASE I, a clear decay is visible, going from 54.89 %, to 31.51 % in the second, 26.89 % in the third and only 3.06 % in the fourth. At the same time, the time the foal spends in the stress level 1, increases from 2.84 %, to 3.90 % in the second training, until 25.10 % in the third, to end up with 39.19 % in the fourth session. The foal is clearly getting used to the trainings and experiences less stress, decay in stress level 3 is indicated in blue in Figure 31.

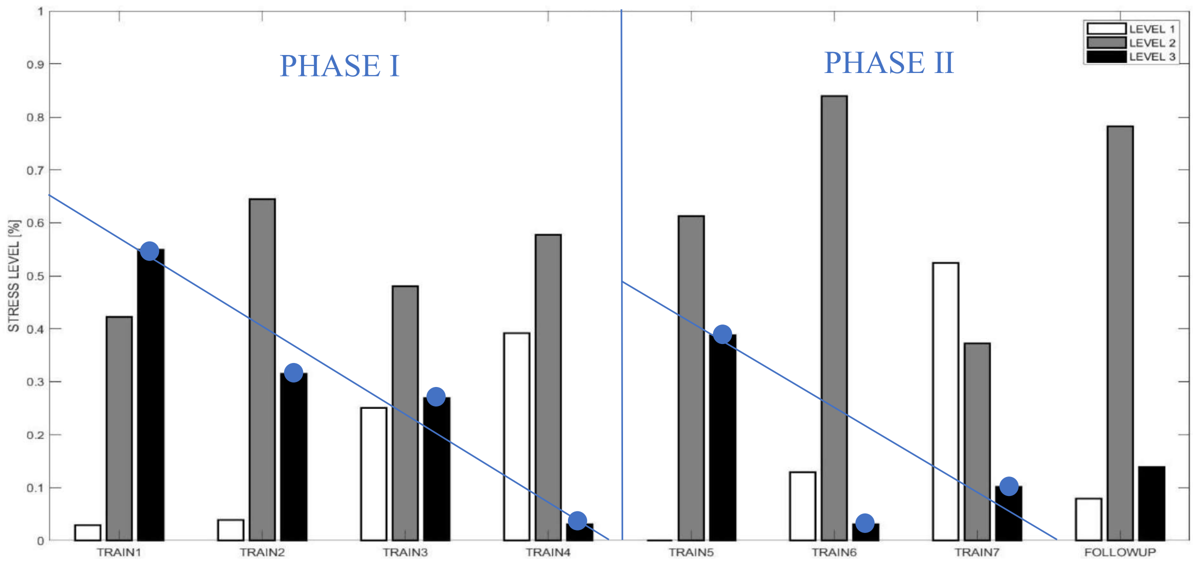


Figure 31 Evolution of gold standard stress levels of entire training of Conchita

In the fifth training, for Conchita the start of PHASE II, the second approach of leading is introduced, indicated with a vertical blue line. This resulted in an explicit increase of stress level 3 until 38.81 %, while the time the foal experiences no stress almost drops to zero. In the sixth training session, the newly introduced exercise causes less time spent by the foal in stress level 3, and most of the time (83.89 %) spent in stress level 2. Although, in the last training session of the initial training program, the stress level 3 percentage rises again from 3.14 % to 10.24 %, it is a nicely balanced training, in which the foal experiences no stress for 52.48 % of the training, while in stress level 2 and 3 for respectively 37.28 % and 10.24 % of the time. The small increase of stress level 3 in the last training session can be explained by the fact that the trainers focus on certain parts of the training that still induce stress in the foal, because, before the foal can graduate from the program, it needs to be comfortable with all the different parts.

In the FU, the foal is most of the time in stress level 2 (78.26 %), and stress free for only 7.84 % of the time. The FU session is performed after spending four months with the herd. The combination of the sudden resumption of the training program and the introduction of the third way of leading is probably the reason for the higher stress levels.

A typical ideal training program, as the one of Conchita, can thus be divided into two phases: PHASE I and PHASE II. In the first phase, from TRAIN1 until TRAIN4 or TRAIN5, the stress level decreases every training session. The start of PHASE II is determined by the introduction of the second way of leading and is associated with a sudden increase of stress in the middle of the program. For most foals, the new exercise is introduced in the fourth training, while for other, like Conchita, it is introduced in the fifth training. In the subsequent trainings, the stress level typically decreases again until the end of the initial training program.

Although it is difficult to recognize the phases in the training progress at group level in Figure 30, by looking at the evolution of the stress levels in the training part TOURS in Figure 32, the typical progress in PHASE I and PHASE II is however visible at group level. The new way of leading is introduced in this part of the training, so the effect is more visible by only considering the stress levels belonging to this part of the training.

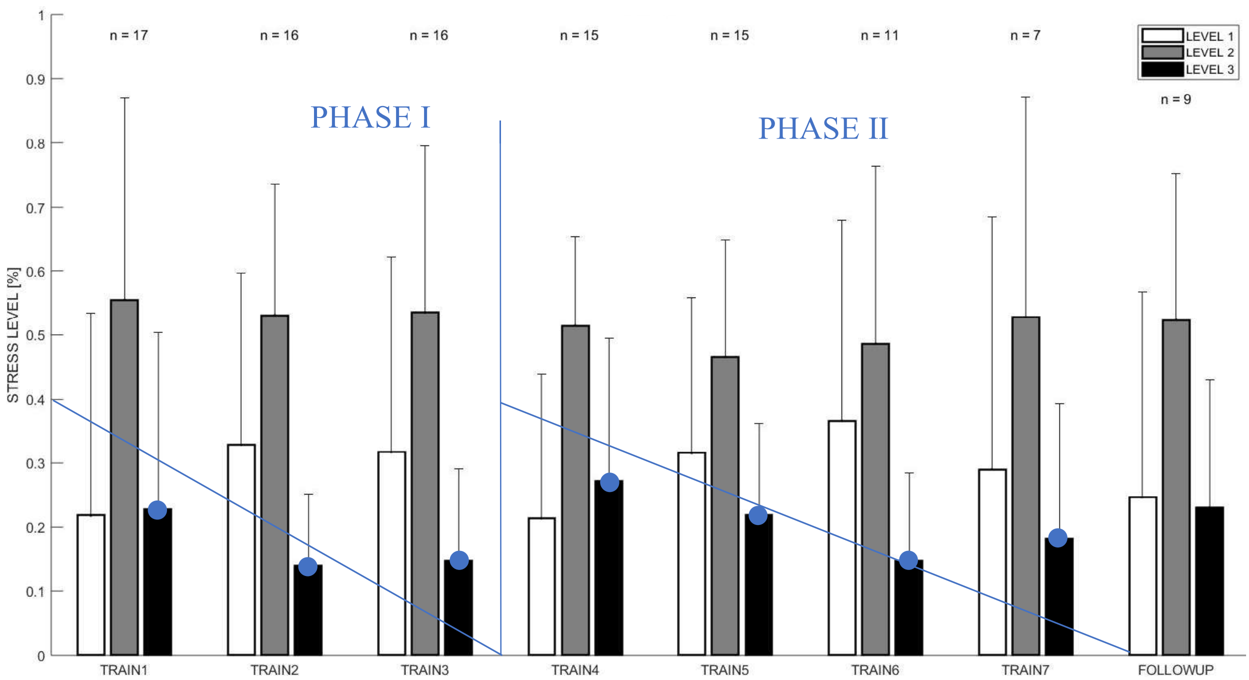


Figure 32 Evolution of gold standard stress levels in TOURS at group level

The slightly increased stress level 3 percentage in TRAIN7 with respect to TRAIN6 might be due to the fact the trainers are focussing on the weaker points of the foal, meaning parts of the training uncomfortable for the foal and thus still inducing stress for the foal.

Unfortunately, not all foals demonstrate the ideal training program, like the one of Conchita. Look for example at the training progress of Code Black in Figure 33. In the first training session, Code Black experiences a lot of stress, spending 63.28 % of the time in stress level 3. Therefore, at a certain moment, the trainers decided to stop the session to resume the training later. In the following training, the foal was handled very gently by the handlers, who spent a lot of time on coping behaviours and incorporating breaks in which the foal has time to release stress before the training is resumed. Due to this gentle approach, the foal spent only 9.10 % of the training in stress level 3. Code Black's unusual stressful reaction when being handled, particularly around the hindquarters and back legs, resulted from the fear of the unknown. Once he felt safe, the over stressful reaction ceased and he improved quickly in training.

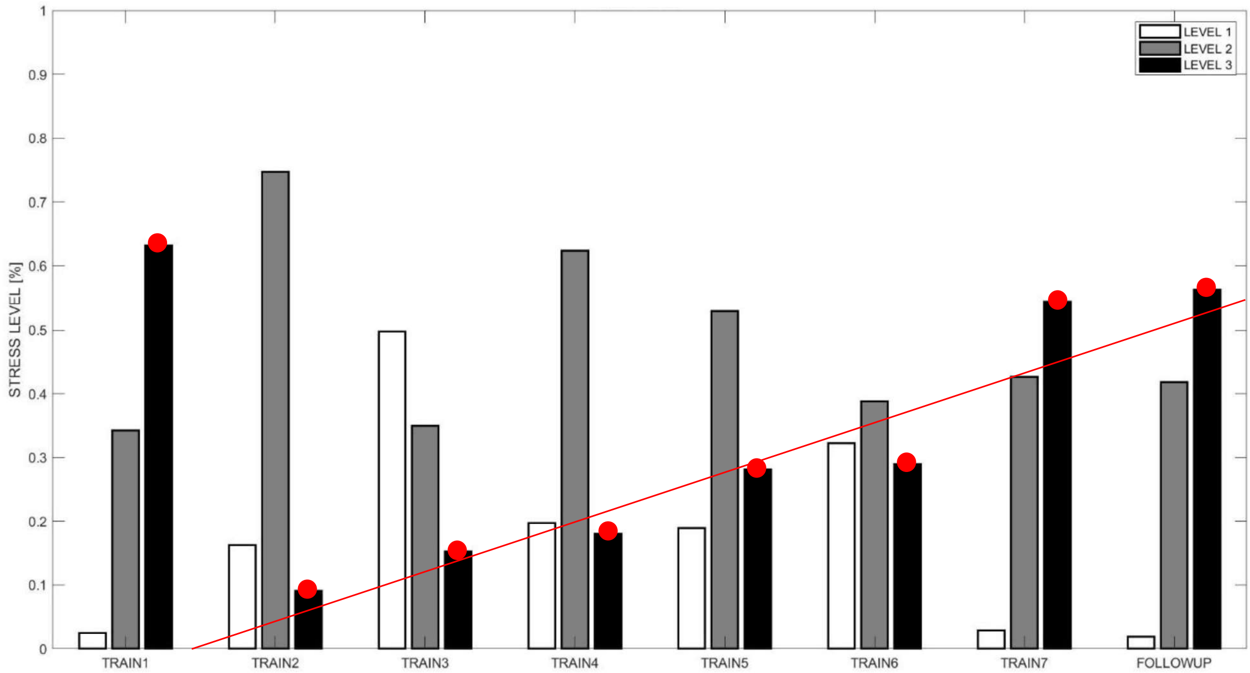


Figure 33 Evolution of gold standard stress levels of entire training of Code Black

In the following sessions, every time he was confronted with an unknown handling, he reacted very intense by going into stress level 3 every time. To enable Code Black to relax, the trainers provided time and space for Code Black to adapt before continuing with the training. With the eye on progressively evolving toward normal training sessions, including less breaks to release the excess of stress, Code Black was progressively challenged in every consecutive training, which is confirmed by the increasing stress level 3 percentages: starting from 15.31 % in the third session, to 18.02%, 28.18 %, 28.99 % and 54.38 % for the respective remaining trainings and 56.21 % for the FU session. It is because some of the foals, for example Code Black, are evolving in a more atypical way, the progress at group level is less clear.

TRAINING PARTS

Besides the progress of the foal's stress level in time, the stress level analysis enables an investigation of the stress levels in the respective parts of the trainings. Figure 34 represents the stress levels of the foals over the respective training parts at group level.

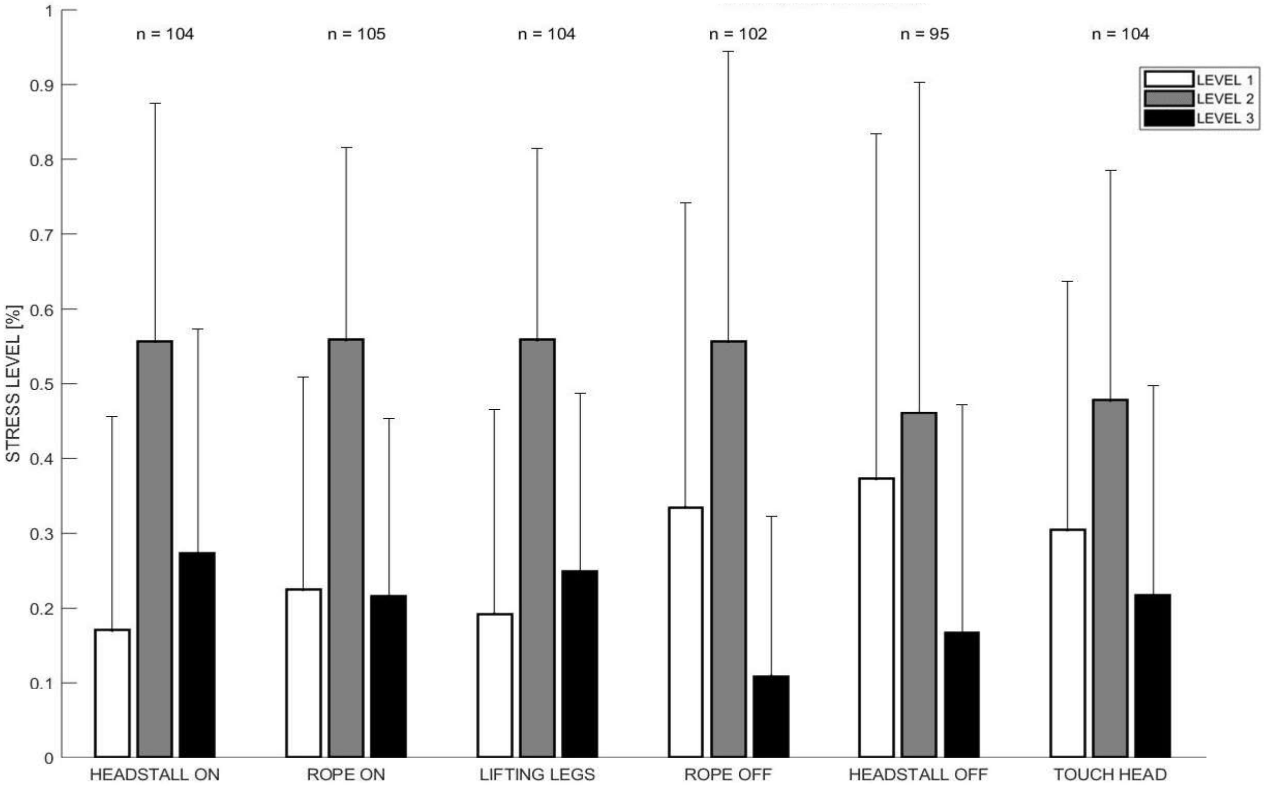


Figure 34 Comparison of gold standard stress levels between training parts at group level

By means of a statistical analysis on the percentages the foals are in stress level 1 and 3, the respective training parts are compared with respect to each other, with the results in Table 10 and Table 11 respectively. By comparing the percentage the foals experience no stress in a particular training part, in Table 10, it is calculated the foals experience no stress for 16.99 % of the time when the headstall is put on, while they it is on average 33.44 % when the rope is taken off and 37.28 % when the headstall is taken off. The significant differences, indicated in bold, between HEADSTALL ON in the beginning of the training session and ROPE OFF and HEASTALL OFF at the end of the training indicates the foals are relieved when the training session is over. While trainers touch the head of the foal in TOUCH HEAD, the foal is on average for 30.46% of the time in stress level 1, while during HEADSTALL ON it is only 16.99 % and during LIFTING LEGS 19.25 %. The significant difference between these training parts, indicates that the foals head is either touched in low stress moments of the training session or the touching of the foals has a calming effect on the foal.

Table 10 Statistical results STRESS LEVEL 1 for TRAIN PARTS at group level

			Welch	MWU	Cohen's d
HEADSTALL ON	x	ROPE ON	p = 0.16600		
HEADSTALL ON	x	LIFTING LEGS	p = 0.56100		
HEADSTALL ON	**x**	**ROPE OFF**	**p = 0.00100**	**p = 0.02673**	**d = -0.46768**
HEADSTALL ON	**x**	**HEADSTALL OFF**	**p = 0.00030**	**p = 0.04537**	**d = -0.53404**
HEADSTALL ON	**x**	**TOUCH HEAD**	**p = 0.00200**	**p = 0.00009**	**d = -0.43488**
ROPE ON	x	LIFTING LEGS	p = 0.40440		
ROPE ON	x	ROPE OFF	p = 0.02640	p = 0.74354	
ROPE ON	x	HEADSTALL OFF	p = 0.00770	p = 0.73042	
ROPE ON	x	TOUCH HEAD	p = 0.06300	p = 0.07900	
LIFTING LEGS	x	ROPE OFF	p = 0.00390	p = 0.36436	
LIFTING LEGS	x	HEADSTALL OFF	p = 0.00120	p = 0.42356	
LIFTING LEGS	**x**	**TOUCH HEAD**	**p = 0.00850**	**p = 0.01369**	**d = -0.36876**
ROPE OFF	x	HEADSTALL OFF	p = 0.53730	p = 0.68520	
ROPE OFF	x	TOUCH HEAD	p = 0.56720	p = 0.35515	
HEADSTALL OFF	x	TOUCH HEAD	p = 0.23700	p = 0.47979	

Table 11 Statistical results STRESS LEVEL 3 for TRAIN PARTS at group level

			Welch	MWU	Cohen's d
HEADSTALL ON	x	ROPE ON	p = 0.12990	p = 0.84314	
HEADSTALL ON	x	LIFTING LEGS	p = 0.51060	p = 0.68086	
HEADSTALL ON	**x**	**ROPE OFF**	**p = 0.00000**	**p = 0.00004**	**d = 0.63021**
HEADSTALL ON	**x**	**HEADSTALL OFF**	**p = 0.01350**		**d = 0.35403**
HEADSTALL ON	x	TOUCH HEAD	p = 0.16720		
ROPE ON	x	LIFTING LEGS	p = 0.32960		
ROPE ON	**x**	**ROPE OFF**	**p = 0.00070**	**p = 0.00000**	**d = 0.47549**
ROPE ON	x	HEADSTALL OFF	p = 0.19930	p = 0.00001	
ROPE ON	x	TOUCH HEAD	p = 0.97540	p = 0.18537	
LIFTING LEGS	**x**	**ROPE OFF**	**p = 0.00000**	**p = 0.00000**	**d = 0.61632**
LIFTING LEGS	**x**	**HEADSTALL OFF**	**p = 0.03660**		**d = 0.30225**
LIFTING LEGS	x	TOUCH HEAD	p = 0.39080		
ROPE OFF	x	HEADSTALL OFF	p = 0.13210	p = 0.76710	
ROPE OFF	**x**	**TOUCH HEAD**	**p = 0.00200**	**p = 0.00221**	**d = -0.43479**
HEADSTALL OFF	x	TOUCH HEAD	p = 0.22000		

By comparing the percentage the foals are triggered into stress level 3, the same trend can be observed. For parts in the beginning of the training session, HEADSTALL ON and ROPE ON, the foals are triggered to stress level 3 for 27.33 % and 21.64 % of the time respectively, while only for 10.89 % and 16.61 % of the time for ROPE OFF and HEASTALL OF, respectively. The significant higher stress level 3 percentages at the beginning of the training, see Table 11, confirm the observations based on stress level 1 percentages, namely parts at the beginning of the training session induces significantly more stress than parts in the middle, like and TOUCH HEAD, or at the end of the training session, like ROPE OFF and HEADSTALL OFF.

During LIFTING LEGS, the foals are 24.85 % of the time triggered into a stress level 3, which is significantly more than parts towards the end of the training session: ROPE OFF and HEADSTALL OFF, indicating LIFTING LEGS triggers the foals significantly more to go into stress level 3. Table 11 also indicates a significant higher stress level 3 percentage (21.75 %) during TOUCH HEAD, with respect to ROPE OFF (10.89 %). Combining this observation with the observations on the stress level 1 percentages, two possible reasonings remain. The touching of the head might be done in a low stressful part of the training, while the touching itself induces a strong stress response. Another reasoning is: the trainers touch the foal's head in highly stressful moments with the eye on reducing their stress level.

While Figure 34 considers all training sessions, Figure 35 and Figure 36 represent the evolution of the stress levels in the respective training parts, going from TRAIN1 until the FOLLOWUP. For the respective trainings, the parts that induce most stress vary from training to training. Although the main trend, namely the higher stress levels in the beginning of the training session towards lower stress levels at the end of the training session is observed in most sessions, no further statistical analysis is performed on the differences within the respective training sessions.

Due to individual differences between the foals, training parts that induce the most stress are different for every foal. For example, some foals experience a lot of stress when the headstall is put on, while other are more triggered during LIFTING LEGS. Also, due to time-variance and the progress of the foals for particular parts in the session, it also changes in time. Therefore, the analysis on group level would not provide addition information. Performing the analysis on every foal respectively, on the other hand, gives an idea about critical points in the training session for that particular foal and its progress in the training program. Another interesting analysis is the evolution of the stress levels in time for a particular part of the training, which can be found in Appendix III.

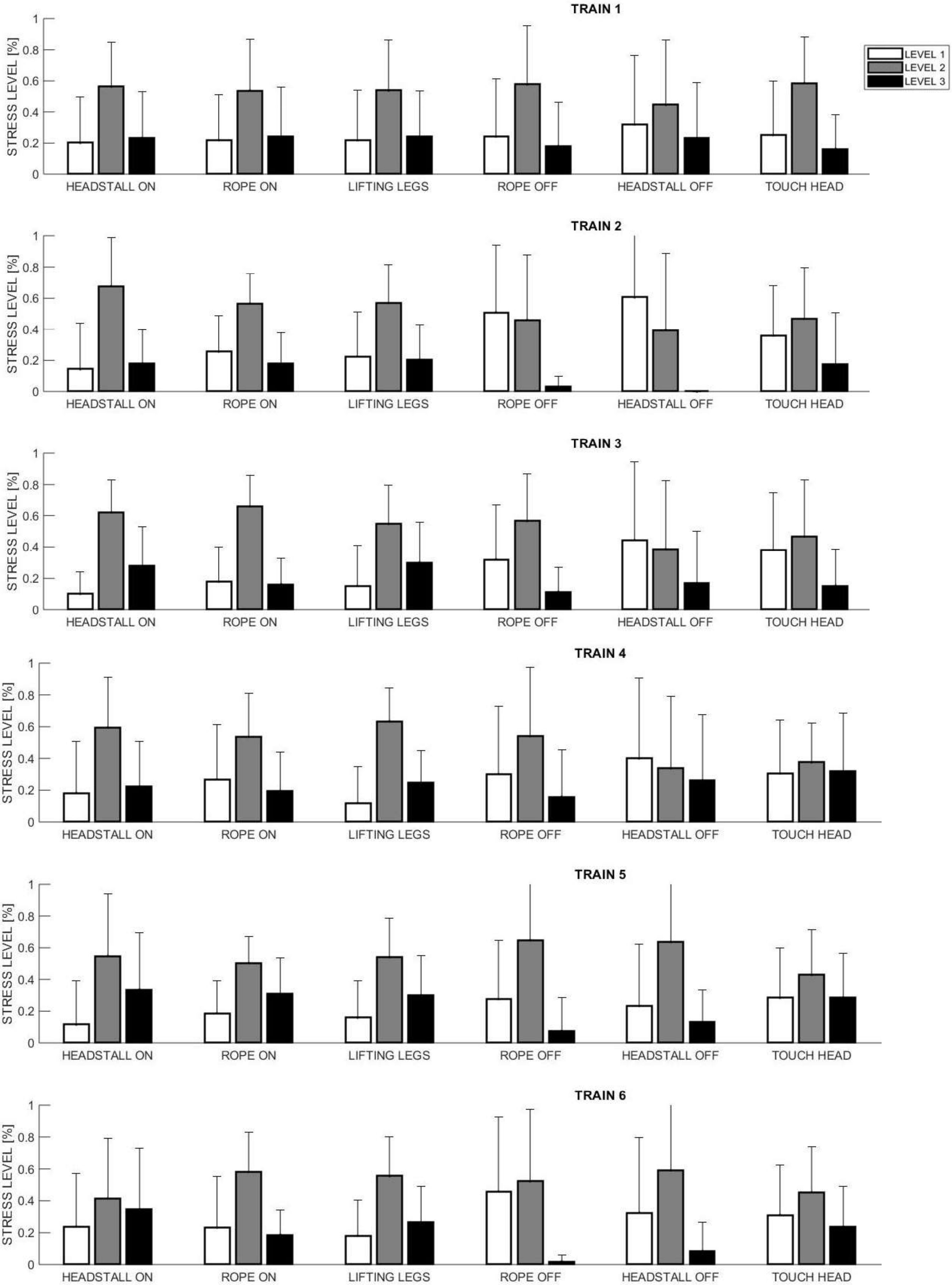


Figure 35 Evolution of gold standard stress levels for respective training parts at group level

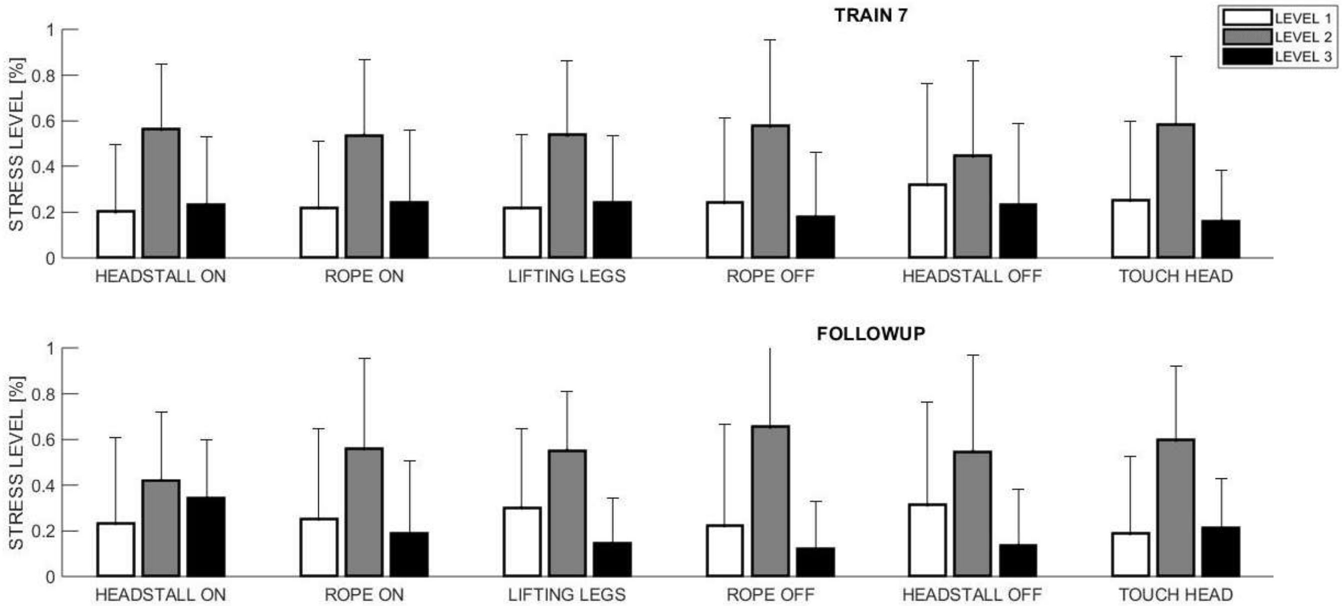


Figure 36   Evolution of gold standard stress levels for respective training parts at group level

MARE

The stress levels of the mare are analysed as well. For the mare, however, no analysis is done on the evolution and on the respective parts of the training since the mare is not actively participating in the training. The research focusses on the foal's stress levels and therefore the mare's analysis is limited to a comparison with respect to the foal's stress levels.

TIME

In Figure 37, the stress levels of the mare are given for TRAIN1 until the FOLLOWUP. By comparing the mare's stress levels in Figure 37 with the foal's stress levels in Figure 30 at group level, it is already clear the mare is less stressed during the training sessions compared to the foal. On average over all training sessions, the mare is experiencing no stress for 58.98 % of the time. Meanwhile, the foal is on average free from stress for only 25.32 % of the time, less than half the time of the mare. The same analysis can be done for stress level 3, giving the result of 4.87 % of the time for the mare and 20.95 % of the time for the foal, which is more than five times the percentage of the mare. The stress levels of the mares are quite constant throughout the program. Since the focus in on the stress level of the foal, no further statistical analysis is done on the comparison between the mare's stress level percentages between the respective training session.

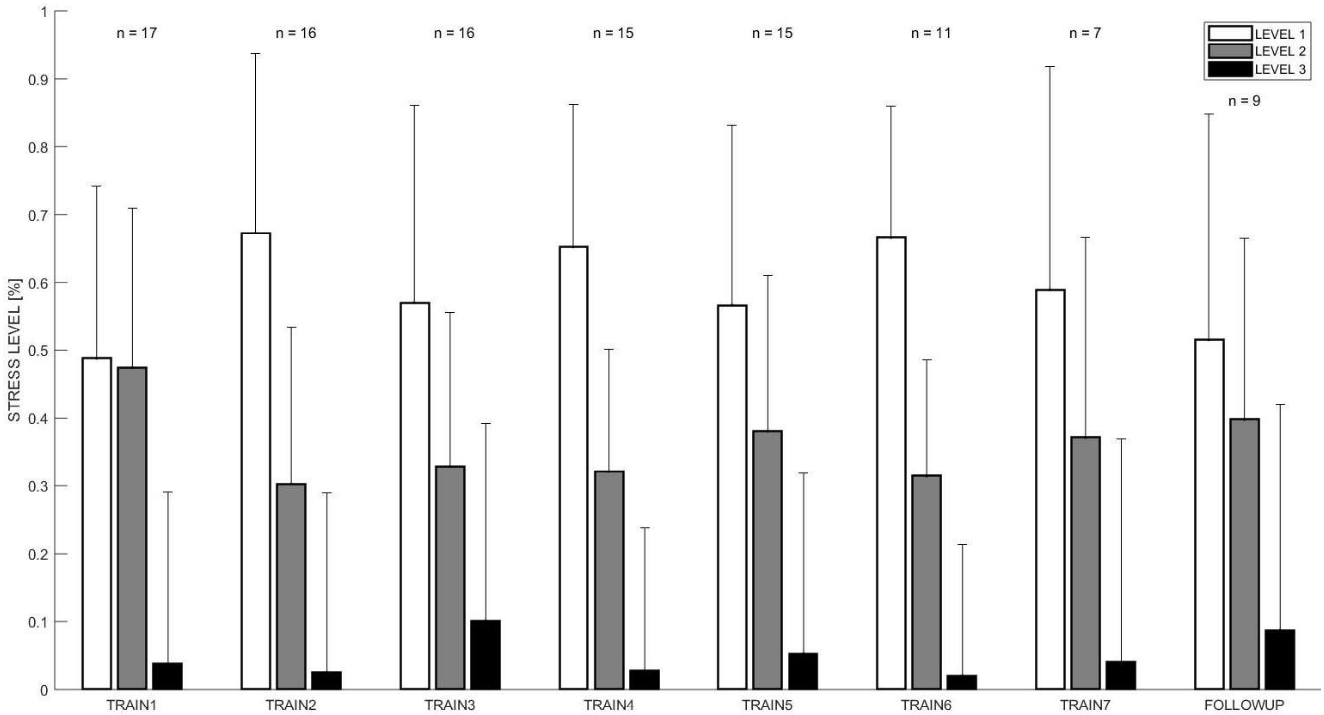


Figure 37 Evolution of the mare's gold standard stress levels of entire training at group level

HR ANALYSIS

In the HR analysis the evolution of the foal's median, mean, min, max and std of the HR is analysed in time and for the respective parts of the training.

FOAL

With the eye on finding HR measures that can be used to evaluate the progress of the foals in the training program, the observations in the HR analysis are compared with the observations in the stress level analysis which represents the gold standard for the stress levels of the foals.

PROGRESS

To make progress in their training program, the foals needs to demonstrate a lower mean stress, a reduction of the occurrence and intensity of moments of high stress levels and consistency of stress throughout the training.

MEAN STRESS LEVEL

The changes in the median, mean and min HR are represented in Figure 38 at group level. As can be seen, the median HR, as well as the mean and min HR decrease over the training session. By estimation regression lines over the respective training sessions TRAIN1 until TRAIN7 (without FOLLOWUP), one can observe a decay of 3.1 bpm per training session for the median HR, 3.3 bpm for the mean HR and 2.1 bpm for the min HR.

