THE USE OF HEART RATE VARIABILITY MEASUREMENTS AS A NON-INVASIVE METHOD OF ASSESSING AFFECTIVE STATE IN HORSES

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Abstract

Domestic horses maintain many of their innate behavioural traits as a group-living, free-ranging herbivore that usually avoids predation by flight. Confinement, isolation, restraint, riding, training and exposure to management practices present a vastly different experience to their natural environment but the psychological need to respond to environmental factors may remain, even when the biological motivation has been removed. This disparity can lead to the development of physiological and behavioural abnormalities indicative of a negative affective state and poor welfare. The concept of animal welfare should include the animals' physical and psychological health and harmony with their environment. There is an increasing call for subjective feelings and the assessment of emotion to be taken into account so that welfare can be enhanced by increasing the incidence of positive experiences and minimising negative ones.

Reliably establishing the affective state of an animal is a challenging task but measuring physiology and behaviour in response to pleasant or unpleasant stimuli can provide evidence for the existence of affective state. Equine research has endeavoured to identify 'reactivity', temperament and emotionality but has not yet investigated underlying affective state in response to different stimuli, the existence of, or factors that might influence positive affective states. As such, there is no strong scientific knowledge of what equine emotional experiences are. Many physiological measures involve invasive procedures that contribute to the stress load of the individual and non-invasive methods often only determine the presence or absence of 'stress' or are dogged by interpretive problems.

Heart rate variability (HRV) is a major growth area in the study of emotion and is an established parameter to quantify the state of the autonomic nervous system. Empirical evidence from human and animal research associates high levels of HRV with positive emotions and vice versa. This research aimed to evaluate the use of HRV alongside behavioural measurements as a non-invasive method of assessing affective state in horses. Equine HRV and behaviour were measured during anticipation of positive rewards (companionship and feeding), during a negatively valenced management procedure (sham-clipping), in four housing systems allowing increasing levels of social contact and potential factors affecting HRV were assessed.

By exposing horses to housing and management conditions differing in their emotional valence it was possible to identify factors that significantly affected HRV and highlight incidences where a significant relationship existed between behaviour and HRV (significant if $p \leq 0.05$). Rewarding and contrasting circumstances were found to be significantly associated with HRV.

Social interaction, 'play' behaviour and the provision of a haylage diet were all associated with high HRV whereas disruption to the horses' leisure time was associated with low HRV. HRV provided an objective physiological measurement for interpreting behaviour and assessing underlying affective state; compliant behaviour in response to an aversive stimulus was not associated with low HRV and specific pre-feeding behaviours (behavioural transitions, ear movement and head nodding) could prove a useful indicator of negative affective state in future studies. Specific social and spatial factors significantly affecting HRV were also identified; tactile contact with neighbouring horses was related to high HRV and hay net position appeared to affect the horses' capacity for environmental monitoring. A natural variation in HRV between equine sexes and between individuals was confirmed and changes in HRV were found to be situation-specific.

It was possible to use these initial data to make suggestions for the establishment of a preferable habitat and management regime for horses. The importance of social interaction was highlighted, particularly 'play' behaviour. Horses housed in confinement and isolation may derive greater benefit from the 'reward' or contrast of social interaction than horses kept in more social conditions. Where negative experiences are encountered, these might be mitigated by rewarding with a preferred forage type and extending visual horizons towards neighbouring horses and the external environment. Ensuring that negative experiences are not prolonged or offsetting them with positive experiences could enhance quality of life.

This study makes a unique contribution to equine welfare research as the use of HRV measurements to assess emotion in horses is a relatively new area of investigation. HRV was found to add vital physiological support to existing findings and although research of this nature is in its infancy, HRV appears to be a promising tool for assessing affective state and interpreting behavioural responses to stimuli in horses. There is extensive scope for further investigation into the use of HRV as a measure of emotional responses to management factors, environmental and ridden/training conditions in order to identify enjoyable or rewarding practices, so that the experience of positive emotion can be incorporated into management and training.

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Chapter One

Literature Review

1.1. INTRODUCTION

The basic behavioural repertoire of the domestic horse remains relatively unchanged from that of 5,000 to 6,000 years ago, but the horse's environment has changed dramatically. A fundamental alteration has occurred in the role of the horse from military, agricultural and transportation use to becoming part of an expanding leisure and sporting industry that has resulted in changes to the way horses are kept and managed (Harris, 1999). As will be discussed throughout this chapter, previous research has identified that management regime is a major factor with the potential to affect welfare in domestic horses.

