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Examining the stress response of horses during equine assisted therapy and subsequent regeneration

Master Thesis

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1 Introduction

Around the world an increasing number of animals serve in animal assisted therapy (AAT) to support human well-being (VanFleet, Fine, O'Callaghan, Mackintosh, and Gimeno 2015, p. 158). By definition, AAT is characterised by structured and goal oriented therapeutic interventions which are conducted by professionals. Thereby, the progress of human well-being achieved by the intervention is documented and measured (International Assoziation of Human-Animal-Interaction Organizations 2018). While the focus is often set on human well-being within AAT, this thesis aims at taking a closer look at the animals involved - more precisely, at therapy horses.

1.1 Equine assisted therapy

The therapeutic implementation of horses has been on the rise since the 1960s and is referred to as equine assisted therapy (EAT) (Latella and Abrams 2015, p.115). There are four dominant sectors of EAT in Austria which differ in their therapeutic orientation: *hippotherapy*, *integrated riding*, *special education and therapy* as well as *ergotherapy* (Verein Österreichisches Kuratorium für Therapeutisches Reiten n.d.). Within the sector of *special education and therapy*, the association *e.motion* (Vienna, Austria) developed a therapy method named *equotherapy* (Gansterer, Fischer, and Poinstingl 2011). *Equotherapy* uses the non-verbal communication between the EAT recipient and the horse to gather additional information about the psychological state of the EAT recipient. The therapist receives this information by observing the reaction of the horse to subtle body language signs of the EAT recipient (Zink, as cited in Gansterer et al. 2011) which otherwise might go unnoticed by humans (Lentini and Knox 2009).
In general, horses seem to be especially suitable for therapeutic interests as they offer unique characteristics compared to other therapy animals (Latella and Abrams 2015, p. 116). By their physical strength they enable humans to sit on horseback (Karol 2007). This circumstance is used in hippotherapy as the multidimensional walking movement of the horse stimulates the rider in a way that is comparable to the human walking movement (Bertoti, as cited in Sterba, Rogers, France, and Vokes 2002). By the rhythmic movement of a horse psychological activity might be induced as well: the rhythm works passively on the rider and walking movement facilitates relaxation for the rider. In contrast, faster gaits (for example trotting) have an activating effect on the rider (Gomolla, Apelles, and Fischer 2011). In any case, the interaction with a horse demands an authentic way of action and the recognition of subtle changes in the horse's behaviour (Karol 2007). The common movement with such a large animal requires a higher level of coordination compared to smaller animals. This movement dialogue between

species takes place on a non-verbal level and offers various connecting points for therapeutic work (Hediger and Zink 2017, pp. 52-53). As horses are prey animals, they communicate primarily through body language (Burgon 2011) and outperform us humans in the identification of subtle changes in mood or intention of our counterparts (Lentini and Knox 2009). Horses as herd animals are also characterised by a distinctive social behaviour. Many humans can identify themselves with horses' instinct to seek safety within a group (Porter-Wenzlaff 2007) and social competences and effective leadership can be practiced (Gehrke 2009).
Clearly, horses possess characteristics which are of great value for EAT. Nevertheless, the therapeutic implementation also comes with challenges for the horse.

1.1.1 Therapy horse welfare

How a therapy horse deals with its task affects its welfare. Broom (1986) defined animal welfare as the animal's state regarding its attempts to cope with the environment (as cited in Broom and Johnson 2000, p. 74). While coping refers to the (physiological and behavioural) efforts of an individual which are necessary to deal with a situation, the amount of input it takes the animal to cope as well as the success of the coping attempts affect the welfare level. In theory welfare is a measurable characteristic and it can reach from very good to very poor. A poor welfare level results from difficulties with coping or an inability to cope (Broom and Johnson 2000, pp. 7, 74-75). Important to note is that the interplay of both mental and physical well-being describes the welfare state of an animal. As EAT has the potential to challenge the horse on both levels (physical and mental) it is of concern for animal welfare.
With regard to the working environment of therapy horses potential challenges might arise from the interaction with a high number of (unknown) people who vary for example in their age, way of movement, way of communication or impulsivity - all factors horses are exposed to during the close contact with EAT recipients. As the EAT recipient's safety is of highest priority, situations might occur during EAT in which it is necessary to postpone the demands of the therapy horse to a later timepoint. Overall, the work asks from the horses a good level of frustration tolerance, an interest in interactions with people and a high level of adaptability (Hediger and Zink 2017, pp. 73-78).
The way how therapy horses deal with these potential challenges differs between individuals. These differences, which are consistent over time and situations, lead to the discrimination between different coping styles. A proactive coping style is characterised by an in general more active response: higher levels of aggression and a greater sympathetic reactivity. A reactive coping style, in opposite, is determined by immobility, less agression and higher parasympathetic reactivity (Koolhaas et al. 1999).Based on individual differences an overload is not necessarily

reflected to the same extent by different horses (Hediger and Zink 2017, p. 74) and as a consequence a similar level of horse welfare is expressed differently between individuals. It is the responsibility of humans to recognise the welfare level of the individual therapy horse and to influence it positively through the underlying human-horse relationship and appropriate management.

The relationship of the horse with the interacting human forms an integral part in the working environment of a therapy horse (Hediger and Zink 2017, p. 79). A relationship of an animal to a human is influenced by the quality and quantity of previous interactions, genetics, the environment and the behaviour of humans in a current interaction. Thus one possibility to improve the relationship of an animal to a human is by more neutral or positive contact. As the underlying relationship to humans influences the provoked emotions which themselves influence the perception of a situation the relationship between humans and animals is affecting the welfare (Waiblinger 2009).

In order to prevent a poor inner state of horses during therapeutic work, Hediger and Zink (2017) suggest implementing methods in the working routine which aim to relief tension from the horse. Examples are the opportunity for wallowing at the end of sessions or to train the horses to communicate an increase in internal tension by snorting. The advantage is that snorting itself decreases tension already and the therapist is given the opportunity to change a specific situation for the horse. The perceived controllability for the horse may be enhanced (Hediger and Zink 2017, p. 80). In parallel to those efforts there is a strong need in research to investigate the welfare of therapy horses and potential consequences by their implementation in EAT.

Animal welfare research serves to address concerns about the quality of life of animals (Fraser, Weary, Pajor, and Milligan 1997) with the scope to provide evidence based guidance on how to treat them. Thereby, the presence or absence of stress is used as one potential indicator of animal welfare. An animal experiences stress if it fails to cope or is likely to fail to cope with a situation (Broom and Johnson 2000, pp. 74, 167, 170).

1.2 Biological stress response and its measurement

A biological stress response follows a perceived threat to an organism's homeostasis. The perceived threat is called a stressor. Encountering stressors is a part of life in all organisms and stressors can be of either good or bad valence. The general model of animal stress consists of three consecutive phases: *recognition of a stressor*, *biological defence against the stressor* and *consequences of the stress response* (Moberg and Mench 2001, pp. 1,3). Important to note is that the perception of a situation, thus an underlying emotion, plays a crucial role in the recognition of stressors (reviewed in Veissier and Boissy 2007). The subsequent biological response

of an individual is expressed in the behaviour combined with changes in certain hormone concentrations or the immune function. The consequences of a stress response determine whether there is a detrimental effect on the welfare of an animal. If either enough resources are available or the intensity and frequency of a negative stressor is low enough, the experience of stress is without negative consequences and without a significant impact on animal welfare (mild stress). Nevertheless the animal might be vulnerable to the effect of a subsequent stressor as the accumulation of negative stressors might limit other bodily functions. If other bodily functions are impaired and the fitness of an individual is diminished (for example its metabolism and reproductive success), the welfare is negatively affected and one refers to distress (Moberg and Mench 2001, pp. 3, 8).
Whether a stressor provokes distress depends on its intensity, duration and frequency. Additionally, its predictability and controllability by the individual strongly influence the consequences of the stress response (Keeling and Jensen 2002).According to Koolhaas, Boer, Coppens, and Buwalda (2010) a biological stress response has a qualitative and a quantitative dimension. Whereas the coping style of an individual describes the quality of a stress response, the intensity and duration of behavioural and physiological parameters inform about its quantity (reviewed in Koolhaas et al. 2010). Although the behavioural and physiological stress response of an individual go hand in hand one can discriminate between both in theory.
The physiological stress response is triggered by the autonomic nervous system (ANS) and the neuroendocrine system. Both systems are interlinked and their effect mechanisms are described in axes. The sympathetic-adrenal-medullary (SAM) axis represents the ANS and the hypothalamic-pituitary-adrenal (HPA) axis reflects the neuroendocrine system. In order to quantify the physiological stress response the state of arousal is measured (Chrousos & Gold, as cited in Glenk and Kothgassner 2017, pp. 100,101). It is important to keep in mind that an increase of physiological arousal is not necessarily always based on an unpleasant cognitive perception but rather serves as a prerequisite to perform behaviour. The recovery, not necessarily the magnitude, of the physiological response is suggested to be important to identify stressors (reviewed in Koolhaas et al. 2011).

1.2.1 SAM axis

The ANS consists of a sympathetic branch and a parasympathetic branch. Both regulate vital function, however, they have an opposing effect on the organism. Sympathetic activation causes an increase in performance, whereas parasympathetic activation furthers digestive and growth processes. Intermeshing perfectly, vital body functions are regulated and the adaptability of the organism to internal or external challenges is secured (Lohninger 2017, pp. 19-28).

If an acute stressor occurs, the ANS is activated. A hormone secreted from the hypothalamus, corticotropin releasing hormone (CRH), activates the locus coeruleus (LC) in the brainstem. As a consequence noradrenaline is released from the brain stem and activates sympathetic fibers. By this activation catecholamines, such as noradrenaline or adrenaline, are secreted from the adrenal medullary. That causes among other things an increase in heart and respiration rate as well as in blood pressure. Within seconds the organism is prepared to either fight or flight (Chrousos & Gold, as cited in Glenk and Kothgassner 2017, pp. 100,101). As adrenaline and noradrenaline are unstable substances the activation of the SAM axis in response to potential stressors is frequently quantified indirectly by measuring characteristics of the heart rate (HR). Differences in electrical activity over time result in the characteristic wave form of a heart beat. Single fluctuations of one heart beat are marked by single letters (see Fig.1) - often referred to as QRS-complex. The R-wave witin the QRS-complex corresponds to the depolarisation of the ventricle. The interval between two R-waves of subsequent beats (RR interval) can be analysed regarding intensity and time. This variation in RR intervals is known as heart rate variability (HRV) (Lohninger 2017, p. 39). It results from the decceleration and acceleration of the heart beat by the ANS. The vagus nerve as the operator of the parasympathetic nervous system predominates at rest. Sympathetic tone increases with increased physical activity or emotional distress. On the basis of both, the HR is continously adapted to inner or outer challenges. A high HRV represents a healthy cardiovascular system (reviewed in Shaffer, McCraty, and Zerr 2014) as well as a flexible ANS (reviewed in Appelhans and Luecken 2006). In contrast, a low HRV caused by autonomic imbalances is linked to various pathologies (reviewed in Thayer, Yamamoto, and Brosschot 2010). Further insight into autonomic regulation is provided by time-domain, frequency-domain and non-linear analyses of RR intervals.

Time-domain analyses express the HR and the RR intervals as a function of time. The number of heart beats during one minute is the HR. In general a low HR during resting periods indicates good health and a well performance potential. The standard deviation of all RR intervals (STDRR) reflects the overall variability of the time between heart beats. A higher STDRR indicates a better interplay of sympathetic and parasympathetic branches of the ANS. The intensity of parasympathetic activity correlates with the root mean square of successive differences (RMSSD) of adjacent RR intervals (Lohninger 2017, pp. 58-61).

Frequency domain analysis describes the influence of specific frequencies on a signal and enables statements about the mode and intensity of the variations in the heart beat. The single frequencies influencing the HRV are high frequency (HF), low frequency (LF), very low frequency and ultra low frequency (Lohninger 2017, pp. 62,66). The HF is merely influenced by the vagus nerve and respiration whereas the LF is influenced by parasympathetic as well as sympathetic activity (Baumert et al., as cited in Lohninger 2017, p. 67). In theory fast and slow fluctuations

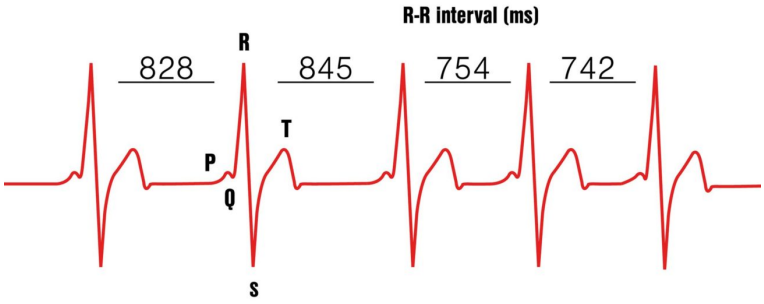

Fig. 1: The variation in electrical activity of the heart is shown over the time of five heart beats. The different fluctuations of one heart beat are marked by letters. The time between one R wave to the subsequent one (RR interval) can be measured [ms] and used for analysis regarding HRV. Source: *https://www.firstbeat.com/de/blog-de/was-ist-die-herzratenvariabilitaet-hrv-und-wieso-ist-sie-wichtig/*

in heart beat sequences are detectable - but the latter ones only in recordings of at least 24 hours (Task Force of The European Society of Cardiology and The North American 1996). The ratio of LF and HF (LF/HF) is used as an indicator of sympathovagal balance (Eckberg, as cited in Lohninger 2017, p. 71). The ratio is highly influenced by respiration and it is recommended to consider the circumstances of data collection for correct interpretations (Task Force of The European Society of Cardiology and The North American 1996).

A scatterplot out of RR intervals is an example for non-linear analysis. Single RR intervals are plotted against the respective subsequent RR interval. The standard deviation of the diameter of the scatterplot (SD1) examines the variability over single beats in the short term. It reflects parasympathetic activity - thus an increase is expressed in a greater SD1 (Mäkikallio, Perkiömäki, and Huikuri 2004, pp. 24,25).

In humans the HRV depends for example on age, gender, individual predisposition, circadian and seasonal rhythm, fitness level, health, environmental influences or psychological stress (Lohninger 2017, pp. 127-128). The possibility to detect relative differences in the activation of the SAM axis by non-invasively measuring characteristics of cardiac activity offers great research opportunities and is also regarded as a suitable approach to identify the level of excitement of an animal (Rietmann et al. 2004). In free-ranging horses seasonal influences on HRV were found. The HR and RMSSD were lower during winter. An increase in activity, raining and humidity positively influenced the RMSSD whereas the ambient temperature negatively affected it. In free ranging horses the parasympathetic activity was higher from 12 a.m. to 6 p.m.