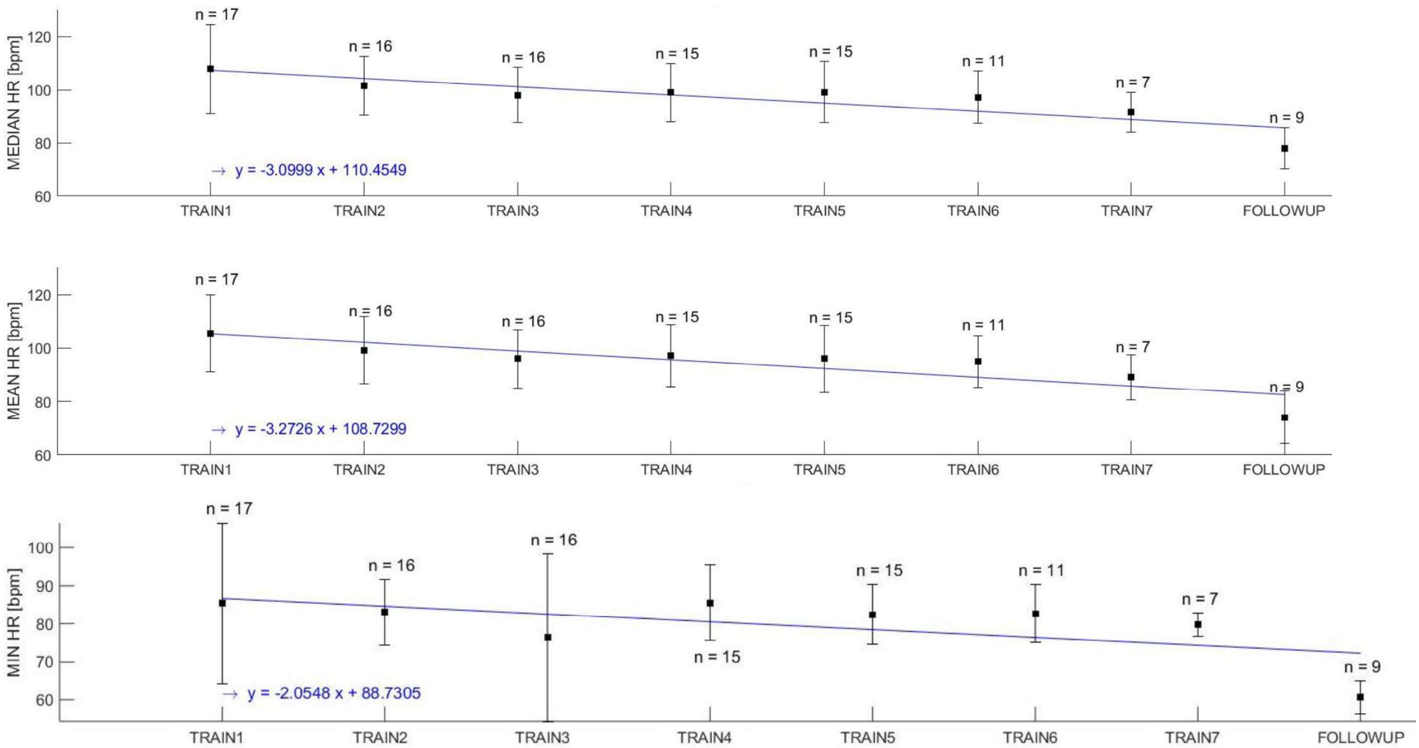


Figure 38 Evolution of median, mean and min HR for entire training at group level

By performing a statistical analysis, significant differences are observed between TRAIN1 versus TRAIN6, TRAIN7 and FOLLOWUP for the mean and median. For the min HR, a significant difference is only found between TRAIN1 and FOLLOWUP. The p-values of the statistical tests are summarised in Table 12, together with the respective Cohen's d and the significant differences are indicated in bold.

By comparing the difference between the mean HR of TRAIN1 and TRAIN3, the Welch's test gives a p-value of p = 0.03490 and Cohen's d = 0.76350, indicating a significant difference. Meanwhile, by comparing the median for the same trainings, the resulting p-value is p = 0.06050, which almost indicates a significant difference. However, the higher variance in TRAIN3 for the min HRs probably causes the higher p-value of p = 0.23820 when doing the same analysis for the min HR.

Table 12 Statistical results of median, mean and min HR analysis at group level

		Welch	MWU	Cohen's d
T1 versus T2	median	p = 0.21290		
	mean	p = 0.18910		
	min	p = 0.67650		
T1 versus T3	median	p = 0.06050		
	mean	**p = 0.03490**		**d = 0.76350**
	min	p = 0.23820		
T1 versus T4	median	p = 0.10620		
	mean	p = 0.06360		
	min	p = 0.97750		
T1 versus T5	median	p = 0.07170		
	mean	p = 0.08000		
	min	p = 0.59570		
T1 versus T6	**median**	**p = 0.04850**		**d = 0.72010**
	mean	**p = 0,02680**		**d = 0.83350**
	min	p = 0.64280		
T1 versus T7	**median**	**p = 0.00330**	**p = 0.00426**	**d = 1.10240**
	mean	**p = 0.00280**		**d = 1.24410**
	min	p = 0.29680		
T1 versus FU	**median**	**p = 0.00000**	**p = 0.00004**	**d = 2.18310**
	mean	**p = 0.00000**		**d = 2.28820**
	min	**p = 0.00020**		**d = 1.41090**

By comparing the observations with the evolution of the gold standard stress levels, the same trends can be observed. In Figure 38, the median, mean and min HR decrease with a slope of almost 3.1, 3.5 and 2.1 bpm per training respectively, indicating a decrease in the stress level throughout the program. The significant differences between the median, mean and min HRs at the end of the training program with respect to TRAIN1 confirm the foals experience less stress towards the end of the program.

However, by comparing the mean, median and min HR of the first training with the third training session, only a significant difference is found for the mean HR and an almost significant difference for the median, the significant difference between the mean HR of TRAIN3 with respect to the mean HR of TRAIN1 confirms the existence of the two phases, as defined in the stress level analysis.

The HR measures thus confirm the trends observed in the gold standard stress level analysis, for example the training program of Conchita in Figure 31. Therefore, these observations give a strong indication that the HR measures, and especially the mean HR, are good measures to represent the mean stress level of a foal in a training session. Therefore, in further investigations, mainly the mean HR is used of represent the mean stress level of the foal.

SHORT HIGH STRESS MOMENTS

In Figure 39, the evolution of the max HR is given. The estimated regression line of the max HR, again estimated without FOLLOWUP, also represents a decreasing trend with a decay of 2.8 bpm per training.

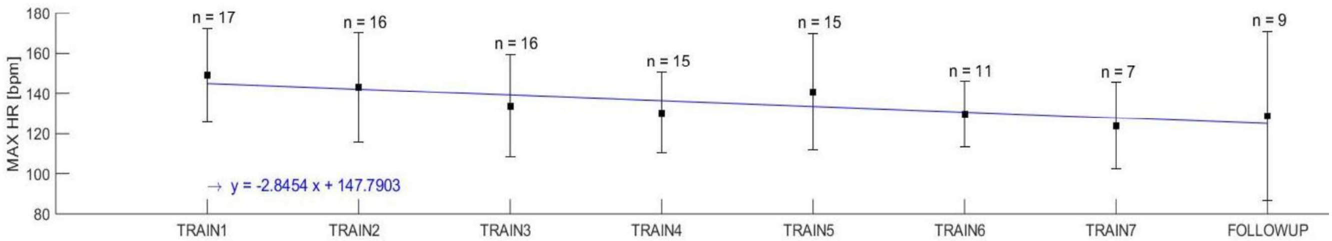


Figure 39 Evolution of max HR for entire training at group level

Performing the same statistical analysis for the max HR, gives the p-values and corresponding Cohen's d in Table 13, with the significant differences again indicated in bold. For the max HR, again significant differences are found between TRAIN1 and trainings at the end of the program.

Table 13 Statistical results of max HR analysis at group level

		Welch	t-test	Cohen's d
T1 versus T2	max		p = 0.50486	
T1 versus T3	max		p = 0.08536	
T1 versus T4	**max**		**p = 0.02331**	**d = 0.84678**
T1 versus T5	max		p = 0.38687	
T1 versus T6	**max**	**p = 0.01640**		**d = 0.91890**
T1 versus T7	**max**		**p = 0.02341**	**d = 1.09400**
T1 versus FU	max	p = 0.11793		

However, no significant difference is found between TRAIN1 and the FOLLOWUP session. This lack of significance might be due to the high variance of the max HRs measured for the different test horses in combination with the small amount of test horses (n = 9) participating in the FOLLOWUP session. The higher variance might be due to the individually different reaction to the newly introduced third way of leading in the FU session: some foals adapt rather easily while other react strongly to new situations. The new training element will induce short moments of high stress and consequently peaks in the measured HR. This will result in a higher max HR measure for that training, while the effect is less pronounced in the median, mean and min HR measures. Therefore, since the max HR measure reflects short moments of stress, the evolution of the max HR can be used as a measure to follow the progress of the amount and intensity of high stress levels.

DYNAMIC RANGE

The same analysis is repeated for the std's of the HRs, illustrated in Figure 40. By estimating a regression line from TRAIN1 to TRAIN7 a decay of 0.15 bpm per training is calculated. For the statistical comparison of the std of the first with respect to the other training session, the results are given in Table 14.

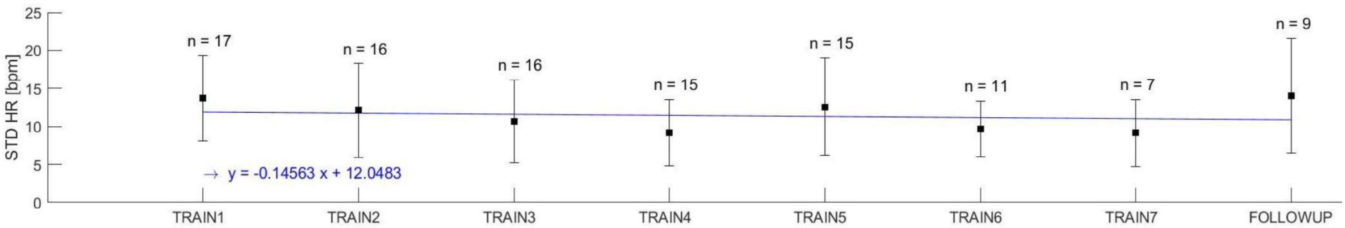


Figure 40 Evolution of std HR for entire training at group level

Significant differences are found between TRAIN1 and TRAIN4, as well as between TRAIN1 and TRAIN6. These significant differences confirm the earlier observations. The std measure represents the dynamic range of the HRs measured in a training session and can thus be used as a measure for the constancy or invariability of the stress levels within the training.

Table 14 Statistical results of std HR analysis at group level

		t-test	Welch	Cohen's d
T1 versus T2	std		p = 0.44070	
T1 versus T3	std	p = 0.12805		
T1 versus T4	**std**	**p = 0.01694**		**d = 0.89580**
T1 versus T5	std	p = 0.60129		
T1 versus T6	**std**	**p = 0.04525**		**d = 0.81400**
T1 versus T7	std	p = 0.06869		d = 0.85970
T1 versus FU	std	p = 0.90365		

The analysis at group level demonstrate a decrease in the median, mean, min, max and std of the HR. While most foals follow the trend, like Conchita given in Figure 41, others deviate, like Celtic Lass in Figure 42. For Conchita, the max, mean and min HR decreases throughout the program with 6.3 bpm, 4.8 bpm and 4.2 bpm per training session respectively. For Celtic Lass, however, the max mean and min HR increases with 2.2 bpm, 0.052 bpm and 1.1 bpm respectively.

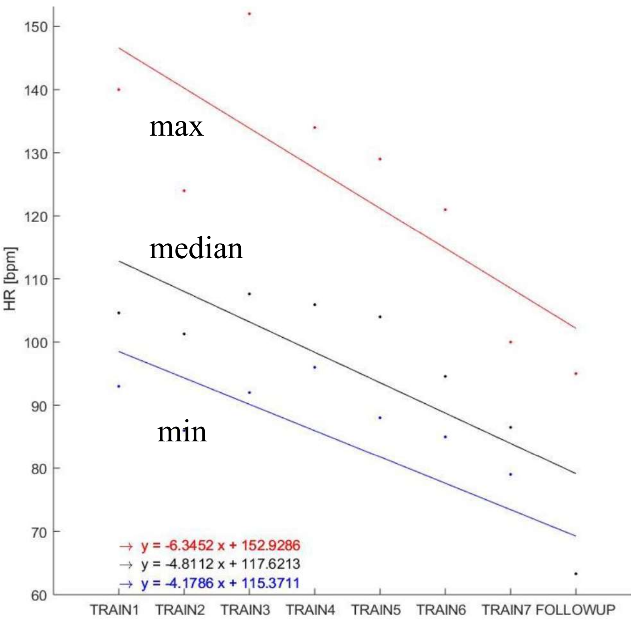


Figure 41 Evolution of HR of Conchita

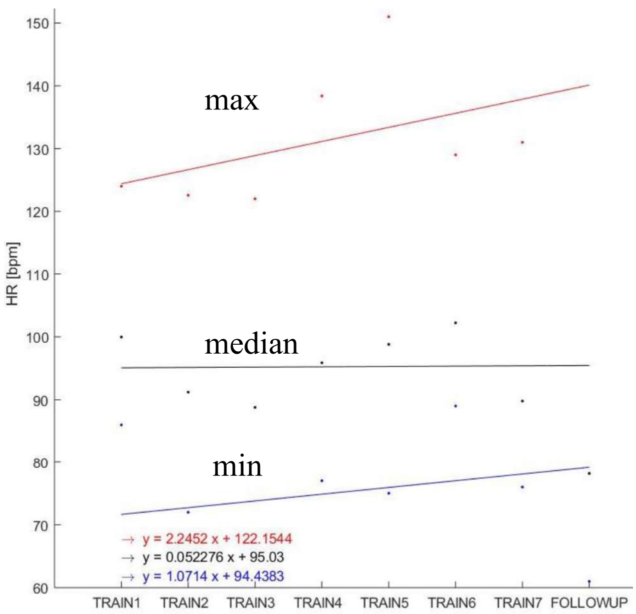


Figure 42 Evolution of HR of Celtic Lass

In Figure 43, the mean HR analysis is repeated for every part of the training respectively, making it possible to analyse the mean stress level of the foals for every particular part of the training respectively. Instead of the mean HR, the evolution of the median and min HR for the respective training parts could have been used to evaluate the mean stress as well. To enable a complete analysis on the progress of the foals in the respective training parts, the max and std HR measures must be analysed as well and are therefore added in Appendix IV, as well as the median, mean and min HR measures. By looking at Figure 43, and the figure in Appendix IV, the decreasing trend of the mean stress level is found in all parts of the training. The slopes of the estimated regression lines for TRAIN1 until TRAIN7 are given in Table 15, while the results of the statistical analysis are presented in Table 16, with the insignificant differences in bold.

Table 15 Slope regression lines mean HR analysis on training parts at group level

PART OF TRAINING	slope
HEADSTALL ON	- 2.2 bpm
ROPE ON	- 1.9 bpm
LIFTING LEGS	- 3.6 bpm
TOURS	- 3.3 bpm
ROPE OFF	- 3.0 bpm
HEADSTALL OFF	- 2.8 bpm
TOUCH HEAD	- 2.3 bpm

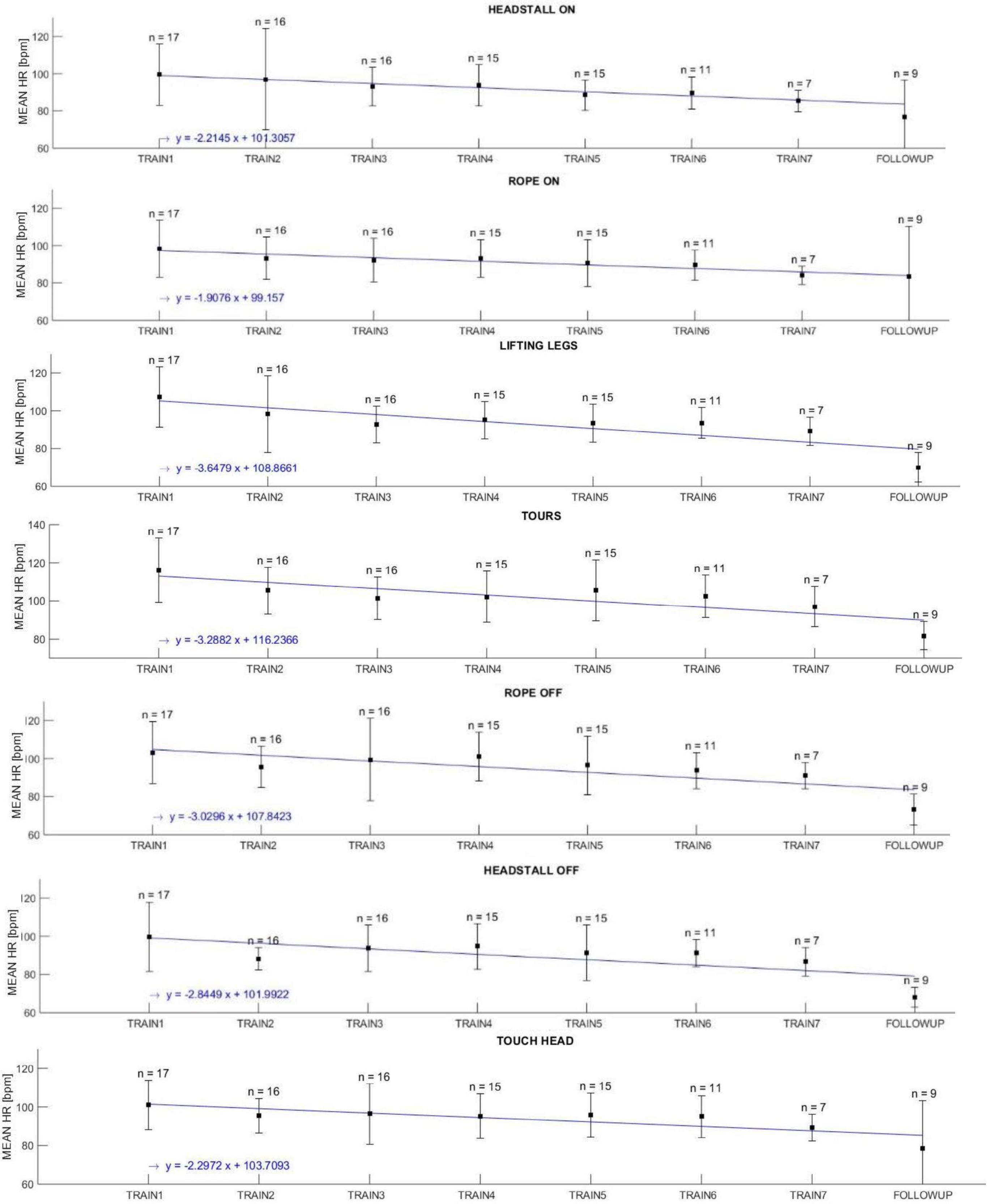


Figure 43 Evolution of mean HR for the respective parts of the training at group level

Table 16 Statistical results mean HR analysis PROGRESS at group level FOAL

	t-test	Welch	MWU	Cohen's d
HEADSTALL ON				
T1 versus T2		p = 0.74630		
T1 versus T3	p = 0.19179			
T1 versus T4	p = 0.24701			
T1 versus T5			**p = 0.04143**	**d = 0.86714**
T1 versus T6	p = 0.06721			
T1 versus T7			**p = 0.00764**	**d = 1.02670**
T1 versus FU	**p = 0.00381**			**d = 1.32040**
ROPE ON				
T1 versus T2		p = 0.28660		
T1 versus T3		p = 0.21400		
T1 versus T4		p = 0.26960		
T1 versus T5		p = 0.11730		
T1 versus T6		p = 0.05550		
T1 versus T7		**p = 0.00260**		**d = 1.06800**
T1 versus FU		p = 0.15510	p = 0.05235	
LIFTING LEGS				
T1 versus T2		p = 0.19660		
T1 versus T3	**p = 0.01221**			**d = 0.96469**
T1 versus T4	**p = 0.02488**			**d = 0.83664**
T1 versus T5			**p = 0.01140**	**d = 0.95936**
T1 versus T6			**p = 0.01444**	**d = 0.92845**
T1 versus T7		**p = 0.00180**		**d = 1.17220**
T1 versus FU	**p = 0.00000**			**d = 2.45210**
TOURS				
T1 versus T2	**p = 0.04770**			**d = 0.73138**
T1 versus T3	**p = 0.00740**			**d = 1.03930**
T1 versus T4	**p = 0.01172**			**d = 0.95084**
T1 versus T5	p = 0.09741			
T1 versus T6	**p = 0.01826**			**d = 0.97468**
T1 versus T7		**p = 0.00190**		**d = 1.34400**
T1 versus FU	**p = 0.00003**			**d = 2.49450**
ROPE OFF				
T1 versus T2	p = 0.15440			
T1 versus T3		p = 0.59700		
T1 versus T4	p = 0.70103			
T1 versus T5	p = 0.27743			
T1 versus T6	p = 0.09953			
T1 versus T7		**p = 0.02830**		**d = 0.82167**
T1 versus FU	**p = 0.00003**			**d = 2.09750**

Table 16 Continued

HEADSTALL OFF				
T1 versus T2		**p = 0.02330**	**p = 0.02655**	**d = 0.80827**
T1 versus T3		p = 0.30620		
T1 versus T4		p = 0.35850		
T1 versus T5		p = 0.16760		
T1 versus T6		p = 0.10350		
T1 versus T7		**p = 0.02160**		**d = 0.81197**
T1 versus FU		**p = 0.00000**		**d = 2.04130**
TOUCH HEAD				
T1 versus T2		p = 0.14790		
T1 versus T3	p = 0.32200			
T1 versus T4	p = 0.16449			
T1 versus T5	p = 0.18401			
T1 versus T6	p = 0.16222			
T1 versus T7	**p = 0.01789**			**d = 1.14930**
T1 versus FU	**p = 0.00057**			**d = 1.71170**

TRAINING PARTS

In the previous part, the evolution of the mean HR is investigated for the respective training parts. Here, the mean HR is used to compare the respective training parts with respect to each other. Figure 44 represents the mean HR for the respective parts including all trainings at group level, while the boxplots for the median, min and max HR measures can be found in Appendix IV.

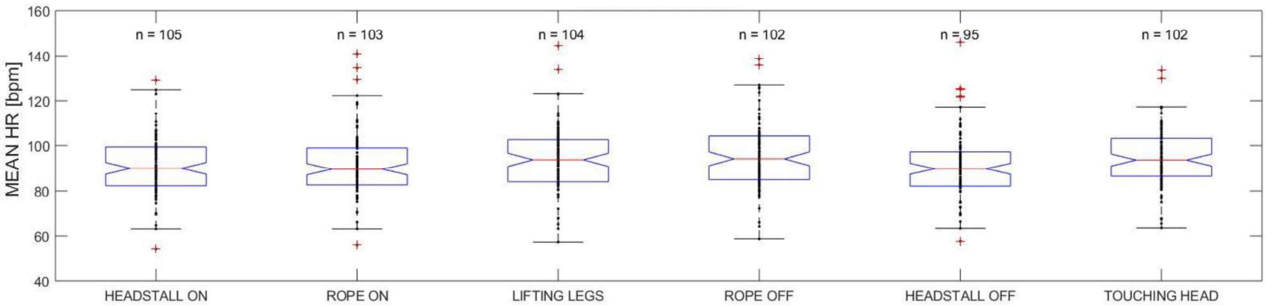


Figure 44 Boxplots comparing mean HR for the respective parts of the training at group level

The mean HR during the training part HEADSTALL ON is 91.9 bpm, for ROPE ON it is 91.6 bpm, while for LIFTING LEGS on average 94.3 bpm is calculated. The mean HR during ROPE OFF is 95.8 bpm and HEADSTALL OFF is on average 90.9 bpm, while for TOUCH HEAD the mean HR is 94.7 bpm. To investigate whether the mean HRs of the respective training parts are significantly different with respect to each other, a statistical analysis is performed with the results are given in Table 17. Significant differences are found between ROPE ON and ROPE OFF ($p = 0.04640$), between ROPE OFF and HEADSTALL OFF ($p = 0.02410$) and between HEADSTALL OFF and TOUCH HEAD, ($p = 0.04730$) and are indicated in bold in Table 17.

Table 17 Statistical results mean HR analysis TRAIN PARTS at group level FOAL

			Welch	Cohen's d
HEADSTALL ON	x	ROPE ON	p = 0.87300	
HEADSTALL ON	x	LIFTING LEGS	p = 0.27930	
HEADSTALL ON	x	ROPE OFF	p = 0.08130	
HEADSTALL ON	x	HEADSTALL OFF	p = 0.62580	
HEADSTALL ON	x	TOUCH HEAD	p = 0.16040	
ROPE ON	x	LIFTING LEGS	p = 0.19370	
ROPE ON	**x**	**ROPE OFF**	**p = 0.04640**	**d = -0.28019**
ROPE ON	x	HEADSTALL OFF	p = 0.72430	
ROPE ON	x	TOUCH HEAD	p = 0.09290	
LIFTING LEGS	x	ROPE OFF	p = 0.50003	
LIFTING LEGS	x	HEADSTALL OFF	p = 0.11200	
LIFTING LEGS	x	TOUCH HEAD	p = 0.57556	
ROPE OFF	**x**	**HEADSTALL OFF**	**p = 0.02410**	**d = -0.32278**
ROPE OFF	x	TOUCH HEAD	p = 0.94233	
HEADSTALL OFF	**x**	**TOUCH HEAD**	**p = 0.04730**	**d = -0.28612**

On average the mean HR during ROPE OFF is 4.2 bpm higher than in ROPE ON and the difference is significant. This observation is in contradiction with the stress level analysis, indicating significant lower stress levels at the end of the training session with respect to training parts at the beginning of the training session. However, during HEADSTALL OFF, typically done directly after ROPE OFF, the mean HR is on average 90.9 bpm, which is 0.7 bpm lower than during ROPE ON. This might indicate the foal is already relaxing while the rope is taken off, because the foal is already demonstrating behaviours indicating stress level 1, but the HR needs some time to recover and lower after a stressful training session.

The significant difference between HEADSTALL OFF and TOUCH HEAD, with the me an HR during TOUCH HEAD 3.8 bpm higher than in HEASTALL OFF, can fit in both theories concerning the TOUCH HEAD part proposed in the stress level analysis. Although it was previously indicated the HR needs time to lower after a stressful situation, it is possible the HR increases much faster than it can decrease. The touching of the head can thus still be done in stressful situations where it is used to lower the stress level of the foal, or in low stressful moment where it increases the stress level of the foal.

Although the significant differences between ROPE OFF and HEASTALL OFF indicates the relaxation of the foal at the end of the training session, the difference is on average only 4.9 bpm. Meanwhile, the 4.9 bpm is on average the largest difference between the respective training parts. The small differences between stress levels and mean HRs for the respective parts, indicate that no part of the training induces extremely more stress than others.

Instead of doing the analysis on average over all training session, the same analysis can be done for the respective training session. In analogy to Figure 35 and Figure 36 in the stress level analysis, the respective training parts are compared with each other based on the mean HR for the respective training session, going from TRAIN1 until the FOLLOWUP, illustrated in Figure 45 and 46 at group level.

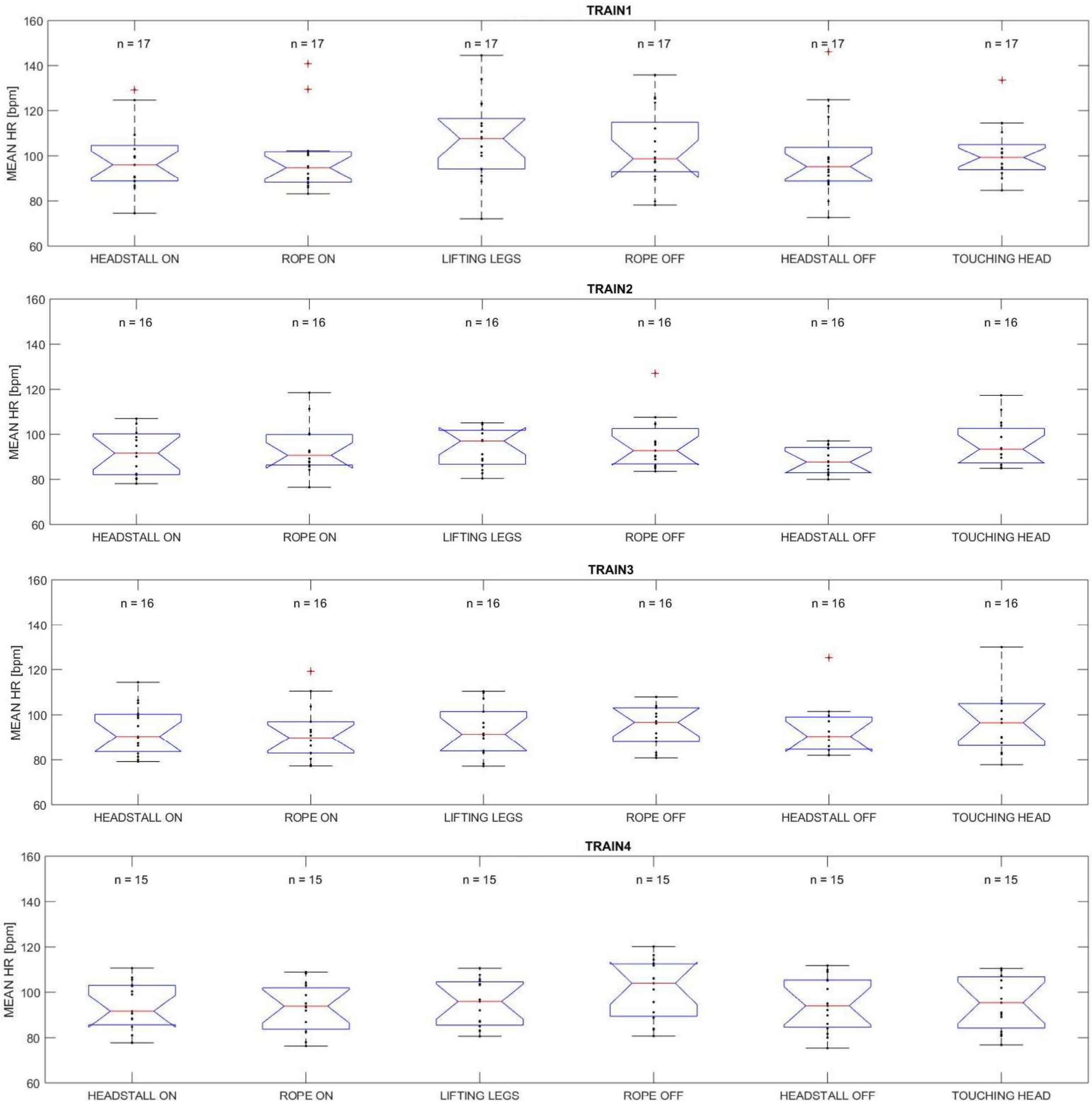


Figure 45 Evolution of HR measures for respective training parts at group level

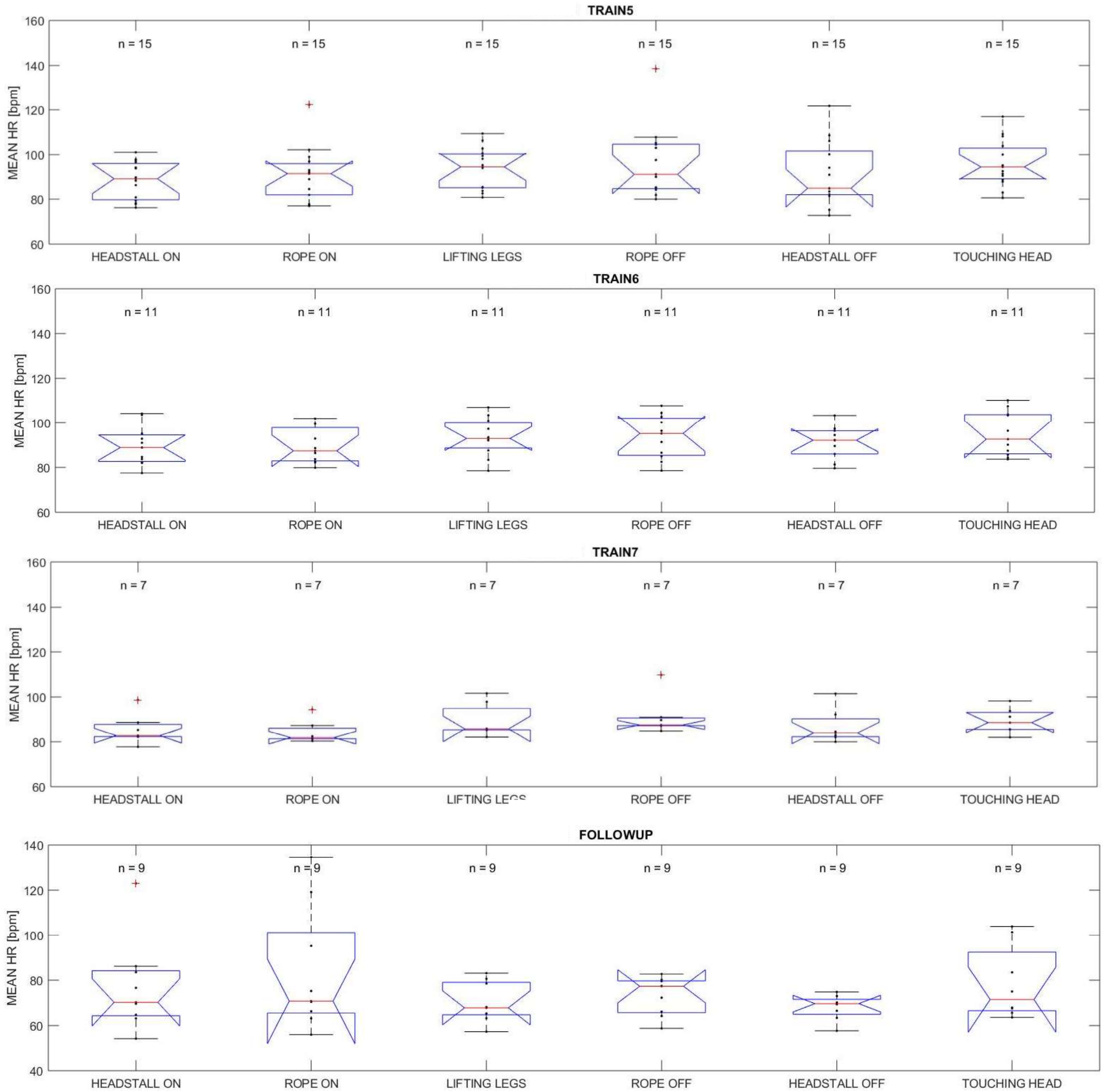


Figure 46 Evolution of HR measures for respective training parts at group level

In analogy to the stress level analysis, no further statistical analysis is performed due to the foal's individual differences. However, the lower HRs measured during HEADSTALL OFF appear in every training session. Meanwhile, it can be observed that the highest mean HRs don't always occur in the same part of the training. For example, in some trainings the highest mean HRs are measured during LIFTING LEGS, while in other trainings in the TOUCH HEAD part.