The fact that horses are a group-living species with complex social structures, potentially rich with interactions between individuals, is often overlooked. Domestic horses are frequently exposed to owner dictated management practices incorporating different housing types, nutrition, handling (Ashley and Holcombe, 2001), training, breeding, social conditions (Mal *et al.*, 1991a; Mal *et al.*, 1991b; Jezierski and Górecka, 1999), unstable grouping and transportation (Strand *et al.*, 2002) all of which expose the horse to situations far removed from their natural environment. Such conditions may prove difficult for the individual to cope with and, where there is a failure to cope and coping mechanisms are overtaxed, the situation becomes stressful (Broom, 1991). Because the process of domestication has not eradicated the natural behavioural repertoire of the horse as a highly social flight animal (McCort, 1984; Goodwin, 1999), any disparity between the natural and domestic environment of the horse may therefore compromise their welfare.

It is unsurprising to learn that physical and behavioural abnormalities are a common occurrence in domestic horses, as evidenced by the presence of stereotypical behaviours (repetitive and apparently functionless behavioural sequences; Cooper and Albentosa, 2005), Equine Gastric Ulcer Syndrome (Thorne *et al.*, 2005) and aggression (Christensen *et al.*, 2002a). Horses may also lack interest in their surroundings, be unresponsive to stimuli, lack motivation or 'withdraw' from their environment and could potentially develop learned helplessness (Morgan

and Tromborg, 2007; Hall *et al*., 2008) as described in laboratory animals by Seligman (1975) and is a feature of depression in humans. Both extremes of abnormal activity or inactivity are undesirable traits and can be considered negative affective states as they fail to achieve homeostasis; maintaining equilibrium between hypoactivity and hyperactivity is preferable. Any advance in methods of identifying, measuring and ultimately minimising anxiety and stress in horses *prior* to the presentation of physical and behavioural abnormalities is therefore invaluable.

Concerns for animal welfare are generally based on the assumption that non-human animals can subjectively experience emotional (affective) states and can suffer or experience pleasure (Boissy *et al*., 2007b). Although there are obvious physiological indicators of compromised welfare in horses (e.g. body condition or endocrinological changes), ascertaining compromised psychological welfare is more complex. The Council of the European Union (2004) refers to animals as sentient beings yet there is no strong scientific knowledge of what their emotional experiences are (Desire, Boissy and Veissier, 2002; Dawkins, 2006).

Animals have evolved systems for recognising harmful or favourable stimuli and as part of these various methods and mechanisms, positive and negative feelings have evolved. It could be argued that in circumstances where behavioural or physical abnormalities are absent there is no proof of poor welfare (Duncan, 2005). Although direct measurement of subjective emotional experiences is not currently possible, the development of accurate measures linking sub-optimal environmental conditions with negative emotions or unpleasant feelings is an important goal in animal welfare science.

Research into farm animal welfare has generally been limited to behavioural and/or physiological responses to aversive stimuli, but it is important to move towards understanding their emotional state and to appreciate that good welfare is not merely the absence of negative experiences; the promotion and recognition of positive emotional experiences should be the

ultimate objective (Duncan, 2005; Boissy *et al.*, 2007b). There are however, few measures of positive affective states, despite these being of increasing interest in animal welfare research.

Concerns surround the ethics of using animals such as horses in sport in general and ethical equitation is coming into sharp focus in equestrian culture (McGreevy, 2007; McGreevy *et al.*, 2011). All international dressage competitions for instance, are governed by the Fédération Equestre Internationale (FEI) whose Code of Conduct declares that the welfare of the horse must be paramount. The FEI has recently introduced the concept of the "happy equine athlete" following criticism regarding the use of certain training methods used in warming-up elite dressage horses (van Breda, 2006, von Borstel *et al.,* 2009; FEI, 2011). Despite problems in identifying the happy horse, the psychological well-being of a riding horse should be a key focus so achieving optimal welfare in all aspects of the horse's lifestyle is an important consideration. Knowledge of what horses perceive as pleasant or unpleasant is limited and has important implications for stable management systems and management practices. Given these issues there is clearly scope for the development of new methods for assessing emotion in horses.