(Pohlin et al. 2017). Therapy horses, in contrast, showed elevated values during the night and lower values during the day (Gehrke, Baldwin, and Schiltz 2011).
Investigations of the SAM axis response in horses were mainly focusing on circumstances like transportation (Medica, Bruschetta, Cravana, Ferlazzo, and Fazio 2017) or competition (Williams, Chandler, and Marlin 2009). In the field of EAT, the examiniation of the SAM axis was used to identify underlying effect mechanisms. Drinkhouse, Birmingham, Fillman, and Jedlickaa (2012) monitored the HR of at-risk youth and horses during EAT. Correlations in HR were found but were most probably a result of external stimuli (Drinkhouse et al. 2012). Naber (2018) and Naber et al. (2019) could reveal correlations of EAT recipients' and equines' HRs during a standardised EAT if the horse was the favourite horse of the EAT recipient. The underlying relation between human and horse seems to influence the inter-species synchronisation in HR (Naber 2018; Naber et al. 2019). Focusing on the horse Gehrke et al. (2011) measured the HR and RR intervals of therapy horses on pasture over 24 hours. In opposite to data obtained in thoroughbred horses by Kuwahara, Hiraga, Kai, Tsubone, and Sugano (1999) the LF and HF components of HRV did not differ significantly between day and night in therapy horses (Gehrke et al. 2011). Further measurements of the HR and parameters of HRV of therapy horses are necessary and will help to quantify and qualify the level of ANS reactivity in the context of EAT. Conclusions about potential stressors during and the short-term consequences of EAT on the ANS of horses are missing.

1.2.2 HPA axis

The neuroendocrine response to stress starts in the hypothalamus. Neurons trigger the secretion of CRH which in turn induces the secretion of adrenocorticotropic hormone (ACTH) from the pituitary. As a consequence of this cascade glucocorticoids are secreted from the adrenal cortex into the bloodstream. Glucocorticoids are steroid hormones and appear as cortisol or corticosterone. In mammals cortisol is the predominant form and serves the provision of energy. Thus, high concentrations of cortisol limit other energy consuming processes such as reproduction, digestion and growth and immune responses are supressed (Glenk and Kothgassner 2017, pp. 100,101).
The HPA axis is regulated by a negative feedback loop. An increase in cortisol concentration downregulates the secretion of CRH and ACTH. The glucocorticoid concentration is subject to circadian rhythm with a peak in the morning in many species (Möstl and Palme 2002; Bohák et al. 2013). Glucocorticoids and their metabolites are secreted in various body fluids such as blood, milk, saliva, urine or feces. Whereas urine and feces sampling has advantages in assessing chronic stress and milk sampling is limited to lactating animals the use of blood and saliva

samples is appropriate for acute stressors (Möstl and Palme 2002). The non-invasive saliva sampling is often preferred over blood sampling as it is less likely that the sampling technique provokes itself a stress response in the animal (Bohák et al. 2013). The cortisol concentration in saliva represents the free and unbound and thus, the biologically active form of the hormone in the body. An increase in the cortisol concentration is detected in saliva approximately up to 20 minutes after the secretion of cortisol into the blood stream (Peeters, Sulon, Beckers, Ledoux, and Vandenheede 2011). Peeters et al. (2011) validated the use of saliva samples in horses. In the context of welfare, glucocorticoids are particularly important if an individual is subjected to stress for prolonged periods of time. Altered and ineffective glucocorticoid function has been linked to the onset and progression of stress-related diseases (Glenk and Kothgassner 2017, pp. 101-107). The cause for increased glucocorticoids as a response to short term mental stress is not yet fully understood in the horse. The magnitude of this neuroendocrine response is often linked to increased metabolic demands, activity levels or physiological loads (König v. Borstel, Visser, and Hall 2017).

With respect to EAT Suthers-McCabe and Albano (2004) found an increase in the blood cortisol concentration after therapy sessions in six out of 33 samples. Worth mentioning is that the sessions seemed not to be standardised, some horses were measured twice and therapy recipients differed in their clinical picture. Fazio, Medica, Cravana, and Ferlazzo (2013) investigated the HPA axis response of horses with regard to sessions performed with riders with disabilities and sessions performed with riders without disabilitites. Lower blood cortisol concentrations were found for sessions performed with riders with disabilites (Fazio et al. 2013). As the use of saliva samples is preferred to blood samples regarding the identification of acute stressors (Möstl and Palme 2002) studies implementing this advice in the field of EAT are missing. A sampling schedule including measurements prior, during and after a standardised EAT seems advisable in order to identify the course of the HPA axis response.

1.3 Regeneration

Regeneration is a broad term with context dependent meanings (McCartney et al. 2017). The term bears a meaning for example in economic, environmental, architectural, social as well as physical matters (SURF 2013). Regarding animals, the term regeneration is used in the context of tissue generation (Brockes and Kumar 2008) but also came to the fore in stress research. Koolhaas et al. (2011) hypothesise that the course of regeneration of the physiological response indicates whether something was perceived as a controllable situation or as a stressor. To Koolhaas et al., the period of regeneration contains more valuable information than the magnitude of a physiological response to identify potential stressors. Earlier studies postulated already that

the speed of regeneration might be linked to the perceived predictability and controllability of a situation. Preexposure to a situation and thus a higher predictability (and controllability) led to a quicker regeneration of the HPA axis (García, Martí, Vallès, Dal-Zotto, and Armario 2000). Very little is known regarding autonomous regeneration. However, Koolhaas et al. (2011) expect the level of the SAM axis response past the exposure to a potential stressor to reveal similiar information about the perception of a situation. An enduring physiological response might indicate that the respective environmental condition exceeds the adaptive capacity of the individual thus is perceived by the individual as uncontrollable. Koolhaas et al. regard a stimulus which exceeds this adaptive capacity of an organism as a stressor. Demands which remain within the adaptive capacity are no stressors (Koolhaas et al. 2011). I refer to regeneration as the process of recovering after a load and specify my investigations on autonomous regeneration after EAT. So far, research of autonomous regeneration in horses is scarce. If performed, the focus is set on the recovery quality after anaesthesia (Tzelos, Blissitt, and Clutton 2015; Sage, Keating, Lascola, Schaeffer, and Clark-Price 2018) or from training (Boffi et al. 2011; Mata 2014). In the field of EAT, lengthy investigations of the ANS activity of horses next to EAT are missing. Malinowski et al. (2018) analysed the activity of the SAM axis over periods of ten minutes prior, in the middle of, directly after and 20 minutes after non-standardised EAT sessions. Mean HR of seven horses revealed differences in measuring time points depending on the day of EAT. On the first day the mean HR was significantly lower during EAT, on the second day only compared to the time prior to EAT and on the third day no significant difference in the mean HR was found. The STDRR and LF/HF did not show a day by time dependency (Malinowski et al. 2018). The RMSSD as a parameter of parasympathetic NS activity was not investigated in the study by Malinowski et al.. Longer investigations of the ANS activity of therapy horses next to therapy sessions were performed as before mentioned by Gehrke et al. (2011). As the measurement started at 8 a.m. and lasted for 24 hours, Gehrke et al. did not aim at quantifying the autonomous regeneration of a single EAT session but rather at providing knowledge about whether 24 hours recordings of therapy horses differ from those of thoroughbred horses.

Many questions regarding the influences of EAT on the ANS activity of horses are still unanswered. Observing the ANS activity directly after EAT for a longer period will increase our knowledge. The speed of recovery of the SAM axis (and in theory also the HPA axis) might contain information about the perception of certain stimuli and help to identify stressors (Koolhaas et al. 2011).

1.4 Aim of the thesis and hypotheses

The objective of this thesis is to examine the physiological stress response of therapy horses in the context of EAT.

The first study (study 1) aims at monitoring the physiological stress response of therapy horses over the course of a standardised EAT session. It is hypothesised that a higher HR, increased sympathetic reactivity (LF/HF) and decreased parameters of HRV (STDRR, SD1, RMSSD) in combination with increased cortisol concentrations during and after EAT parallel an acute stress reaction of horses due to EAT.

A second study (study 2) aims at monitoring the autonomous regeneration of therapy horses after everyday therapeutic work by measuring the HR and RR intervals. It is hypothesised that a substantial influence of EAT on the ANS of therapy horses past the time of EAT can be excluded if no significant difference in the mean HR and parameters of HRV exists between measurements prior compared to after EAT. It is further hypothesised that the duration of the autonomous regeneration after EAT can be quantified if the mean HR and parameters of HRV differ significantly for a certain period between measurements prior compared to after EAT. To conclude, it is hypothesised that a substantial influence of EAT on the ANS of therapy horses past EAT can be excluded if no significant difference in the mean HR and parameters of HRV exists between measurements under the experimental and the control condition.

2 Material and Methods

All methods and procedures used in these studies were discussed and approved by the institutional ethics and animal welfare committee in accordance with GSP guidelines and national legislation (reference number: ETK-05/01/2018; ETK-11/09/2018). All data were collected at the association *e.motion* (Baumgartner Höhe 1, 1145 Vienna, Austria).
The participating horses were housed in open stables within a herd of either eight or ten horses. All horses had access to hay and water *ad libitum*. In their daily routine, each horse has a reference person who is responsible for the horse and its balanced training in addition to the therapeutic workload. All horses were accustomed to the experimental environment already prior to data collection.

2.1 During EAT

The data collection during EAT (study 1) was conducted as an integral part of the study by Naber (2018), who aimed at the identification of patterns of synchrony in HR, HRV and salivary cortisol between the EAT recipient, the therapist and the horse during EAT. The study by Naber covers the effects of EAT on the EAT recipient and was approved by the Ethics Committee of the University of Vienna (reference number: 00303).

2.1.1 Experimental design

Data were collected in February 12-25, 2018 in the riding hall with an ambient temperature of 2,5 $\pm 1,8$ °C and a humidity of 64,8 $\pm 11,9$ %. The sessions were scheduled within 1:30 to 5:30 pm. Ten females (21.8 $\pm$3.39 ($\bar{x}\pm$SD) years) participated in the study as EAT recipients. All visited the association on a regular basis and were experienced with EAT for 12.8 $\pm$4.71 years. All recipients had previously received a diagnosis of intellectual impairment (Naber 2018).
Two mares and two geldings (N=4) of the breed criollo with an average age of 14,8 $\pm$5,6 years and 9,4 $\pm$6,6 years of experience in EAT served as therapy horses (see Tab. 1). At the days of data collection the horses were kept in their usual management and housing routine but no additional EAT sessions were performed.

All standardised sessions were conducted by the same female therapist in the riding hall of the association *e.motion* and followed the subsequent pattern. At the beginning of each session a saliva sample (s) was collected (s1). HR measurement of horse, recipient and therapist was started simultanously right after s1 and lasted throughout the session. Baseline values were obtained over five minutes by humans sitting on a chair and the horse standing right next to them without interaction (baseline 1). After baseline 1 the EAT recipient led the horse in walking pace

Tab. 1: Characteristics of participating horses. The age and experience in EAT are expressed in years.

horse	sex	age	breed	EAT experience
1	♀	9	criollo	3
2	♂	11	criollo	5
3	♀	20	criollo	14
4	♂	19	criollo	14

for two rounds along the riding area. The therapist walked next to them and subsequently secured the mounting of the horse by the recipient at a ramp. While the horse stood still at a place in the middle of the riding hall five minutes of guided relaxation for the EAT recipient began on horseback (relaxation 1). The therapist remained next to the horse and instructed for example breathing exercises, relaxation of single body parts or dream journies. After that the EAT recipient faced simple cognitive-motor tasks on top of the horse for five minutes (challenge), for example mastering a track and obstacle course by riding in walk. The therapist announced the tasks from a distance and interfered as few as possible in the interaction between EAT recipient and horse. After challenge the next saliva sample was collected (s2), followed by another period of relaxation (relaxation 2). As a next step, the EAT recipient dismounted the horse at the ramp and the session ended with another baseline measurement (baseline 2) and saliva sample (s3). All measuring devices were removed and after 30 minutes of rest back in the stable a last saliva sample was collected (s4) from the horse (see Tab. 2).

Tab. 2: Course of a standardised session under the experimental condition (E) and the control condition (C). The order of saliva samples (s1-s4) and activity phases (baseline, relaxation, challenge) within one session is described in the first row. An EAT recipient was present only under E. The vertical lines indicate the end of the session in the riding hall.

	s1	baseline 1		relaxation 1	challenge	s2	relaxation 2		baseline 2	s3			s4
E	✓	5 min	2 rounds, mounting	5 min	5 min	✓	5 min	dismounting	5 min	✓	‖	30 min	✓
C	✓	5 min	2 rounds	5 min	5 min	✓	5 min		5 min	✓	‖	30 min	✓

The temporal sequence for each session was documented in Mircosoft®Excel®2016 MSO and used in further analysis to identify the five minute periods of baselines, relaxations or challenge in the tachogramm.

Each horse performed two standardised sessions per EAT recipient resulting in four or six sessions per horse. Due to limitations in time each horse performed only one control session (see Tab. 3). These control sessions followed the same pattern but with no EAT recipient present.

During the challenge under the control condition the therapist demanded from the ground the same tasks from the horse as the EAT recipient did from horseback under the experimental condition. Due to reasons of feasibility the control conditions were performed towards the end of the data collection period.
Sessions were generally scheduled so that horses performed not more than one experimental session per day - as an exception, it happened once that a horse performed two experimental sessions at the same day with a gap of three hours in between. Around 30 minutes prior to each session horses were cleaned and prepared for the HR measurements by the same person in a single box which was situated next to and in visual contact with the group stable. On top of the electrodes horses were saddled with a pad and voulting girth. Halters were used to which a lead string or reins (prior to mounting) were attached - snaffle bits were never used. Horses remained in the single box until the onset of the session.
The interacting humans differed regarding the intensity of the relationship (IR) with the horse. The therapist was the reference person of two horses resulting in a higher (+) intensity of the relationship between therapist and horse (IRT) for those horses. The intensity of the relationship between EAT recipient and horse (IRR) was high (+) for five recipients who participated with their favourite horse and low (-) for the remaining five recipients who participated with an unknown horse (see Tab. 3).