MARE

Also, the HR measurements of the mare are analysed. Here only the mean and max HR are discussed, with the additional results of the median, min, and std HR added in Appendix VI. The analysis of the mare is restricted to the HR measures calculated over the full training because the mare is not actively participating in the training, meaning a HR analysis on the respective parts of the training would not provide additional information.

TIME

In Figure 47, the regression lines for the mean and max HR for TRAIN1 until TRAIN7 are plotted. The mean and max HR decrease with slope of 1.5 bpm and 3.1 bpm per training respectively. The statistical analysis to identify significant difference between the respective training sessions is given in Table 18, with significant differences indicated in bold.

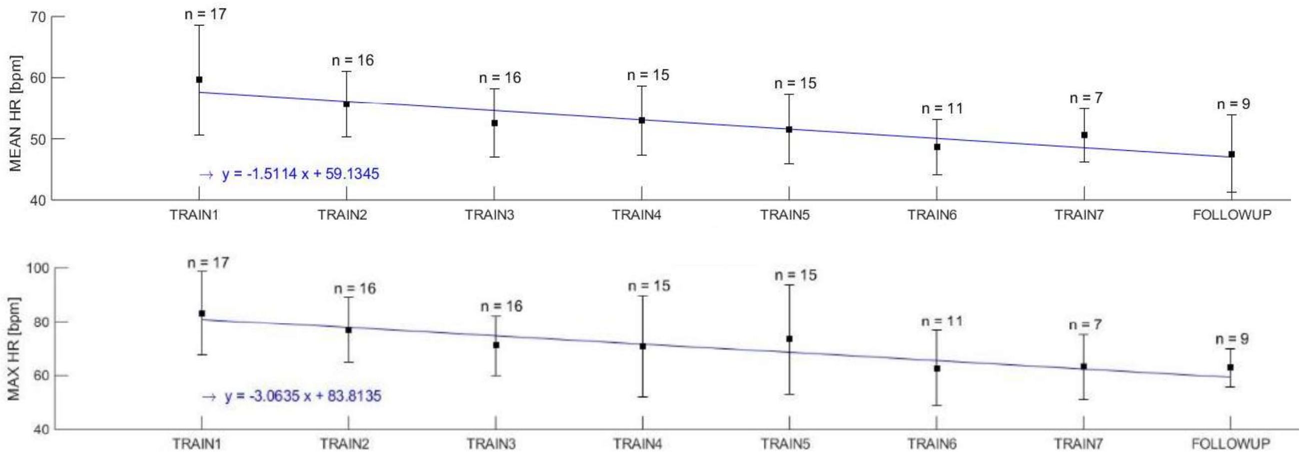


Figure 47 Evolution of the mare's mean and max HR in time at group level

Table 18 Statistical results mean HR analysis PROGRESS at group level MARE

		t-test	Welch	MWU	Cohen's d
T1 versus T2	mean			p = 0.22753	
	max	p = 0.21453			
T1 versus T3	**mean**		**p = 0.00920**	**p = 0.00469**	**d = 0.96386**
	max	**p = 0.01612**			**d = 0.88652**
T1 versus T4	**mean**	**p = 0.01728**			**d = 0.89277**
	max	p = 0.05433			d = 0.70941
T1 versus T5	**mean**	**p = 0.00526**			**d = 1.06620**
	max		p = 0.14560		
T1 versus T6	**mean**	**p = 0.00078**			**d = 1.47090**
	max	**p = 0.00158**			**d = 1.36520**
T1 versus T7	**mean**	**p = 0.01573**			**d = 1.17540**
	max	**p = 0.00612**			**d = 1.36160**
T1 versus FU	**mean**	**p = 0.00102**			**d = 1.54050**
	max			**p = 0.00091**	**d = 1.52270**

The mean HR of the mares in TRAIN1 is on average 59.6 bpm, while in the FOLLOWUP only 47.5 bpm, being significantly different than the mean HR in TRAIN1. The mean stress level of the mare is thus higher in the first training session and lowers throughout the program, just like the foal. The mean HR of the mares in TRAIN1 is already significantly different from the mean HR in TRAIN3, indicating the mare is getting used to the trainings after only two sessions.

The decrease in the max HR, being per 3.1 bpm per training session, is even stronger than the decrease in the mean HR of 1.5 bpm per training. The mare thus experiences more short moments of high stress level in the first training session, than towards the end of the program. The significant difference between TRAIN1 and TRAIN3, but the lack on a significant difference of TRAIN1 versus TRAIN4 and TRAIN5 for the max HR, indicate the mare is again experiencing more short moments of high stress when the new way of leading is introduced in the training program of the foals. This observation confirms the interaction between the foal and mare during training [2].

The decrease in the mean stress level of the mares was not identified during the behaviour-based stress level analysis. This might be due to the generally low stress level of the mare, making it probable the mares did not change their behaviour, or the slight changes were too small to recognise. In the stress level analysis, however, the stress level percentages of the mare were compared with respect to the stress levels of the foals. It was indicated the foals are experiencing explicitly more stress than the mares, which is quite logic since the mares are not the ones being trained. The mean HR of the foals during training is 96.5 bpm, while it is only 52.4 bpm for the mares. Indeed, the lower HR of the mare can, on one hand, be explained by the lower stress levels of the mares, but on the other hand, the HR of a horse might also decrease with age, the way it does in humans [104]. The decrease of the HR with age is not notable on the short period of time, being maximally three weeks, in which the initial trainings are performed but might be noticeable when comparing the HRs of the initial training sessions and the FU session. Therefore, it is interesting to consider the changes in the resting HR of the foals. The HR analysis can for example be performed on the difference between the measured HR and the resting HR of the foal instead of using the absolute values of the HR measures. This will also enable a better comparison of the foals with respect to each other because every horse is individually different and has therefore a different resting HR at a particular age. In this experiment, it was impossible to measure the resting HR since the equipment was put on just before the start of the training session, leaving no time to return to their resting HR before the start of the training session.

The HR measures defined in this research, being median, mean, min, max and std HR, are suitable to evaluate the stress level of the foal and thus the progress of the foals throughout their training program. From the median, mean and min HR, the median and mean are found to be the better measures to evaluate the mean stress level, while the max and std HR can be used to evaluate the occurrence and intensity of short moments of high stress and the dynamic range of the measured HRs, respectively.

In further research, however, it might be interesting to investigate the use of other HR measures to evaluate the stress levels, for example the HRV [105]. The HRV is defined as the variability of time intervals in consecutive heartbeats [106] or the variability of the RR-intervals. For the calculation of the RR-intervals, the electrocardiogram (ECG) is needed, which was not available for this research.

According to Von Borell, the HRV represents the SNS activation during a stress response and therefore an excellent measure to evaluate stress. However, due to the CITD characteristics of the horse [84], it is important to take into account the baseline HRV of a horse. Not only the resting HR but also the baseline HRV varies strongly between individuals because of numerous of factors, for example genotype, behaviour, temperament or food intake. To account for the variability the, it might be interesting the difference between the measures HRV and the baseline instead of the absolute HRV values. Meanwhile, analysing the resting HR or HRV baseline itself can already contribute information about the overall stress level of an individual horse.

Irregular intervals between two heartbeats or HRV, is considered as a sign of healthy cardiac activity [107]. According to Lovallo, keeping homeostasis is also present at organ level [45]. Using feedback loops, the heart itself is constantly keeping track of its current cardiovascular homeostatic balance and in case of small perturbation with respect to the homeostatic balance, the heart will adapt to restore the balance. For example, to keep a constant blood pressure, the HR will vary the frequency and intensity of its pulsations according to present blood pressure. If the current blood pressure is too low, the heart will compensate by contracting earlier, which results in a measurable HRV. In case of stress, the induced perturbations in the homeostatic balance are too large for the heart to restore the balance by adapting by itself. Instead, the NC takes over control and the small variations in the HR disappear [45].

Unfortunately, as it was the case for cortisol levels as a measure for stress, there is no consensus on the relation between the HRV and a decreased PSN or increased SNS activity [108]. According to Lovallo and Von Borrel, a decreased HRV is correlated to the SNS activity during a stress response [109], while Ahmed found no proof for a direct relation between the SNS activation and the HRV [110]. Porges, on the other hand, was able to link the PNS activity to the HRV without problems [111]. According to Tiller, psychological stressors induce a change in HRV, while they are often not reflected in changes in HR or RR [112], denoting the HRV as a better measure for stress than the HR measures.

In literature, different techniques are used to assess the stress level of horses, including blood or salivary cortisol concentrations [113], [114], absolute HR measures [113], [114], HRV [113], [114], [115], SDRR [113] RMSSD [113]. Depending on the situation, some measures are more suitable to evaluate stress, while in other circumstances, another might be more useful. In the specific case of the foal trainings of Foal NZ, it is interesting to further investigate the use of HR measures and HRV to evaluate the stress during training sessions and follow the progress of the foals in their training program. In contrast to taking cortisol samples, ECG signals can easily be measured during training without disturbing the training session of the foals.

MODELLING

With the eye on the development of real-time stress monitoring during foal training, off-line stress monitoring is performed. Based on the model parameters of estimated black box models, features are calculated and used to monitor stress. In the modelling procedure, different approaches are used to estimate the black box models. Both the opportunities of time-invariant and time-variant models are investigated. Due to the individual differences and time-variance of a living system, the models needed to be estimated for every horse and every training respectively.

TIME-INVARIANT MODELS

The modelling procedure started by investigating the possibility to model a time-invariant SISO model in a non-stressful part of the training with a step input for ACT, indicated as a CONDITION I. The goal was to use the SISO model to simulate the changes in HR as a cause of variations in the ACT. The ΔHR = measured HR – simulated HR then represents the HR changes due to stress, meaning ΔHR can be used as a measure to monitor stress.

SISO MODELS

To facilitate the modelling procedure a Modelling program was written in Matlab. The program consisted of different steps, including filtering, data selection, model estimation and selection of the best model based on the YIC and R2 identifications criterions. To select part of the data, on which the model will be estimated, that meets the criteria of CONDITION I, a summarising plot of the training session is created. In Figure 48, the example of the FOLLOWUP session of

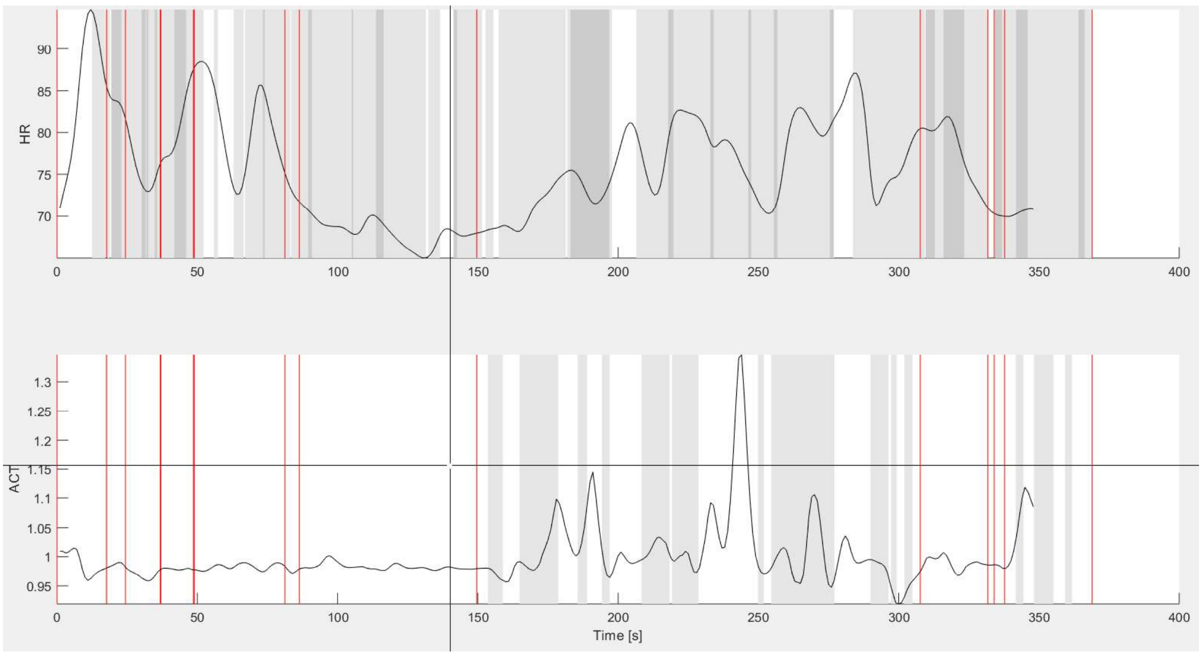


Figure 48 Black Beauty FOLLOWUP data selection in modelling program

Black Beauty is given. In the first subplot, the output HR is plotted, with in grey scale the gold standard stress levels: white indicates stress level 1, light grey indicates stress level 2 and dark grey stress level 3. On the second subplot, the measured input ACT is plotted with on the background the labelled activity. The red vertical lines in both subplots indicate the start and end times of the respective training parts.

In the process, however, some difficulties were encountered in estimating proper SISO models. As can be seen in Figure 48, it is challenging to find parts in the training session answering the required conditions of CONDITION I. First, the times the foals are in stress level 1 during the training session are short and limited, while most of the time even non-existent. Meanwhile, the ACT during the trainings is very low and variable. The foals only walk a few tours during the training sessions, in which they randomly start, stop, start again with, and so on, meaning the activity also lacks the presence of a step up or step down, followed by a steady state. The training and thus the activity is mainly determined by the foal itself.

In Figure 49, the selected data part used to estimate the SISO model for the FOLLOWUP of Black Beauty is plotted. In the first subplot, the input ACT is plotted, while the second subplot includes the measured output HR and the estimated model. The estimated model is characterised with a first order for the autoregressive polynomial, a fourth order for the input polynomial, while the YIC = - 0.44496 and R^2 = 0.84939. As can be seen in Figure 49, the badly estimated model is unable to obtain a good fit for the data on which the model is estimated, making it useless and unsuitable to use it to do a HR simulation. The bad estimation might be due to the low and variable activity and the lack of a step input, followed by a steady state. As a next step in the modelling procedure, the SISO modelling was performed on the labelled activity vector instead of the measured activity. The labelled activity vector is one when the foal is moving and zero when it is not moving and therefore contains more data parts in the training where a clear step up or step down is present. However, experience learned that using the labelled activity allows better

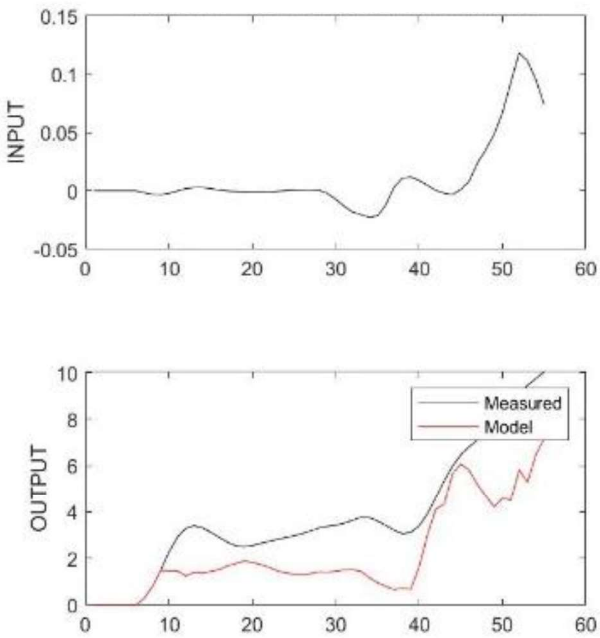


Figure 49 Example data selection and model estimation in modelling program

models than the measured activity, for most of the training sessions it was impossible to find a step up or step down in the ACT in CONDITION I followed by a steady state. Therefore, it is decided to reorient the focus of the modelling procedure to the investigation of time-variant models.

TIME-VARIANT MODELS

For the time-variant modelling, it was decided to keep on working with the labelled activity by using the two vectors WALKING and STANDING as inputs.

MISO MODELS

The time-variant modelling procedure started by the identification of the model order. Manually testing different model orders for the input and output polynomials, indicated that higher order models did not provide a better fit. The goodness of fit is represented by the average standard error (AvSE) or the mean difference between the measured HR and the modelled HR, illustrated in Figure 50. In Figure 51, the AvSEs for different models with model order 1 for the autoregressive polynomial and model orders 1, 2, 3, 4 and 5 for the input polynomial(s) are given. The mean AvSE for models with fifth order input polynomial(s) is 2.56 bpm, while the mean for models with first order input polynomial(s) is 2.63 bpm, which is only 2.7 % higher than for the fifth order input polynomial(s). The estimated DARX models with first order autoregressive polynomials and two first order input polynomials result in the estimation of three time-variant model parameters a_1, b_1 and b_2. An example is given in Figure 52, being the estimated first order DARX model of Code Black TRAIN1.

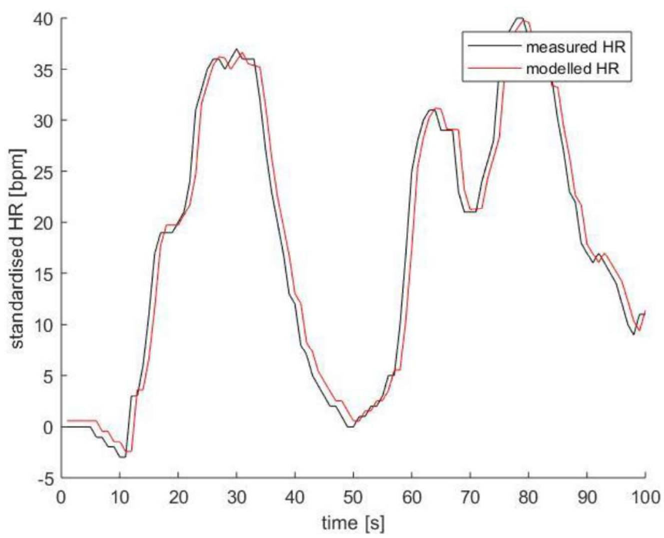


Figure 50 Measured versus modelled HR

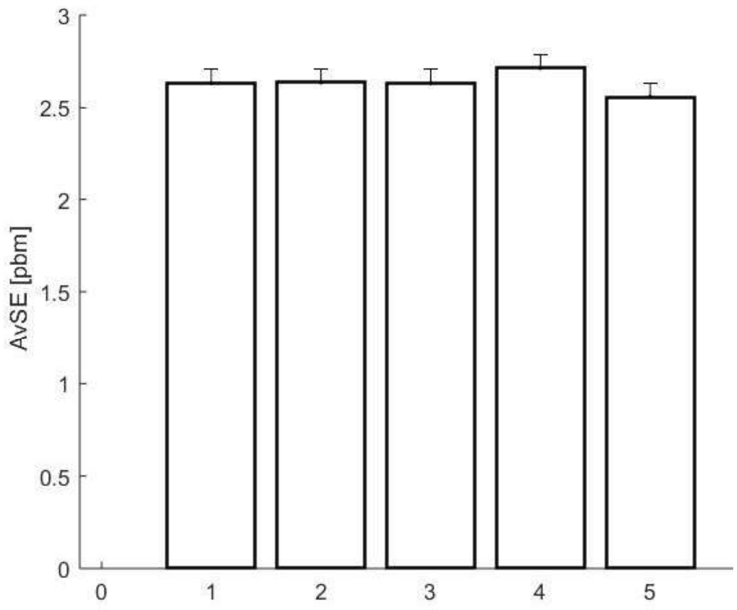


Figure 51 AvSE for different model orders

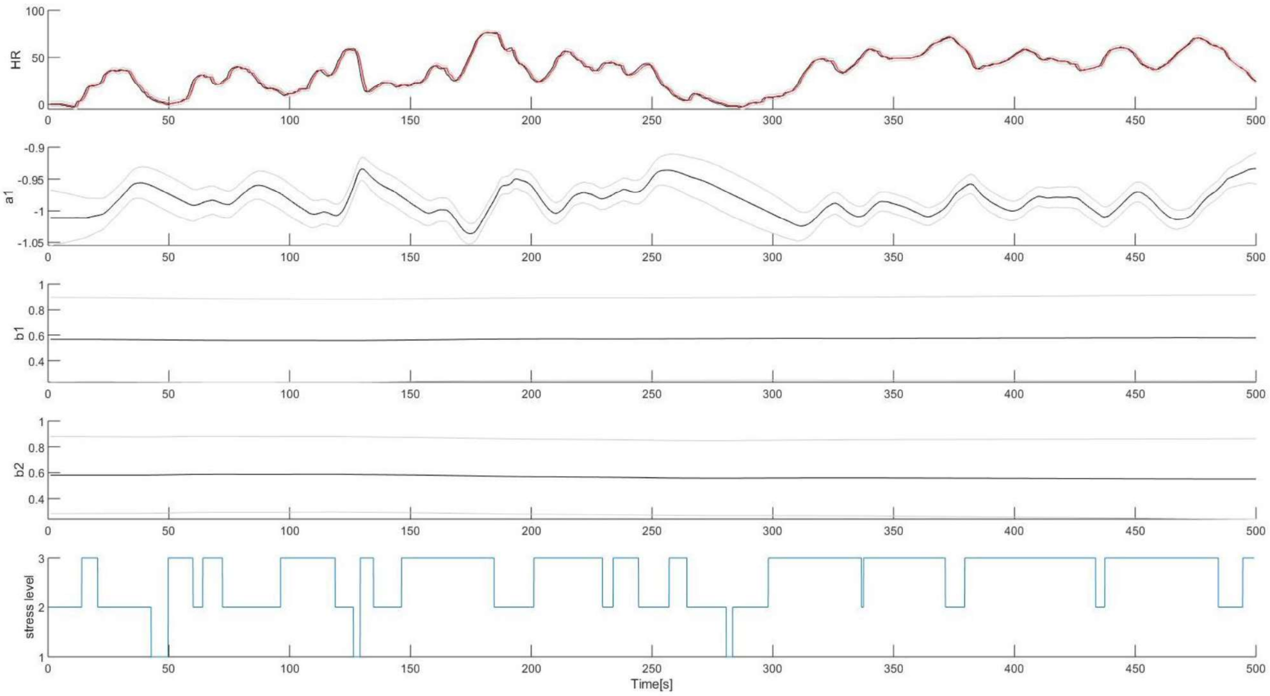


Figure 52 Estimated DARX model with model parameters a_1, b_1, b_2 of Code Black TRAIN1

In Figure 52, the first subplot contains the measured HR (black) and the estimated HR (red), the next three subplots give the estimated model parameters a_1, b_1 and b_2 (black) and the respective estimations error, while the last subplot contains the gold standard stress levels (blue), making it possible to compare the model parameters with the HR and gold standard stress levels. Since the foals are CITD, the DARX models are estimated for all trainings of all foals, with the example of Code Black TRAIN1 in Figure 52 a representative example. For all the estimated DARX models, the time-variant b_1 and b_2 model parameters only slightly changed, while they most of the time can be considered as constant. Apparently, the b_1 and b_2 parameters, referring to the influence of the input variables WALKING and STANDING, provide no addition information concerning the stress level of the foals, which might also be due to the low activity of the foals.

Because the activity input WALKING and STANDING in the MISO models provide no added value, the modelling procedure was reallocated towards time-variant autoregressive models without any other inputs.

AUTOREGRESSIVE MODELS

The modelling procedure is continued with time-variant or dynamic autoregressive models, in short DAR models. By estimating a DAR model, a time-variant parameter a_1 is estimated. The time-variant $a_1$parameter represents the current output in function of the previously measured output. Because of the time-variance and individual differences between the foal, the models are again estimated for all training sessions of all horses separately. The results of the DAR modelling procedure are given by means of two representative case studies. In the first case study, Black Beauty is used as a good representative for the foals that experiencing normal amounts of stress during the training. The second case study is used to give a representative example of a foal experiencing relatively high amounts of stress.

CASE STUDY 1

All training sessions of Black Beauty, including the FU session, are illustrated in Figure 53, with in the first subplot the normalised measured (black) and modelled (blue) HR and in the second subplot the estimated DAR model parameter a_1 (black) with in grey the estimations errors. To enable an investigation of the meaning of the a_1 parameter, the gold standard stress levels are plotted in the third subplot.

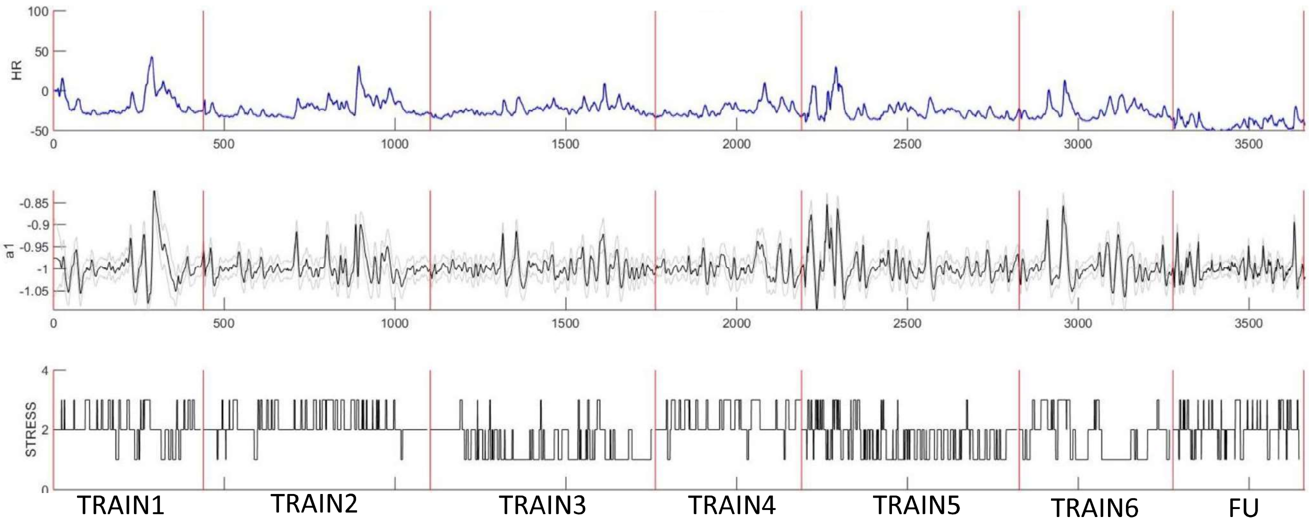


Figure 53 Results DAR modelling with model parameter a_1 for all trainings Black Beauty

In the further analysis on the $a_1$model parameter it is investigation whether it can be used to calculate features to monitor stress. For every training session of Black Beauty, the changes in the a_1 parameter are analysed and compared to the changes in the gold standard stress level. To enable the comparison, Matlab is used to synchronise the video of the training session with the changes in the a_1 parameter and the gold standard stress levels. While the video is playing, a red line is used to run over a_1 parameter and the gold standard stress levels. To illustrate this, nine snapshots of the analysis are given in Figure 54.

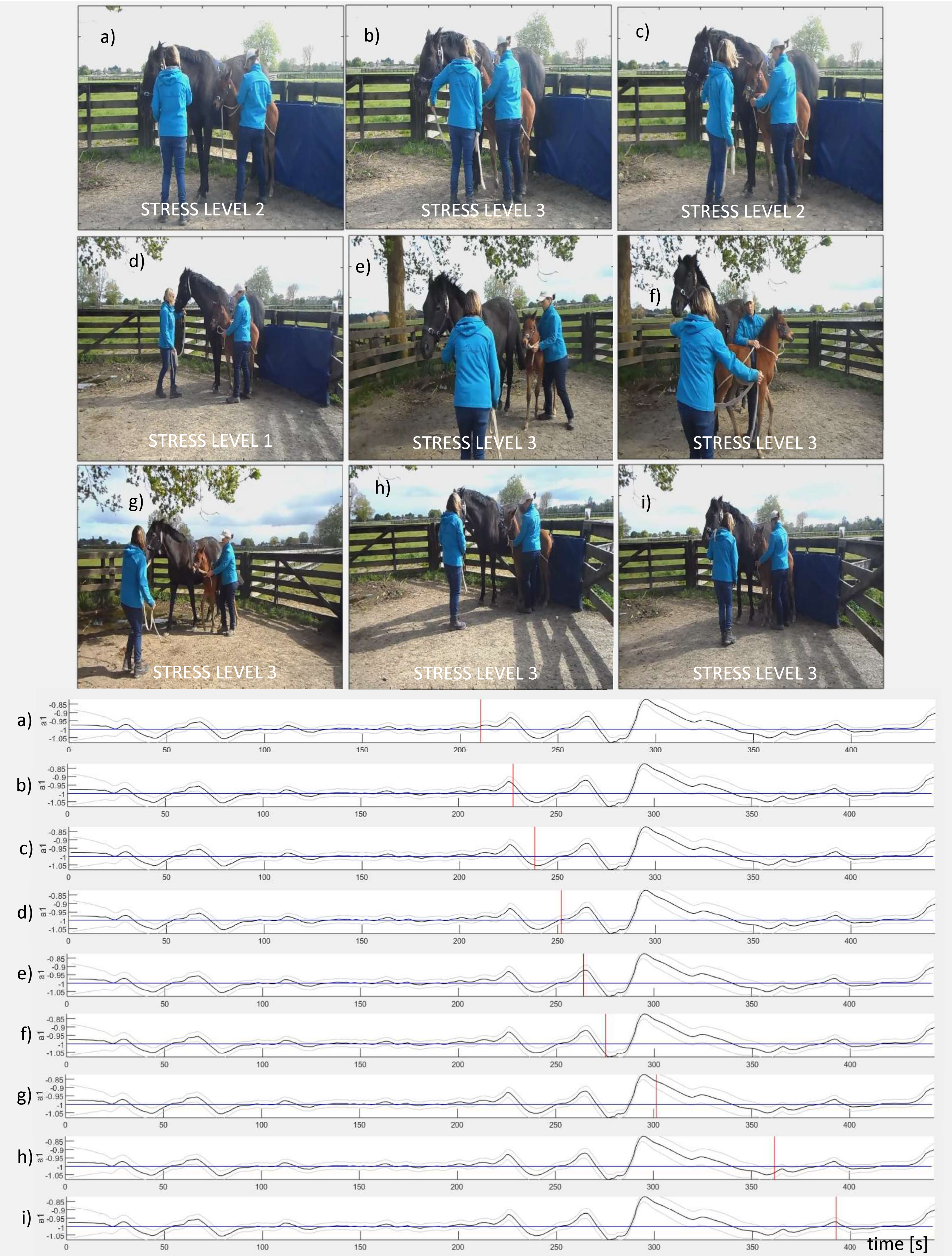


Figure 54 Analysis of a_1 parameter on nine critical points Black Beauty

In Figure 55, the measured and modelled HR, estimated a_1 parameter and gold standard stress levels are again illustrated for Black Beauty TRAIN1. By looking at the snapshots in Figure 54 and the plots in Figure 55, it can be observed that the further Black Beauty's a_1 parameter deviates from -1, the more the foal tends towards stress level 3. This observation indicated that the deviation from the a_1 parameter from -1 might be a good feature to represent the stress levels of the foal. The usability of the feature is investigated in the next section.