If it is possible to identify behavioural activity or tendencies, reinforced by physiological evidence which represent different emotions and/or changes in emotion, then this information can be used not only as a way of assessing the horses' response to different management, social and housing practices but ultimately as a tool to improve welfare and enhance well-being. Assessing emotion in animals is a relatively new area of research, with very little equine-specific information available from published work. Methods of assessing underlying emotional states and emotional reactions in horses have therefore not been conclusively developed and require further investigation.

This research project will use behavioural and physiological measures to investigate whether the emotional reaction of horses can be assessed in response to different environmental, social and

management conditions as well as determining the underlying affective state of individual horses. This series of studies will also attempt to identify key features of equine management systems that appear to relate to positive well-being with the ultimate aim being the improvement of welfare by increasing the incidence of positive experiences.

1.2. THE NATURAL EQUINE LIFESTYLE

The association between horses and humans spans several thousand years, but the evolving horse-human relationship from predation to companionship has resulted in conflicts of interest for both species. It is therefore necessary to understand the nature and origin of these conflicts in order for equine management practices to be developed that minimise the deleterious impact on the welfare of the horse (Goodwin, 1999).

There is a lack of information about truly wild horse behaviour but a number of studies such as van Dierendonck *et al*. (1996), Christensen *et al*. (2002b) and Boyd and Keiper (2005) have been carried out on feral populations of horses (once captive horses that have returned to the 'wild') which document the social behaviour of herds in semi-maintained conditions. Studies of free ranging domestic and feral populations provide an insight into the natural state of the horse, where behaviour is subject to evolutionary and environmental pressures, with minimal human intervention and provide valuable knowledge for improving the welfare of captive or domestic counterparts e.g. Cooper and Albentosa (2005).

As summarised by McCort (1984) and Goodwin (1999), horses are naturally free-ranging, grazing herbivores with a structured social environment. Most feral horses live in small stable groups called bands that inhabit large, overlapping geographic portions of their environment, known as home ranges, that make use of the best available food from season to season and can vary in size from 0.6 to 78 km^2 (Boyd and Keiper, 2005). This area remains constant over a period of months to years and incorporates grazing sites, water holes, shade, wind breaks and refuges from insects (McCort, 1984).

In unmanaged feral populations the most commonly observed type of band is the harem or family band, composed of a stable core of sexually mature mares three years or older, their immature offspring and one or more adult stallions (Klingel, 1975). These cohesive bands are not usually restricted in their movements so may roam over vast areas of land and stay together even in the absence of the stallion. Other kinds of bands include all-male bachelor bands and bands of mares and their offspring. Family band sizes range from 3 to over 30 horses, influenced by a number of factors including the quality and quantity of food, availability of dry land and water resources within the home range (Pacheco and Herrera, 1997).

Horses divide their time between activities that allow them to satisfy their basic requirements for food, water, movement and rest. The time budget is the amount of time invested by animals in each of these activities and varies according to the characteristics of the environment, season and age/sex of the animal (Boyd and Keiper, 2005). Horses are polyphasic animals as regards sleep and rest periods, i.e. sleeping more than twice in a 24-hour period. During the day the horse is awake and alert over 85% of the time; the horse will normally graze continuously for several hours then rest for longer or shorter periods depending on the weather conditions and distances that must be travelled to obtain water and sufficient forage (Arnold, 1985; Boyd and Bandi, 2002; Houpt, 2010). Typically, free-ranging horses spend between 50 and 63% of their time grazing depending on the quantity and quality of resources, 8% of their time moving, 26% of their time standing and 8% lying down (Duncan, 1980).

Group-living in a 'band' serves as a strategy for defence against predators and as such, horses have evolved a variety of behaviours that maintain group cohesion and stability (Goodwin, 1999). Most horses have one or more preferred associates with whom they maintain close proximity more than with other herd members and are observed engaging in affiliative behaviour such as mutual grooming, as described by Bennett and Hoffmann (1999) and Goodwin (1999) and observed by Feh and De Mazieres (1993).