Tab. 3: The IRT and IRR for the respective horse is indicated. The number of EAT recipients (n(R)) and the resulting number of experimental sessions (n(E)) and the number of control sessions (n(C)) per horse are listed.

horse	IRT	n(R)	IRR	n(E)	n(C)
1	-	3	+/-	6	1
2	+	3	+	6	1
3	+	2	-	4	1
4	-	2	-	4	1

2.1.2 HR, RR interval measurement and data processing

HR and RR intervals were measured simultanously in horse, EAT recipient and therapist. Three Polar®V800 telemetric devices were used which were combined with a chest strap for humans or an equine H7 HR sensor electrode base set (Polar®Electro Oy, Kempele, Finland) for horses. The positive electrode pad was placed on the left side underneath the withers under the pad, the negative one on the left side under the girth. Good contact between the electrode pads and the horse's skin was ensured by wetting the horse's hair from the places of electrode pads and the

use of contact gel. The device works with a sampling frequeny of 1000Hz.
The collected data was transferred to a PC and further processed in a commercial software (Kubios HRV Standard 3.1.0, University of Eastern Finland, Kuopio, Finland). For data analysis the five minute sequences were chosen according to the activity phases shown in Tab. 2: baseline 1, relaxation 1, challenge, relaxation 2, baseline 2. The sequences were inspected for artifacts and if necessary corrected with a very low threshold artifact correction. The applied very low threshold identifies all RR intervals that differ more than 0,45 s compared to the local average. This is an appropriate correction level for HRs of up to 60 bpm. A cubic spline interpolation was used to replace these artifacts (Aranda, de La Cruz, and Naranjo 2017). Each five minute sequence was analysed regarding time domain (HR, STDRR, RMSSD), frequency domain (LF/HF) and non-linear parameters (SD1) of HRV. Frequency domain analysis was computed using an autoregressive model. Following Gehrke et al. (2011) frequency bands from 0.04-0.15 Hz for LF and 0.15-0.40 for HF, as usually chosen in humans, were also applied to the horse data. As we were interested in changes in the LF and HF, any disturbing low frequency trend component was removed from the RR interval series by the method "smoothn priors" (λ = 500) resulting in a cut of frequency of 0,035 Hz (Tarvainen, Niskanen, Lipponen, Ranta-Aho, and Karjalainen 2014).

2.1.3 Saliva sampling and processing

Saliva samples were collected using Salivettes (Sarstedt AG Co. KG, Nümbrecht, Germany). The sampling device was gently put in the horse's mouth for 30-40 seconds (rubber gloves were used to avoid contamination) and stored in a saliva retainer tube. Sample collection took place by previously trained people and was tolerated by all horses without restraint.
Saliva samples were taken at timepoint s1, s2, s3 and s4 during the experimental and the control condition. The sampling schedule takes the time delay between plasma cortisol and salivary cortisol levels into account. To control for the circadian rhythm of horses, saliva samples were additionally taken between 7-9 h, 12-14 h and 18-20 h on two control days (with at least one day in between) with no therapeutic intervention.
All samples were directly put on ice and stored in a freezer at -20°C at the association *e.motion* until further processing. In preparation for analysis, samples were thawed and subsequently centrifuged for ten minutes with 3750 rpm at 20°C (Department of Physiology, Pathophysiology and Experimental Endocrinology, University of Veterinary Medicine, 1220 Vienna). Further analysis took place at the University of Vienna (Department of Behavioural Biology, University of Vienna, 1090 Vienna). 10 µl of saliva were used for analysis. A highly sensitive cortisol EIA as discovered by Palme & Möstl was applied (as cited in (Schmidt et al. 2010)). Other studies

have proven a successful implementation of this method for equine saliva (Schmidt et al. 2010; Aurich et al. 2015). Each sample was assayed twice. The respective 10 µl were diluted with assay buffer in a ratio 1:10. The average interassay precision of equine samples was less than 15%.

2.1.4 Data analysis

As the individual horse was considered as the statistical unit data of various EAT recipients and sessions had to be further summarised by calculating mean values. First, the two experimental sessions per EAT recipient were averaged. In a next step, the parameters were averaged per horse (over different clients). Data processing was performed in Mircosoft®Excel®2016 MSO and data analysis in IBM SPSS Statistics 23.0. Data were analysed using descriptive statistics and are presented, unless otherwise indicated, as mean values with standard deviation ($\bar{x} \pm$SD). At first, the baseline values of the HR and the parameters of HRV were compared at the beginning (baseline 1) and at the end (baseline 2) of EAT under the experimental condition. Afterwards, the intensity of the HR, STDRR, RMSSD, LF/HF and SD1 was compared over the course of a session (considering also relaxation 1, challenge and relaxation 2). Additionally, each activity phase under the experimental condition was compared to the respective one under the control condition.
The mean cortisol concentration was compared between samples resembling HPA axis stimulation prior (s1) and subsequent (s4) to EAT. All samples, including the ones at the beginning of EAT (s2) and during EAT (s3), were compared regarding their change in intensity and were also compared to the control condition.

Influences of the IR on the physiological response
Horses were grouped after a high (+, N=2) and low (-, N=2) IRT. Within this grouping the sessions of each horse were additionally split according to a high (+) or low (-) IRR. This resulted in comparisons on the basis of individual horses - with the exception of a low IRT (-) in combination with a low IRR (-). These data are subsumed of two different horses (traceable in Tab. 3).

2.2 Autonomous regeneration

2.2.1 Experimental design

For the second study (study 2), data were collected during the normal operation of the association *e.motion* in November and December 2018 in an ambient temperature of 6,5 $\pm 4,4$°C and

a humidity of 63,4 $\pm 11,0\%$ at the onset of measurements and 4,2 $\pm 3,8$°C with a humidity of 82,0 $\pm 11,3\%$ later in the evening. Ten therapy horses of various breeds with a mean age of 17,7 $\pm$7,54 years and 8,8 $\pm$6,8 years of experience in EAT participated in the study (see Tab. 4).

Tab. 4: Characteristics of horses participating in study 2. The age and experience in EAT are expressed in years.

horse	sex	age	breed	EAT experience
1	♀	9	criollo	3
4	♂	20	criollo	15
5	♀	23	shetland pony mix	17
6	♀	20	criollo	12
7	♀	29	shetland pony	18
8	♂	25	icelandic horse	15
9	♂	8	criollo	1
10	♂	8	warmblood	1,5
11	♀	11	criollo	5
12	♀	24	camargue	1

The measurements took place in the respective open stable within the herd. Each horse was measured once under the experimental condition and once on a control day. Under the experimental condition, the HR and RR intervals were measured for one hour prior to EAT and to a maximum of three hours after EAT (see Tab. 5). The measurements after EAT were shortened, if it collided with the closing time of the association *e.motion*. The EAT sessions under the experimental condition took place between 2 p.m. and 6:15 p.m., thus each horse was measured at least for one and a half hours after EAT. If possible, the measurements were continued for three hours after EAT, which was considered a feasible time span to study autonomous regeneration.

Tab. 5: The measurement schedule under the experimental condition (E) and the control condition (C) is shown. The HR and RR intervals were recorded continously before and after EAT but divided into 30 minutes sequences for analysis. EAT only took place under E (✓). Each horse was measured at least up to one and a half hours after the ending time of EAT (indicated by vertical lines), only few up to three hours.

	-60 min	-30 min	EAT	+0 min	+30 min	+60 min	+ 90 min	+ 120 min	+ 150 min
E	30 min	30 min	✓	30 min	30 min	30 min	30 min	30 min	30 min
C	30 min	30 min		30 min	30 min	30 min	30 min	30 min	30 min

One EAT group session of 60 minutes served as the EAT session under the experimental con-

dition. Sessions were carried out with two to four children with a mean age of 9.4 (± 3.3 SD) years who did not show any motor impairment but exhibited impaired cognitive or social functioning. The sessions were conducted by different female therapists with at least one other therapy horse present. A low intensity of movement for the horse, mainly standing or walking gait, characterised the performed EAT units. That way, the comparability between conditions could be ensured best as these were the most observed activity patterns in the open stable. Sessions included for example guided rides in the surrounding of the association, relaxation exercises for the EAT recipient, dressing up the horses or free work in the riding hall. On control days, the time of measurement equalled the respective one under the experimental condition, but the horse remained in the open stable instead of performing EAT. The measurements were scheduled so that half of the horses started with the experimental and the other half with the control condition. In general, no additional EAT services, riding or training activities took place on the days of data collection.

During data collection the activity of the horse in the open stable was logged on a per second basis in Microsoft®Excel®2016 MSO to identify periods of standing for later analysis. Walking (as opposed to standing) was defined as a fluent movement of all legs in the same direction. Additionally, the time a horse spent eating or potential disturbances (people entering, noises) were noted even if the disturbances did not necessarily cause noticable changes in the horse. To log the activity pattern the experimenter remained within sight with the horse but kept distance and did not interact. If the experimenter lost sight of the respective horse it was logged and the experimenter changed place accordingly. After each 30 minute sequence the horse was approached calmly by the experimenter to check the data transmission of the measuring device.

2.2.2 HR, RR interval measurement and data processing

The HR and RR intervals were measured by a Polar®V800 telemetric device combined with an equine H7 heart rate sensor belt set (Polar®Electro Oy, Kempele, Finland). As the H7 belt set is a wireless device, it was deemed more suitable for measurements in the open stable compared to the electrode base set from study 1. The measurement device was always attached to the horse in the open stable. The horse's coat was wetted, which in addition to electrode gel ensured good contact. An equine chest protector (normally used against grating) was put on top. A small sewed-on pocket at chest height stored the V800 monitor during measurements close to the sensor. The data collection (-60 min) began after a ten minutes acclimatisation period to the measurement equipment - at +0 min the data collection started as soon as possible after the attachment to not lose potentially interesting data.

After each measurement the data were transferred to a PC. The activity protocol revealed that

each horse stood at least for 20 minutes in each 30 minutes sequence. To minimise the influence of motion, 20 minutes of standing were chosen for HRV analysis within each 30 minutes sequence. For this purpose the activity protocol of each horse was inspected and, starting from the beginning of each 30 minutes sequence, only periods of standing that lasted at least for 30 seconds were included to merge the 20 minutes sample. If a disturbance was noted in the activity protocol, a time span lasting from 5 seconds before to 30 seconds after the disturbance was excluded. If a disturbance lasted noticeably longer than 30 seconds, the frequency and kind of disturbance during the respective 30 minutes sequence was noted down in the analysis as a confounding factor. The time which a horse spent eating while standing was calculated within each 20 minutes sample.
For HRV analysis the respective 20 minutes of standing were chosen in Kubios and merged to one sample per 30 minutes. A very low threshold level was chosen to detect artifacts. The percentage of replaced beats was noted for each 20 minutes sample. The frequency bands and further settings of HRV analysis were chosen as described in subsection 2.1.2. The characteristics of each merged sample (mean duration of the single periods of standing and the percentage of replaced beats by the artifact correction) were noted.

Feasibility of data collection

The winter coat of the horses demanded careful moistening prior to attachement. The H7 belt can easily be adjusted to the various sizes of horses. A low battery status in the sensor unit of the measuring device led to abnormal HRs during the first six measurements. This fact became clear after the data collection was finished. As a consequence, the first six days of data collection had to be excluded from the analysis resulting in a reduced sample size. During the period of data collection, three other measurements were detected as insufficient and were repeated successfully.

2.2.3 Data analysis

Only 30 minutes sequences which were measured for all horses were included in the analysis (-60 min, -30 min, +0 min, +30 min, +60 min). Due to the loss of some data the final sample consisted of seven horses (N=7; exclusion of horse 4,6,12) regarding the comparison within the experimental condition and four horses (N=4; additional exclusion of horse 1,9,11) regarding the comparison between conditions. Within the experimental condition each parameter (HR, STDRR, RMSSD, LF/HF, SD1) at each timepoint was tested for normal distribution by the Shapiro-Wilk test. The timepoints before EAT (-60 min, -30 min) were compared to the timepoints after EAT (+0 min, +30 min, +60 min), as well as once with each other. This was done by an

ANOVA for repeated measures or a Friedman test as a non-parametric alternative. A Bonferroni correction was applied resulting in an alpha level of $\alpha = 0,0125$.
The comparison of each parameter at one timepoint (-60 min/-30 min/+0 min/+30 min/+60 min) between conditions was performed by a paired samples t-test or a Wilcoxon signed-rank test as a non-parametric alternative. The alpha level remained at $\alpha = 0,05$.

3 Results

3.1 During EAT

3.1.1 HR and parameters of HRV

The HR at the end of EAT (baseline 2: 38,70 ±8,59 bpm) was slightly increased compared to the measurement in the beginning of EAT (baseline 1: 35,39 ±4,47 bpm). The highest HR was found during the challenge (50,67 ±9,37 bpm). In both periods of relaxation for the EAT recipient the HR was elevated to around 40 bpm - this is slightly increased in comparison to baseline values. Differences in the HR occured comparing both conditions. Under the control condition, the HR remained on a low level over the course of measurement and was only elevated during the challenge (see Fig. 2 and Tab. 6).

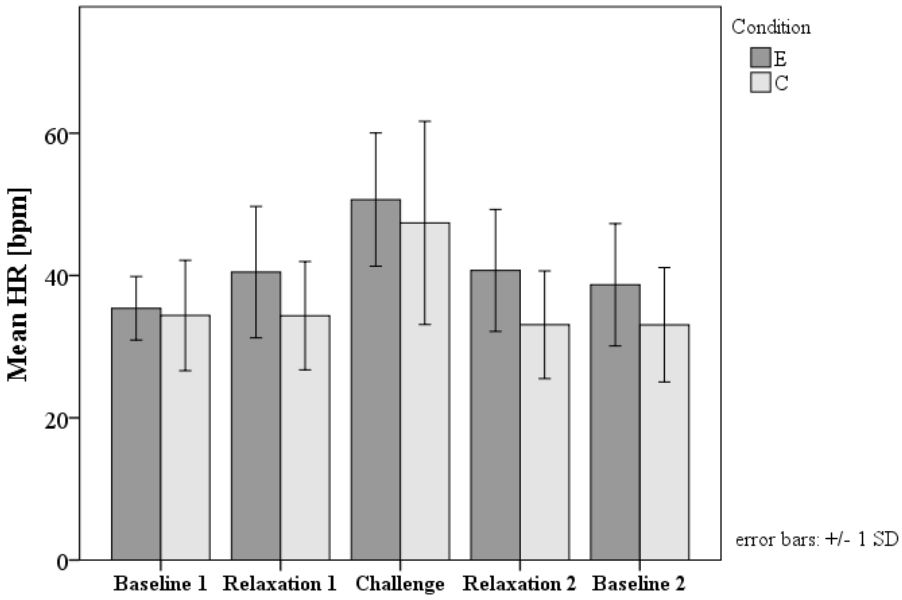

Fig. 2: HR (±SD) of horses (N=4) compared between the experimental condition and the control condition over the course of a standardised EAT.