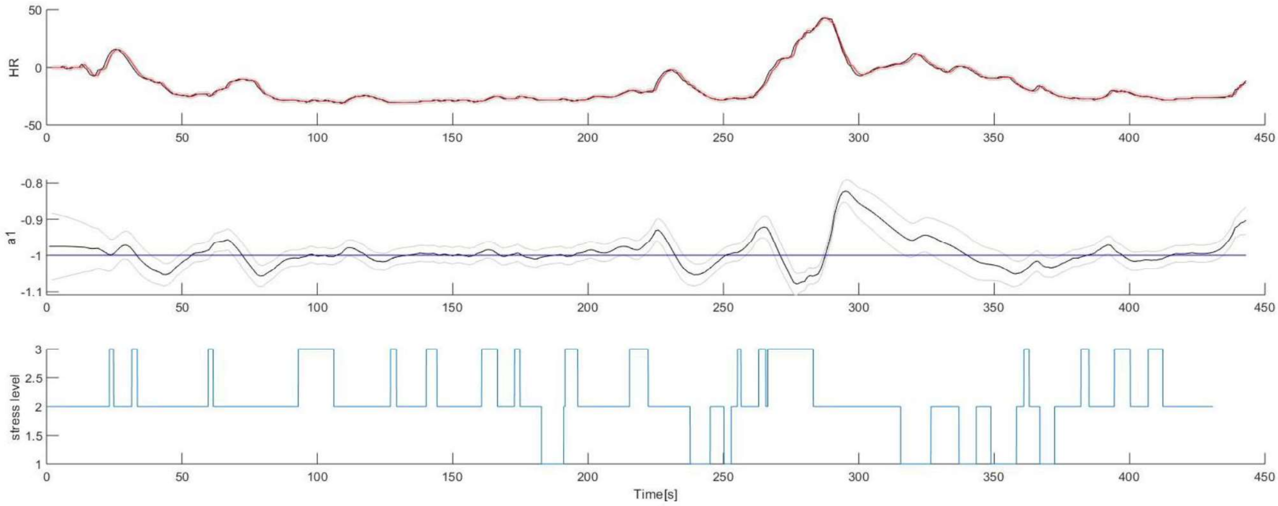


Figure 55 Results DAR modelling with model parameter a_1 for Black Beauty TRAIN1

CASE STUDY 2

In the second case study, changes of the a_1 are analysed for a training in which the foal is experiencing relatively high amounts of stress, for example Code Black. For all training sessions of Code Black, including the FU session, the normalised HR, estimated a_1 model parameter and gold standard stress levels are plotted in Figure 56.

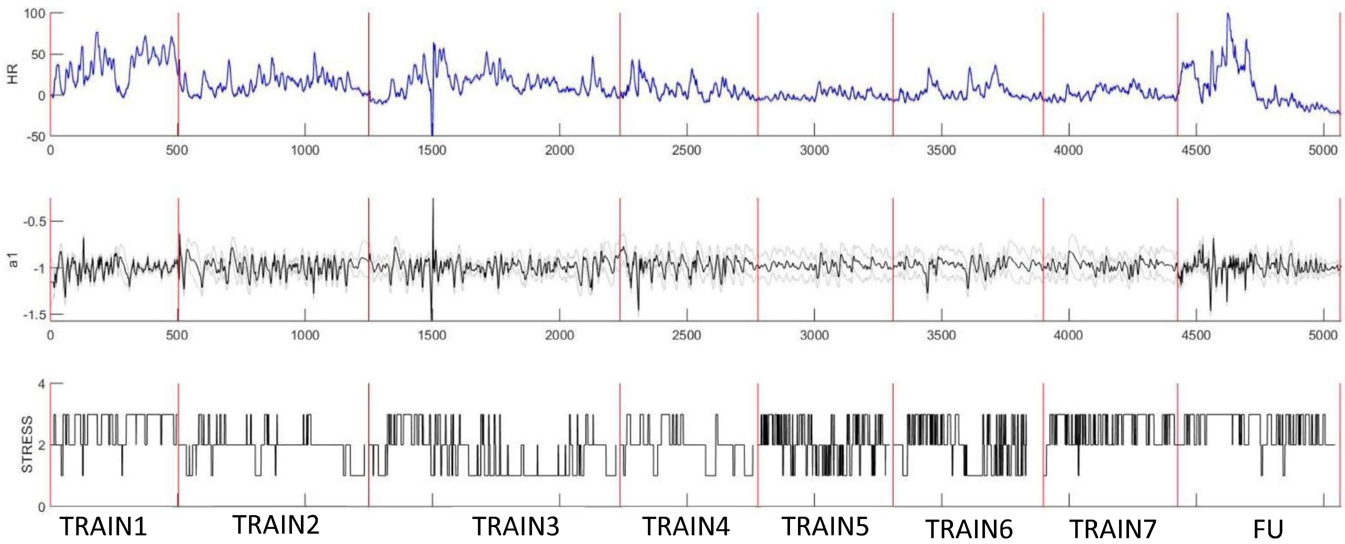


Figure 56 Results DAR modelling with model parameter a_1 for all trainings Code Black

Since especially during the first training session, Code Black is experiencing extreme amounts of stress, being for more than 60 % of the time in stress level 3, the changes in the a_1 parameter are analysed for Code Black TRAIN1, with snapshots of the analysis given in Figure 58.

By analysing the changes of the a_1 parameter with respect to the gold standard stress levels in Code Black TRAIN1 illustrated in Figure 57, it can be observed that when the a_1 parameter is higher than -1, Code Black is in stress level 1. When, however, the a_1 parameter is around the value of -1, the foal is in stress level 2 and when lower than -1, Code Black is in stress level 3.

In contrast to Black Beauty, not the deviation from -1 but the absolute value relatively to -1 is linked with stress for Code Black. This observation indicates that the value relatively to -1 might be a good feature to represent the stress levels of the foals experiencing relatively high amounts of stress.

The different link between changes in the a_1 parameter and the stress levels for foals experiencing either normal amounts or relatively high amounts of stress, might suggest that the link is not only horse-specific due to the CITD characteristic, but also depending on the general stress level within the training session.

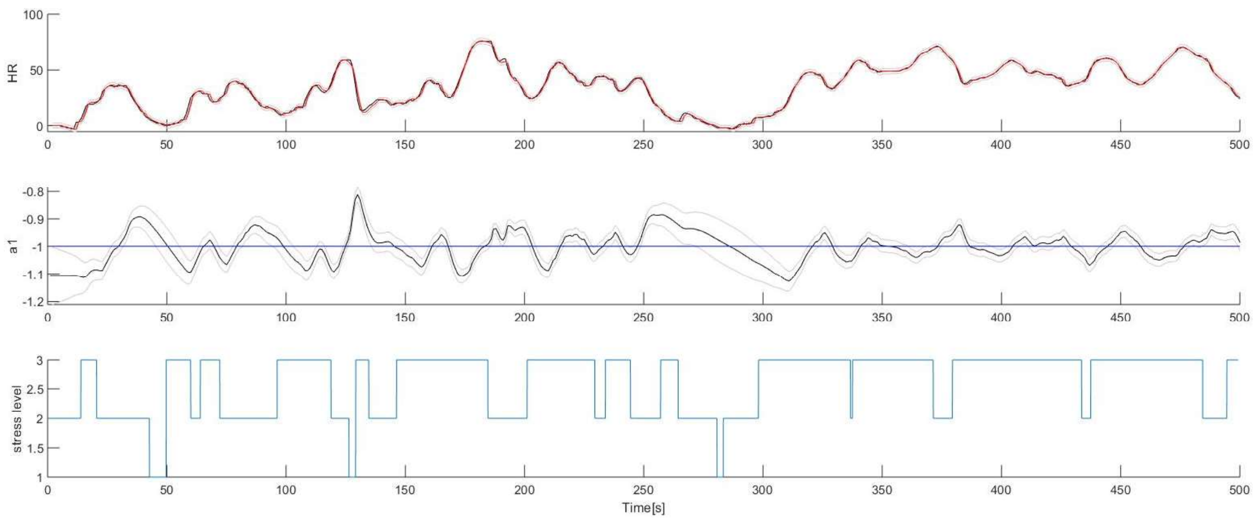


Figure 57 Results DAR modelling with model parameter a_1 for Code Black TRAIN1

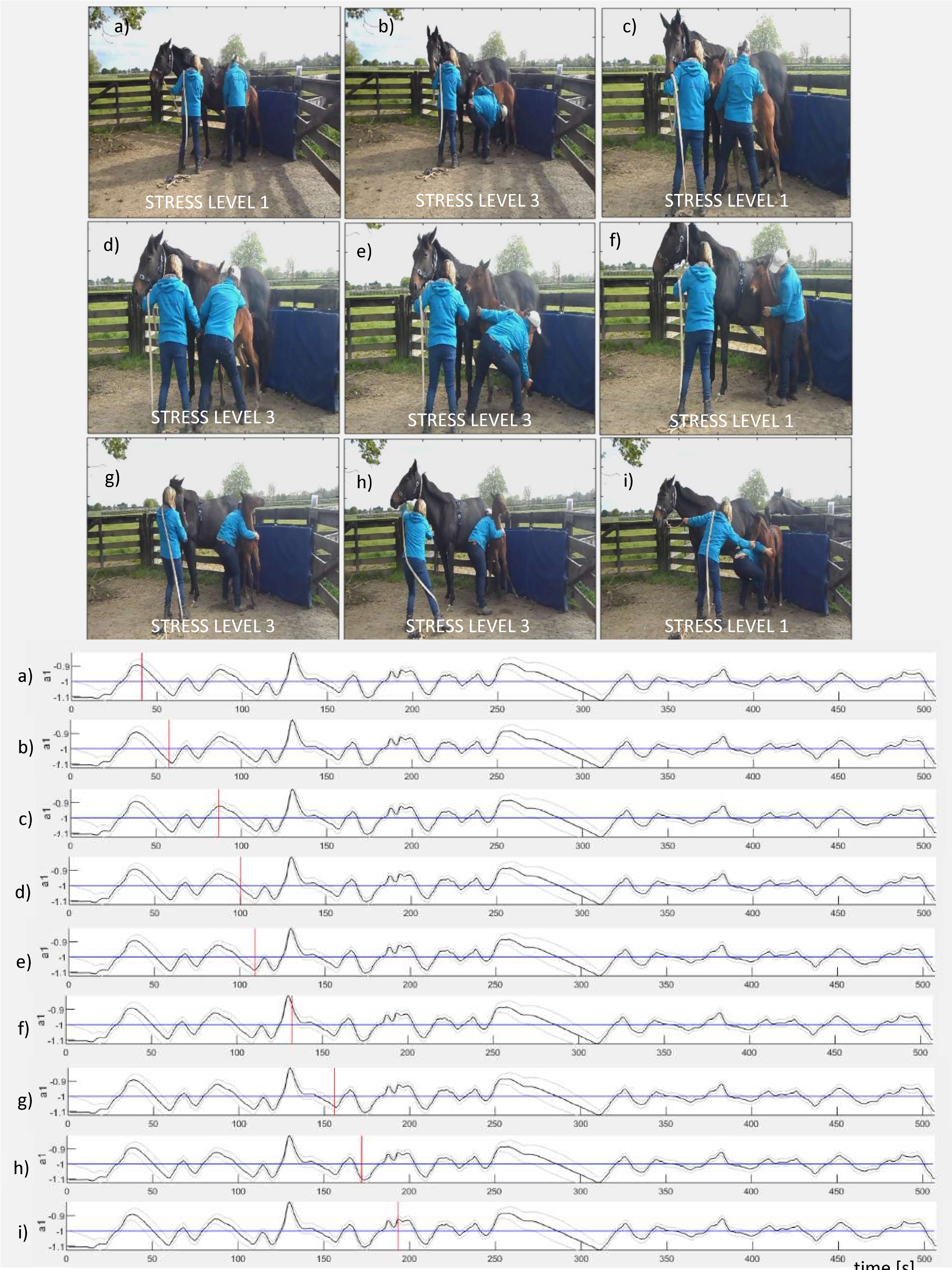


Figure 58 Analysis of a_1 parameter on nine critical points Code Black

MONITOR STRESS

Based on the observation of the changes of the a_1 parameter in relation to the gold standard stress levels, four different approaches are used in Algorithm I for the calculation of features that will be used to monitor stress off-line.

The first approach is based on the change of the a_1 parameter in the case study of Code Black. For Code Black, a decreasing a_1 parameter is associated with an increase in stress, either stress level 2 or 3. The first feature F1 is defined to indicate stress when the a_1 parameter decreases. In the same training, it was also observed that when the foal is in stress level 3, the a_1 parameter was lowered below -1. Therefore, the second feature F2 is indicating stress level 3 when the a_1 parameter is decreasing and below -1. It was also seen that, sometimes, although the a_1 parameter started increasing after moments of high stress levels, the foal stayed in stress level 3 as long as the a_1 parameter was below -1. Therefore, a third feature F3 is defined as an adapted version of the second feature. The F3 feature indicate stress at the same moments in time as in the F2 features, but for F3 the stress persists until the a_1 parameter crosses the threshold of -1 again.

Based on the observations in the first case study of Black Beauty, the fourth feature F4 is defined as the derivation of the a_1 parameter from -1. If the deviation of the a_1 parameter from -1, exceeds a threshold 1, F4 is indicating stress level 2, while if it exceeds a second threshold 2, the feature indicates stress level 3.

For Black Beauty TRAIN1 and Code Black TRAIN1, the four calculated features are plotted in Figure 59 and Figure 60 respectively.

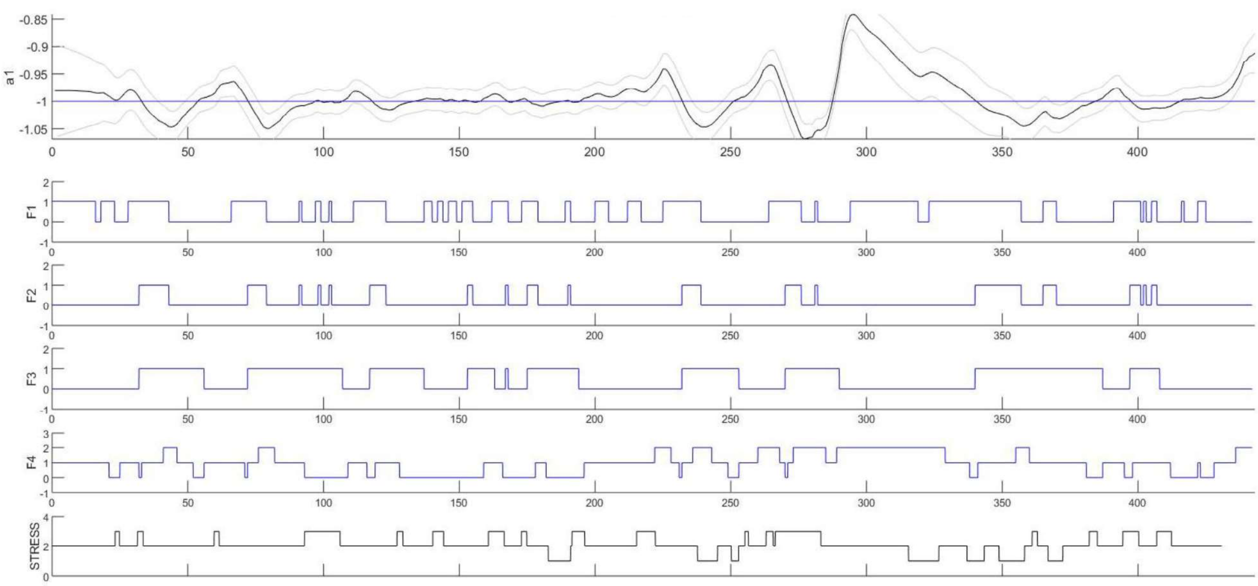


Figure 59 Features to monitor stress off-line Black Beauty TRAIN1

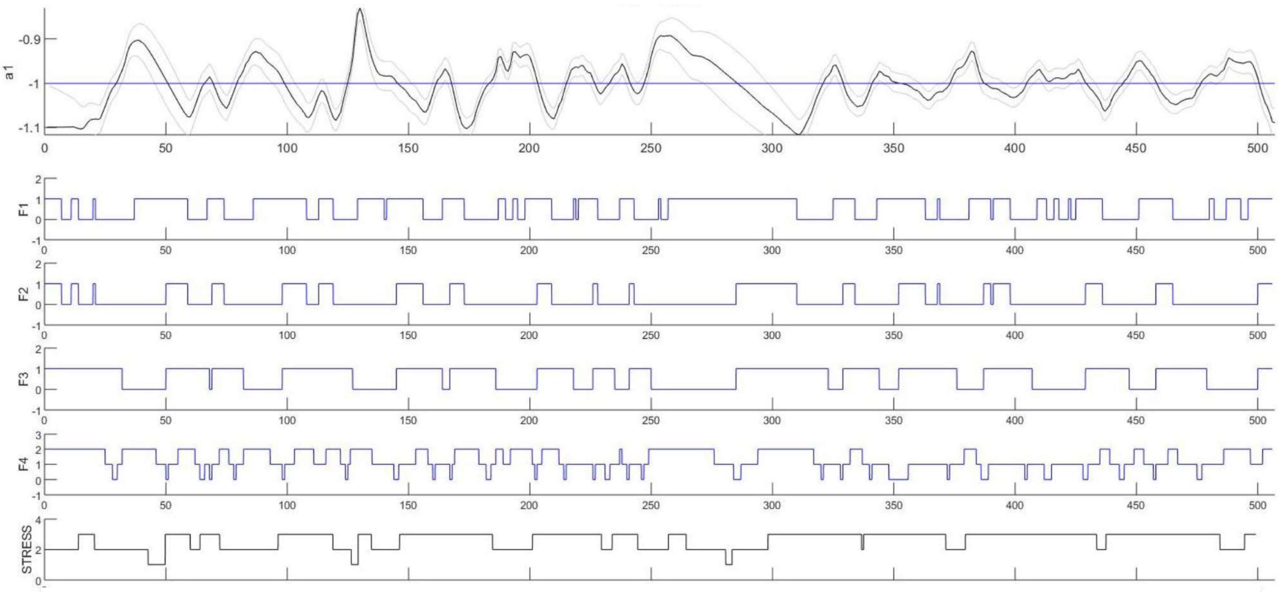


Figure 60 Features to monitor stress off-line Code Black TRAIN1

The first feature F1 is used to indicate stress, being either stress level 2 or stress level 3. The second and third features F2 and F3 indicate the moments the foal is in stress level 3, while the fourth feature F4 estimates when the foal is in stress level 1, 2 and 3. To clarify this description of the features, Figure 29 of the Materials and methods section is repeated here, see Figure 61.

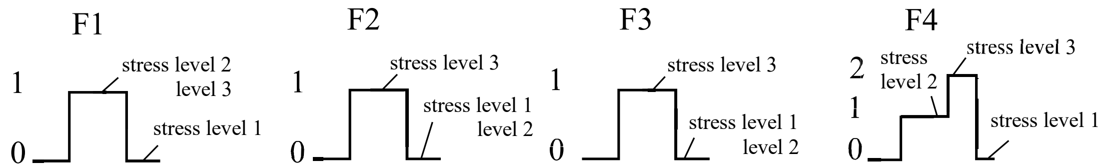


Figure 61 Comparison of F1, F2, F3 and F4 with gold standard stress levels

The quality of the calculated features is tested in Algorithm II and represented by the efficiency at which the features are able to estimate the stress level. In Table 19 the efficiency of the respective features is given for Black Beauty TRAIN1, illustrated in Figure 59 and Code Black TRAIN1, illustrated in Figure 60.

Table 19 Efficiency of features

Black Beauty		Code Black	
F1	47.2 %	F1	54.1 %
F2	69.5 %	F2	51.9 %
F3	53.4 %	F3	63.6 %
F4	47.1 %	F4	31.9 %

Next, the features are calculated for all the other trainings of all foals, with the quality of the respective features represented in Figure 62. The first feature F1 can correctly identify stress in the training session for on average 55.7 % and maximally 69.8 %. In the off-line detection of stress level 3, feature F2 performs with an efficiency of 69.0 %. An example for feature F2 of 84.1 % is given in Figure 63, containing the calculated features for Go South TRAIN5. The third feature F3 is able to identify stress level 3 with an efficiency of 57.4 %, while the fourth feature F4 is has an efficiency of 35.0 %.

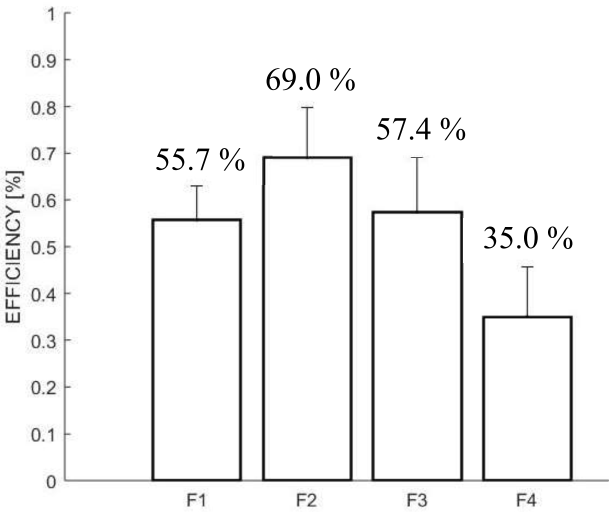


Figure 62 Feature efficiency

The off-line stress monitoring in this research uses features that are defined based on the model parameters of autoregressive models. Although for all the horse in this research, the same definitions and thresholds are used to calculate the features, one of the features F2 was already able to monitor stress level 3 with an efficiency of almost 70%. Although the definition of this feature is quite simple: F2 indicate stress level 3 when the autoregressive model parameter is decreasing and lower than the -1 threshold, monitoring stress level 3 with F2 gives already acceptable results at group level. Meanwhile, for some of the foals, F2 even reaches efficiencies of almost 85 %.

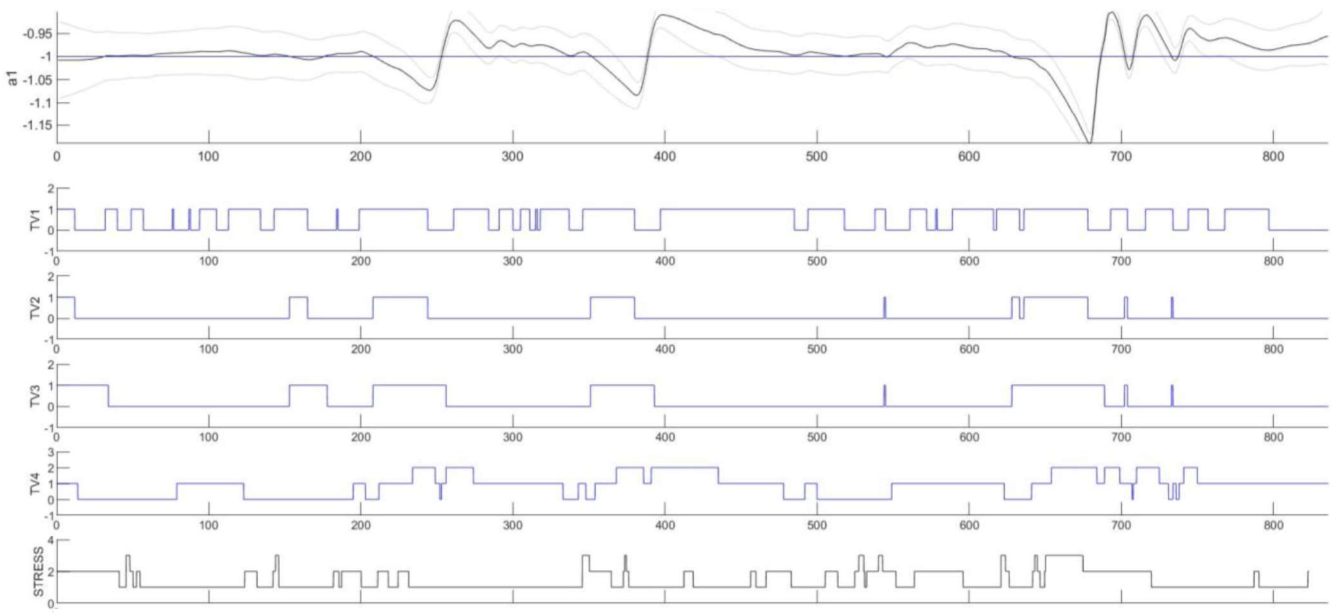


Figure 63 Features to monitor stress off-line Go South TRAIN5

If a feature is already able to correctly detect almost 70 % of stress level 3 without taking into account the individual differences and time-variance of the foals, imagine the efficiencies that can be accomplished when the features consider the CITD characteristics of the foals [116].

The enhance the quality of the off-line stress monitoring, it is recommended to perform further research on the optimisation of these features. The optimisation can be done by optimising the estimated model that represent the horses as a biological system or by adapting the definition of the feature to every respective horse and situation. In this way the optimised features are horse-specific and take into account the time-variance characteristic of the horses.

Researchers were already able to model biological systems [117], [118], and use these models to control the biological system [119]. A horse is a biological system and by measuring its physiological responses to particular (a) input(s) a model can be estimate that represents the horse as a biological system. Using the model, the status of the horse can be monitored or controlled [84]. Depending on the application, it is more appropriate to use time-invariant models or dynamic models. With the eye on the development of real-time monitoring, it is preferred to use dynamic models [120].

In the specific case of stress monitoring in horses, earlier research has been done on estimating models for the horses based on HR and ACC measurements [116]. Hereby, it is important take into account the CITD characteristics of living systems: the response of a horse to a particular input is horse-specific and time-variant [84]. During the modelling procedure, it is important to estimate a separate model for every horse respectively [116] and make the features adaptable to the situation by using dynamic algorithms [116] or artificial intelligence [121].

The enhance the quality of the off-line stress monitoring, further research can be done on the optimisation of the estimated model to represent the horse as a biological system [84]. In the context of stress monitoring during the foal training sessions of Foal NZ, it is recommended to investigate in other techniques to measure the ACT. In this research, the ACT is measured by using one three-dimensional ACC sensors on the left side of the horse, measuring only the movements of the trunk. However, it might be interesting to measure full body movement by obtaining also the movement of the legs and feet, head, neck and tail.

The obtain full body moment data, different techniques can be used. Some technologies use ACC sensors on the respective parts of the body [122], [123], [124], for example the MVN Biomech technology [125], and analyse the kinematic data in specifically developed software [125]. Another possibility is to use optoelectronic systems [126], [127], for example the Vicon system [125]. In an optoelectronic system, reflective markers are places on the respective body parts and their position is analysed by detecting the reflected light on cameras and perform video analysis [125], [127].

The additional ACC information of the full body movements might be an added value and enable the estimation of better models. Another way to improve the quality of the models is to include prior knowledge about the physiological stress response in the models and work towards the development of a grey box model [84]. Better models allow more representative model parameters for particular physiological variables.

For the application of stress monitoring during foal trainings by Foal NZ, practical considerations are important as well. Ideally, the measuring technologies are easy to put on and off, non-invasive and must not disturb the training of the foals, making wearable technologies ideal and have already been used successfully in the context of stress monitoring in horses [116]. Beside the optimisation of the stress monitoring, for Foal NZ it will also be interesting to investigate the interaction between the foal and mare. The interaction exists in two directions [2]. Further research can answer the question whether the mare can be used more effectively to control the stress level of the foal.

CONCLUSION

Biological responses can be used to monitor the status or conditions of biological systems. After estimating a model that represents the biological system, the model parameters can be used to define features used for monitoring a status of the biological system. The HR is a biological response that is directly linked with the physiological stress response. By estimating dynamic autoregressive models on the HR measurements of foals, it was possible to define a feature that is able to monitor high stress levels with an accuracy of almost 70 %.

The HR recordings also enabled the calculation of HR measures that can be used to evaluate the progress of the foals in their training program. In a particular time interval, the median, mean and min HR can be used to assess the mean stress level of the foals in that time interval. The max HR can be used to indicate the occurrence of short moments of high stress levels, while the dynamic range of the HR can be used to evaluate how consistent the stress level is in the time interval. Be observing the evolution of the different HR measures in time, the progress of the foals in their training program can be evaluated. To attain a positive progress, the mean stress level must decrease, indicated with a decreasing median, mean and min HR, and a more consistent stress level throughout the training session is needed, resulting in a lower dynamic range. Meanwhile, short moments of high stress levels must be avoided which is represented by a decreasing max HR measure.

The foal training program offered by Foal NZ is effective in training the foals. The first training session typically induces stress in the foals because of the abrupt change in their environment and the confrontation with training for the first time. During training, they learn some basic behaviours and become more comfortable with the trainings throughout the program. Every time a new exercise is added to the training, the stress level of the foals increases but decrease again in the consecutive sessions. During training, Foal NZ also teach the foals some proper coping behaviours by stimulating them to lick or chew and thereby prevent the development of destructive coping behaviours. By combining the training of basic behaviours with the development of appropriate coping strategies, Foal NZ is not only able to improve the welfare and performance of the horse, but also the safety for the people working with them.

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APPENDIX I HORSE PHYSIOLOGY

For a better understanding of the stress response, basic knowledge about the physiology of a horse is beneficial. In general, the nervous system (NS) is responsible for the initiation and coordination of the physiological processes of the stress response, while the endocrine system (ES) is responsible for the regulation of the physiological response [45].

Based on the anatomical position, the NS can be subdivided into different parts: the central nervous system (CNS) and the peripheral nervous system (PeNS). The CNS consists of the brain, brain stem and spinal cord, underlined in Figure I1, and is the central controlling system of the body. The PeNS, on the other hand, consists of the nerves in between the CNS and the periphery of the body and is responsible for the communication of the CNS and the environment [45].

Depending on the part of the CNS where the peripheral nerves depart, cranial PeNS nerves and spinal PeNS nerves can be distinguished, both indicated in a frame in Figure I1. Depending on the direction of the transmitted signals, a difference is made between afferent and efferent signals. A stimulus, received by a receptor organ, is transferred into an afferent signal. The afferent signal is sent through the PeNS to the CNS. The afferent signals are then further processed in the CNS. As a respond to the stimulus, the CNS will send out an efferent signal, which will be passed through the PNS to an effector organ. For example, if light is captured by receptors in the eyes, the signals is transferred into an afferent signal and sent through the PeNS to the CNS. If the light is too bright and the CNS sends out signals an efferent signal to the eyes to close them [45].

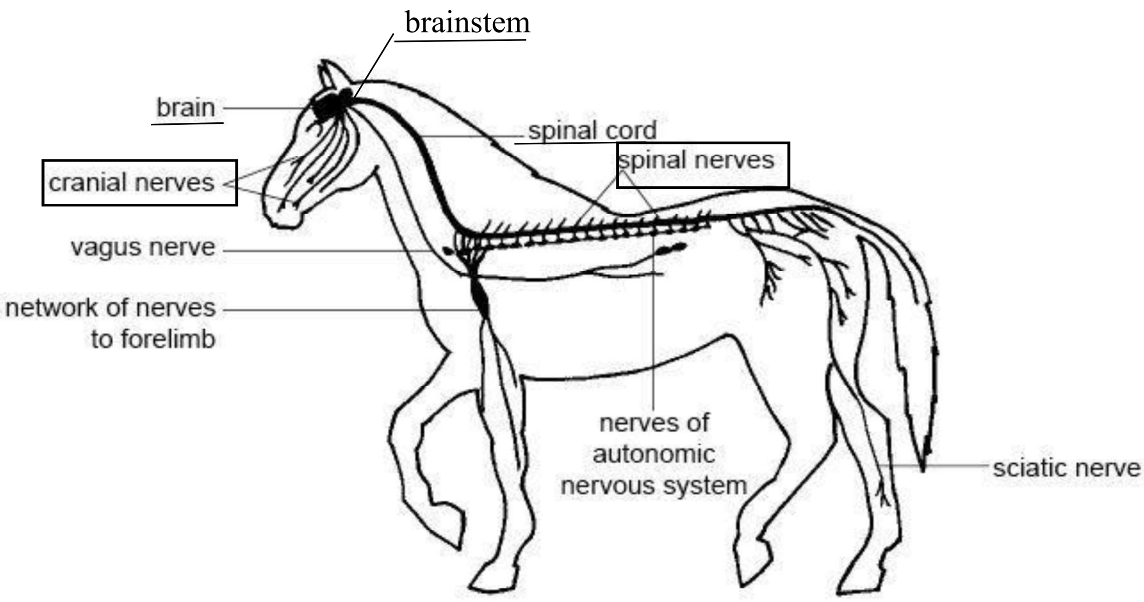

Figure I1 Nervous system of a horse [215]

The NS can also be subdivided based on its function, into the somatic nervous system or voluntary nervous system (VNS), involved in consciously steered processes and the autonomous nervous system (ANS), involved in the primarily regulation of the body without being aware of it. The VNS refers to the parts of the NS that can be controlled by conscious control and consist basically of the motor parts of the NS responsible for body movements [45].

FUNCTION

The function of the NS is threefold, namely sensing, processing and responding. Before the body can adapt to changes, the body needs to be able to sense changes both inside the body and the environment. For example, eyes sense changes in light frequency and intensity and the ear detects changes is pressure waves in the environment. Inside the body, for example, biochemical receptors react to changes in concentration of ions or for example pH. After sensing, the received information is synchronised, analysed and processed in the CNS. The CNS is also able to store to information, which enables the ability to remember and learn from earlier experiences. Once the received stimulus is processed in the CNS, an appropriate response is initiated, for example muscle contraction or gland secretion as a reaction to the stimulus [45].

MORPHOLOGY

The NS is a complex network of basic building blocks, known as neurons. A neuron consists of three main parts: the cell body, the axon and dendrites, as illustrated in Figure I2.