Although equids are highly adaptable, which is why they have been successfully domesticated, little of their species-specific behaviour has changed (van Dierendonck *et al*., 1996; King and Gurnell, 2007) and there is a limit to their ability to adapt to some of the circumstances and environments in which they are placed, as evidenced by the existence of behavioural problems in stabled horses (Winskill *et al*., 1995; Cooper and Albentosa, 2005; Casey, 2010). The importance of social companionship has been demonstrated in a study by Schatzmann (1998). When given the option to select from (a) individual stall with hay and straw, (b) hay outside, (c) firm or soft ground surfaces and (d) to be alone or in company, the highest priority was always to choose the company of, or view contact with, other horses. The horses also showed a preference for being outside and eating grass, regardless of the weather conditions (Schatzmann, 1998).

It is generally accepted that husbandry practices have the greatest effect on the development of stereotypies in stabled horses (Luescher, McKeown and Halip, 1991; Houpt and McDonnell, 1993) and that management changes that allow a horse to perform actions within their behavioural repertoire will lead to a reduction in stereotypic expression (Cooper, McDonald and Mills, 2000). It seems reasonable to propose that a captive animal should be able to perform its full complement of behaviours at levels similar to its free-living relatives, thus improving welfare by meeting the horse's need to perform certain behaviours and behavioural patterns identified from studies of feral populations.

The assumption seems to be that a healthy wild or feral horse is likely to have adequate welfare and that a captive horse, in an environment in which certain behavioural patterns cannot be expressed, is likely to have a welfare problem. In fact this may not necessarily be the case as the feral horse's life is subject to the perils of predation, parasites, disease, injury, harsh weather conditions and scarce resources, conditions which make survival precarious. Allowing horses to express their full behavioural repertoire could mean exposing them to extremes of heat and cold, hunger or predatory stimuli, all of which may include negative experiences that are

considered poor welfare and normally avoided in a captive environment (Hughes and Duncan, 1988). Therefore if the time-budget of a domestic horse is different from its wild counterparts, this does not necessarily represent a welfare issue.

1.3. THE EFFECT OF DOMESTIC MANAGEMENT PRACTICES ON EQUINE WELFARE

The concept of animal welfare is not easily defined in scientific terms but Duncan (2005) suggests that a description of welfare should consider the animal's mental and physical health, harmony with the environment, the ability to adapt to an artificial environment without suffering and should take the animal's feelings into account. The Animal Welfare Act (2006) makes specific provisions for the promotion of welfare in the UK. The Act requires that the person responsible for an animal should take into account the animal's need for a suitable environment, to be able to exhibit normal behaviour patterns and its need to be housed with, or apart from, other animals.

The term 'welfare' often refers to a characteristic of an individual which must vary over a scale from very good to very poor and be measurable scientifically using a range of behavioural and physiological assessments (Broom, 1991). One of the most useful definitions of stress is 'when adverse effects in the environment or management system force an animal to make abnormal or extreme adjustments of physiology or behaviour in order to avoid physiological malfunctioning, thus, assisting the animal in coping with its environment' (Stull, 1997). The closely associated concepts of welfare and stress may be considered as opposites since welfare cannot be achieved under stress and vice versa. The relationship between these two terms has been summarised by Broom (1998) "the welfare of any stressed individual is poor, but poor welfare can exist without stress if an individual is coping, but with considerable difficulty" yet this largely depends on the degree and duration of stress as without it there is no evolutionary pressure for growth or adaptation.

The daily life of a free-living animal may be interrupted and disarranged by a range of variable and novel events or environmental factors, for example weather conditions, location and quality of resources, or the presence or absence of predators. Organisms have adapted to and survive in unstable conditions and it is likely that some environmental instability, or uncertainty, is necessary in order to avoid boredom and to optimise individual vigilance. Variation or fluctuation of events that cause uncertainty and arousal are part of the natural conditions to which even domesticated animals are adapted. A baseline occurrence of behavioural and physiological stress responses is therefore normal or natural and does not necessarily reflect adverse or unacceptable conditions (Wiepkema and Koolhaas, 1993).

Stress responses or negative affective states are typically evoked when an individual is unable to respond behaviourally or control negative or positive events (Wiepkema and Koolhaas, 1993). The amount of stress experienced may vary from low to severe; 'severe' implying low levels of predictability and controllability of relevant events, where stress is severe and welfare absent. 'Low' stress therefore implies high predictability and controllability values, but does not guarantee good welfare. While high and enduring predictability and controllability of events maximises certainty, it implies the near absence of environmental novelty and may introduce boredom associated with decreased vigilance. In a review of the topic of stress and animal welfare Wiepkema and Koolhaas (1993) conclude that predictability and controllability should ideally have intermediate or optimal value and not be too high or too low for long periods of time.