The STDRR decreased at the end of EAT (baseline 2: 82,97 ±48,50 ms) compared to the measurement in the beginning (baseline 1: 116,20 ±70,12 ms). It was highest during the challenge (144,38 ±68,23 ms) and higher during relaxation 2 (117,31 ±64,80 ms) compared to relaxation 1 (92,82 ±39,07 ms). The control condition revealed a higher STDRR for each activity phase. The largest difference between conditions occured at baseline 2 (see Tab. 6).
The intensity of the parasympathetic nervous system activity was higher in the beginning of EAT

(RMSSD of baseline 1: 149,17 ±100,77 ms) compared to the end (RMSSD of baseline 2: 105,09 ±66,20 ms). The RMSSD was highest during the challenge (162,08 ±84,61 ms) but remained similarly high during relaxation 2 (157,63 ±90,36 ms). During relaxation 1 the intensity of parasympathetic activity was similiar to baseline 2. The RMSSD under the control condition was higher compared to the experimental condition at each activity phase, with the most pronounced difference in baseline 2 (see Fig. 3 and Tab. 6).

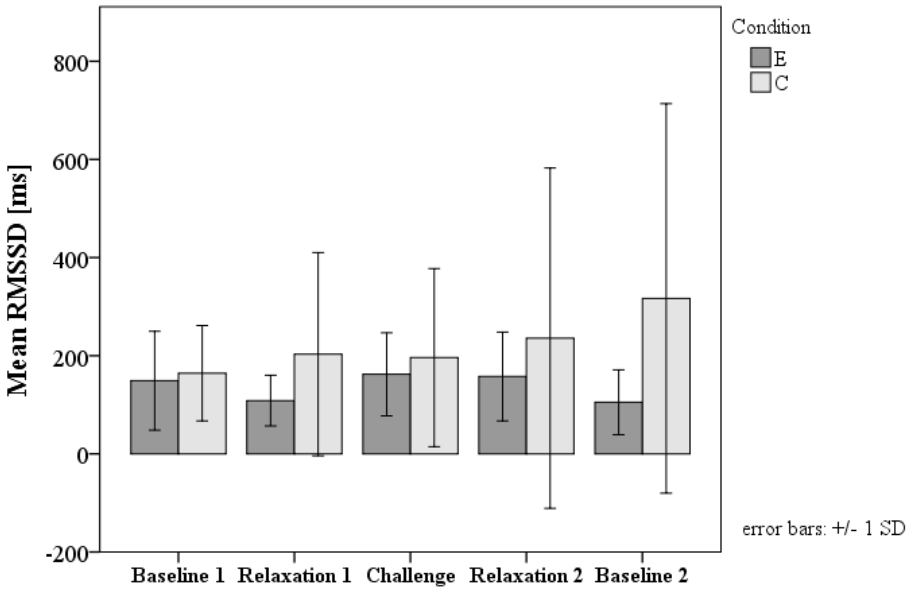

Fig. 3: RMSSD ($\bar{x}$±SD) of horses (N=4) over the course of one standardised session (x-axis) compared between the experimental condition (E) and the control condition (C).

The LF/HF was lower at the end of EAT (baseline 1: 3,12 ±2,26; baseline 2: 2,17 ±0,44). The challenge provoked the highest increase in the LF/HF (3,84 ±1,53) whereas periods of relaxation led to a lower LF/HF (relaxation 1: 1,68 ±0,46; relaxation 2: 1,78 ±0,55). Differences in the LF/HF occured between conditions. In baseline 1 and challenge the LF/HF under the experimental condition noticeably rose above the one under the control condition. In opposite, periods of relaxation and baseline 2 showed a lower LF/HF under the experimental condition. Values of both conditions converged most in baseline 2 (see Tab. 6).

The short term HRV indicated by the SD1 was higher in the beginning of EAT (baseline 1: 105,81 ±71,49 ms) than after (baseline 2: 74,49 ±46,91 ms). Over the course of a standardised session, the SD1 was highest during the challenge (114,83 ±59,92 ms), but similarly high in relaxation 2 (111,72 ±64,05 ms). The SD1 at relaxation 1 (76,87 ±36,55 ms) was nearly as low as at baseline 2. As described for STDRR and RMSSD, the SD1 under the control condition exceeded the

values under the experimental condition in each activity phase. The most pronounced difference occured in baseline 2 (C: 224,83 ±281,63 ms; see Tab. 6).

Tab. 6: HR and parameters of HRV of horses (N=4) compared between the experimental condition (E) and the control condition (C). Values are expressed as $\bar{x}$ ±SD. Each activity phase (baseline 1, relaxation 1, challenge, relaxation 2, baseline 2) over the course of a standardised session lasted five minutes.

	baseline 1	relaxation 1	challenge	relaxation 2	baseline 2
HR [bpm]					
E	35,39 ±4,47	40,48 ±9,23	50,67 ±9,37	40,71 ±8,58	38,70 ±8,59
C	34,38 ±7,764	34,35 ±7,62	47,39 ±14,28	33,07 ±7,57	33,07 ±8,03
STDRR [ms]					
E	116,20 ±70,12	92,82 ±39,07	144,38 ±68,23	117,31 ±64,80	82,97 ±48,50
C	152,58 ±75,90	184,08 ±170,20	199,81 ±151,97	157,71 ±214,20	231,99 ±257,44
RMSSD [ms]					
E	149,17 ±100,77	108,46 ±51,59	162,08 ±84,61	157,63 ±90,36	105,09 ±66,20
C	164,31 ±97,17	203,09 ±207,15	196,07 ±181,37	235,76 ±346,75	316,84 ±396,84
LF/HF					
E	3,12 ±2,26	1,68 ±0,46	3,84 ±1,53	1,78 ±0,55	2,17 ±0,44
C	1,44 ±1,74	2,95 ±1,77	2,49 ±2,66	2,63 ±1,37	2,65 ±1,34
SD1 [ms]					
E	105,81 ±71,49	76,87 ±36,55	114,83 ±59,92	111,72 ±64,05	74,49 ±46,91
C	116,57 ±68,96	144,08 ±146,98	138,96 ±128,55	167,27 ±246,02	224,83 ±281,63

3.1.2 Salivary cortisol

The cortisol concentration after a standardised EAT session (s4: 0,48 ±0,19 ng/ml) was on a comparable level to the sample prior to EAT (s1: 0,38 ±0,12 ng/ml). The cortisol concentration at the beginning of a standardised EAT session (s2: 0,34 ±0,25 ng/ml) remained similarly low. During the session the cortisol concentration increased (s3: 1,13 ±0,61 ng/ml). The control condition revealed a lower cortisol concentration for s3 (0,53 ±0,63 ng/ml), but showed higher concentrations for all other samples - with a pronounced increase in s4 (1,72 ±2,36 ng/ml; see Fig. 4 and Tab. 6).

The cortisol concentration measured on control days in the stable ranged from 0,46 ±0,64 ng/ml at noon to 0,19 ±0,19 ng/ml in the evening. The highest concentration (0,49 ±0,36 ng/ml) was measured in the morning (see Fig. 5).

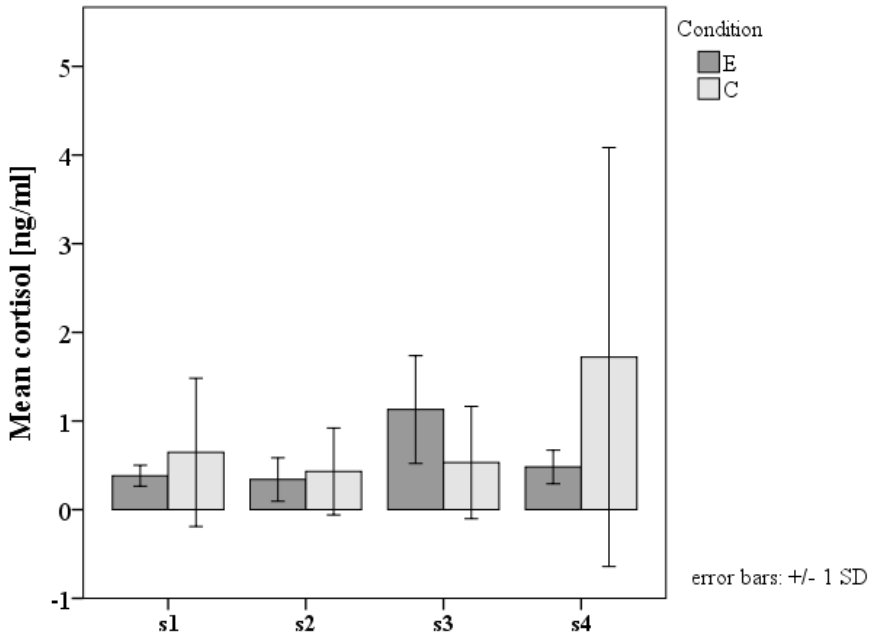

Fig. 4: Salivary cortisol concentrations ($\bar{x}\pm$SD) of horses (N=4) prior to (s1), at the beginning (s2), during (s3) and shortly after (s4) the experimental condition (E) or the control condition (C).

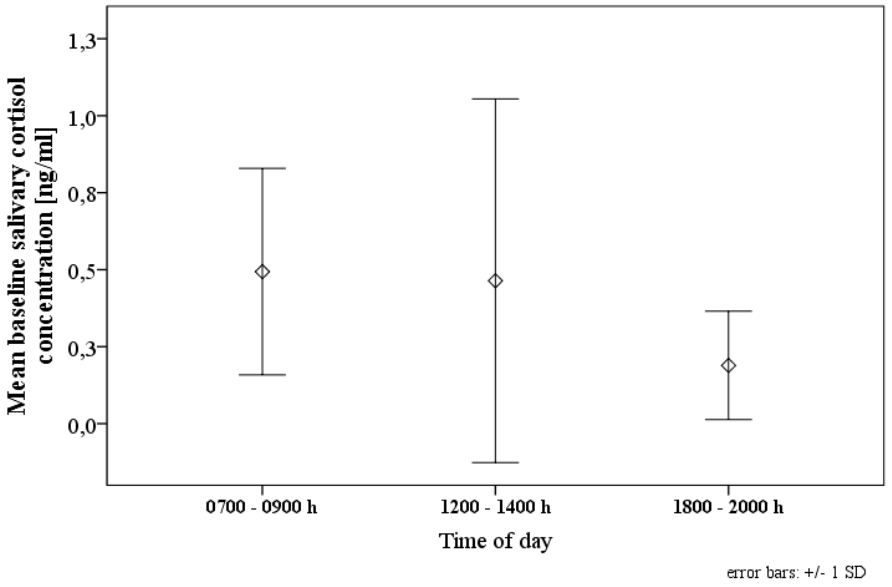

Fig. 5: Baseline salivary cortisol concentrations ($\bar{x}\pm$SD) of samples collected in the stable at three different timepoints (0700-0900 h, 1200-1400 h, 1800-2000 h) in therapy horses (N=4).

3.1.3 Influences of the IR on the physiological response

By grouping horses according to the underlying IR with the interacting humans differences in the HR and parameters of HRV occurred.

The HR was most elevated over the course of a standardised session for a horse characterised by a high IR with both interacting humans (+IRT; +IRR). Interestingly, the HR of horses with a low IRR (-IRR) expressed a similar pattern independent of the IRT: the HR was elevated during the challenge, but on a nearly stable level for all other phases. In opposite, horses with a high IRR (+IRR) had higher HRs also during relaxation 1 and relaxation 2 (see Fig. 6 and Tab. 7).

The STDRR was highest during the challenge for horses with a high IRR (+IRR), independent

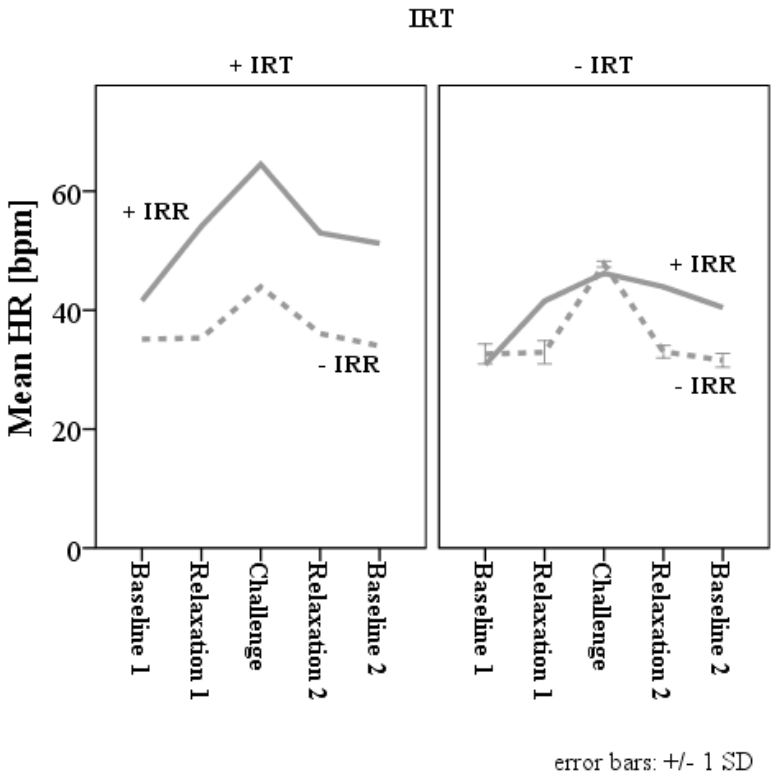

Fig. 6: HR ($\bar{x} \pm$SD) of horses shown over the course of the experimental condition (x-axis: baseline 1 - baseline 2). Horses were grouped after a high (+) or low (-) IRT. Within this grouping solid lines indicate a high (+IRR), dashed lines a low (-IRR) IRR. Each line consists of measurements of one individual horse (N=1), except for the dashed line representing a low IRT in combination with a low IRR (-IRT and -IRR; N=2).

of the IRT. Horses with a low IRR but a high IRT (+IRT and -IRR) showed an elevated STDRR during the baseline 1 and the relaxation 2. The STDRR remained on a constant lower level over the course of the experimental condition for horses with a low IRR in combination with a low

IRT (-IRT and -IRR; see Tab. 7).

A similiar pattern was found for the intensity of the parasympathetic nervous system activity (RMSSD) and short term HRV (SD1). The RMSSD and SD1 of horses with a high IRR (+IRR) was highest during the challenge. A horse with a low IRR in combination with a high IRT (+IRT and -IRR) showed high RMSSD and SD1 in baseline 1 and relaxation 2. A horse with a low IRT and a low IRR (-IRT and -IRR) showed the lowest RMSSD and SD1 over the course of EAT, except for baseline 2 (see Tab. 7; for RMSSD see also Fig. 7).