NEURON

The cell body forms the controlling centre of the neuron and contains the nucleus, Golgi apparatus, mitochondrion and the endoplasmic reticulum. The dendrites and axon, on the other hand, have the function of making connections between the cell bodies of neighbouring neurons. Dendrites receive incoming signals and convert them into a cross-membrane potential in the cell body. If the cross-membrane potential, resulting from multiple dendrite, is larger than

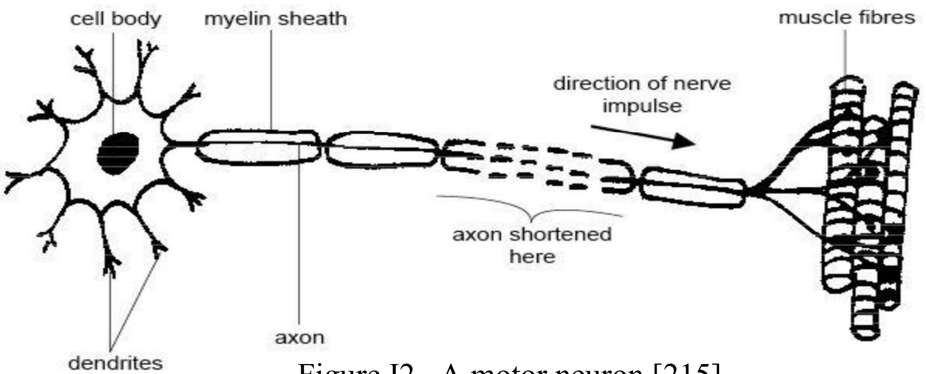

Figure I2   A motor neuron [215]

a certain threshold, the neuron is activated and an action potential is generated and transmitted along the axon. At the end of the axon, the signal is, on his turn, transmitted to the dendrites of other neighbouring neurons. The end of the axon forms branches, called telodendrions, making it possible for an axon to connect with dendrites of multiple neurons [128].

NEURON TYPES

Depending on the position and the signal that is being transferred in the neuron, three different neurons can be distinguished: a sensory neuron, a relay neuron and a motor neuron. A sensory neuron is a neuron that transmits signals from a sensory organ, to the CNS. In Figure I3, the example is given for a pin that pricks in the skin of a horse. The pain receptors in the skin will be triggered and will send a sensory signal towards the spinal cord. In the spinal cord the signal is transferred from the sensory neuron to a relay neuron of the spinal cord. A relay neuron is a neuron located in the spinal cord or the brain that forms the connection between sensory and motor neurons. The motor neuron then transmits the signal from the relay neuron to a muscle of gland to stimulate muscle contraction of gland secretion [45].

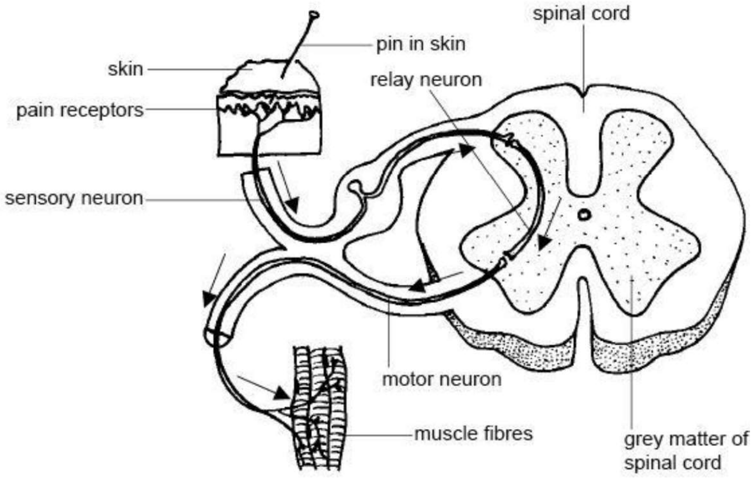

Figure I3 Sensory, relay and motor neurons [215]

SIGNAL TRANSDUCTION

Within a neuron the cross-membrane potential is transmitted as an action potential in the axon, while the transmission of the signal from one neuron to another, is synaptic, as illustrated in Figure I4. At the end of the axon, in the synaptic terminals of the telodendrion, the action potential triggers the presynaptic membrane to excrete neurotransmitters and release them into the synaptic cleft. After crossing the synaptic cleft, the neurotransmitters are recognized by neurotransmitter receptors on the postsynaptic membranes of the dendrites. Then, an electrical signal is generated and transmitted through the dendrites to the cell body of the next neuron. In this way, signals can be transferred from one neuron to another. Therefore, when extending to a larger scale, signals can be sent through the nervous system [128].

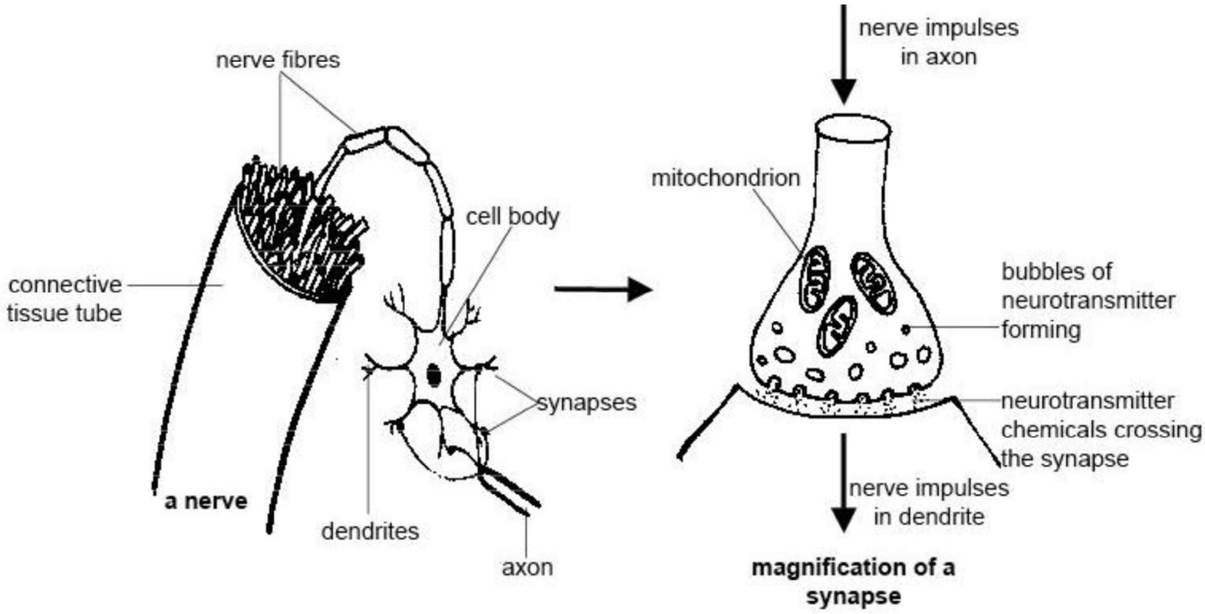

Figure I4 Nerve and magnification of a synapse [215]

NEUROTRANSMITTERS

Different kinds of neurotransmitters exist. Depending on the function of the particular part of the nervous system, the neurons use other neurotransmitters. For example, in the SNS the neurotransmitter norepinephrine (NE) is used for excitation, while acetylcholine (Ach) is used by the VNS and the PNS to restore homeostasis. Some neurotransmitters are released in different parts of the NC all over the body, but others are, for example, only released in the CNS, like dopamine (DA), serotonin (5-HT) and gamma aminobutyric acid (GABA) only released in the brain and spinal cord [45].

NOREPINEPHRINE

During a stress response, the production of the neurotransmitter NE is increased, both in the brain and in the postganglionic SNS innervations of critical organs. NE, used in a positive feedback loop, enables a general excitation of the body, preparing it for a possible fight-or-flight response [45].

ACETYLCHOLINE

To be able to relax after a stress response, the body needs other neurotransmitters to be released with an opposite effect on the body. To relax, the neurotransmitter Ach is released in the preganglionic junctions of the ANS and in the postganglionic innervations of the PNS, which results in a general relaxation of the body. The body will restore the homeostatic balance and focusses again on maintain vital body functions [45].

CENTRAL NERVOUS SYSTEM

The CNS, being the brain, brainstem and spinal cord consists of white matter, containing bundles of axons, and grey matter, containing the cell bodies of the neurons [45].

BRAIN

The brain consists of different regions that are responsible for different functions. The brain of a horse consists of four main parts: the fore brain, the mid brain, the cerebellum and the hind brain, as illustrated in Figure I5 on the dorsal and lateral view [55].

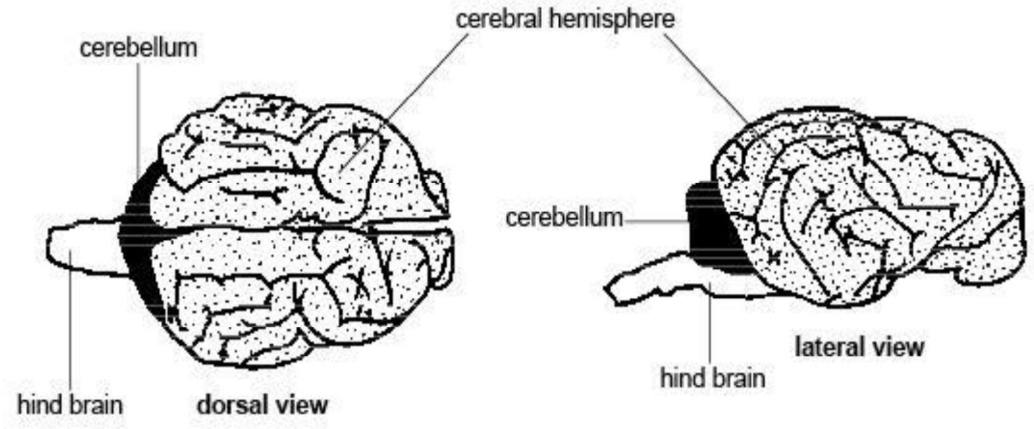

Figure I5 Dorsal and lateral view of the brain of a horse [215]

FORE BRAIN

The large sagittal section in Figure I6, clearly illustrates the different parts of the fore brain, consisting of the two cerebral hemispheres. The left and right cerebral hemisphere are separated by a deep hemisphere groove but are still interconnected with a bundle of nerve fibres in the middle of the brain. The grey matter, containing of the cell bodies of the neurons, are located at the outside of a cerebral hemispheres, referred to as cerebral cortex, and is responsible for the functionality of the cortex. The inside of the cerebral hemispheres, called cerebrum, consists of white matter and includes the axons of the neuron, being responsible for signal transduction. The cerebral cortex is responsible for the processing of incoming signals, coordination of movement, intelligence, memory and reasoning. Specific regions in the cortex are related with particular functions [55]. For example, the sensory cortex is responsible for the processing of vision, hearing, taste and smell, while the motor cortex is responsible for the coordination of movement of the fore-limbs, hind-limbs or tail [84]. For example, when a horse sees and smells food, sensory impulses pass via the optic and olfactory nerve to the sensory cortex and are interpreted. If the horse is hungry and decides to walk towards the food, the motor cortex initiates and coordinates the movement towards the food [55].

MID BRAIN

The brain parts that play a central role in the initiation and coordination of the stress response, however, are located in the mid brain, including the thalamus, hypothalamus, hippocampus and pituitary gland. The hypothalamus forms the bridge between the nervous system and the endocrine system since it is located in the base of the mid brain and at the same time the central controlling centre for the regulation of hormone balances in the body [45], [55].

The hypothalamus produces some hormones itself, but it mainly controls the production and release of hormones in the pituitary gland. Hereby, it is responsible for the regulation of physiological processes in the body. It ensures the maintenance of the homeostatic balance, a constant body temperature, blood pressure and HR, while it coordinates the physiological stress response [45], [55].

CEREBELLUM

The cerebellum, or little brain, is important for muscle contraction, keeping posture and balance while walking or running. By receiving information from the organ of balance and stretch receptors in tendons and muscles, the cerebellum coordinates movement [45], [55].

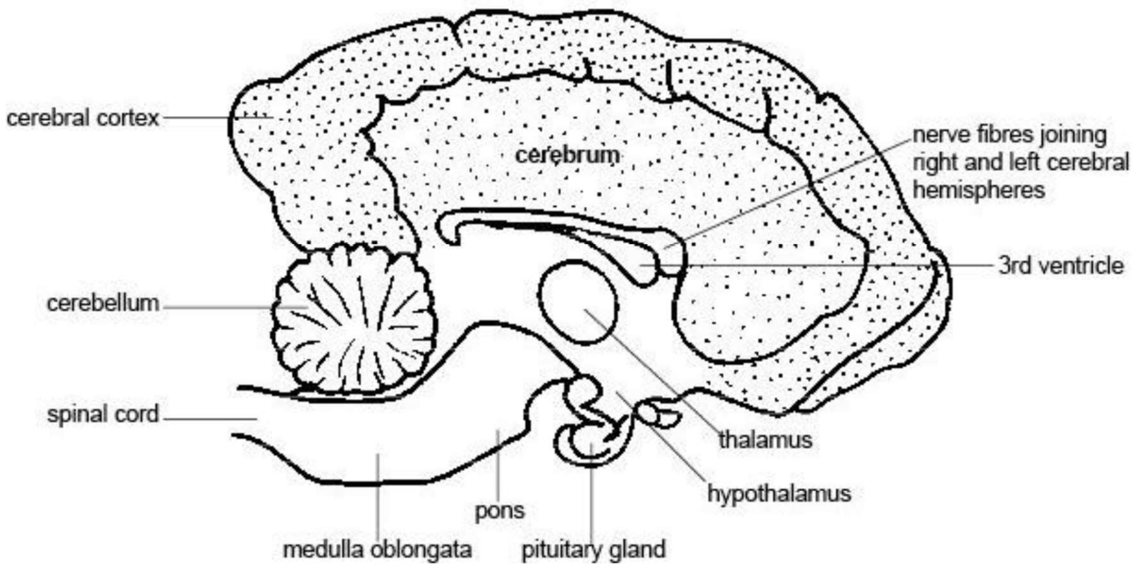

Figure I6 Longitudinal section of the brain of a horse [215]

AUTONOMOUS NERVOUS SYSTEM

The ANS consist of the sympathetic (SNS) and parasympathetic (PNS) nervous system [45] and is responsible for the primarily regulations in body processes, like working of the intestines, glands and smooth muscle function, that are occurring unconsciously [45]. It ensures the homeostatic balance, even when the inner or outer environment is changing. Keeping homeostasis is a matter of balancing processes and obtaining a steady state. The ANS contains two subsystems that work contradictorily: the sympathetic autonomous nervous systems (SNS) and the parasympathetic autonomous nervous system (PNS). Both systems are affecting the same organs but have an opposite effect [45]. Generally, the SNS has a stimulating effect, while the PSNS has more a calming effect, as illustrated in Figure I7.

SYMPATHETIC NERVOUS SYSTEM

In case of stress, the SNS enables a stress response in which the HR, BP, COV and RR automatically increase. For example, when a horse is confronted with a predator, the ANC will automatically increase the HR and RR to increase the energy flow in the body. It will dilate the blood vessels to provide more blood to the muscles, while the amount of blood flowing to less critical regions, like the gut and skin, reduced. It will stimulate the liver to release more glucose. The horse is automatically prepared to flee from the predator without consciously being aware of it [45].

PARASYMPATHETIC NERVOUS SYSTEM

Once the horse is safe, the PNS enables the horse to relax again. Without the possibility to counteract the activation of the SNS, the body will not be able to recover to a resting steady state. After the stimulation of the SNS, the PNS will lower the activation of the heart muscle, smooth muscles, intestines and glands again. The body will restore its normal functioning and restore the resting homeostatic balance. The normal functions of a relaxed body are for example an effective digestion and defaecation and urination, with a regular heartbeat and respiratory rate. Therefore, the SNS is responsible for a stress response and the PNS for calming down after a stress response [45].

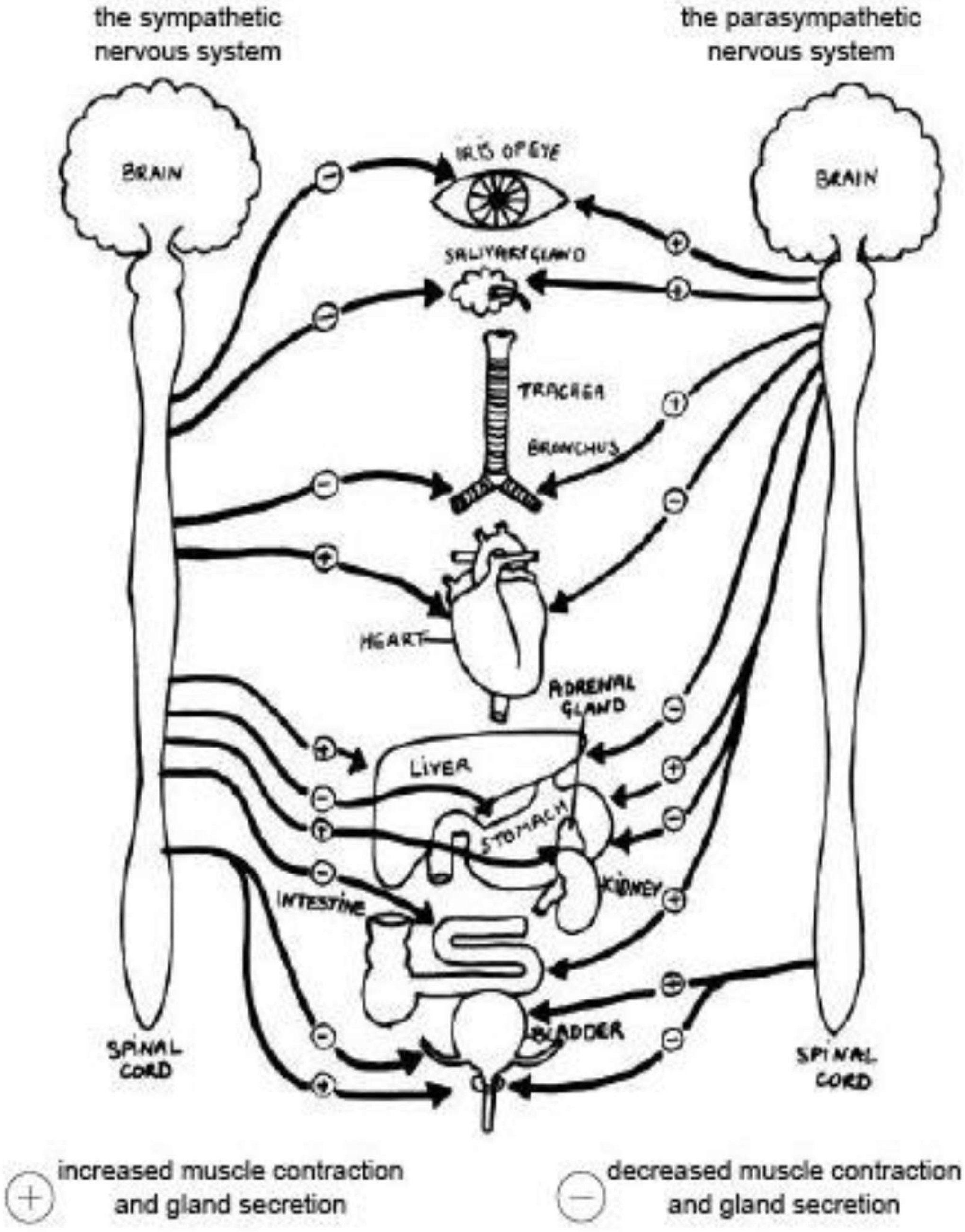

Figure I7 Function of the sympathetic and parasympathetic nervous systems [215]

APPENDIX II STRESS ETHOGRAM

Table II1 Stress ethogram with signs of tensed uncomfortable feeling

SIGNS OF TENSED UNCOMFORTABLE FEELING					
BODY PART	STRESS INDICATOR	STRESS			EXPLANATION
FACIAL EXPRESSIONS		1	2	3	
eyes					
	neutral	1			eyes are relaxed, no wrinkles are visible
	rounded lid	1			top lid of the eye is rounded and not triangulated
	blinking		2		blinking too rapid or slow
	wrinkles		2		wrinkles around the eyes
	tense eyes		2		Top lid of the eye is triangulated
	looking away		2		looking away and averting eyes from trainer
	wide open eyes			3	white of the eyes is visible
	eyes closed			3	(half) closed eyes while horse is dissociated
ears					uni- or bilateral
	neutral	1			ears relaxed at the base
	forward		2		ears pointing forward with little movement
	backwards		2		ears pointing backwards with little movement
	rapid moving		2		rapidly moving back and forth
	flattened			3	ears entirely flattened
mouth					
	relaxed	1			jaws are relaxed and lip is relaxed, no wrinkles
	gaping		2		horse opens/closes mouth repetitively
	tight		2		mouth and chin closed and lips tight
	wrinkles		2		wrinkles on lips
	jaw twisting		2		jaw twisting
	grinding		2		horse grinds teeth
	tense jaws			3	tense jaw and no movement
	exposed teeth			3	teeth exposed as threat to bite
	biting			3	horse is biting you or snapping at you
tongue					
	tongue out	1			tongue is hanging out of the mouth
	lolling		2		horse is moving with the tongue
nose					
	neutral	1			nostrils are relaxed
	tense nostrils		2		nostrils are tense
	wide nostrils			3	nostrils very wide open
	narrow nostrils			3	nostrils very narrow

Table II1 Continued

BODY PARTS					
head and neck					
	neutral	1			head and neck are relaxed and held stable
	tensed neck		2		neck muscle are tenses or neck arches
	turning away		2		turning head away
	startle reflex			3	head held high, known as startle reflex posture
	tilting head			3	horse tilts head to one side
	tossing			3	horse moves in a quick forward-upward motion
	shaking			3	horse shakes head in a quick left to right motion
	threatening			3	head held low and in great tension as a threat
legs					
	paw ground		2		horse paws the ground as displacement behaviour
	stomping		2		horse stomps on the ground or objects
tail					
	neutral	1			tail is held in low neutral position
	tense tail		2		stiffness in base of the tail
	high tail		2		tail is elevated, higher than horizontal line of back
	swishing		2		horse is swishing tail irregularly
	swishing			3	wringing and exaggerated movement of the tail
	pressed tail			3	tail is pressed to the body
posture					
	relaxed	1			horse has relaxed barrel
	tense barrel		2		horse exhibits a tension in the barrel
	weight shifting		2		weight shifted on hind end or front end
MOVEMENT					
gait					
	smooth gait	1			active and smooth gait, purity of movement
	smooth transitions	1			transitions of gaits are smooth and relaxed
	rhythmic	1			horse moves with rhythm
	choppy stride		2		choppy stride
	hitting ground		2		hooves hit the ground hard
	tensed transitions		2		transition of gait looks like they are an effort

Table II1 Continued

velocity					
	neutral velocity	1			horse moves in constant desired velocity
	hesitant		2		horse hesitates to do movements
	moving		2		difficult to stand still
	freeze		2		freeze or difficulty to move or “lazy” appearance
	halt		2		cessation of movement of all four feet
	backwards		2		horse moves backwards
	abrupt halt			3	abrupt cessation of movement/going forward
	fast backwards			3	fast backward movement, usually after abrupt halt
	quick movements			3	horse becomes quick in its movement
sudden movements					
	jumping aside		2		horse jumps aside
	refusing		2		horse refuses to execute an order
	defaecation		2		increased defaecation or urination
	bucking			3	horse is bucking
	rearing			3	horse is rearing
	kicking			3	horse is (threatening to) kick/strike out
	fleeing			3	chases away from an object or side of an area
	turning			3	horse suddenly stops and turns around
	back-and forward			3	moving fast back-and forward
FOCUS					
	easy to shift	1			easy to shift horse’s focus and attention
	soft focus	1			not hard focus into somewhere in the distance
	alert		2		all sense on alert
	hard to shift		2		harder to shift focus
	moving			3	fixated on moving away
	unresponsive			3	horse does not respond to trainer
SOUND					
	snorting	1			snorting sound of exhalation with flutter pulsation
	blowing		2		non-pulsated sound by expulsion trough nostrils
	sighing		2		horse makes a sighing sound
	coughing		2		horse makes a coughing sound
	groaning			3	horse makes a groaning sound
	stridor			3	high pitch sound caused by turbulence in airway

Table II2 Stress ethogram with coping behaviours

COPING BEHAVIOURS			
	BEHAVIOUR	STESS LEVEL	EXPLANATION
	licking	2	licking its lips
	rubbing	2	rubbing or scratching head or other body parts
	chewing	2	chewing on objects or licking
	yawning	2	horse yawns more than often
	chest biting	2	biting at chest or shoulders
	return to comfort	3	horse returns to other horses, barn, pasture, …

APPENDIX III STRESS LEVEL ANALYSIS

In Figure 35 and Figure 36 in the main text, evolution of the stress levels in the respective training parts, going from TRAIN1 until the FOLLOWUP is given. Here, the evolution of the stress level percentages in time is given for every training part respectively. By performing this analysis for every foal respectively, the progress of a foal in every part of the training session can be followed.

HEADSTALL ON

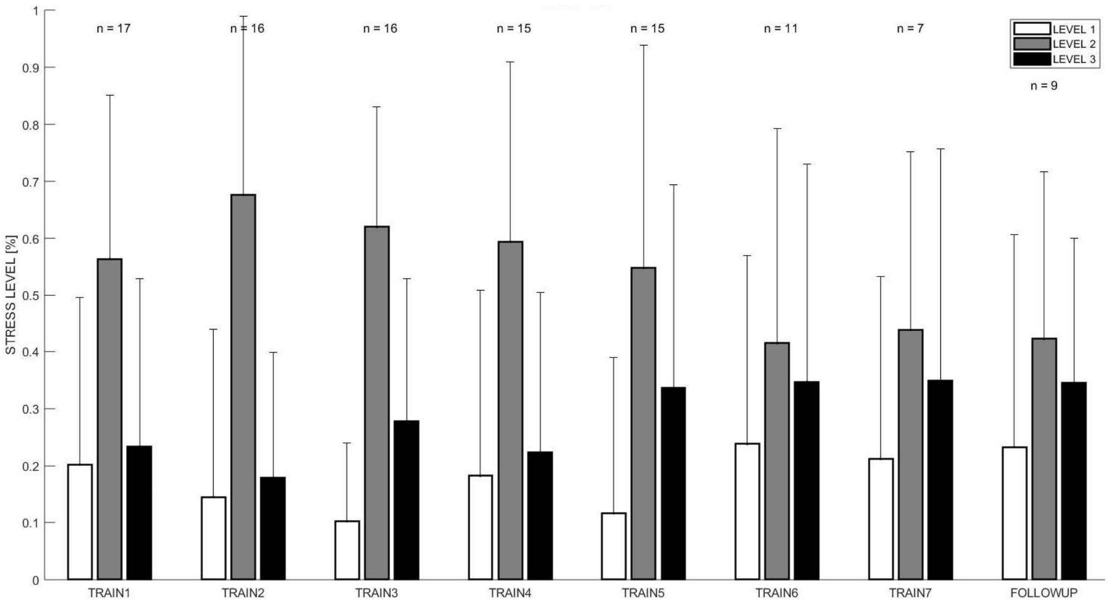

Figure III1 Evolution of gold standard stress levels HEADSTALL ON at group level

ROPE ON

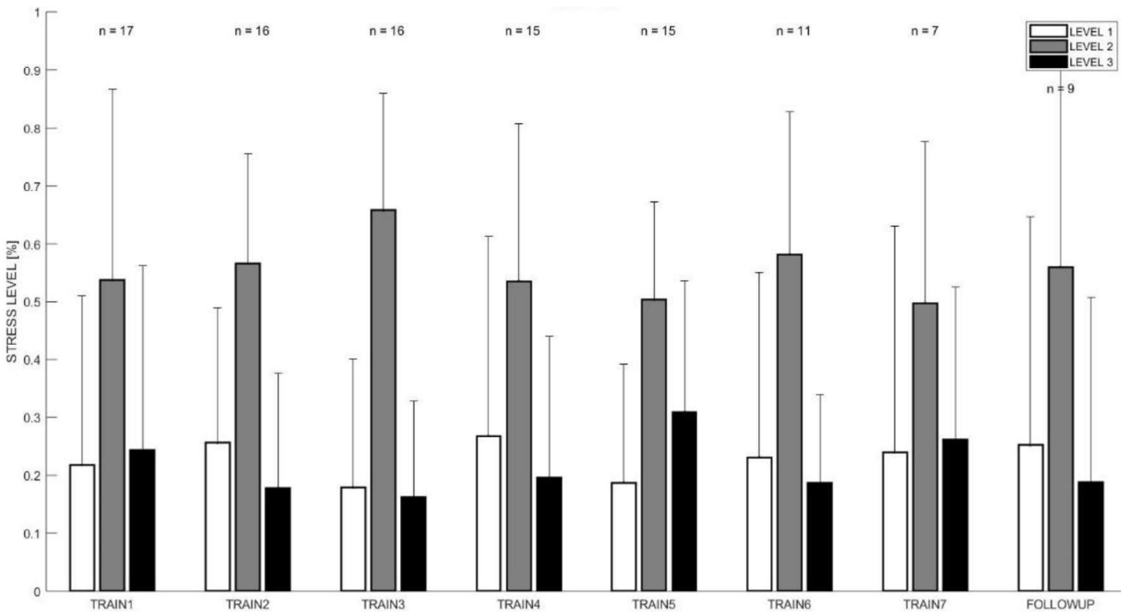

Figure III2 Evolution of gold standard stress levels ROPE ON at group level

LIFTING LEGS

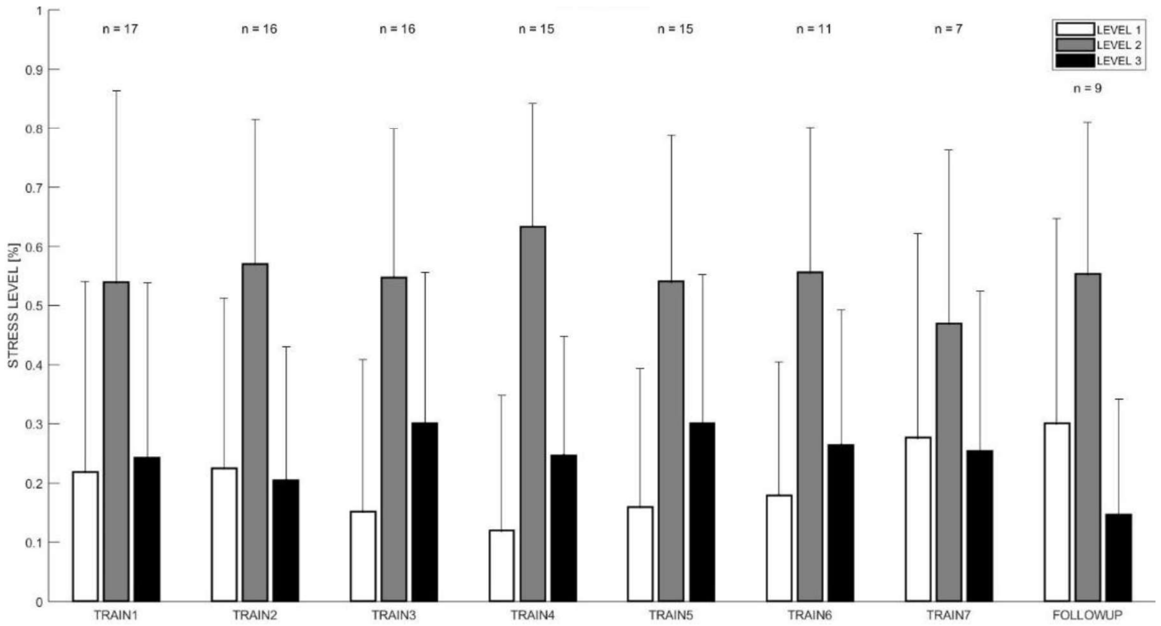

Figure III3 Evolution of gold standard stress levels LIFTING LEGS at group level

ROPE OFF

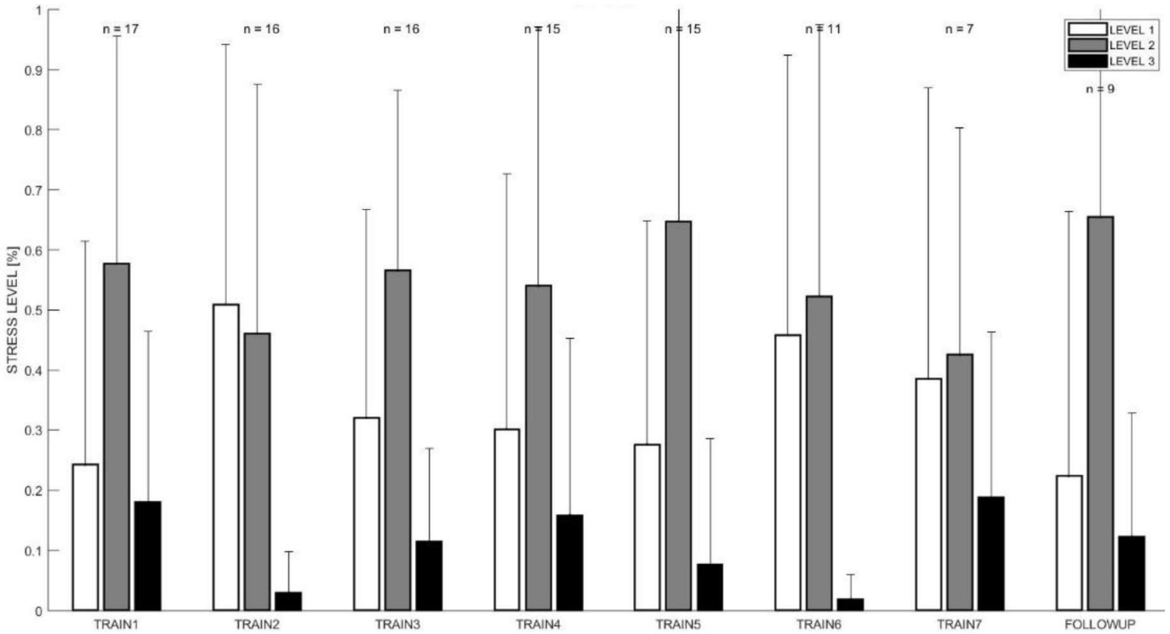

Figure III4 Evolution of gold standard stress levels ROPE OFF at group level

HEADSTALL OFF

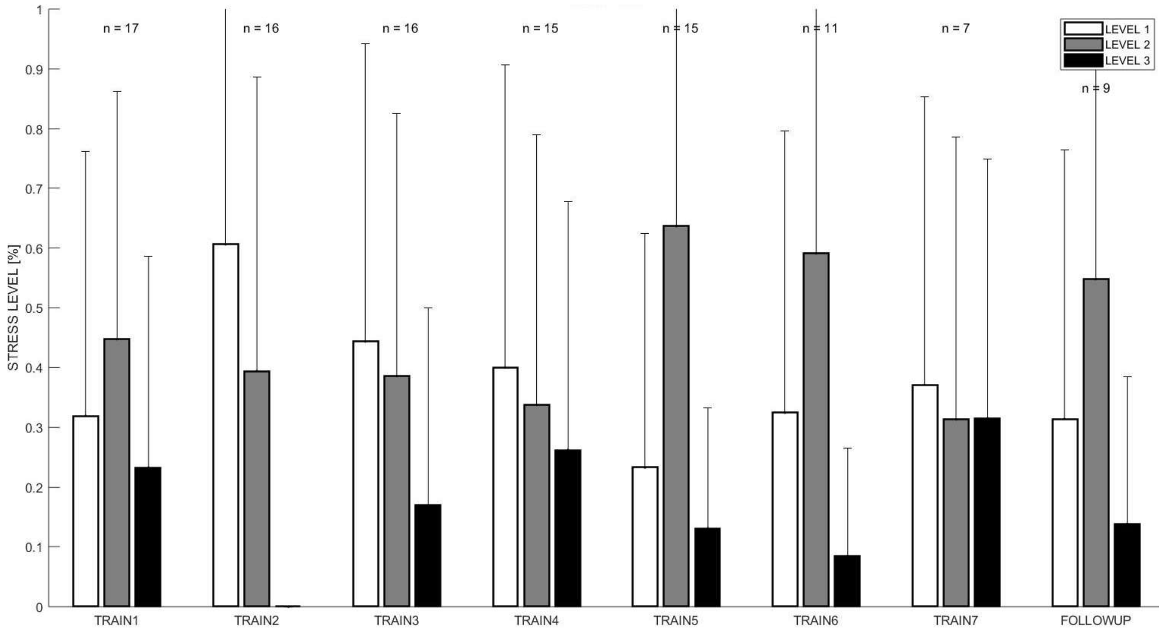

Figure III5 Evolution of gold standard stress levels HEADSTALL OFF at group level