During the challenge the LF/HF was elevated for horses with a low (-IRR) over those with a high

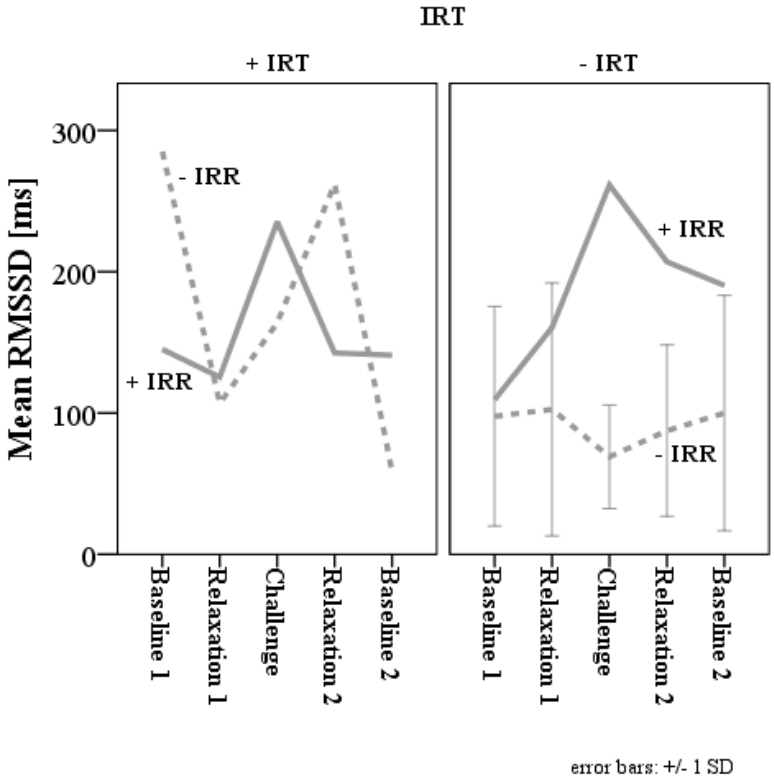

Fig. 7: RMSSD ($\bar{x} \pm$SD) of horses shown over the course of the experimental condition (x-axis: baseline 1 - baseline 2). Horses were grouped after a high (+) or low (-) IRT. Within this grouping solid lines indicate a high (+IRR), dashed lines a low (-IRR) IRR. Each line consists of measurements of one individual horse (N=1), except for the dashed line representing a low IRT in combination with a low IRR (-IRT and -IRR; N=2).

(+IRR) IRR independent of the IRT. A remarkable increase of LF/HF occured during baseline 1 for a horse with a high IRT in combination with a low IRR (+IRT and -IRR; see Tab. 7).

The cortisol concentration did not seem to be influenced by the underlying IR with the interacting humans. Both a horse with a high IRT and a high IRR (+IRT and +IRR) and a horse with a

Tab. 7: HR and parameters of HRV ($\bar{x}$ ±SD) shown over the course of the experimental condition (baseline 1 - baseline 2). Horses were grouped according to a high (+) or a low (-) IRT and IRR. "N" indicates the number of horses in the respective category.

	IRT	IRR	N	baseline 1	relaxation 1	challenge	relaxation 2	baseline 2
HR[bpm]	+	+	1	41,59	54,10	64,54	52,97	51,23
		-	1	35,12	35,31	43,91	36,05	33,98
	-	+	1	30,81	41,55	46,21	43,92	40,44
		-	2	32,64 ±1,68	32,90 ±1,95	47,74 ±0,51	33,02 ±1,06	31,56 ±1,15
STDRR [ms]	+	+	1	111,04	106,70	200,37	104,46	113,31
		-	1	210,24	115,21	143,83	191,80	50,68
	-	+	1	93,10	108,13	219,60	153,93	143,00
		-	2	81,46 ±57,64	81,43 ±66,34	81,90 ±47,24	68,95 ±45,80	75,33 ±59,17
RMSSD [ms]	+	+	1	145,02	125,43	235,40	142,48	140,96
		-	1	285,11	106,85	163,87	262,06	58,54
	-	+	1	109,40	160,51	261,47	206,95	190,31
		-	2	97,69 ±77,68	102,58 ±89,58	69,03 ±36,73	87,47 ±60,73	99,97 ±83,37
LF/HF	+	+	1	1,23	1,48	2,10	1,21	2,50
		-	1	6,32	1,47	5,79	2,12	2,35
	-	+	1	2,10	1,56	3,14	1,69	2,06
		-	2	2,23 ±1,16	1,75 ±0,88	4,63 ±1,67	1,65 ±1,01	1,38 ±1,32
SD1 [ms]	+	+	1	102,79	88,85	166,70	100,93	99,86
		-	1	202,27	75,76	116,13	185,77	41,52
	-	+	1	77,62	113,74	185,28	146,66	134,88
		-	2	69,30 ±55,11	72,76 ±63,54	48,92 ±26,03	62,04 ±43,08	70,92 ±59,14

low IRT and low IRR (-IRT and -IRR) showed a stronger increase in the cortisol concentration in s3 followed by a clear decrease in s4. The lowest cortisol concentration in s3 was measured for a horse with a high IRT and a low IRR (+IRT and -IRR) - this horse had the highest values in s4 (see Tab. 8).

Tab. 8: Salivary cortisol concentrations ($\bar{x}$ ±SD) of horses shown prior to (s1), at the beginning (s2), during (s3) and after (s4) the experimental condition. Horses were grouped according to a high (+) or a low (-) IRT and IRR. "N" indicates the number of horses in the respective category.

	IRT	IRR	N	s1	s2	s3	s4
Cortisol [ng/ml]	+	+	1	0,32	0,21	1,78	0,59
		-	1	0,25	0,07	0,32	0,62
	-	+	1	0,24	0,86	0,94	0,25
		-	2	0,67 ±0,20	0,30 ±0,21	1,39 ±0,15	0,32 ±0,28

Dividing up the horses according to the underlying IRT and IRR under the control condition (although no EAT recipient was present) did not reveal the same pattern in the intensity of HR, HRV parameters and cortisol concentration. Although a horse with a high IR (+IRT; +IRR) again

showed the highest HR level throughout the session, the HR remained lower during periods of relaxation for all horses independent of the IRR. Furthermore, a horse with a low IR (-IRT; -IRR) did not have constantly low STDRR, RMSSD and SD1 throughout the control session as it was observed under the experimental condition (see Appendix, Tab. 11). No horse had the highest cortisol concentration at s3 under the control condition (see Appendix, Tab. 12).

3.2 Autonomous regeneration

All parameters ($\bar{x}$ ±SD) were compared between the different measuring times within the experimental condition (see Tab. 9). A one-way repeated measures ANOVA with a Greenhouse-Geisser correction (as Mauchly's test of sphericity showed that this assumption was not met) revealed no statistically significant main effect of the measuring time on the HR of horses within the experimental condition (F(4, 24) = 1,93, p = 0,177, η_p^2 = 0,249, see Fig. 8). Dependent on the measuring timepoint there was neither a statistically significant difference of the STDRR (Friedman test: $\chi^2(4)$ = 4,91, p = 0,296) and RMSSD (Friedman test: $\chi^2(4)$ = 2,63, p = 0,622) nor of the LF/HF (Friedman test: $\chi^2(4)$ = 6,74, p = 0,150) within the experimental condition. The SD1 did also not show a statistically significant difference depending on the measuring timepoint within the experimental condition (Friedman test: $\chi^2(4)$ = 2,62, p = 0,622).

Tab. 9: HR and parameters of HRV of horses (N=7) were measured at timepoints before (-60 min; -30 min) and after (+0 min; +30 min; +60 min) EAT. The average ($\bar{x}$ ±SD) was calculated over 20 minutes as of the respective timepoint. The vertical lines indicate the timepoint at which an EAT group session took place.

N = 7	-60 min	-30 min	+0 min	+30 min	+60 min
HR [bpm]	36,45 ±6,35	35,97±5,97	35,22 ±6,40	33,94 ±5,86	34,27 ±5,46
STDRR [ms]	97,42±39,96	111,72±40,58	111,31 ±61,04	110,06±53,00	109,16±46,38
RMSSD [ms]	115,46±58,26	137,53±66,10	134,25±82,99	135,12 ±70,11	130,76±69,01
LF/HF	2,83±0,96	2,19±0,77	2,95 ±1,33	2,57 ±0,96	3,37 ±1,59
SD1 [ms]	81,71 ±41,24	97,32 ±46,78	95,01 ±58,74	95,62 ± 49,62	92,56 ±48,86

Additionally, each parameter ($\bar{x}$ ±SD) at each timepoint was compared between the experimental and the control condition (see Tab. 10). The pairwise comparison of the HR between conditions by a paired samples t-test revealed no statistically significant difference neither at the measurements prior to EAT (-60 min, -30 min) nor after EAT (+0 min, +30 min, +60 min). The same applies to the STDRR, RMSSD and SD1 at each respective measuring timepoint compared between the experimental and the control condition (for test statistics see Appendix, Tab. 13 and for RMSSD Fig. 9). The Wilcoxon Signed-rank test revealed no statistically significant

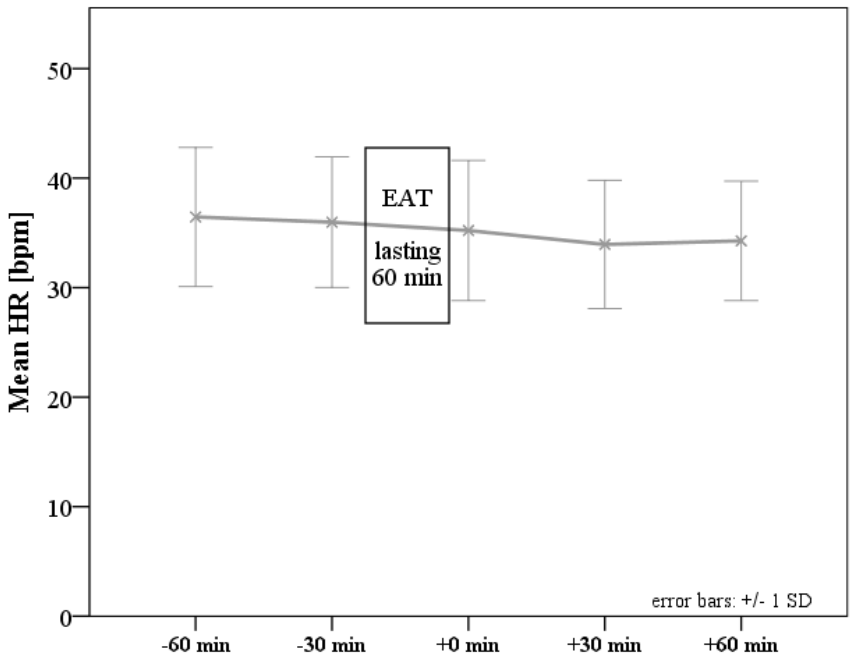

Fig. 8: HR ($\bar{x}$ $\pm$SD) of horses (N=7) at different timepoints (x-axis) under the experimental condition. The HR was calculated over 20 minutes as of each timepoint. The box indicates the time when an EAT group session took place, but does not correspond to the scale of the x-axis.

difference in the LF/HF compared between conditions (-60 min: Z = -1,6, p = 0,109; -30 min: Z = -5,4, p = 0,593; +0 min: Z = -7,3, p = 0,465; +30 min: Z = 0,0, p = 1,000, +60 min: Z = -3,7, p = 0,715).

The descriptive statistics of the characteristics of each 20 minutes sample revealed that horses seemed to stand longer at a time and spent less time eating directly after the EAT session (+0 min) compared to before (-60 min, -30 min) within the experimental condition. The longest mean duration of standing at a time was shown under the control condition (+30 min). Horses spent less time eating at other timepoints (see Appendix, Tab. 14).

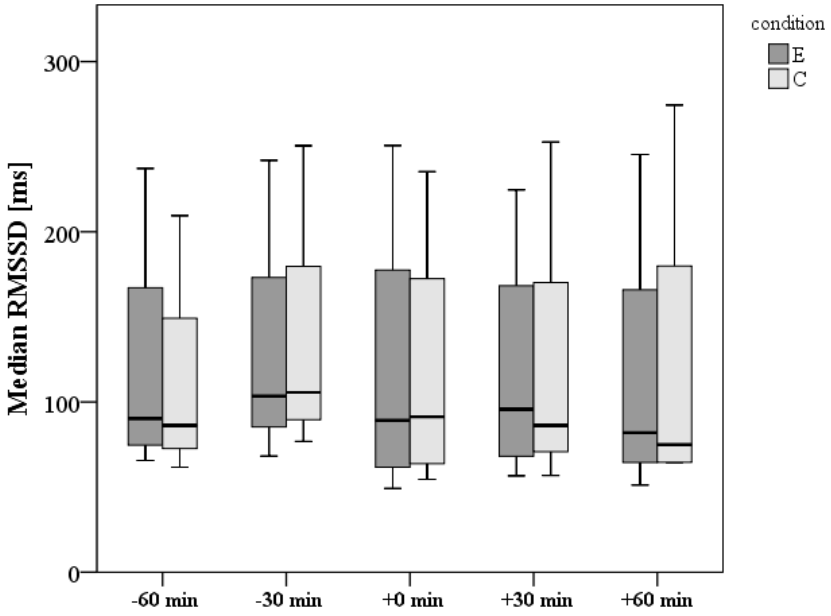

Fig. 9: Median RMSSD of horses (N=4) compared at different timepoints (x-axis) between the experimental condition (E) (dark grey) and the control condition (C) (light grey). Under E, an EAT group session took place right before measuring time "+0 min".

Tab. 10: HR and parameters of HRV of horses (N=4) calculated over 20 minutes ($\bar{x}$ ±SD) at five different time points (-60 min; -30 min; +0 min; +30 min; +60 min) under the experimental condition (E) and the control condition (C). The vertical lines mark the timepoint at which either an EAT group session took place (under E) or the horse remained in the open stable (under C).

N = 4		-60 min	-30 min	+0 min	+30 min	+60 min
HR [bpm]	E	38,55 ±6,47	37,42 ±6,75	37,59 ±7,02	36,16 ±6,30	37,04±4,64
	C	37,11 ±4,37	35,16 ±5,60	35,02 ±4,75	33,18 ±5,43	35,14 ±4,89
STDRR [ms]	E	105,61±53,43	109,61±51,36	98,70 ±60,64	98,22±54,60	93,99 ±55,86
	C	97,60±44,30	109,96±42,62	93,83 ±53,63	97,64±62,57	99,97±65,51
RMSSD [ms]	E	120,86±78,64	129,26±76,93	119,59±90,24	118,18±74,49	115,17±88,12
	C	110,89±66,79	134,67±78,52	118,13±81,43	120,47±89,25	122,20±101,93
LF/HF	E	3,09±1,06	2,55±0,81	2,84 ±1,44	2,98±1,11	3,58±1,95
	C	2,37±0,89	2,30±0,98	2,37±0,99	4,87±5,59	3,62±3,27
SD1 [ms]	E	85,52±55,66	91,47 ±54,45	84,63±63,87	83,63± 52,72	81,50±62,36
	C	78,47±47,26	95,30±55,57	83,60±57,63	85,26±63,17	86,48±72,14

4 Discussion

4.1 During EAT

By therapeutically implementing horses in EAT for their purposes, humans bear the responsibility for the equine well-being. The quantification of the horses' physiological stress response in the context of EAT contributed to uncover the status of equine well-being and served as a general basis to support and to secure it.

The measurement of the HR revealed a higher HR at the end (baseline 2: 38,70 $\pm$8,59 bpm) in comparison to the onset (baseline 1: 35,39 $\pm$4,47 bpm) of a standardised EAT session. Changes in HR are seen as a suitable psychophysiological parameter in animal sciences by indicating the sum of physical and psychological challenges (König v. Borstel et al. 2017). In addition, changes in HR are also linked to energy expenditure and metabolic rate (Pohlin et al. 2017). The baseline measurements served to monitor the HR and RR intervals before and after EAT in a standing condition within the research environment but without interaction with humans. In both baseline measurements the HR remained within the range of 25-40 bpm, which was desribed by Evans as a normal equine resting HR (as cited in Rietmann et al. 2004). At such moderate exercise levels, the parameters of HRV quantify changes in parasympathetic or sympathetic modulation of the ANS and might add information about the valence of an arousal state (König v. Borstel et al. 2017). The slight increase in HR at baseline 2 in comparison to baseline 1 was accompanied by a decrease in RMSSD, STDRR and SD1 as well as a decrease in LF/HF (see Results, Tab. 6). According to König v. Borstel et al. (2017), an increase in HR in combination with a decrease in STDRR and RMSSD might indicate an increased level of stress. As the LF/HF also decreased, the slightly elevated HR was probably not caused by an increase in sympathetic activity. An increase in HR can also result from a decrease in parasympathetic activity (reviewed in von Borell et al. 2007). Thus the observed decrease in RMSSD might account for the increase in HR at baseline 2. As the increase was very small, it does not seem that the horse is subjected to a high level of stress while standing next to humans at the end of EAT (baseline 2). Malik & Camm connected a decrease in HRV to either autonomic withdrawal or a high level of sympathetic input (as cited in Task Force of The European Society of Cardiology and The North American 1996). As the LF/HF itself decreased, a high level of sympathetic input did not seem to have caused the decrease in HRV. As a consequence, the results might rather suggest autonomic withdrawal. The horse might be less reactive at the end of EAT (baseline 2) compared to the onset and first encounter of the EAT recipient (baseline 1). It is likely that the frequent repetition of a standardised session led to an anticipation of the end of interaction by the horse with the beginning of baseline 2. An anticipatory physiological response has already been observed in

horses (Becker-Birck et al. 2013). However, the control condition provoked higher values for all parameters of HRV in baseline 2. As the control condition was not performed equally often, it cannot be excluded that a more frequent repetition would also have provoked a decrease in ANS reactivity at the end of a control session. In general, by repeated testing habituation or sensitisation to the research environment might influence the responsiveness of the animal (reviewed in Waiblinger et al. 2006). Therefore the difference in the number of sessions per horse under the experimental condition but also between conditions should be balanced better in future studies. To conclude so far, an initial comparison of both baseline measurements did not show signs of acute distress, but did also not show signs of increased relaxation for the horse at the end of EAT. To complete the picture, the physiological response over the course of a standardised EAT session is discussed subsequently.

The periods of relaxation provoked a higher HR compared to baseline measurements (relaxation 1: 40,48 $\pm$9,23 bpm; relaxation 2: 40,71 $\pm$8,58 bpm). The fact that both periods of relaxation followed after walking (relaxation 1: 2 rounds of walk; relaxation 2: challenge) might explain a slight increase in HR during periods of relaxation as an aftereffect of the previous walking movement. But as the previous walking did not provoke an increase in HR during periods of relaxation under the control condition (relaxation 1: 34,35 $\pm$7,62 bpm; relaxation 2: 33,07 $\pm$7,57 bpm) it seems unlikely that the walking itself accounts for the elevated HR observed during periods of relaxation under the experimental condition. In any case, the difference in HR compared between conditions suggests a higher energy expenditure under the experimental condition as a consequence of greater physical or mental challenges. The difference between both conditions is based on the presence of an EAT recipient under the experimental condition only. However, if the mere presence of an EAT recipient could account for the additional load under the experimental condition, it would already have provoked an increase in the HR during baseline 1. As the increase in HR started by the onset of relaxation 1 and lasted during relaxation 2 it seems plausible that the mounting of the horse and by this the increased physical workload caused a higher metabolic demand and thus a higher HR. This explanation is supported by König v. Borstel et al. (2017), who claim that the movement and weight of a rider are a source of physical stress in equitation. To reduce the influence of the EAT recipient's weight, it would have been advisable to better balance the physical demands for the horse between conditions. In order to do so, future studies could either investigate EAT which is performed from the ground (without riding) or add weight on horseback during control conditions. However, as study 1 was performed as an integral part of the study by Naber (2018) it was essential that the EAT recipients sit on horseback. As it is rather complex to account for the respective weight of different clients by artificial weight it was not used in this study. Although this imbalance in physical load between conditions cannot be denied, the control condition proved that the mere walking prior to

periods of relaxation or the change of place to the middle of the riding hall during relaxation 1 and relaxation 2 do not account for the elevated values under the experimental condition. In order to identify the underlying valence of this slight increase in arousal under the experimental condition, the changes in HRV are considered in the following.

While the state of HR and HRV during relaxation 1 resembled the one of baseline 2 (increase in HR, decrease in STDRR, RMSSD, LF/HF, SD1 in comparison to baseline 1) relaxation 2 provoked higher levels of parasympathetic activity accompanied by a decrease in LF/HF (see Results, Tab. 6). Clearly, relaxation 2 did not provoke a distress response in the horse. Although both periods of relaxation made the same demands on the horses, the animals seemed to be more relaxed in relaxation 2. Possible explanations are that either the predictability of the situation was higher for the horses facing it a second time or the standing was more pleasant after the active challenge period. Interestingly, the data by Naber (2018) show that the EAT recipient's HRV changed differently comparing relaxation 1 and relaxation 2: EAT recipients showed a slight increase in sympathetic activity and a decrease in parasympathetic activity at relaxation 2 compared to relaxation 1, thus the opposing direction to the observations seen in horses. The HR and parameters of HRV between EAT recipients and horses were not significantly correlated (Naber 2018).

Another potential influencing factor during periods of relaxation might have been the occasional stroking of the horse by an EAT recipient. As it was not forbidden or standardised (in order to offer the EAT recipients an authentic experience with the animals) it cannot be specified how much it influenced the SAM axis response of the horse. Previous research has shown that the HR of horses is sensible to the stroking of humans depending on their underlying attitude (Hama, Yogo, and Matsuyama 1996). Briefly summarised, periods of relaxation for the human also have the potential to increase relaxation for the horse. The appropriate timing of these periods of relaxation over the course of an EAT session, for example subsequent to more active phases, might enhance the added value for the horse. A higher predictability seems to positively influence the level of relaxation. A practical implementation which might increase the predictability for the horse could be the constant use of fixed (visually marked) resting areas during periods of relaxation. One well-known example is the Equiplace®.

Over the course of a standardised session the highest HR, STDRR, RMSSD, LF/HF and SD1 was provoked during challenge. The pronounced increase in HR during challenge (50,67 $\pm$9,37 bpm) can be explained by a change in the activity level from standing to walking. The HR during challenge was typical for walking movement and was lower in comparison to the HR of twenty warmblood horses during forward walking (57 $\pm$8,46 bpm, Rietmann et al. 2004). However, the increase in activity during challenge has to be considered when comparing this activity period to other baseline or relaxation measurements which were performed on standing. As the increase in HR was accompanied by an increase in all parameters of HRV the results

do not suggest an acute distress response during challenge under the experimental condition. The increase in RMSSD, STDRR and SD1 during walking is in line with Pohlin et al. (2017) who reported that moving positively influences the level of relaxation. However, the additional simultanous increase of the HR and LF/HF rather suggest a generally increased ANS reactivity than mere relaxation during challenge. The simultanous increase of both branches of the ANS is known in literature by the fullfilment of a task which provokes a flow experience (Peifer, Schulz, Schächinger, Baumann, and Antoni 2014). Whether such an experience could also account for therapy horses at work needs further investigation.

In comparison to the control condition, the HR and LF/HF were higher and the STDRR, RMSSD and SD1 lower (see Results, Tab.6). Again, the EAT recipient's weight on horseback and thereby the increased metabolic demand under the experimental condition may account for the difference in HR. As the challenge under the experimental condition was accomplished by the EAT recipient with the horse and under the control condition by the therapist with the horse a direct comparison of the SAM axis reponse between conditions remains difficult. The control condition was performed only by the therapist and the horse due to reasons of feasibility. In future studies another person should join the control condition in order to better balance the number of individuals between conditions. Referring to how it was performed in study 1, the distance between horse and therapist during challenge was greater under the experimental compared to the control condition (during which the horse was led by the therapist). Not only the distance of the horse to the therapist differed at challenge between conditions but also the attitude of the interacting humans to the challenging situation was not properly controlled for. As the therapist was equine experienced and probably not challenged by the tasks, the challenge under the control condition rather controls for the equine performance of the tasks itself. The tasks itself did not provoke a distress response in the horse as the parameters of HRV remained on a similiar level compared to relaxation 1 (see Results, Tab. 6). Nevertheless, an influence of the human stress level (probably different between therapist and EAT recipient) on the equine SAM axis response cannot be excluded and might additionally account for differences in the level of STDRR, RMSSD, SD1 and LF/HF of horses at challenge compared between conditions. Naber (2018) revealed that the HR and parameters of HRV were not significantly correlated between EAT recipient and horse as, in opposite to horses, EAT recipients showed a decrease in STDRR, RMSSD and SD1 from relaxation 1 to challenge. So far, the observations reveal no acute distress response during challenge under the experimental condition.

The argument that the EAT recipient's weight on horseback caused a higher energy demand and physiological challenge for the horse is supported by the change in cortisol concentration over time. Generally, saliva samples have been valued to quantify an acute stress response in horses. Important to note is that while an increase in cortisol concentration can follow from physical

stress for example enhanced arousal, activity or physical demand, it is not yet fully understood to which extent mental stress influences the HPA axis response (König v. Borstel et al. 2017). The pronounced increase in cortisol concentration was only detectable at s3 under the experimental condition, which reflects a point in time at which the horse was mounted. The mere presence of the EAT recipient did not cause an enhanced HPA axis response (s2). These circumstances suggest that a higher physical demand during EAT rather than an acute mental stress provoked the increase at s3. The fact that the cortisol concentration decreased shortly after EAT (s4) indicates that the greater challenge under the experimental condition diminished quickly - most probably after demounting. The saliva sample collection on control days without EAT showed higher levels of cortisol in the morning with a decrease over the day. As data collection took place as of 1 p.m. the circadian rhythm does not account for the elevated cortisol concentration at s3. A short term increase in cortisol might then be induced by increased arousal (König v. Borstel et al. 2017). Furthermore, it can be excluded that the research environment per se and the demanded tasks caused the increase at s3 under the experimental condition as no such increase was observable under the control condition.

In summary: a standardised EAT did not provoke a physiological response of the therapy horses that indicates acute distress. A higher workload during EAT was identifiable compared to the control condition. The quick recovery of the HPA axis within 30 minutes after EAT (s4) suggests that EAT forms a controllable condition for trained horses and remains within their adaptive capacity (reviewed in Koolhaas et al. 2011).

Interestingly, splitting the horses according to the underlying IR with the interacting humans revealed different patterns in the SAM axis response. The highest HR throughout a session was observed in a horse with a high IRT and IRR (+IRT; +IRR; see Results, Tab.7). As this pattern was observed under both conditions, interindividual differences rather than the underlying IR explain the higher level in HR of this particular horse. But it became obvious that only horses with a high IRR independent of the IRT showed elevated HRs during periods of relaxation under the experimental condition - not the control condition. The same horses revealed a higher STDRR, RMSSD and SD1 compared to horses with a low IRR during challenge under the experimental condition. As the horses are trained in a way that reinforces the spatial and emotional bonding with the EAT recipient (referred to as *stick to client*; Hediger and Zink, p. 79), it is likely that a higher IRR includes a stronger emotional bonding between the horse and the EAT recipient compared to horses with a low IRR. A stronger emotional bond might explain the higher HRs during periods of relaxation and might facilitate relaxation for the horse during challenge (interaction primarily between EAT recipient and horse). Under the control condition, the intensity of the STDRR, RMSSD and SD1 of horses with a high IRR did not follow the pattern of the experimental condition: the values exceeded and fell below the values of the experimental condition or

in comparison to values of horses of a low IRR (see Appendix, Tab.11). However, the control condition is not suitable to control for this claim as the network of relationships was changed by the absence of an EAT recipient - the therapist was the only person the horses could stick to. Further research is necessary to prove that claim. So far, Merkies, McKechnie, and Zakrajsek (2018) found that the behavioural and physiological responses of horses seem to depend strongly on the underlying equine-experience of humans. Merkies et al. (2018) suggest that a higher reactivity of horses towards equine-experienced people might be based on an expectation to work. Although the level of experience of the different clients (12,8 $\pm$4,71 years, Naber (2018)) was not further taken into account, it might be possible that horses with a high IRR had greater expectations to work as they knew the EAT recipients already from former sessions. But an increased expectation to work as a cause for elevated HRs during periods of relaxation does not explain why the HR was not already elevated during baseline 1 (the first encounter of the interacting humans). Although the cause for the higher HR of horses with a high IRR could not be identified ultimately, the data show that the significant correlation in HR which was identified by Naber (2018) and Naber et al. (2019) between EAT recipients and their favourite horses (high IRR) might result partly from a higher HR of those horses during periods of relaxation.
Further in line with the claim that the underlying IR influences the SAM axis response during EAT is the observation that horses with a low IR to all interacting humans (-IRT; -IRR) had the lowest STDRR, RMSSD and SD1 throughout an EAT session (except at baseline 2; see Results, Tab. 7). This was the case only under the experimental and not under the control condition (see Appendix, Tab. 11). As the quantity of previous interactions is one influencing factor of the relationship between humans and animals, it is assumed that horses with a high IRT or IRR (more previous interactions) had a better relationship to the therapist or EAT recipient. As a better relationship provokes more pleasant emotions and thereby influences the perception of a situation positively, horses with a high IRT or high IRR outperform those of low IRT and low IRR regarding the intensity of STDRR, RMSSD, SD1. These observations suggest that the intensity of the underlying relationship is a previously underrated modulator of physiological arousal and horse welfare during EAT. It seems recommendable that therapy horses should be familiar to at least one of the interacting humans. Waiblinger (2009) demonstrated already that a good relationship influences the welfare and performance of animals. Although the quality of the relationship was not tested in study 1 beforehand, it is tempting to assume that it was of a good quality, given that more previous interactions furthered relaxation. The results support the claim of Waiblinger that a good relationship seems to alleviate other potential sources of stress. The measurement of equine oxytocin might have revealed additional differences in oxytocin concentration according to the underlying IR. As study 1 used only non-invasive techniques, blood sampling to determine oxytocin concentrations was not included. Malinowski et al. (2018) did

measure oxytocin concentrations of therapy horses in response to EAT but did not find a change in oxytocin levels. However, as ten different clients participated in study 1, a modulating effect of personality and an influence on the human-horse relationship beyond the described IRR cannot be excluded. So far, the results suggest that the IR to interacting humans positively influence the ANS response during EAT. The cortisol concentration did not change according to the underlying IR, indicating that it rather reflects the physical demand (see Appendix, Tab. 12).