TOUCH HEAD

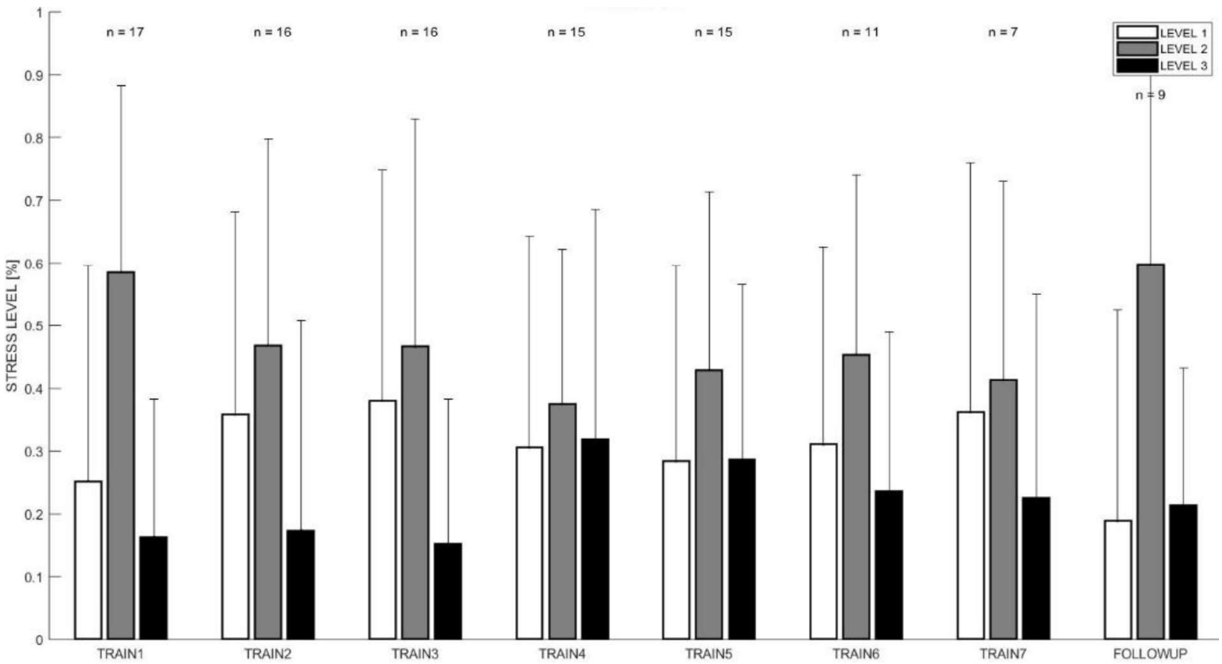

Figure III6 Evolution of gold standard stress levels TOUCH HEAD at group level

APPENDIX IV HR ANALYSIS

In Figure 43 in the main text, the mean HR analysis is repeated for every part of the training respectively, making it possible the analyse the progress of the foals within a particular part of the training. Here, besides the mean HR, the same analysis is repeated for the median, min, max and std HR analysis. The median, mean and min HR are measures used to represent the evolution of the mean stress level of the foal throughout its training program, while the max and std HR are used to investigate the evolution of short moments of high stress levels and the constancy of the stress levels, respectively.

HEADSTALL ON

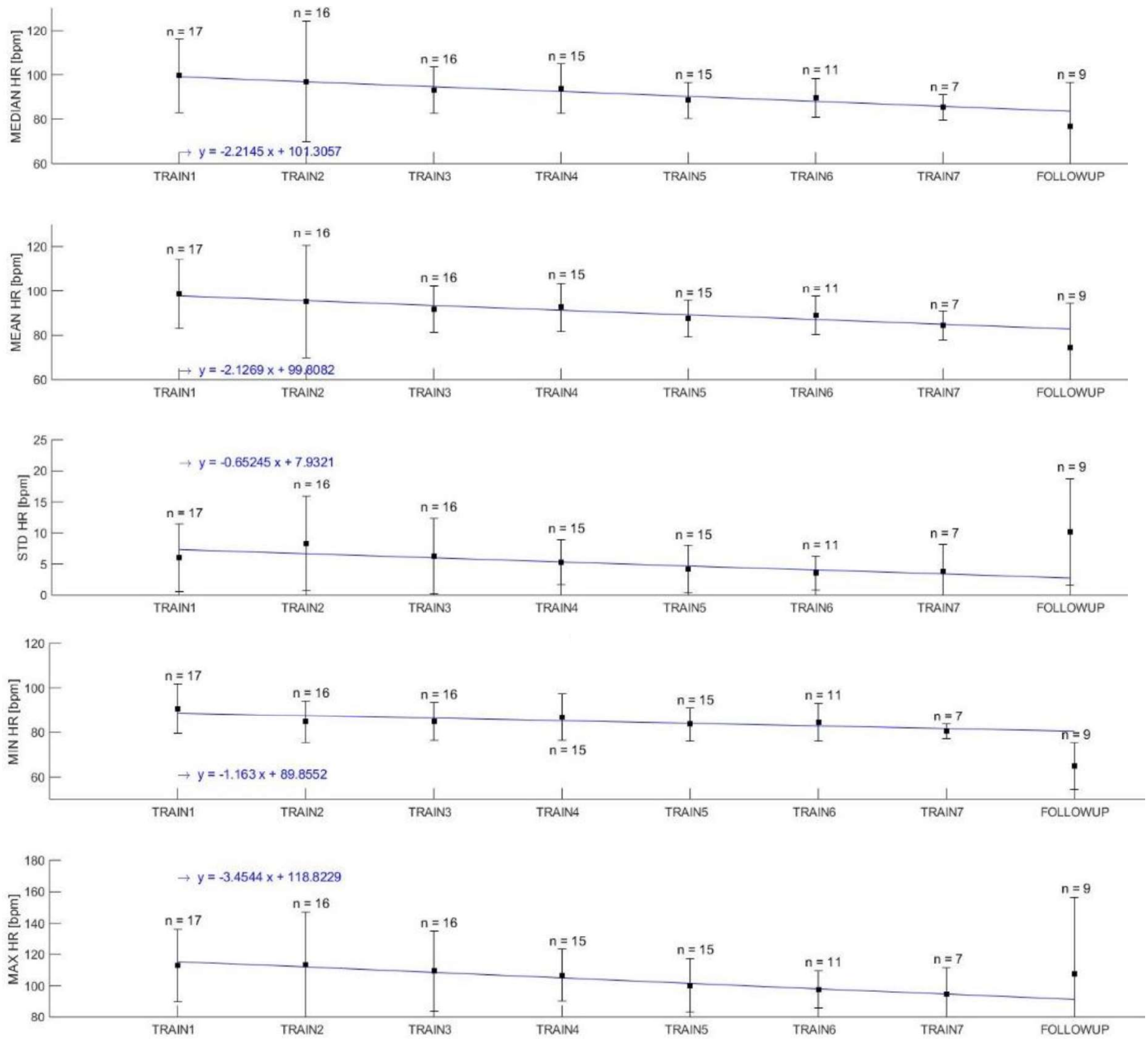

Figure IV1 HR analysis HEADSTALL ON at group level

ROPE ON

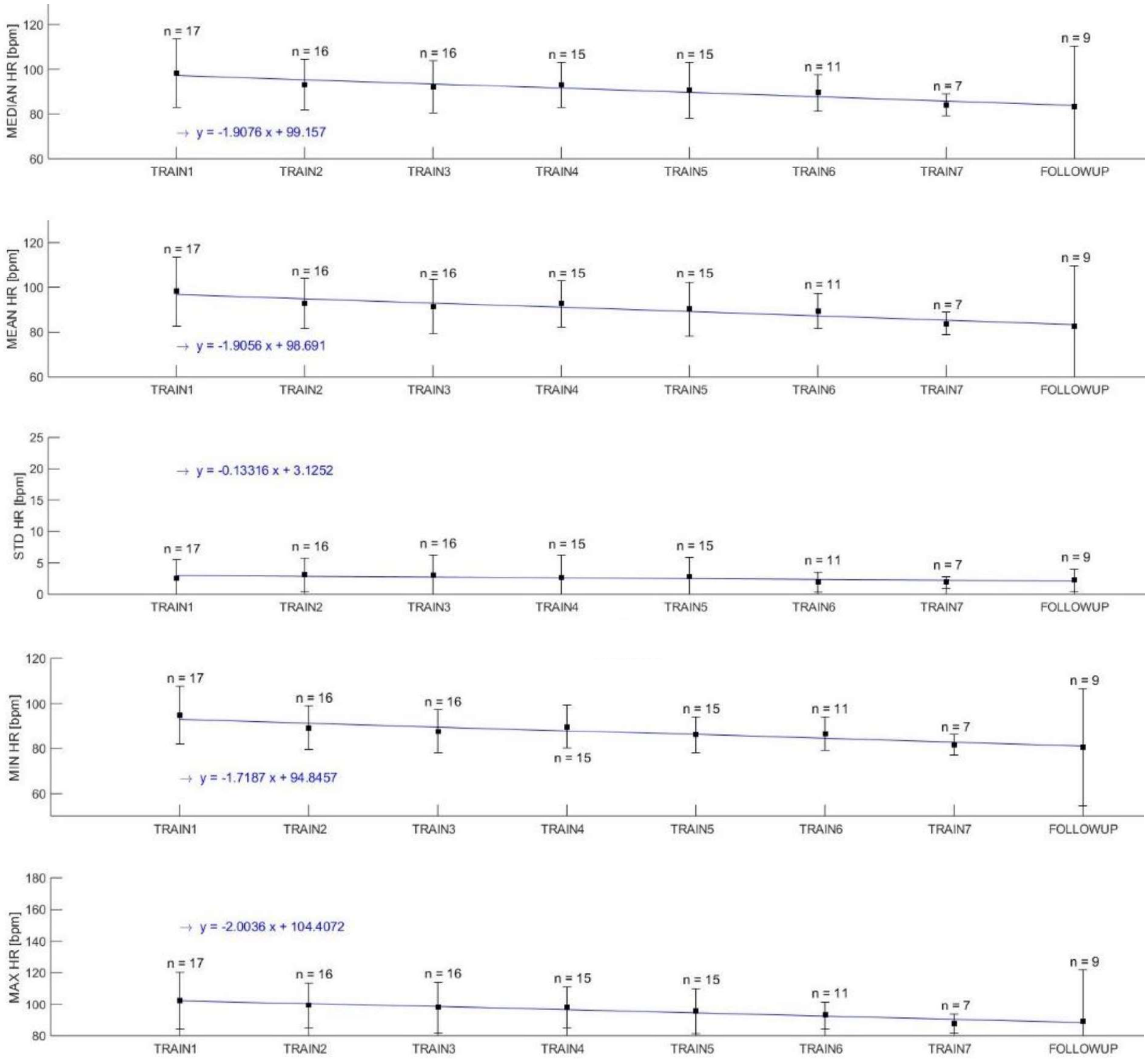

Figure IV2 HR analysis ROPE ON at group level

LIFTING LEGS

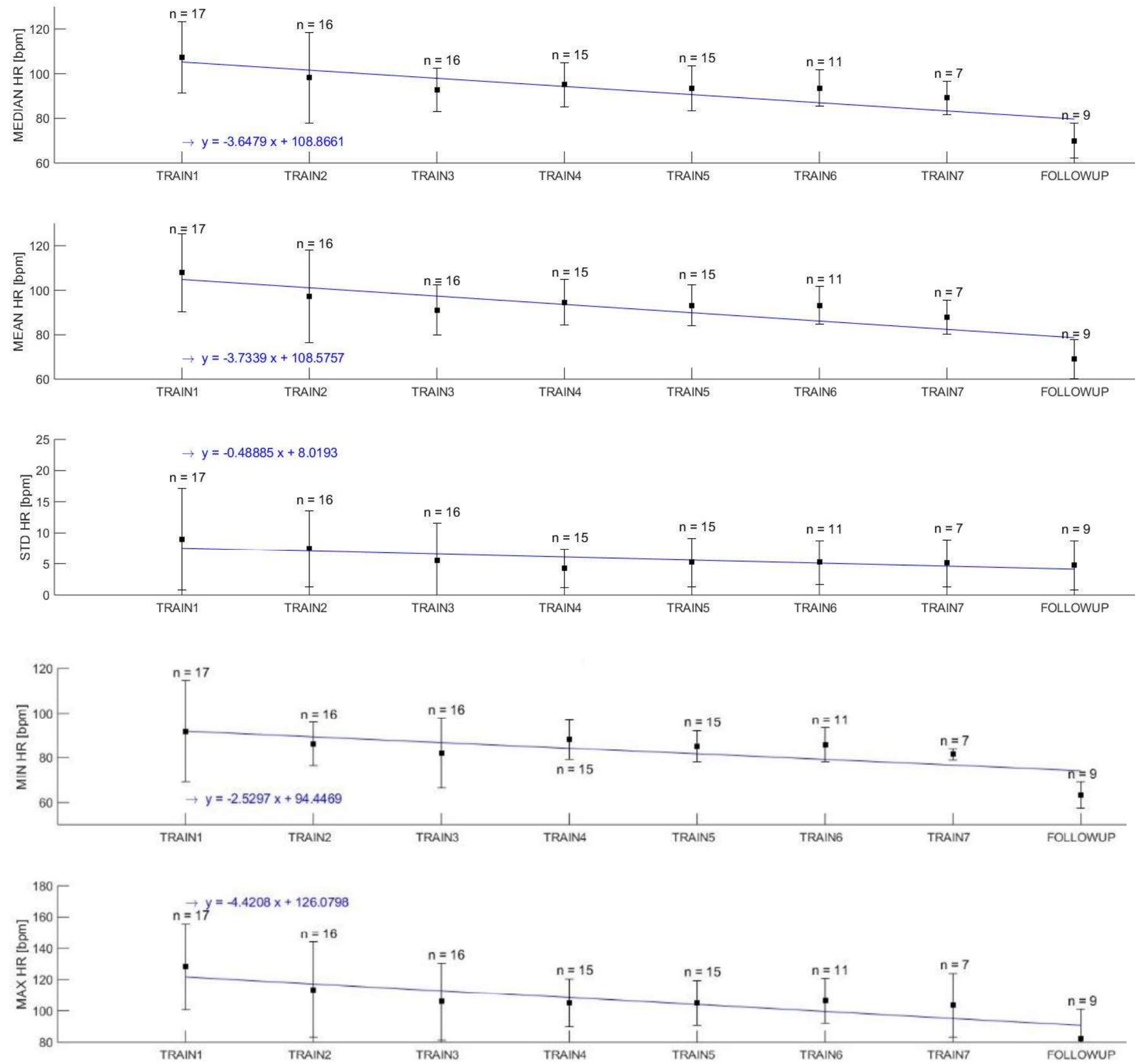

Figure IV3 HR analysis LIFTING LEGS at group level

ROPE OFF

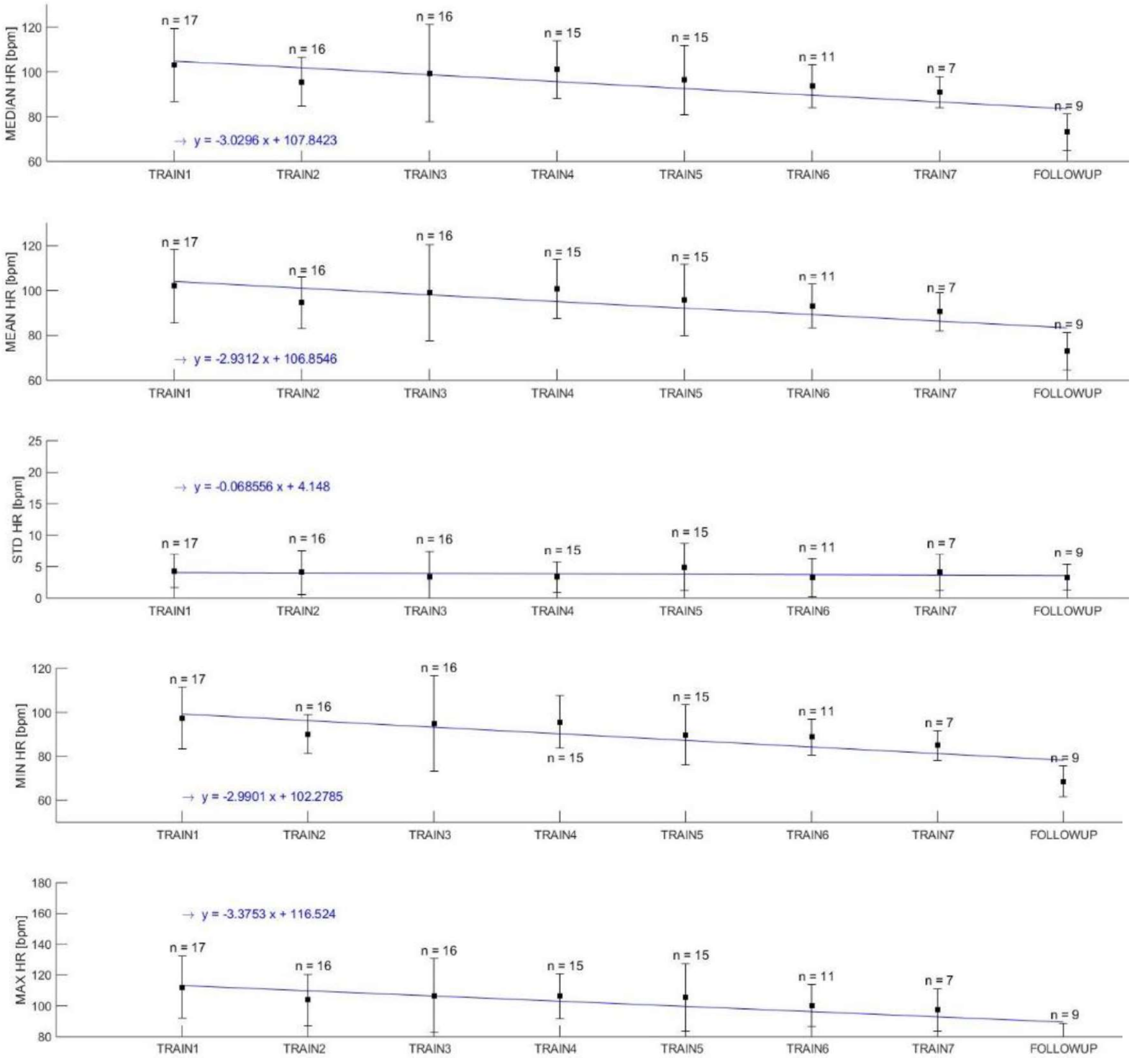

Figure IV4 HR analysis ROPE OFF at group level

HEADSTALL OFF

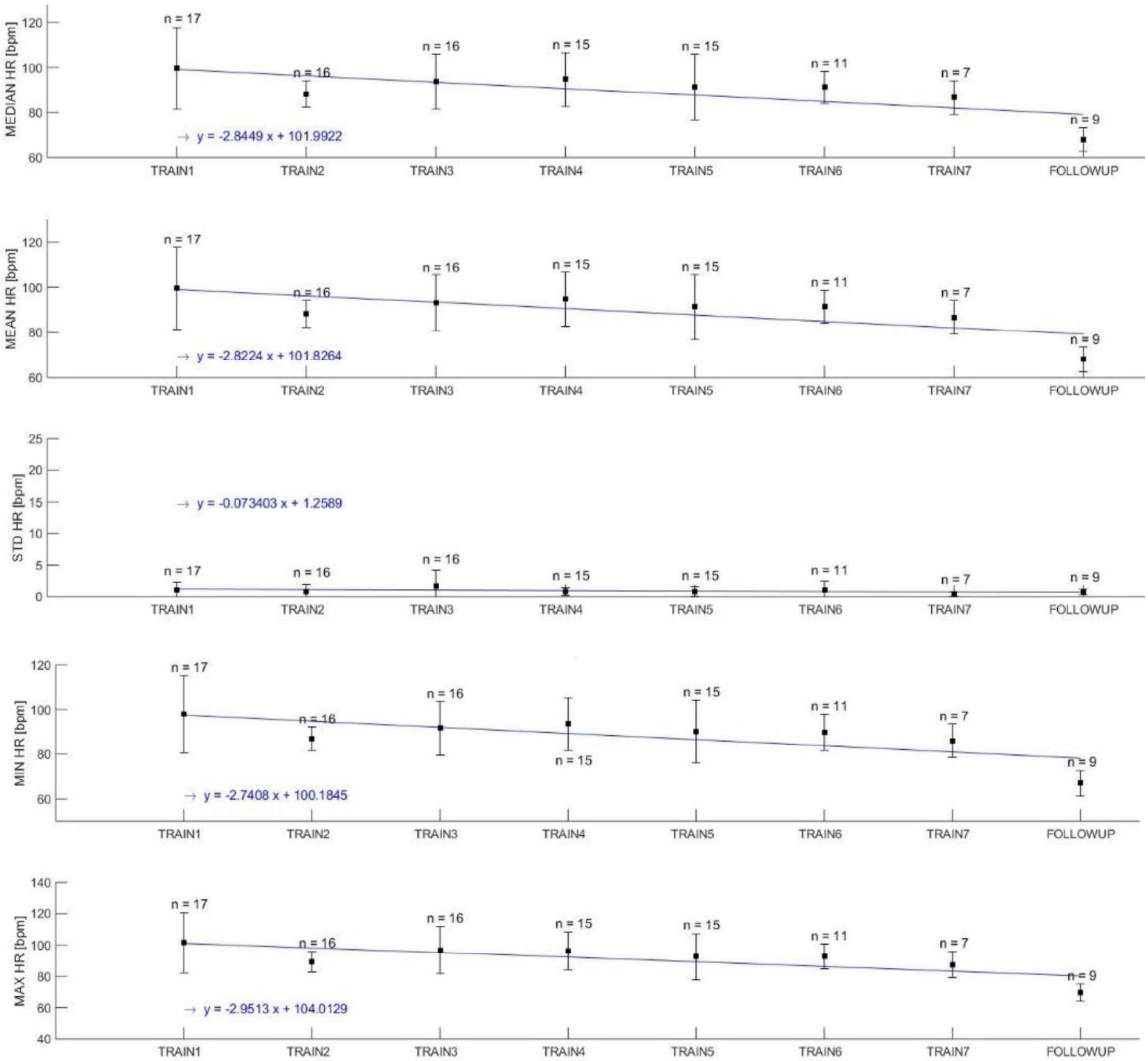

Figure IV5 HR analysis HEADSTALL OFF at group level

TOUCH HEAD

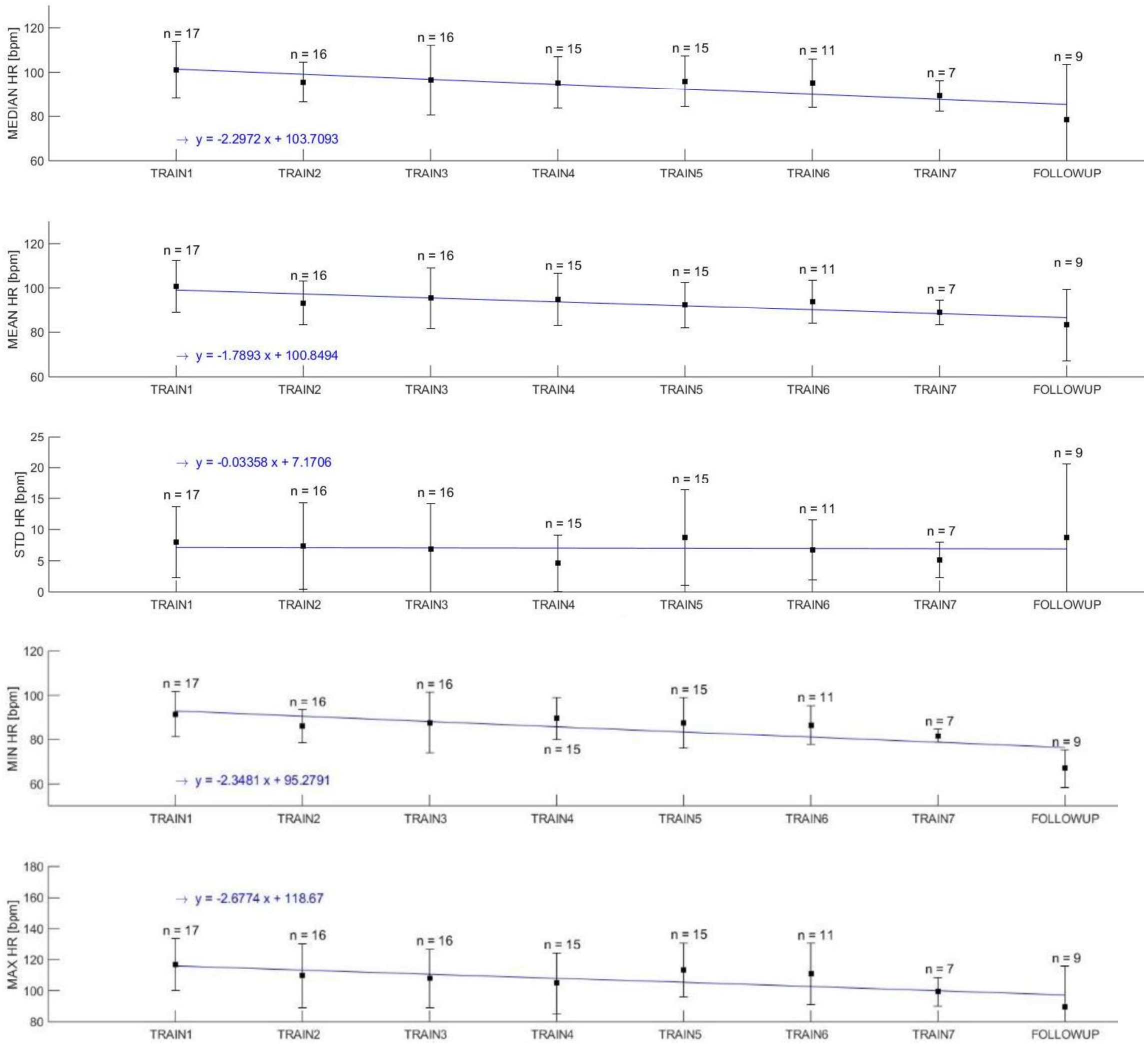

Figure IV6 HR analysis TOUCH HEAD at group level

APPENDIX V HR ANALYSIS

Figure 44 in the main text represents the mean HRs for the respective parts of the training at group level. Here, the boxplots for the median, min and max HR measures are represented. The mean, median and min HR can be used to compare the mean stress level in the respective training parts, while the max and std HR are used to investigate the difference in moments of high stress levels and the dynamic range of the measured HRs.