In a more general reflection, the measurement of the SAM and HPA axis is regarded as useful to assess difficulties in coping in the short term (Broom and Johnson 2000, p. 95). Nevertheless, an additional observation of behavioural parameters would have brought further insight in the valence of different arousal states (König v. Borstel et al. 2017) over the course of EAT. A suitable alternative to physiological measurements are preference tests or latency to approach tests (Moberg and Mench 2001, p. 140). Behavioural observations are missing in study 1 due to limited resources. However, a combination of neuroendocrine and cardiovascular measurements, as done in study 1, is still of advantage (Broom and Johnson 2000, p. 108). For all measurements, behavioural and physiological, the underlying coping style influences the extent of the response (König v. Borstel et al. 2017). As behavioural as well as physiological measurements are necessary for the identification (Koolhaas et al. 1999) it is not possible to draw conclusions about underlying coping styles and the extent of influence on the obtained data. To date, the interpretation of physiological as well as behavioural responses remains difficult. The discrimination between a successful adaptive or a distress response and the interpretation of results is to some extent experimenter biased (Koolhaas et al. 2011).

Overall, a generalisation of the results is limited due to a small sample size. The findings prove that the described circumstances did not provoke a distress response in these four therapy horses rather than allowing extensive conclusions about the use of horses in EAT in general. The whole environment at the association *e.motion* (such as open stables, living in a herd, constant reference persons, balanced training) might have indirectly influenced the results and cannot be directly transferred to therapy horses who work in a different environment. It was not possible to exclude those factors in this on-farm research. However, the whole concept of the association *e.motion* might serve as a positive example of how to train and handle therapy horses during and aside of EAT. At the association *e.motion*, the controllability and predictability for the horses during training but also in the daily routine is actively enhanced through measures such as snorting as a matter to communicate an increased internal tension, or by constantly addressing the horse's behaviour and adjusting the own accordingly. In general, the training at the association *e.motion* aims at confirming the horses in their ability to influence interactions with humans to counteract learned helplessness in the longterm (Hediger and Zink 2017, p. 80). The mode of training and experience of the participating horses cannot be considered in isolation from the

obtained results as the perceived controllability and predictability influence the perception of a situation by the individual (Keeling and Jensen 2002) - thus also the perception of a standardised EAT session in a research environment. The obtained results might reflect a realistic setting on-farm as more and less (9,4 ±6,6 years) experienced horses were included in the study. However, it is likely that the measurement of differently experienced therapy horses would have altered the outcome. Thus the general value of the results might be limited, as a variation in the perception of the research environment might have differed by experience (Waiblinger et al. 2006).

In addition to the subjective experience of an animal, the frequency of EAT services is of relevance. Study 1 investigated the physiological stress response in the context of one standardised EAT session. The daily routine of therapy horses may include more than one session. As any stimulus can become a stressor if it occurs often enough (Moberg and Mench 2001, p. 164) the physiological response might vary with more frequent or longer lasting EAT services.

The temperature, season, age, sex, training and health condition are all parameters which influence the physiology of an individual (Lohninger 2017, pp. 127,128) and cannot be excluded as confounding factors in this study. As the circadian rhythm has influencing effects on the cortisol concentration (Möstl and Palme 2002; Bohák et al. 2013) and cardiac activity (Gehrke et al. 2011; Pohlin et al. 2017) all measurements were restricted to the second half of the day. The sessions of different EAT recipients were not performed at exactly the same time in an afternoon. Subsequently, the control session did also not match in time exactly with all experimental sessions. To further diminish an influence of the time of the day it would be optimal to adjust this matter within one individual and between horses in future studies. Furthermore, the order of control and experimental sessions should be better balanced in future approaches. Due to a small sample size, only descriptive analysis could be performed. Future studies with a bigger sample size should investigate the described claims using statistical analysis.

In comparison to previous research in the field of EAT, the physiological response observed in study 1 showed both similiarities and discrepancies. The comparison of the cortisol level before (s1) and after (s4) EAT showed a minimal increase. This is not in line with the findings of Suthers-McCabe and Albano (2004), who reported a decrease in plasma cortisol after EAT compared to before in most of the horses tested. McKinney, Mueller, and Frank (2015) took saliva samples before, after 30 minutes and after 60 minutes under a resting, a therapeutic riding and a traditional hunt seat lesson program. No significant differences in cortisol concentration were found between conditions or sampling time. The median cortisol concentrations decreased throughout the therapeutic riding condition. As six children in the age of eight to 14 years participated in the therapeutic riding group (McKinney et al. 2015) their weight on horseback might have been a lower physical demand for the horses compared to adult females

participating in study 1. Working with riders with disabilities in study 1 did not provoke an extended HPA axis response at s4. This coincides with research by Fazio et al. (2013), who found a decreased cortisol concentration in horses after sessions with riders with disabilities compared to sessions with unexperienced recreational riders.
In study 1, the HR during relaxation 1 and relaxation 2 is similar to values by Pyle (2006) who reported a mean HR of 42 bpm over three hippotherapy sessions. Pyle also reported higher values during the experimental compared to the control condition. As the methodology differed strongly to study 1, it is difficult to draw further parallels. Another study with veterans by Malinowski et al. (2018) found neither signs of increased stress (plasma cortisol, HR, RR-interval, LF/HF, STDRR) nor of well-being (oxytocin) due to an EAT service. By observing physiological (i.e. HR, LF/HF) and behavioural (ear movement, head movement, snorting, defication) parameters in therapy horses in 58 EAT sessions, Mendonça et al. (2019) concluded as well that EAT forms a neutral rather than a negative or positive event for horses. The quick recovery of the cortisol concentration at s4 and an increase in parasympathetic modulation of the ANS (at challenge and relaxation 2) and overall and short-term HRV (at challenge) during the standardised session in study 1 support the claim by Malinowski et al. and Mendonça et al. (2019) that EAT does not provoke an acute distress response in the horse. As all parameters of HRV declined at baseline 2, the results of study 1 do also not show signs of increased relaxation or well-being at the end of EAT and are again in line with findings by Malinowski et al. and Mendonça et al.. More research is needed to monitor the short term consequences of EAT also on the SAM axis (including RMSSD). As Koolhaas et al. (2011) suggested, the focus should additionally be put on the speed of recovery of the HPA and SAM axis to identify stressors. Regarding the HPA axis, study 1 could exclude a prolonged activation in response to EAT. In future studies, shorter sampling intervals of cortisol might reveal more detailed insight on the speed of recovery of the HPA axis.
Study 1 demonstrated that a standardised EAT did neither provoke an acute distress response during EAT nor a prolonged activation of the HPA axis past EAT in four therapy horses. It suggests an influencing effect by the intensity of an underlying relationship between humans and horses on the level of arousal, not only of EAT recipients (Naber 2018; Naber et al. 2019), but also of therapy horses.

4.2 Autonomous regeneration

Study 1 revealed decreased parameters of HRV at the end of one standardised session (baseline 2) and left questions open regarding the short-term consequences of EAT on the SAM axis. This second, independent study approach (study 2) with a focus on the autonomous regeneration of

therapy horses subsequent to EAT was conducted to comply with the request by Koolhaas et al. (2011) to more strongly consider the speed of recovery of a physiological response in order to draw conclusions about potential stressors. It revealed no statistically significant differences in HR, STDRR, RMSSD, LF/HF and SD1 between measurements before and after a group EAT session. A comparison of each parameter at each measuring timepoint between the experimental and the control condition did not show any statistically significant differences.

The obtained results do not suggest that the service in a group EAT session was perceived as a stressor by the horses. No period of an altered physiological response after EAT was identifiable at all. The intensity of the HR and parameters of HRV directly after an EAT session was within the range of the measurements prior to EAT. Only the LF/HF directly after EAT (+0 min: 2,95) slightly exceeded the measurement before (-60 min: 2,83). At later timepoints after EAT the HR (+30 min; +60 min) fell slightly below and the LF/HF (+60 min) exceeded to a small extent the values obtained before EAT (see Results, Tab. 9). As these deviations were neither statistically significant nor occurred in a consistent manner (for example during all measurements after EAT), they cannot be considered short-term consequences of EAT.

The comparison between conditions did not reveal a distinct pattern in HR or parameters of HRV that could be attributed to the difference in performance. The only consistent disbalance between conditions was a higher HR under the experimental compared to the control condition (see Results, Tab. 10). This difference in HR of around 2 to 3 bpm was not statistically significant. As it occurred already in the measurement before EAT (-30 min), it cannot be considered as autonomous regeneration due to EAT. In general, all obtained HRs remained within the range of a normal equine resting HR (25-40 bpm) as described by Evans (as cited in Rietmann et al. 2004), and suggest good health and a good performance potential (Boudoulas, Borer, and Boudoulas 2015).

The absence of differences in quality or quantitiy of the SAM axis response subsequent to an EAT group session excludes a substantial influence of EAT on the ANS of therapy horses past the time of the EAT service. If a stimulus has a strong effect, a time interval is identifiable before the physiological response returns to control values (Task Force of The European Society of Cardiology and The North American 1996). As no such time interval was identifiable, the duration of autonomous regeneration, if any, was shorter than the time it took to bring the horses back into the stable. Furthermore, no statistically significant differences were measured between the experimental and the control condition which confirms the absence of a prolonged influence of an EAT session on the ANS.

In general, horses are less stressed if they either experience fewer stressors or have the ability to recover more quickly from stressors (Norton, Piette, Exadaktylos, and Berckmans 2018). This means that the therapy horses either did not perceive the EAT session as a stressor at all

or recovered within a few minutes on the way back to their stable. Both cases suggest that such an implementation in therapeutic work does not cause an alarming situation. However, the results apply only for the performance in one group session and cannot be generalised to more frequent or prolonged service times within one day. It cannot be excluded that a stronger stimulus would be perceived as stressful (Moberg and Mench 2001, p. 164). Thus more frequent or longer lasting sessions could provoke identifiable changes on the SAM axis response in the short-term. Very likely, the frequent preexposure to therapeutic work in experienced therapy horses led to a higher predictability and controllability of the situation, and might have led them to experience dependent variations in the perception of an EAT session. Due to the potential influence of the underlying experience on the perception, the general value of the results might be biased (Waiblinger et al. 2006). Koolhaas et al. (2011) highlighted this connection between previous experience and the speed of recovery of the HPA axis and suggested that the SAM axis most probably is affected in a similar way. However, even if one assumes that the EAT session provoked a strong physiological response during the time of service, it was not prolonged after a session was over. Koolhaas et al. (2011) interprete a physiological response that diminished after the stimulus disappeared as an environmental condition that does not exceed the adaptive capacity of an animal. Remaining within the adaptive capacity means that a stimulus is not perceived as a stressor (Koolhaas et al. 2011). As a consequence, the performance in one EAT group session cannot be interpreted as stressful for the therapy horses.

The strength of study 2 lies in its high practical relevance. The horses were monitored during their daily routine and in a realistic setting. As studying an animal in most cases affects to some extent what is studied (Moberg and Mench 2001, pp. 125-127), it cannot be ruled out that the attaching of the measurement equipment or observation of their activity pattern did influence the horses and their physiological response. It was not optimal that the acclimatisation period to the measuring devices (ten minutes) could only be applied at -60 min and not at +0 min. The data collection at +0 min started as soon as possible to avoid the loss of important data after EAT. As no difference in the parameters at +0 min occured compared to earlier measurements and the procedure was identical between conditions, it is not necessary to further discuss a potential influence by adjusting the measuring devices. In general, measuring an animal in a familiar environment reduces influences due to an artificial, unknown environment (Waiblinger et al. 2006), but also comes with some drawbacks. As the sessions in the daily routine were not standardised, the reproducibility of this study is diminished. However, the single sessions followed certain specifications to counteract a weak reproducibility. The influence of people entering the open stable, herd dynamics or environmental conditions on the results cannot be exactly determined. Waiblinger et al. (2006) raise awareness of the potential influence of herd mates on the behaviour of the tested individual. To counteract this, such incidences were noted at the time

and excluded from the analysis as far as possible. As the horses could move freely within the open stable, the experimenter had to change place accordingly to remain within range of vision to the horse. Despite attempts to use similar observation points, the variance in distance between experimenter and horse possibly influenced the intensity of the experimenter effect. However, by protocolling the activity pattern, it was feasible to conduct the measurements within the open stable and no restriction of the horses was necessary.

Generally, it is difficult to find appropriate conditions for HRV measurement in animals and requirements differ from those in humans. On one hand, HRV measurements in animals can be confounded by a restriction of movement as it might enhance sympathetic modulation of the ANS, and on the other hand, movement itself creates a background noise (Vitale et al. 2013). To account for these difficulties, the horses were measured in the open stable free to move and only periods of standing were included in the analysis. The measurement of free moving horses was already successfully conducted in a study by van Vollenhoven, Grant, Fletcher, Ganswindt, and Page (2016), who obtained more reliable HRV measurements over five different days on pasture than on stock. As the horses in study 2 had access to hay or trees continously, eating as a potential influencing factor could not be excluded, but the duration was noted. To account for these weaknesses the duration of eating, the frequency of potential disturbances and the mean length of standing periods at a time (which where merged to one 20 minutes sample) were noted per measuring time (see Appendix, Tab. 14). Our data show that horses stood more at a time and spent a shorter time eating directly after EAT compared to measurements taken before. This might indicate resting. However, as horses stood longer at a time under the control condition (+30 min) and spent less time eating at other times, the timepoint rather than the intensity of the observed behaviours differed between conditions. The frequency of disturbances was within a similiar range between conditions. These disturbances consisted under both conditions of people cleaning the stables and outside area and the preparation for or the conduction of other EAT services in the riding hall. Such incidents occur on a regular basis in the daily routine of the horses and did not provoke an obvious response. To reduce the influence of the circadian rhythm, measurements were scheduled on the second half of the day. Although it was ensured that the horses did not perform EAT or balanced training on the days of data collection, the sessions on preceding days were not standardised. In dogs, a higher number of sessions per week led to an enhanced secretion of cortisol (Kirchengast and Haubenhofer 2007). An influence of preceding sessions on the intensity of the SAM axis response can also not be excluded in horses. Another potential influence on the physiological response could have come from the order of experimenal and control sessions. Although the measurements were planned in a way that half of the horses (N=5) started with the experimental condition and half of the horses (N=5) with the control condition, the loss of data created an imbalance in this order. In the final sample,

two out of the seven remaining horses performed the experimental condition first. However, regarding the comparisons between conditions, the balance was reestablished (N=4): two horses started with the experimental and two with the control condition. Furthermore, it is important to note that most of the control sessions were performed on Sunday for reasons of feasibility. On these days, no services were offered at the association, which might have led to a quieter environment under the control condition, furthering relaxation for the horses.

In general, the accuracy of the HRV measurement and analysis method influences the outcome. It is recommended to discard data that include more than five percent of corrected beats (Stucke, Große Ruse, and Lebelt 2015). As it turned out during the analysis process that six measurements were unusable due to a low battery in the beginning of data collection, all remaining measurements were included in the analysis independent of their number of replaced beats. In order to retrace the quality of data, the percentage of replaced beats is listed in Tab. 14 per measuring timepoint (see Appendix). For future measurements one should consider to change the battery in prevention to contain data loss and to be able to follow the five percent limit of replaced beats. The original sample size of ten horses (measured once per condition) would likely have yielded more reliable data and should not be undercut in future study approaches. Independent of the sample size, the question of the most appropriate artefact correction to keep the daily variance but remove background noise and artefacts is still not solved in HRV analysis. In study 1 and 2, the RR intervals were corrected by a very low threshold artefact correction. The applied method followed van Vollenhoven et al. (2016), who advise to not use a very strong correction factor and, more importantly, to report which one was applied. In fact, van Vollenhoven et al. showed that all remaining correction factors led to a similiar pattern of HRV. In addition, the applied autoregressive model seems suitable for the analysis of heart beats as it estimates the spectral distribution of intervals between biological signals (Bernasconi, Messmer, Bernasconi, and Tholen 1998). It has already been applied to measurements in humans by Bernasconi et al. (1998) and horses by Rietmann et al. (2004). In general, HRV analysis are also of concern regarding mathematical bias. This claim is based on a non-linear connection between RR intervals and the underlying HR. This non-linear connection explains why similar changes in HR lead to greater RR intervals at low average HRs compared to high average HRs (Sacha 2013). To prevent this, Sacha (2014) suggests normalization of HRV analysis by dividing the RR intervals by the respective average HR. As the measured level of HR in the studies 1 and 2 never exceeded 60 bpm, and thus remained within the range of low average HRs according to Sacha, the suggested normalisation was not applied and an influence on the analysis of this data might be negligible.

In summary, study 2 succeeded in quantifying the ANS reactivity subsequent to EAT. As the HR and parameters of HRV did not differ within the experimental condition or between conditions,

no indications of autonomous regeneration after EAT were found. A substantial influence of a single EAT group session on the HR and parameters of HRV after EAT could be excluded.

5 Conclusion

The measurement of the physiological stress response during a standardised EAT session did not indicate an acute distress response in experienced therapy horses. Although the physiological response reflected a higher demand during EAT compared to a control setting, the therapeutic implementation of trained horses seemed to resemble a controllable condition for the horses. In a second study approach, a substantial influence of a single EAT session on the ANS of therapy horses past the time of service was excluded. This leads to the conclusion that the performance in a single EAT session laid within the adaptive capacity of trained therapy horses. This being said, it is important to keep in mind that the physiological response in the context of EAT showed not only no signs of acute distress but also no signs of increased relaxation at the end of an EAT session. However, the level of equine relaxation during EAT seemed to be positively affected by a more intense relationship with at least one interacting human. Besides this, periods of relaxation benefited the horses more as they encountered them a second time.
These findings give not only hope to the idea that it is possible to conduct EAT in the spirit of the one health concept, but also put pressure on scientists to continue work on the well-being of therapy horses in the context of EAT. More scientifically proven knowledge about how to implement an increased level of controllability and predictability for the therapy horses in their everyday life but especially during EAT sessions is needed. This information would benefit caretakers, therapists, therapy recipients - but in particular therapy horses.

6 Summaries

The physiological stress response of therapy horses in the context of equine assisted therapy was successfully monitored. Measurements during a standardised therapy session revealed neither signs of acute distress nor of enhanced relaxation in four therapy horses. Higher levels of cortisol and heart rate during a session suggest a greater physical demand caused by carrying a therapy recipient on horseback. As parameters indicative of the intensity of parasympathetic activity and heart rate variability were highest during a session and the cortisol concentration quickly declined after a session, the results do not suggest that the horses perceived the therapy service as a substantial stressor. Interestingly, the intensity of the parasympathetic nervous system activity and heart rate variability remained lowest during a session for horses interacting with less familiar humans. The intensity of the underlying relationship between humans and horse seems to be a previously underrated modulator of physiological arousal with the potential to influence the welfare of horses during equine assisted therapy. Comparing the end of a session to the beginning, the equine heart rate was slightly higher accompanied by a decrease in sympathetic and parasympathetic nervous system reactivity as well as heart rate variability. An additional measurement of the heart rate and RR intervals of seven horses before and after equine assisted therapy in the daily routine revealed no statistically significant differences depending on the measurement timepoint or in comparison to control days. A prolonged influence of one group session of equine assisted therapy on the heart rate and parameters of heart rate variability past the time of therapeutic service could be excluded. To conclude, the implementation of horses in one session of equine assisted therapy seemed to remain within their adaptive capacity and therefore unlikely represented an acute distress response.

Die physiologische Stressreaktion von Therapiepferden im Rahmen pferdegestützter Therapiearbeit konnte erfolgreich gemessen werden. In Messungen während einer standardisierten Therapieeinheit zeigten vier Therapiepferde weder eine akute Disstressreaktion noch Anzeichen vermehrter Entspannung. Ein Anstieg in Herzfrequenz und Kortisolkonzentration während der Therapieeinheiten lässt auf eine vermehrte körperliche Anstrengung, vermutlich verursacht durch den Patienten auf dem Pferderücken, schließen. Dadurch, dass sowohl die Parasympathikusaktivität als auch die Herzfrequenzvariabilität innerhalb der therapeutischen Einheit die höchste Intensität aufwiesen und auch die Kortisolkonzentration nach dem Ende der Einheit innerhalb kürzester Zeit zurückging, kann nicht davon ausgegangen werden, dass die Pferde die therapeutische Arbeit als beeinträchtigenden Stressor wahrnahmen. Während einer Therapieeinheit wiesen interessanterweise die Pferde, die mit ihnen weniger vertrauten Menschen arbeiteten, die geringste Parasympathikusaktivität und Herzfrequenzvariabilität auf. Scheinbar bildet die Intensität einer zugrundeliegenden Beziehung zwischen Mensch und Therapiepferd einen bisher unterschätzten Wirkfaktor auf das Erregungsniveau, wodurch auch das Wohlergehen des Pferdes während einer Therapieeinheit beeinflusst werden könnte. Die Herzfrequenz der Pferde zeigte am Ende der Therapieeinheit im Vergleich zum Anfang eine leichte Steigerung, während sowohl die Sympathikus- und Parasympathikusaktivität als auch die Herzfrequenzvariabiltät niedrigere Werte aufwiesen. Bei einer zusätzlichen Messung der Herzfrequenz und RR-Intervalle von sieben Therapiepferden vor und nach der therapeutischen Arbeit im Alltag konnte kein statistisch signifikanter Unterschied in Abhängigkeit der Messzeitpunkte oder im Vergleich zu Kontrolltagen festgestellt werden. Ein anhaltender Einfluss einer pferdegestützten Therapiegruppenstunde auf die Herzfrequenz und auf Parameter der Herzfrequenzvariabilität über die Zeit der Therapiearbeit hinaus konnte ausgeschlossen werden. Abschließend lässt sich zusammenfassen, dass für Pferde die therapeutische Arbeit im Ausmaß einer Therapieeinheit innerhalb ihrer Anpassungsfähigkeit zu liegen scheint und keine akute Disstressreaktion hervorruft.

List of Abbreviations

AAT animal assisted therapy

EAT equine assisted therapy

ANS autonomic nervous system

SAM sympathetic-adrenal-medullary

HPA hypothalamic-pituitary-adrenal

CRH corticotropin releasing hormone

LC locus coeruleus

HR heart rate

HRV heart rate variability

STDRR standard deviation of all RR intervals

RMSSD root mean square of successive differences

HF high frequency

LF low frequency

LF/HF ratio of LF and HF

SD1 standard deviation of the diameter of the scatterplot

E experimental condition

C control condition

s saliva sample

IR intensity of the relationship

IRT intensity of the relationship between therapist and horse

IRR intensity of the relationship between EAT recipient and horse

n(R) number of EAT recipients

n(E) number of experimental sessions

n(C) number of control sessions

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Appendix

Tab. 11: HR and parameters of HRV ($\bar{x}$ ±SD) shown over the course of the control condition (C) (baseline 1 - baseline 2). Values are expressed as $\bar{x}$±SD. Horses were grouped according to high (+) or low (-) IRT and IRR. "N" indicates the number of horses in the respective category.

	IRT	IRR	N	baseline 1	relaxation 1	challenge	relaxation 2	baseline 2
HR [bpm]	+	+	1	45,35	45,75	68,45	44,33	45,06
		-	1	30,49	29,98	38,26	29,97	29,05
	-	+	1	27,69	31,36	44,06	27,85	28,15
		-	2	30,84 ±4,45	30,84 ±,73	41,42 ±3,74	29,00 ±1,62	29,08 ±1,32
STDRR [ms]	+	+	1	40,38	42,48	202,17	39,33	40,4
		-	1	177,25	308,14	82,67	478,76	594,02
	-	+	1	207,63	353,02	413,69	57,83	237,69
		-	2	196,34 ±15,97	192,85 ±226,51	257,21 ±221,3	56,37 ±2,07	146,77 ±128,58
RMSSD [ms]	+	+	1	39,97	38,44	146,46	39,59	41,3
		-	1	269,52	471,99	71,35	755,19	888,43
	-	+	1	202,30	260,24	464,17	83,38	282,82
		-	2	173,87 ±40,19	150,97 ±154,54	283,23 ±255,88	74,13 ±13,08	168,81 ±161,23
LF/HF	+	+	1	4,00	5,48	2,09	4,46	4,14
		-	1	0,09	2,51	6,35	1,28	2,40
	-	+	1	0,97	1,37	0,55	1,96	0,94
		-	2	0,84 ±0,17	1,90 ±0,75	0,76 ±0,30	2,40 ±0,63	2,03 ±1,54
SD1 [ms]	+	+	1	28,33	27,24	103,73	28,06	29,27
		-	1	191,21	334,87	50,59	535,80	630,47
	-	+	1	143,57	184,61	328,98	59,18	200,70
		-	2	123,36 ±28,58	107,10 ±109,62	200,75 ±181,34	52,61 ±9,29	119,79 ±114,42

Tab. 12: Salivary cortisol concentrations ($\bar{x}$ ±SD) shown over the course of the control condition (C) (s1 - s4). Values are expressed as $\bar{x}$±SD. Horses were grouped according to high (+) or low (-) IRT and IRR. "N" indicates the number of horses in the respective category.

	IRT	IRR	N	s1	s2	s3	s4
Cortisol [ng/ml]	+	+	1	0,05	1,12	0,28	0,11
		-	1	1,83	0,11	0,34	1,64
	-	+	1	0,64	0,06	0,04	0,05
		-	2	0,35 ±0,41	0,25 ±0,26	0,75 ±1,0	2,57 ±3,56

Tab. 13: The results of the paired samples t-test are shown. The intensity of the HR, STDRR, RMSSD and SD1 at different timepoints (-60 min; -30 min; +0 min; +30 min; +60 min) was compared between experimental condition (E) and control condition (C).

		T	df	p
HR [bpm]	-60 min	0,49	3	0,658
	-30 min	1,03	3	0,379
	+0 min	1,20	3	0,317
	+30 min	2,30	3	0,105
	+60 min	0,99	3	0,40
STDRR [ms]	-60 min	1,61	3	0,205
	-30 min	-0,08	3	0,942
	+0 min	1,31	3	0,281
	+30 min	0,08	3	0,950
	+60 min	-0,89	3	0,441
RMSSD [ms]	-60 min	1,62	3	0,204
	-30 min	-2,64	3	0,078
	+0 min	0,30	3	0,784
	+30 min	-0,20	3	0,854
	+60 min	-0,78	3	0,495
SD1 [ms]	-60 min	1,62	3	0,204
	-30 min	-2,63	3	0,078
	+0 min	0,30	3	0,79
	+30 min	-0,20	3	0,854
	+60 min	-0,78	3	0,495

Tab. 14: Potentially confounding characteristics of each 20 minutes sample at each timepoint (-60 min to +60 min) are listed: The mean duration of standing at a time and eating, the mean frequency of disturbances and the mean % of replaced beats by artifact correction. The vertical lines indicate the time of either an EAT session under the experimental condition (E) or an equally long time gap under the control condition (C). Seven horses were included regarding the analysis within E. The comparison between conditions consisted of fewer horses (N=4).

N=7		$\bar{x}$	-60 min	-30 min	+0 min	+30 min	+60 min
E	standing	[mm:ss]	04:12	03:31	10:06	07:43	05:10
E	eating	[mm:ss]	09:02	12:00	05:25	04:05	05:14
E	disturbances	frequency	0,43	0,43	0,14	0,43	0,29
E	replaced beats	%	5,2	9,7	11,6	8,2	8,4
N=4		$\bar{x}$					
E	standing	[mm:ss]	03:24	03:35	08:55	07:23	04:35
C			07:00	03:31	03:16	11:28	04:24
E	eating	[mm:ss]	06:30	07:11	04:49	02:09	04:39
C			12:42	07:37	11:32	02:35	02:42
E	disturbances	frequency	0,5	0,75	0,25	0,75	0
C			0,25	0,75	0,5	0	0,25
E	replaced beats	%	7,6	11,5	11,4	10,1	8,9
C			3,3	6,3	7,6	12,1	13,1

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