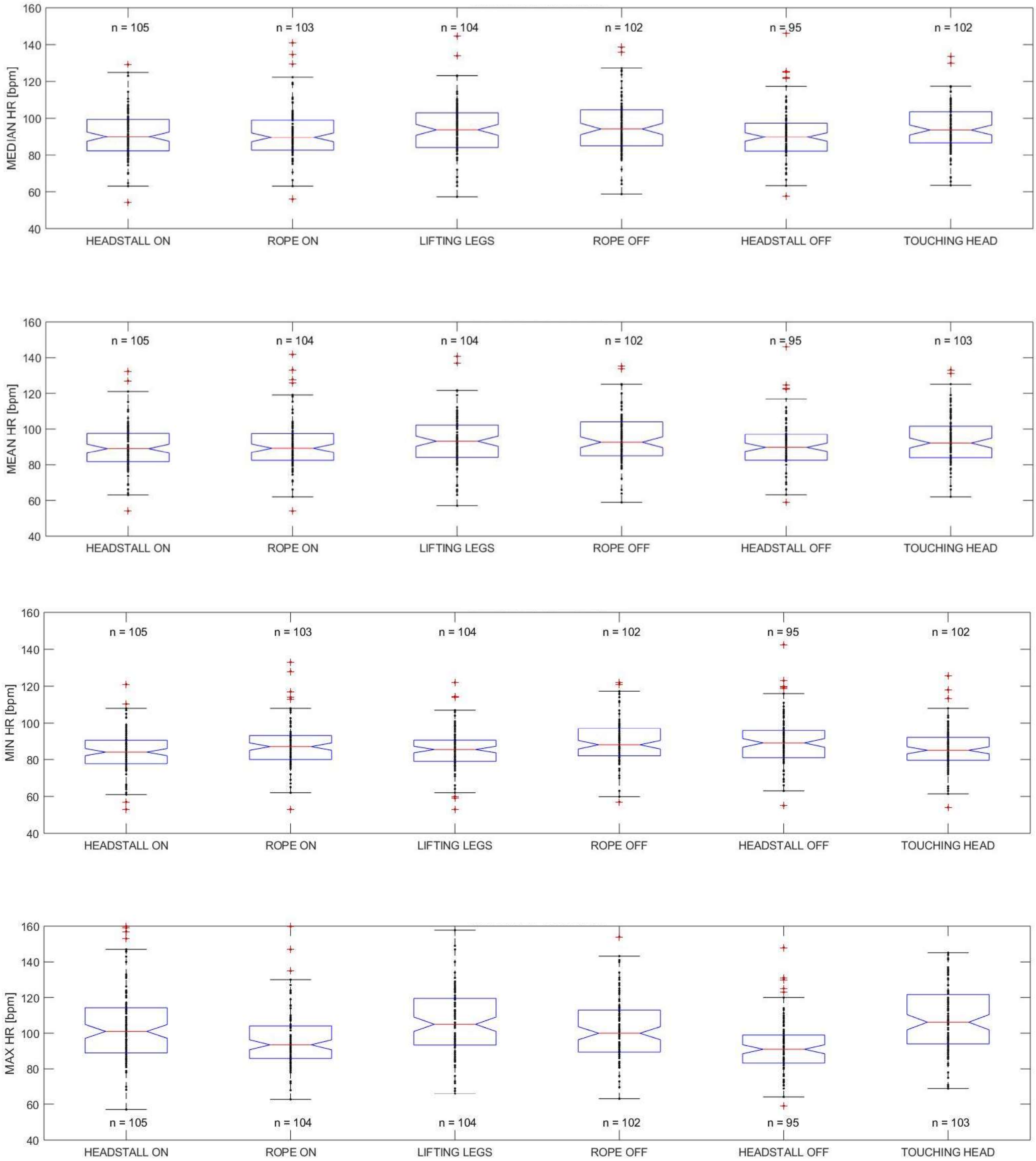

Figure V1 Boxplots comparing HR measures for respective training parts FULL TRAIN

TRAINING 1

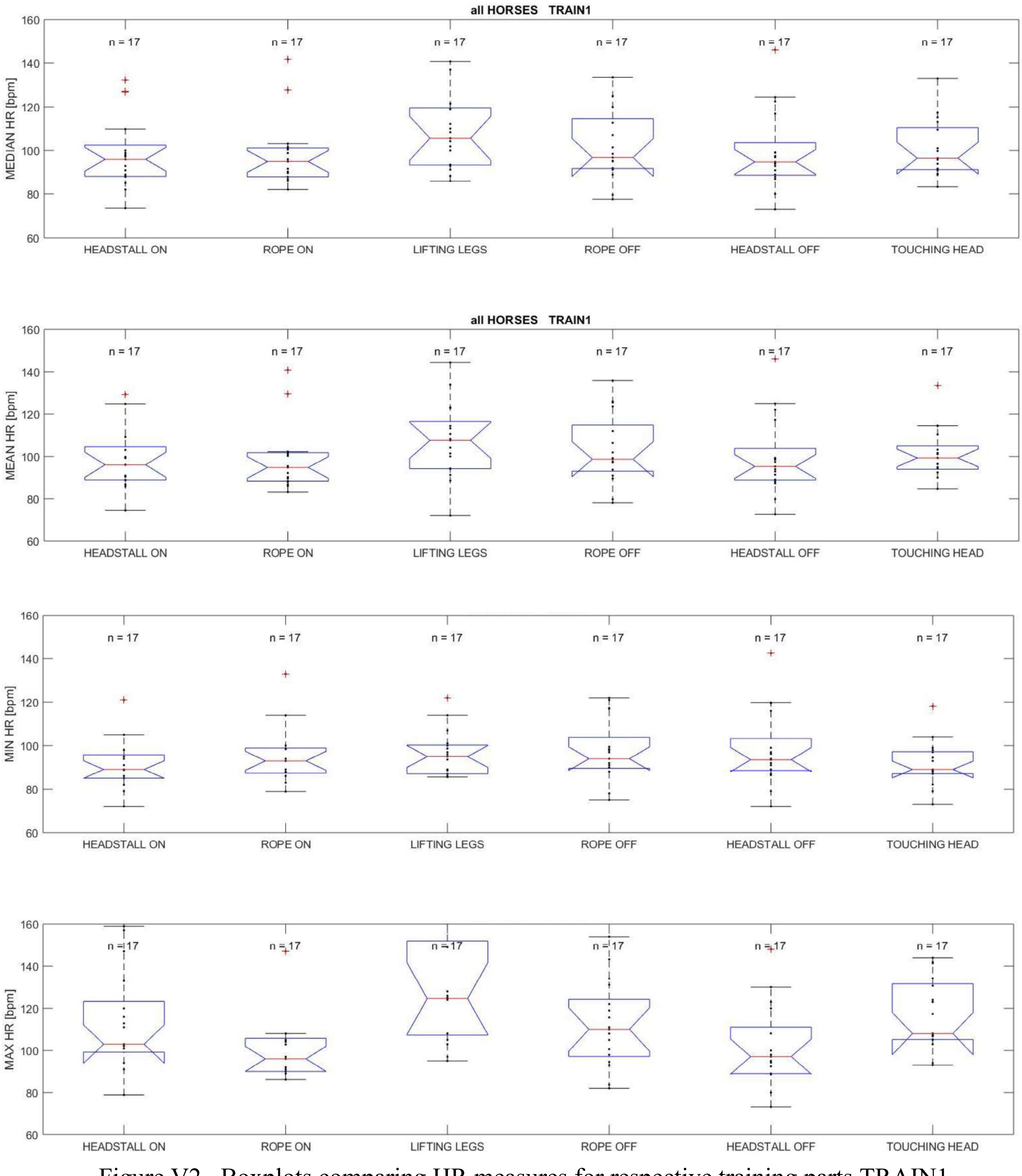

Figure V2 Boxplots comparing HR measures for respective training parts TRAIN1

TRAINING 2

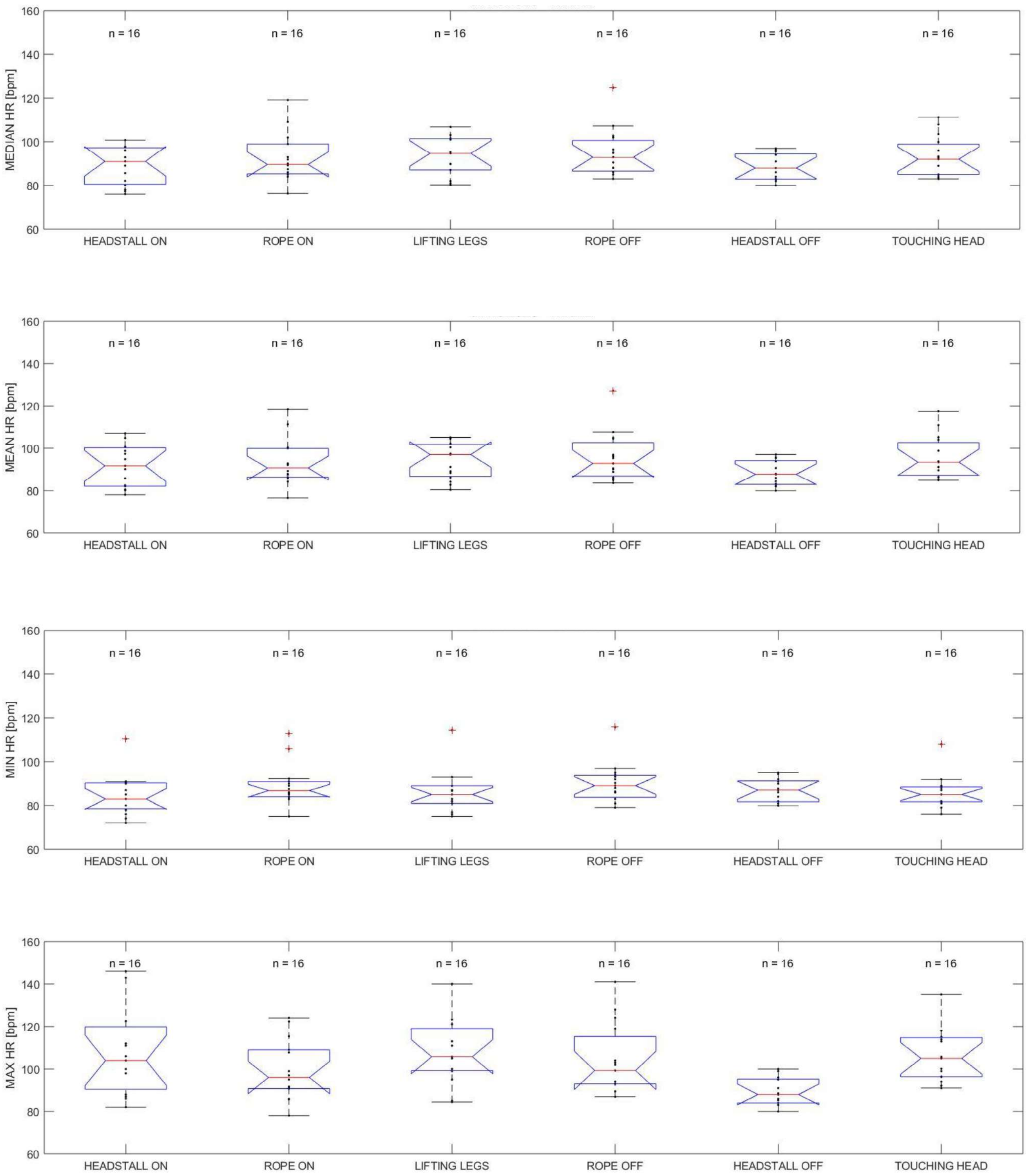

Figure V3 Boxplots comparing HR measures for respective training parts TRAIN2

TRAINING 3

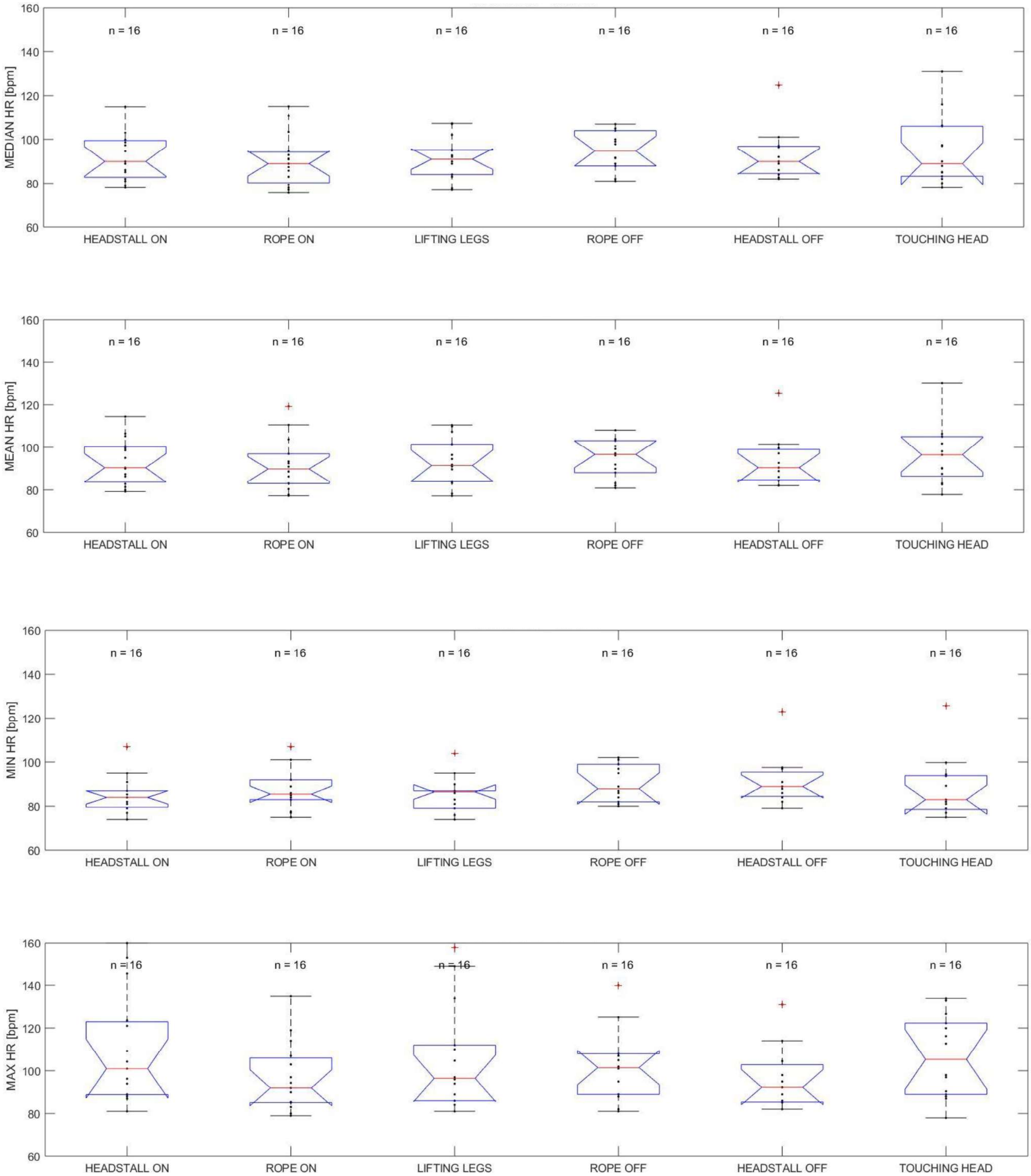

Figure V4 Boxplots comparing HR measures for respective training parts TRAIN3

TRAINING 4

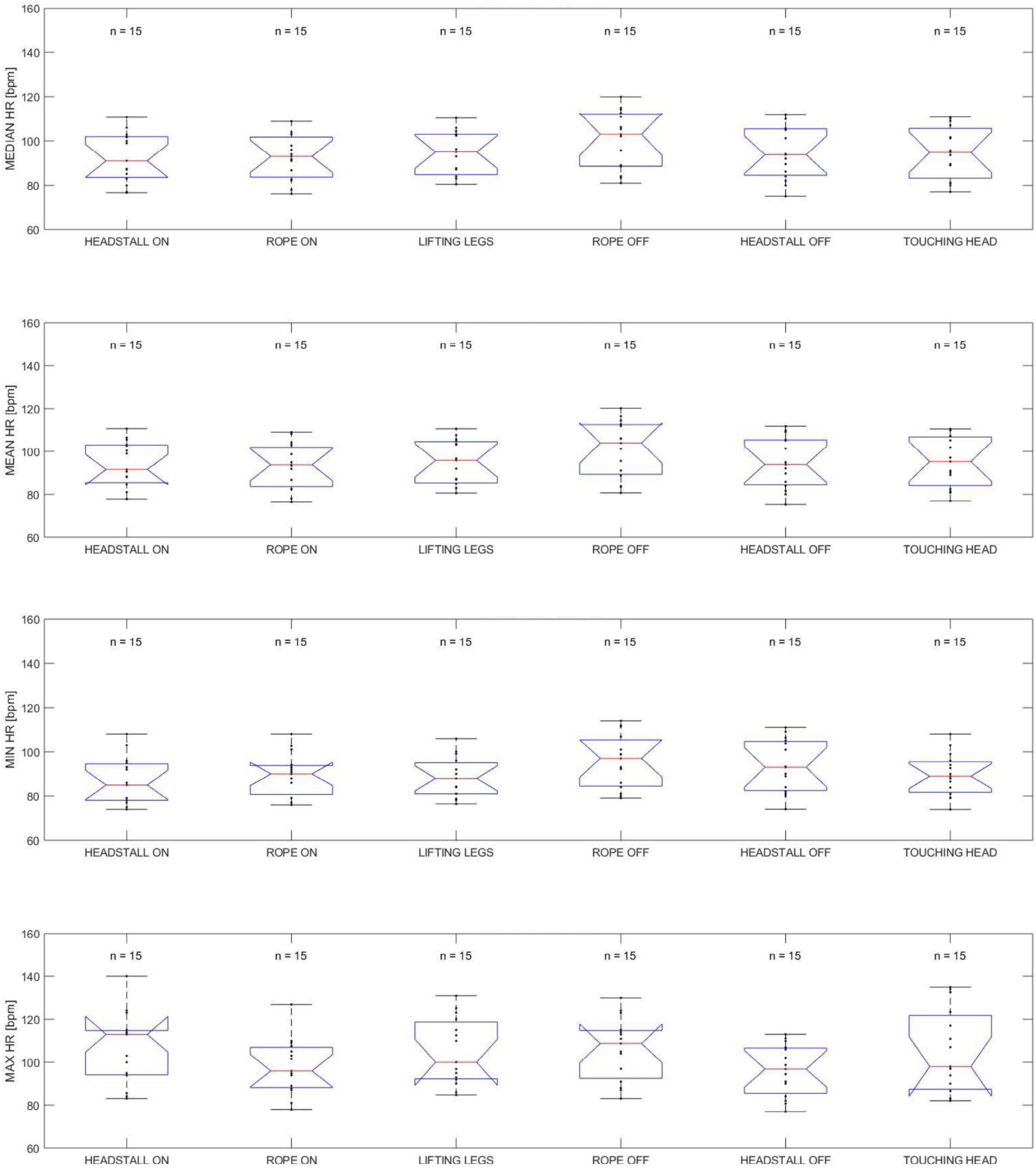

Figure V5 Boxplots comparing HR measures for respective training parts TRAIN4

TRAINING 5

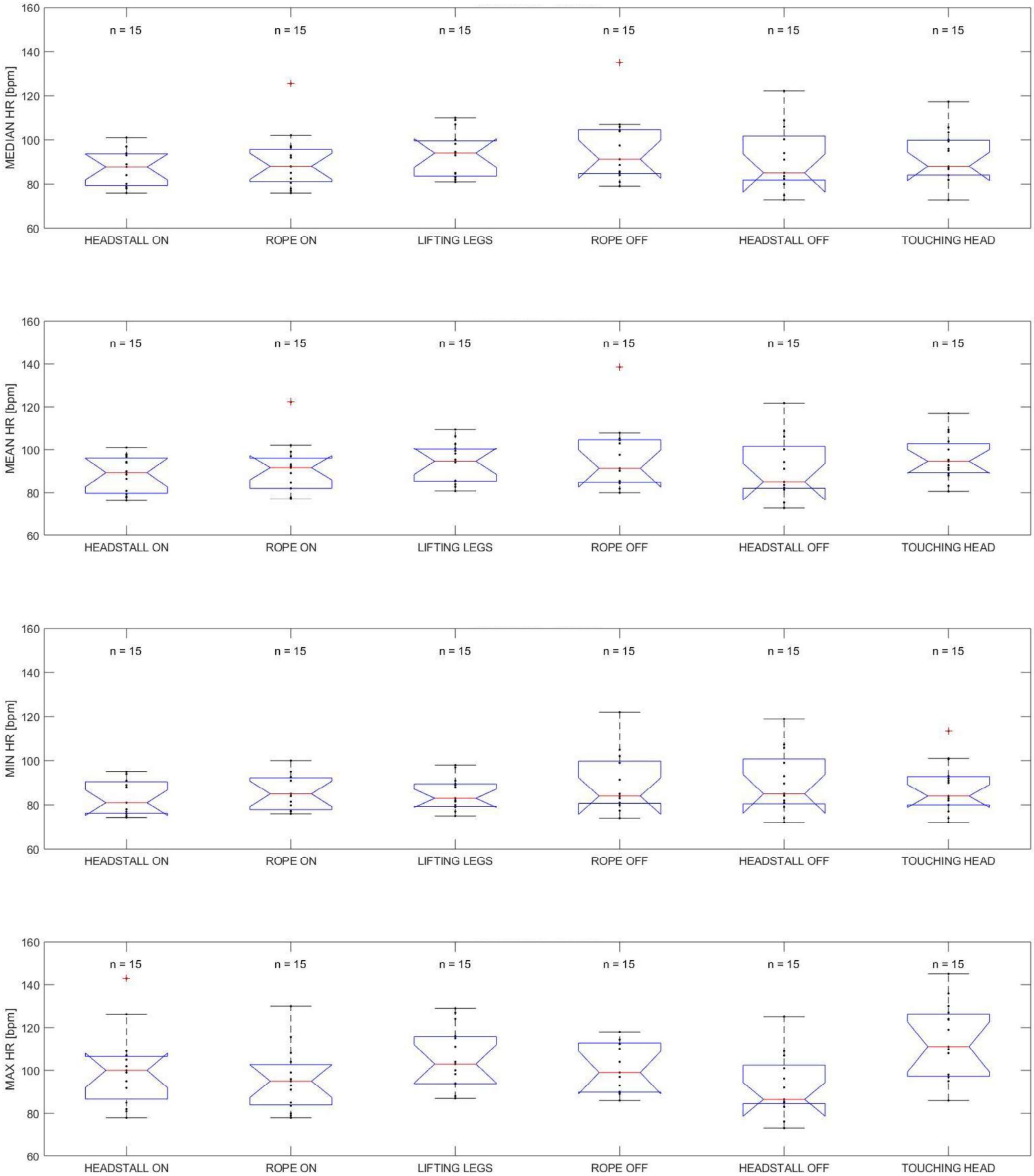

Figure V6 Boxplots comparing HR measures for respective training parts TRAIN5

TRAINING 6

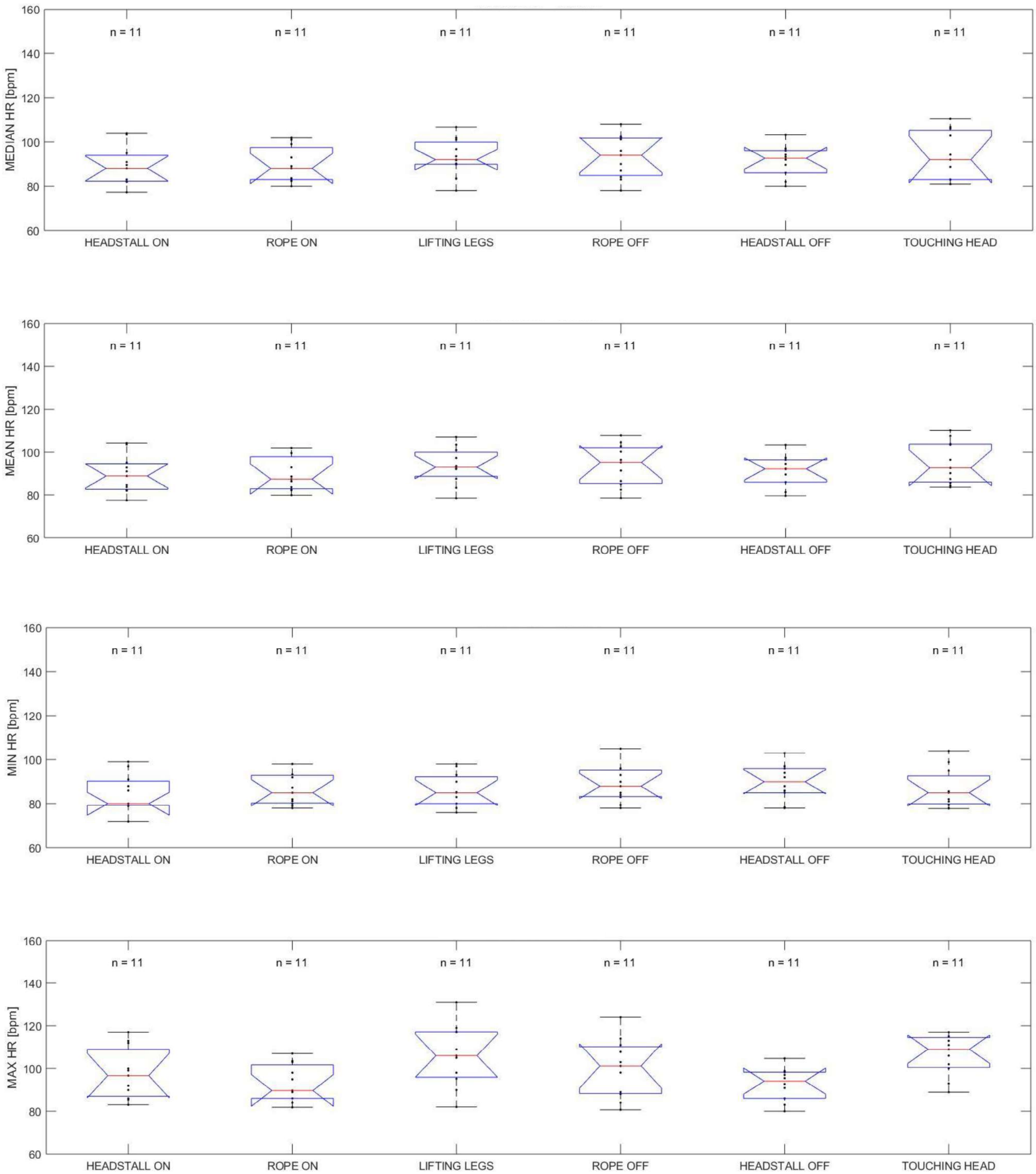

Figure V7 Boxplots comparing HR measures for respective training parts TRAIN6

TRAINING 7

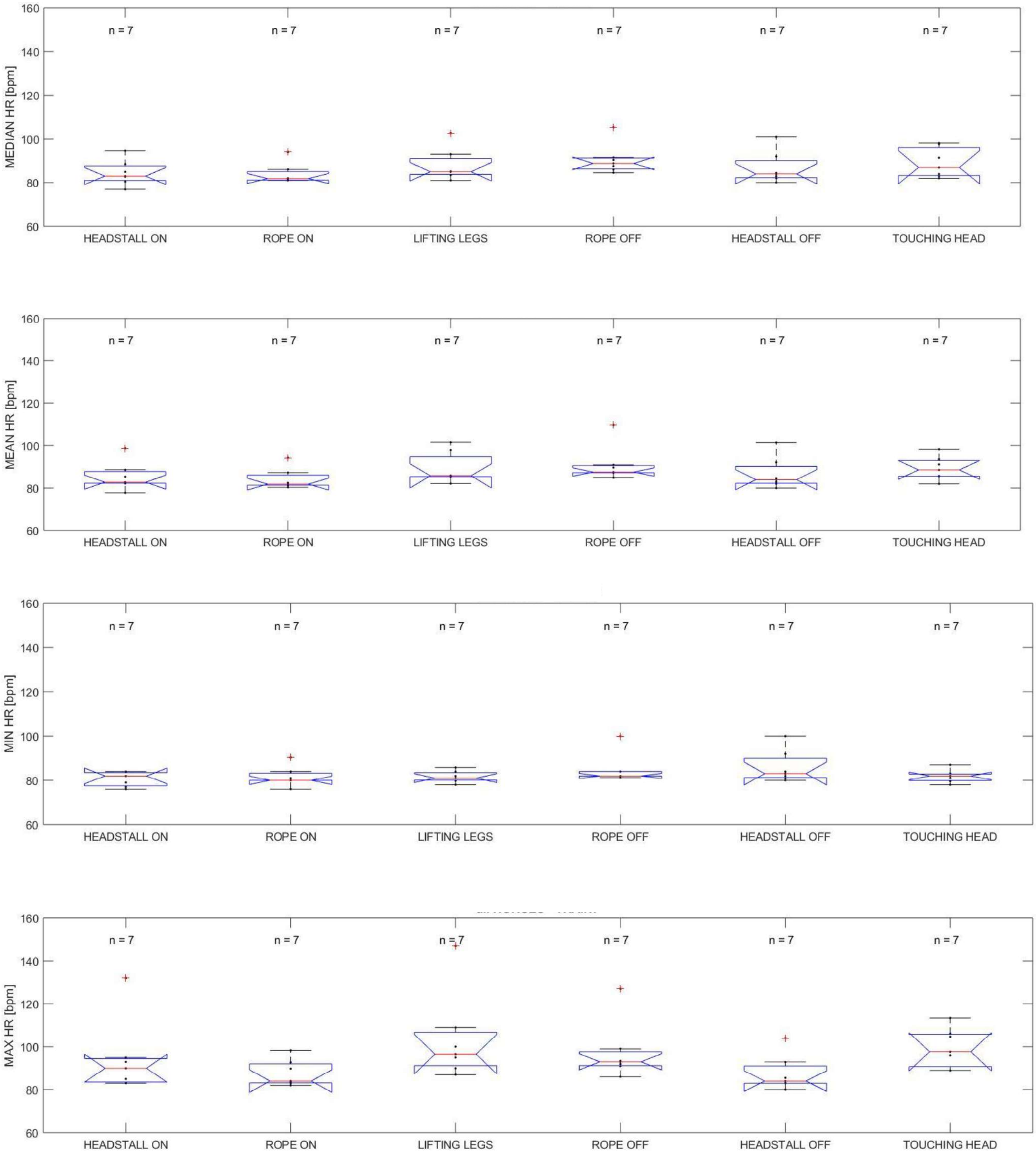

Figure V8 Boxplots comparing HR measures for respective training parts TRAIN7

FOLLOWUP

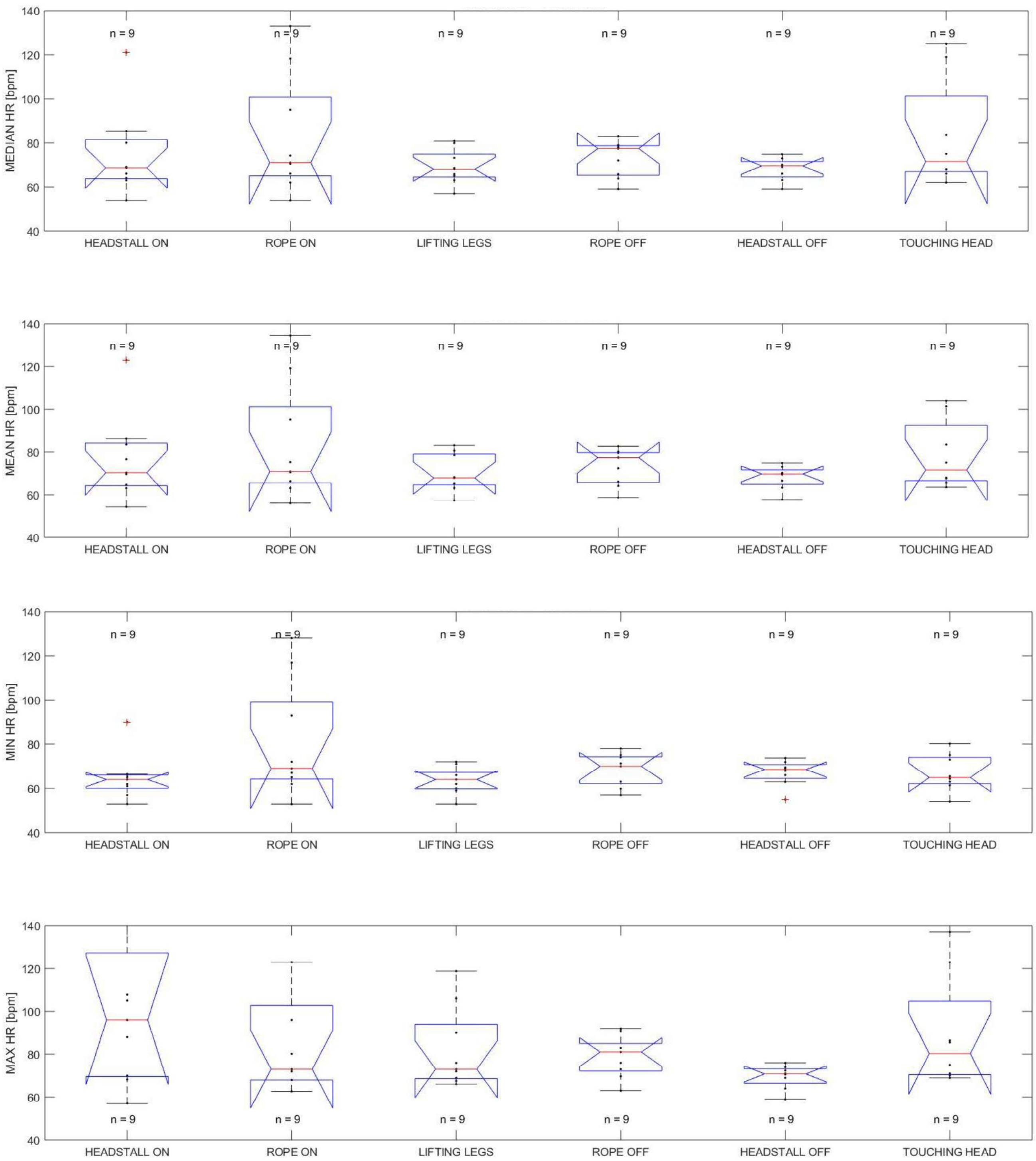

Figure V9 Boxplots comparing HR measures for respective training parts FOLLOWUP

APPENDIX VI MARE STRESS

In the HR analysis of the mare, only the mean and max HR are analysed, given in Figure 47 in the main text. Here the results of the median, min, max and std HR are given as well.

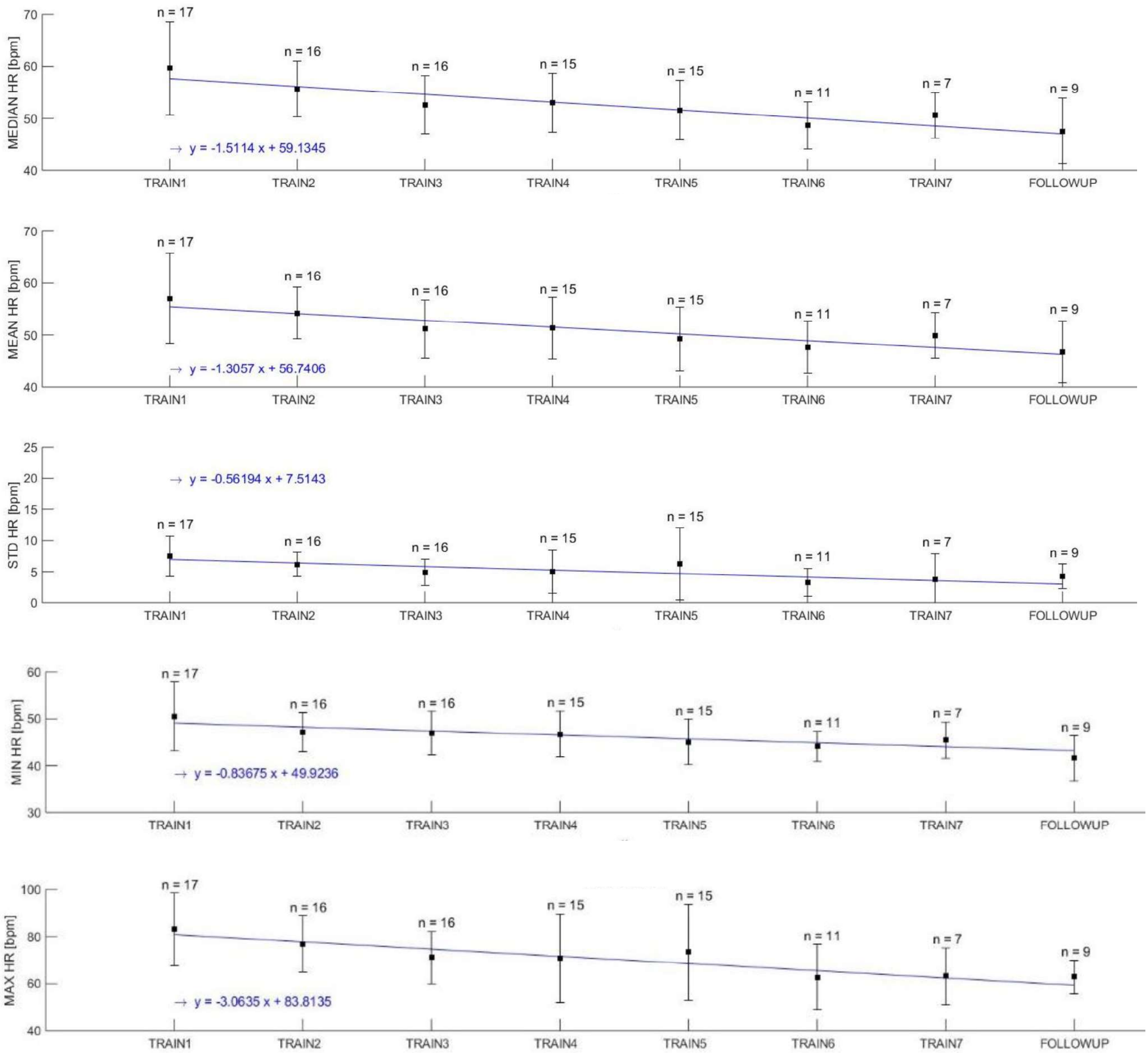

Figure VI1 Evolution of the mare's HR measures in time at group level

APPENDIX VII APPROVAL ETHICAL COMMISSION

AE Application 14302 ~ (Status=APPROVED)(Applicant=culluma) Foal Education Collection of Data

Group	Line	Question	Answer
		THERE IS CONFIDENTIAL INFORMATION IN THIS APPLICATION **Do not discuss this work outside the context of this application (Project planning or AEC deliberations). Confidentiality also precludes indirect use of the information in any other forum.**	**The algorithms used by KU Leuven University for analyzing the heart rate and accelerometer data**
		ASSOCIATED Documents	
		Attached pdf 7824	10 ISES Principles Equus Education
		Attached pdf 7825	Equus Education
		Attached pdf 7826	Stress Assessment
		Attached pdf 7845	Signatures Leigh Wills, Sally King
		0. ADMINISTRATIVE Details	
0	1	**Title**	Foal Education Collection of Data
0	3	**Applicant**	Leigh Wills (application by Ali Cullum for Leigh)
0	4	**Institution**	AgResearch Limited
0	5	**Business Address**	Equus Education (NZ) PO Box 690 Cambridge 3450
0	6	**Phone**	07 8235688
0	7	**Mobile phone**	0274378870
0	8	**Email**	Leigh@equuseducation.com
0	9	**Contact details**	
0	10	**Name of person responsible for the animals during manipulations (MUST be named on and sign the personnel page). If more than one facility is being used, all persons responsible for animals must be named and sign this Application.**	Leigh Wills, Sally King
0	11	**Phone**	0274378870
0	12	**Mobile phone**	0274378870

0	13	**Email**	Leigh@equuseducation.com
0	14	**Person responsible for entry of Trial Drug and animal manipulation data into Animal Tools database. (AgResearch requirement only ~ MUST be named on and sign the personnel page). If no AgR facilities are being used please enter Not Applicable**	Not Applicable
0	15	**Program Manager (MUST be named on and sign the personnel page)**	Leigh Wills
0	16	**Biometrician (MUST be named on and sign the personnel page - or hard [paper] copy if no computer access)**	Harold Henderson
0	17	**Facility Manager - If more than one facility is being used, all Managers must be named and sign this Application. (MUST be named on and sign the personnel page - or hard [paper] copy if no computer access)**	Windsor Park Stud, Little Avondale Stud, Mapperley Stud, Rich Hill Stud, Cambridge Stud
0	18	**Is this a new experiment?**	Yes
0	19	**Project Dates ~ PLEASE enter all dates in the form requested [dd/mm/yy] AND ensure that the start date you enter occurs after the next meeting of your committee. If you do not expect to renew this Application please do not enter a Renewal Date**	
0	20	**Start Date (dd/mm/yy)**	20/09/2017
0	21	**Finish Date (dd/mm/yy)**	20/09/2018
0	22	**Renewal Date (dd/mm/yy) PLEASE only enter a date in this field if you genuinely expect a need to renew this application after the indicated finish date. THE DATE ENTERED SHOULD BE 3 MONTHS PRIOR to the end date of this experiment to allow for processing time.**	
0	23	**Are there FOOD SAFETY issues under the Animal Products Act, or Regulatory approval, associated with this project (PLEASE NOTE: The FOOD CHAIN includes food for**	NO

		either HUMAN CONSUMPTION or PET FOOD)? If you are using Laboratory Rodents and/or other species that are never used for human consumption or pet food, or if the animals in the project will be safe to enter the food chain, please type the word NO and then proceed to the next question. However, if some or all of the animals must be excluded from EVER entering the food chain because of the manipulations, drugs they will be given or because of Regulatory Approval requirements, please type YES and then explain why. AgResearch animals that may not enter the food chain must be PINK TAGGED.	
0	24	**Maori Consultation. If there is any uncertainty, it is the applicant's responsibility to approach local Māori representatives for clarification.**	
0	25	**Do any aspects of this project require consultation with Maori?**	NO
0	26	**If Yes please indicate what aspects are of interest to Maori. If necessary, evidence of approval from iwi or hapu must be appended as a pdf**	
0	27	**Are you requesting URGENT consideration? Please only tick YES if the AEC needs to consider this Application before the next scheduled meeting.**	No
0	28	**If YES, please state why the Application is urgent**	
0	29	**Database Administration**	
0	30	**From the Dropdown, AgResearch users please indicate the name of your Section; Parented users please select EXTERNAL USER**	EXTERNAL USER
		1. AEC JURISDICTION	
1	1	**Which committee are you applying to? (Use Dropdown box)**	Ruakura

1	2	**Some aspects of this work will also be undertaken under the jurisdiction of the AgResearch AEC at (Use Dropdown box)**	
1	3	**Some aspects of this work will also be undertaken under the jurisdiction of the AgResearch AEC at (Use Dropdown box)**	
1	4	**Some aspects of this work will also be undertaken under the jurisdiction of the AgResearch AEC at (Use Dropdown box)**	
1	5	**Are staff, animals or facilities of another organisation(s) which has an Animal Ethics Committee involved in this project?**	Yes
1	6	**If YES Name other organisation(s) involved in this project**	KU Leuven University Belgium
1	7	**Does the Ethics committee of that institution(s) have a copy of this application?**	No
1	8	**Has this application, a similar or largely similar application been submitted to another Ethics Committee on a previous occasion?**	Yes
1	9	**If YES, please provide the name of the committee that the project has previously been submitted to and details of the submission**	Massey University.AEC/15 (Amended 07/10) The training we measured is the same. We filled out written forms. This time we are adding a heart rate monitor and an accelerometer.
		2. REGULATORY APPROVAL	
2	1	**Any regulatory approval must be obtained before submission and proof that all necessary approval(s) have been granted must be attached**	
2	2	**Are approvals required from an outside body(ies? Please answer either YES or NO**	NO
2	3	**If any aspects of this proposal require approval from a regulatory body(s) please select from list below**	
2	4	**Approval is required from (Use Dropdown box)**	

2	5	**Approval authority ~ Please supply approval number, and note that a pdf of approval has been attached**	
2	6	**Approval is also required from (Use Dropdown box)**	
2	7	**Approval authority ~ Please supply approval number, and note that a pdf of approval has been attached**	
2	8	**Approval is also required from (Use Dropdown box)**	
2	9	**Approval authority ~ Please supply approval number, and note that a pdf of approval has been attached**	
2	10	**If Other please specify ~ Please supply approval number, and note that a pdf of approval has been attached**	
		4. LAY SUMMARY	
4	1	**Confidentiality**	
4	2	**Is information in this application commercially sensitive?**	Yes
4	3	**If YES, which aspects are commercially sensitive?**	The algorithms used by KU Leuven University for analyzing the heart rate and accelerometer data
4	4	**PROJECT SUMMARY ~ Please use language that lay people can understand. The answers on each line must contain fewer than 7800 characters (including spaces). The size is not checked until you save and when the limit is exceeded the current input is rejected. Enter your information in small inputs and save regularly. If your answer becomes too large you will be prompted to insert an extra line to save your current input**	
4	5	**Objectives**	To observe stress behaviour during routine handling of up to 300 foals. To measure the interaction between 30 mares and foals along with 30 foals stress levels with a heart rate and accelerometer during the Equus Education (NZ) Ltd foal training process.
4	6	**Introduction**	The horse training company, Equus Education, is contracted by Windsor Park Stud, Cambridge Stud, Little Avondale Stud, Mapperley Stud and Rich Hill Stud to train up to 300 young foals. Between the age of 3 to 20 weeks, foals are habituated and trained for 6-9 sessions at 1-3 day intervals for up to 30 minutes (including time from taking out of paddock to return to paddock) comprising approximately 15 minutes individual training time per session. Sessions are conducted in a padded yard with the mare beside the foal. Positive and negative reinforcement are utilized to shape the desired behaviours of leading from a halter only (without the assistance of a tail/bum rope) and balancing on 3 legs whilst the 4th is raised. Habituation to

			people, equipment and handling occurs via flooding and systematic desensitization. This training is part of the normal preparation of foals. We are recording the outcomes for research. The alterations from normal foal preparation due to this study/experiment will be the presence of a person videoing, and the attachment of the heart rate monitor and accelerometer on a surcingle that will be added to 30 mares and 30 foals during training sessions only, to measure the above system. How do we justify that we know the heart rate data we collect will show that the horse is stressed or not stressed? https://lirias.kuleuven.be/bitstream/123456789/588858/1/Presenting+a+methodology+for+continuous+monitoring+of+mental+state+in+horses.pdf
4	7	**Methods ~ (If you have a Table to present, please append it to the Application as a PDF)**	Please see Equus Education Ethics Committee Document pdf 7825 attached, and Stress Document pdf 7826
4	8	**Design ~ (If you have a complicated diagram to present, please append it to the Application as a PDF)**	Please see International Society Equitation Science (ISES) 10 Principles attached pdf 7824
4	9	**Timetable of events ~ (If you have a Table or complicated diagram to present, please append it to the Application as a PDF)**	Observations and measurements will be made during the routine handling. Apart from the addition of the surcingle to 30 mares and 30 foals, the routine handling procedure will be unaltered from normal commercial foal preparation.
4	10	**Expected outcomes**	Handled foals will learn new behaviours. Stress of mares and or foals will be reflected in their heart rate and accelerometer measurements.
4	11	**Contingency Plans**	If an animal is uncomfortable with the handlers and surroundings, the training will be stepped back a stage. If they continue to be unhappy, they will be returned to the paddock. If animals are sick or injured, the owner and veterinarian will be immediately consulted, and appropriate action taken.
4	12	**SYNOPSIS ~ for evaluation of the Ethical Cost/Benefit. The grades are A, B, C, D and E. If you have any doubts check the MPI guidelines link in the HELP File.)**	
4	13	**Briefly summarise the number of groups of animals, number of animals per group, manipulations (and Grading) to be done on animals in each group, drugs/chemicals to be administered to each group and the main benefit expected from this work.**	Grading B: 30 x thoroughbred mare and their 30 foals. Total = 60 with heart rate and accelerometer equipment attached during handling period only on girth or surcingle encircling trunk of body behind shoulders Grading A: Up to 270 mares and 270 foals = 540, which are observation only in accordance to ISES Principles Total 600, made up of 300 mares, 300 foals Main benefit is to elucidate stress levels during handling of mares and foals. This will enable better future training methods.
		5. PROJECT TYPE	
5	2	**This application is a (Use Dropdown box)**	New application
5	3	**If this Application is a renewal of a previous approval, please complete this page before moving to section 6.**	
5	4	**Previous number(s):**	

5	5	**Previous title:**	
5	6	**Lay summary of results from previous year:**	
5	7	**Significance of these results:**	
5	8	**Reasons for continuing:**	
5	9	**If any aspects of the original application have been modified, please list the modifications**	
		6. SCIENCE Justification	
6	1	**Immediate Goal of this project:**	To measure stress levels in foals during foundation training.
6	2	**Purpose of your experimental programme:**	Understand stress levels in foals during foundation training.
6	3	**Longer term benefits of the research programme:**	How stress levels effect horses' racing careers.
6	4	**Has this application been reviewed by your peers**	NO, but have had consultation with KU Leuven University
6	5	**If YES please detail how the project has been peer reviewed**	
6	6	**Are you aware of similar/previous work in this field either in NZ or overseas?**	NO
6	7	**If YES, please detail how similar/previous work in this field either in NZ or overseas relates to your proposal:**	
		7. ANIMAL USE Justification	
7	1	**Total number of animals being used**	600
7	2	**What is the highest MPI grading of manipulations that will apply to this project (use dropdown) - The grades must reflect the summed impacts of both the initial state of the animal and the induced effect of the experimental procedure, not the induced effect alone**	B (LITTLE IMPACT)
7	3	**How many animals will be in this grade?**	60

7	4	**How did you choose which species/breed/strain of animal to use for this project?**	Racehorses are the breed we work with.
7	5	**Why is it necessary to use sentient animals to achieve the goal(s) of this work?**	We are measuring foals normal training of foundation behaviours required to be a race horse. This can only be measured in live foals and their dams.
7	6	**[REDUCTION] How have you reduced the number of animals you propose to use to the minimum compatible with achieving the purpose of the work?**	We have not reduced the numbers per se as these mares and foals would go through the same process of training with or without our observation research.
7	7	**[REFINEMENT] In what ways have you sought to minimise the noxiousness of the procedures you propose to use?**	The mares and foals are in their normal environment. If the mare or foal are uncomfortable with the training, we would stop our process. The mare and foal are kept together throughout the training.
7	8	**[REPLACEMENT] What alternatives to using animals have been considered and why have they been rejected?**	Not applicable for this study as live mares and foals are required for the training.
		8. PUBLICATION	
8	1	**How will the results of this work be published or disseminated**	If an opportunity is presented we may showcase this research in an international forum.
		9. BIOMETRIC EVALUATION	
9	1	**Was there a Power Based assessment of the adequacy of sample size(s)**	NO
9	2	**Main Hypothesis of interest**	Stress Assessment of foals
9	3	**Main variable of interest**	Stress as analysed using algorithm of heart rate and accelerometer data.
9	4	**Experimental design**	Fill out a stress form from above (9.3) data.
9	5	**Biometricians comments**	From discussion with Leigh: 6-9 sessions with each 30 (mare/foal) pair from approval until end October and then repeat up to 5 sessions in January with each (mare/foal) pair. This should provide sufficient data to examine relationships among behaviour data from video, heart rate and accelerometer data from the various sessions for the 30 mare/foal pairs to be investigated.
9	6	**Data used in Power analysis**	
9	7	**Standard deviation (sd) of the main variable**	
9	8	**Where was the sd obtained from**	
9	9	**Minimum true difference of interest**	
9	10	**Desired power of the experiment**	
9	11	**Desired level of significance**	

9	12	**Resulting sample size**	
9	13	**If a power analysis has not been performed please explain why here**	See Biometrician's comments in 9.5
		10. ANIMAL INFORMATION	
10	1	**Animal Details (Use Dropdown boxes)**	
10	2	**Species ~ Breed or strain**	Horses ~ Thoroughbred
10	3	**Common name ~ Gender**	Racehorse ~ Mixed sex
10	4	**Reproductive status ~ Age**	Normal and Non-pregnant ~ mixed age
10	5	**Number being used ~ Health status**	600 ~ healthy
10	6	**Additional Animal Details (Use Dropdown boxes)**	
10	7	**Additional Species ~ Breed or strain**	
10	8	**Additional Common name ~ Gender**	
10	9	**Additional Reproductive status ~ Age**	
10	10	**Additional Number being used ~ Health status**	
10	11	**1 Additional Animal Details (Use Dropdown boxes)**	
10	12	**1 Additional Species ~ Breed or strain**	
10	13	**1 Additional Common name ~ Gender**	
10	14	**1 Additional Reproductive status ~ Age**	
10	15	**1 Additional Number being used ~ Health status**	
10	16	**2 Additional Animal Details (Use Dropdown boxes)**	
10	17	**2 Additional Species ~ Breed or strain**	
10	18	**2 Additional Common name ~ Gender**	

10	19	**2 Additional Reproductive status ~ Age**	
10	20	**2 Additional Number being used ~ Health status**	
10	22	**3 Additional Animal Details (Use Dropdown boxes)**	
10	23	**3 Additional Species ~ Breed or strain**	
10	24	**3 Additional Common name ~ Gender**	
10	25	**3 Additional Reproductive status ~ Age**	
10	26	**3 Additional Number being used ~ Health status**	
10	27	**4 Additional Animal Details (Use Dropdown boxes)**	
10	28	**4 Additional Species ~ Breed or strain**	
10	29	**4 Additional Common name ~ Gender**	
10	30	**4 Additional Reproductive status ~ Age**	
10	31	**4 Additional Number being used ~ Health status**	
10	32	**Previous use of animals**	
10	33	**Have any of the animals been used in previous experiments (i.e. with a different Project number)**	NO
10	34	**If YES What were the animals used for previously?**	
10	35	**When and where did the previous use occur?**	
10	36	**What were the effects of the previous use?**	
10	37	**Please present information to justify re-using these animals**	
		11. ANIMAL WELFARE	

11	1	**Please detail the animal husbandry and welfare principles that will be applied throughout the project**	
11	2	**In preparation for the experiment**	At normal grazing. Please see attached 10 ISES principles for handling and care.pdf 7824
11	3	**While in the experiment**	Please see attached 10 ISES principles pdf 7824
11	4	**After recovery/use (feeding, post-operative care, analgesia, antibiotics, etc.)**	Return to normal grazing.
11	5	**At the end of the experiment**	Return to normal grazing.
11	6	**Please detail any other animal welfare considerations (e.g. transport)**	Animals will be handled in quiet surroundings, always with the foal being close to its mother.
11	7	**Monitoring - any animals that die during the experiment from causes not immediately obvious from clinical signs MUST BE POST-MORTEMED and the AEC notified immediately**	
11	8	**Please detail how the Facility, Animal welfare, Operators etc: of this project will be monitored**	If the trainers feel the mares and foals are unsuitable for intensive monitoring they will be left out of the study.
11	9	**Please indicate the signs/behaviours you will monitor and how frequently they will be monitored**	
11	10	**Body weight ~ Frequency**	No
11	11	**Weight loss ~ Frequency**	No
11	12	**Water intake ~ Frequency**	No
11	13	**Food intake ~ Frequency**	No
11	14	**Posture ~ Frequency**	Yes ~ throughout training
11	15	**Gait/movement ~ Frequency**	No ~ throughout training
11	16	**Coat condition ~ Frequency**	No
11	17	**Vocalisation ~ Frequency**	No
11	18	**Respiration ~ Frequency**	No
11	19	**Faecal consistency ~ Frequency**	No
11	20	**Vaccination site reaction ~ Frequency**	No

11	21	**Fistula cleanliness ~ Frequency**	No
11	22	**Fly strike ~ Frequency**	No
11	23	**Parasites ~ Frequency**	No
11	24	**Haemorrhage ~ Frequency**	No
11	25	**Oedema/swelling ~ Frequency**	No
11	26	**Infection ~ Frequency**	No
11	27	**Self mutilation ~ Frequency**	No
11	28	**CNS signs ~ Frequency**	No
11	29	**Other**	
11	30	**If you detect adverse effects, how will they be managed? - any animals that die during the experiment from causes not immediately obvious from clinical signs MUST BE POST-MORTEMED.**	If the trainers feel the mares and foals are unsuitable for intensive monitoring they will be left out of the study. If there are any problems with the health and welfare of the dam or foal, the owner and (if appropriate) a veterinarian will be consulted immediately.
11	31	**Disposal of animals - How will the animals be disposed of? (Use Dropdown box)**	Returned to farm
11	32	**Disposal other**	
11	33	**If sold or retained, where will the animals be located to (Facility, location, conditions etc)**	
11	34	**If animals are to be euthansed indicate method of euthansia or slaughter**	
11	35	**Electric stun and exsanguinate ~ captive bolt/exsanguinate**	No ~ No
11	36	**Anaesthetics overdose ~ Cervical dislocation**	No ~ No
11	37	**CO2 chamber ~ Guillotine**	No ~ No
11	38	**Other**	
11	39	**If animals are to be euthanased or slaughtered, please supply details (Facility, location, conditions etc)**	
11	40	**Who will perform the procedure (Detail experience and SOP numbers)**	

11	41	**How will carcasses be disposed of?**	
11	42	**Treatment endpoints: Provide details of endpoints that will result in cessation of treatments**	
11	43	**Not applicable**	No
11	44	**Loss of weight ~ Details**	No
11	45	**Intervention trigger (e.g.FE) ~ Details**	No
11	46	**Tumour size ~ Details**	No
11	47	**Irritation by implant/device ~ Details**	No
11	48	**Metabolic upset ~ Details**	No
11	49	**Death ~ Details**	No
11	50	**Euthanasia of moribund animals ~ Details**	No
11	51	**Euthanasia on clinical threshold ~ Details**	No
11	52	**Other ~ Details**	No
11	60	**CONTINGENCY PLANS ~ How do you plan to deal with an emergency/unforseen circumstances that may affect the welfare of the animals.**	
11	61	**Have you made any contingency plans?**	YES
11	62	**Please detail how you will deal with unexpected events, or alternatively why you think such plans are unnecessary.**	If the trainers feel the mares and foals are unsuitable for intensive monitoring they will be left out of the study. If there are any problems with the health and welfare of the dam or foal, the owner and (if appropriate) a veterinarian will be consulted immediately.
11	65	**ADVERSE EVENTS**	
11	70	**Any adverse events that impinge on animal welfare are to be reported to the AEC immediately.**	
		12. NON-SURGICAL Manipulation	
12	1	**SAMPLING**	
12	2	**If you are collecting samples, CHOOSE SAMPLE TYPE FROM DROPDOWN below**	

12	6	**If there is an SOP approved by your AEC for the SAMPLING manipulation(s) please cut the name and number from the appropriate file found on the RVM/SOP page and paste in the space below. If no approved SOP is available please describe the methodology.**	pdf 7824 10 ISES Principles Equus Education pdf 7826 Stress Assessment
12	7	**DRUG administration**	
12	8	**Intramuscular ~ Intracardiac**	No ~ No
12	9	**Subcutaneous ~ Per os by mouth**	No ~ No
12	10	**Intraperitoneal ~ Drug in water bottle**	No ~ No
12	11	**Intravenous ~ Other**	No ~ No
12	12	**If Other DRUG administration method please list**	
12	13	**If there is an SOP approved by your AEC for this manipulation please cut the name and number from the appropriate file found on the RVM/SOP page and paste in the space below. If no approved SOP is available please describe the methodology.**	
12	14	**BEHAVIOUR and HANDLING**	
12	15	**Observation only ~ Enforced activity**	Yes ~ No
12	16	**Physical restraint ~ Nutritional regime**	Yes ~ No
12	17	**Other**	Yes
12	18	**If Other BEHAVIOUR and HANDLING manipulation please list**	heart rate and accelerometer equipment attached during handling period only on girth or surcingle encircling trunk of body behind shoulders
12	19	**If there is an SOP approved by your AEC for the BEHAVIOUR and HANDLING manipulation(s) please cut the name and number from the appropriate file found on the RVM/SOP page and paste in the space below. If no approved SOP is available please describe the methodology.**	heart rate and accelerometer equipment attached during handling period only on girth or surcingle encircling trunk of body behind shoulders
12	20	**EXPOSURE to**	

12	21	**Parasite ~ Micro-organism**	No ~ No
12	22	**Off-licence drug ~ Off-licence chemical**	No ~ No
12	23	**Biological product ~ Radiation**	No ~ No
12	24	**Electrical stimulation ~ Abnormal environment**	No ~ No
12	25	**Other**	No
12	26	**If Other EXPOSURE please list**	
12	27	**If there is an SOP approved by your AEC for the EXPOSURE manipulation)s) please cut the name and number from the appropriate file found on the RVM/SOP page and paste in the space below. If no approved SOP is available please describe the methodology.**	
12	28	**WILD (Non Domesticated) ANIMALS**	
12	29	**Handling ~ Capture**	No ~ No
12	30	**Marking, Tagging or Branding ~ Transmitter attachment or implantation**	No ~ No
12	31	**If there is an SOP approved by your AEC for this manipulation please cut the name and number from the appropriate file found on the RVM/SOP page and paste in the space below. If no approved SOP is available please describe the methodology.**	
12	32	**If other use of WILD (Non Domesticated) ANIMALS please describe**	
12	33	**If you are performing manipulations not listed above, please detail**	
12	34	**If there is an SOP approved by your AEC for the WILD ANIMAL manipulation(s) please cut the name and number from the appropriate file found on the RVM/SOP page and paste in the space below. If no approved SOP is**	

		available please describe the methodology.	
12	35	**Have you made contingency plans for major risk factors to the WILD ANIMALS**	
12	36	**If yes detail your contingency plans**	
		13. SURGICAL Manipulation	
13	1	**Animals will survive surgery ~ Animals will NOT survive surgery**	
13	2	**Manipulations for which SOPs have been approved**	
13	3	**If there is an SOP(s) approved by your AEC for this manipulation please cut the name and number from the appropriate file found on the RVM/SOP page and paste in the space below. If no approved SOP is available please describe the methodology.**	
13	4	**For all manipulations where an SOP has not been approved, please enter the following information:**	
13	5	**ASEPTIC TECHNIQUE**	
13	6	**Give details of Aseptic techniques to be used, including preparation**	
13	7	**ANAESTHESIA and ANALGESIA**	
13	8	**Will the animals receive pre-emptive analgesia**	
13	9	**If not why will the animals not receive pre-emptive analgesia?**	
13	10	**Detail how the effectiveness of analgesia will be tested**	
13	11	**Depth of Anaesthesia - Please indicate how this will be tested**	
13	12	**Thermal methods ~ Electrical methods**	
13	13	**Mechanical methods ~ Chemical methods**	
13	14	**Metabolic methods ~ Local inflammatory reactions**	

13	15	**Deep pain reflex response**	
13	16	**MONITORING**	
13	17	**Duration of monitoring period**	
13	18	**List of parameters to be monitored**	
13	19	**Will you be using standard monitoring sheets**	
13	20	**If YES who will perform the monitoring**	
13	21	**If NO why are you not using standard monitoring sheets**	
13	22	**POST-OPERATIVE support**	
13	23	**Not applicable, animals do not recover**	
13	24	**Conservation of body heat ~ Monitoring sheets**	
13	25	**Fluid administration ~ Recovery in individual cages**	
13	26	**Administration of analgesics ~ Other**	
13	27	**If Other POST-OPERATIVE support please list**	
13	28	**ENDPOINTS**	
13	29	**The endpoint of these manipulations is (Use Dropdown box)**	
13	30	**If Euthanasia upon clinical threshold or Other please specify**	
		14. DRUGS and CHEMICALS	
14	1	**List all drugs and Chemicals to be administered to animals during this project below.____________________ PLEASE NOTE: You are required to complete a separate IDAO for EACH DRUG/CHEMICAL that is to be used in this project and is not an OTC compound or being administered by a registered veterinarian.**	

14	2	**If the drugs have been ACCEPTED by the AEC in terms of label information as appropriate for the routine use you intend please cut the name of the drug and its coversheet number from the appropriate file(s) found on the RVM/SOP page and paste in the space below. IF NO DRUGS ARE TO BE ADMINISTERED PLEASE DO NOT WRITE IN THIS SPACE**	
14	3	**If you are using drugs that do not have a COVERSHEET, please go to HELP on this page and use the link to download the template (second line below the green box). Complete one sheet for each drug and/or chemical. Send the completed form electronically to Mariette Komene. These will be made available in the RVM/SOP function for future reference.**	
14	4	**Have you had to make up new RVM COVERSHEET(s) for this project**	No
14	5	**If YES please name the drugs you have created the new RVM COVERSHEET(s) for**	
14	6	**UNREGISTERED Drugs or Chemicals**	
14	7	**Name of Drug or Chemical**	
14	8	**Composition**	
14	9	**Route of Administration**	
14	20	**Will ANY drugs used in this project (other than OTC Products) be administered by ANY PERSON who is NOT a registered Veterinarian (please answer either YES or NO)__________________This question is an internal trigger for IDAO creation, names are not required here.**	NO
		21. TISSUE COLLECTION	
21	1	**If you are requesting permission to collect tissue following euthanasia, even if you are not manipulating the animals before they are**	

		euthanased you must complete this page	
21	2	**If this is an application primarily to collect animal tissue only, you must complete a full application**	
21	3	**Species (Use Dropdown box)**	
21	4	**If more than one species are being used please list additional species here**	
21	5	**How many animals of each species will be used?**	
21	6	**What tissue(s) are to be collected?**	
21	7	**If the animals are being euthanased for the purpose of tissue collection, please indicate what method of euthanasia will be used**	
21	8	**Have any other staff who might be interested in other tissues been advised?**	
21	9	**If tissue is to be collected from live animals please state how it will be collected**	
21	10	**If the animals will be manipulated prior to collection of the tissue please detail the manipulations**	
		97. SOURCE(s) and TRIAL SITE(s)	
97	1	**This section allows statistics to be gathered for MPI (Animal use information) and data transfer into Animal Tools for AgR animals.**	
97	2	**Please ensure you have selected the correct Trial Site as information is transferred to the Animal Tools database. Please note that you may select more than one Trial Site where relevant depending on the fate of the animals. If the endpoint is returning/transporting animals to a AgR Farm ensure you add this as an additional Trial Site.**	
97	3	**HORSES**	Source = Private Farm(s) Trial Site = Private Farm(s) Number = 600

		98. NOTES ~ Read only	
98	1	**Status Change**	(culluma 17/09/2017) SUBMIT
98	2	**Committee Decision**	(21/09/2017 APPROVEDgrahamf)
		99. PERSONNEL and Sign off	
99	1	**Committee**	RUAKURA
99	1	**APPLICANT: All personnel who will manage the animals or perform manipulations on them, The Programme leader, Facility manager Biometrician and the person responsible for entering info on the animal database must sign this application.** **PLEASE Describe clearly what each person's role in the project is.** **All personnel who will perform manipulations on the specified animals must:** **A - Have been trained and be competent in the manipulations specified (unless this is a specified training protocol)** **B - Be conversant with the AgResearch Code of Ethical Conduct.** **TICKING THE APPROVE BOX IMPLIES THAT YOU HAVE:** **A - read and understood this application** **B - agreed to comply with all statutory requirements regarding the use of animals in experiments** **C - agreed to comply with all statutory requirements regarding the use of any Restricted Veterinary Medicines specified for use in this project.** **Any person whose name is NOT selected from a dropdown list may not be recognised by the auto email function in the database.**	

99	99	**AEC_DRY_SheahanA ~ Job () Location (;)**	please do not sign
99	99	**CULLUMA ~ approved ~ Job (Veterinarian and Animal Welfare Officer) Location (Ruakura; An Phys; Animal Welfare Officer)**	Veterinarian
99	99	**HENDERSONH ~ approved ~ Job (Senior Statistician) Location (Ruakura; North Wing, Ground floor)**	Statistician
99	99	**Wills, Leigh ~ Job () Location (;)**	Applicant, trainer

SUMMARY

For centuries, thoroughbred horses have been dominating the race horse industry, which is not surprising since the thoroughbred horse is bred with the eye on the development of the ultimate race horse by combining physical strength with mental power. Unfortunately, the thoroughbred's strong temperament is accompanied with a high sensitivity for stress, causing the typical problems the race horse industry is facing nowadays. The high stress sensitivity of the thoroughbreds is not only impairing the horse's performance on the racing track, but also involves health risks on the long term. To improve their welfare and performance, the thoroughbred industry is investing in foal training. Learning the foals appropriate techniques to cope with their stress at young age, enables them to cope properly with high amounts of stress later in life, making their competitive life as a race horse easier. To obtain a progressive foal training, it is important to keep the stress level of the foals as low as possible. If the foal is experiencing too much stress, the training must be paused and adjusted accordingly, hence the need for the development of real-time stress monitoring during foal training.

During training, physiological parameters linked with stress can be measured and further processed to calculate features that can be used to monitor stress. Here, as a first step in the development of real-time stress monitoring, HR measurements are used to monitor stress off-line. The features used in the off-line stress monitoring are calculated based on time-variant model parameter of the dynamic autoregressive modelling of the foal's HR. Besides monitoring, HR measurements enable also the evaluation of the foal's progress in its training program. The respective HR measures are used to represent the mean stress level of the foal, the occurrence and intensity of short moments of high stress or how constant the stress level is throughout the training. Comparing the HR measures of the respective training session, enable the evaluation of the progress of the foals, where positive progress is defined as a decrease in the mean stress level, short moments of high stress and a more consistent stress level throughout the training. Meanwhile, comparing the HR measures for respective parts of the training unable the analysis of eventual differences between the parts.

Thus, by measuring the HR and proper processing, a lot of information can be obtained about the stress level and the performance of the foal. If the stress level can be monitored in real-time, the trainers are able to adjust their training based on the current stress level and by keeping in mind the stress inducing training parts – which is different for every foal since they are individually different – the trainers are even able to anticipate.