Naturally occurring behavioural and physiological stress responses are often associated with emotional expressions and are normally short in duration (Dantzer, 1988; Desire, Boissy and Veissier, 2002; Veissier *et al*., 2009). Providing the individual is able to solve the conflict (seeking company or shelter, finding food, avoiding a predator) naturally existing stress or welfare problems should fall within the normal adaptive range of the organism and may be considered desirable even under domestic conditions.

Under management conditions where species-specific behaviour is prevented and predictability and controllability of events is low, a variety of physiological and behavioural symptoms may arise indicating chronic stress (Beerda *et al.*, 1997). One of the most serious stressors an animal can experience is the loss of control over its environment (Winskill *et al.*, 1995) yet it is the 'norm' for captive horses to be exposed to management and environmental conditions in which they lack both choice and control over their environment, activity levels and diet and where species-specific behaviour is prohibited. When prevented from avoiding aversive stimuli or from seeking out a more appropriate environment such conditions may at the very least induce a negative affective state or could even be beyond the horse's ability to cope, meaning their welfare is seriously compromised (Luescher, McKeown and Halip, 1991; Cooper and Albentosa, 2005).

Although equids are highly adaptable, which has partially contributed to the success of domestication, studies of domestic horse behaviour in semi-managed conditions indicate that their behaviour has changed little through domestication; much of the behaviour observed in wild or feral populations of horses can be seen in free-ranging domestic populations (Crowell-Davis, 1993; Waran, 1997; Christensen *et al.*, 2002b; van Dierendonck, 2006). Time budget analysis of feral and pasture-kept horses has uncovered the importance of intraspecific social contact (Houpt and Houpt, 1988; Kiley-Worthington, 1990; Schatzmann, 1998), exercise and lengthy grazing times (Crowell-Davis, Houpt and Carnevale, 1985; Houpt *et al.*, 1986; van Dierendonck *et al.*, 1996; Benhajali *et al.,* 2009). When given ad-lib hay and the opportunity to see and touch adjacent horses, the time budget of stabled horses is very similar to free-ranging horses in regard to time spent eating and resting (Sweeting, Houpt and Houpt, 1985; Sweeting and Houpt, 1987; Houpt and Houpt, 1988; Kiley-Worthington, 1990).

Many of the traits which ensured survival of its ancestors are difficult to accommodate in the domestic environment of the horse; their unique behavioural repertoire evolved in response to the challenges faced in their evolutionary niche of a herd-forming, grazing herbivore that

usually avoids predation by flight (Goodwin, 1999). In its natural environment, the horse is a social animal, spending most of its time in close contact with conspecifics, confinement and isolation in a stable therefore offers the horse a vastly different experience than a natural, pasture-like environment.

A recent study by Lee *et al.* (2011) discovered that horses expressed a greater preference for turn out in company than turn out alone even after two days of confinement in their stall. Nine horses were given a series of two choice preference tests to determine how long they preferred to be in a paddock: either to navigate through a maze to access a paddock, or avoid the maze and access their stall. After 15 minutes in the paddock the horses were re-tested, but all chose the paddock when released into a paddock with three other horses. They were retested every 15 minutes until they chose to return to their stalls. They chose to navigate through the maze to stay out for 35 ± 6 minutes when other horses were in the paddock but for only 17 ± 2 minutes when they would be alone. When deprived of stall release for 48 h the horses chose to remain in the paddock with other horses for 54 ± 6 minutes, but for only 16 ± 4 minutes when they were alone.

Compared to their feral relatives the diversity of behaviours observed in stabled horses has been dramatically altered due to the confining nature of the domestic husbandry system. In the captive environment, many of the horse's daily challenges have been removed; they are provided with food, water and shelter but their choice of feed, social interactions and movement are limited, conflicting with the evolutionary processes which determined the behaviour of their predecessors. The major aspects of equine management with the potential to affect welfare are the quality, amount and type of space, amount and duration of movement restraint, artificial feeding regimes and sensory deprivation imposed on the animals.

Optimal captivity conditions should result in more natural behaviour; horses kept in poor conditions such as individual housing or even small enclosures are deprived of opportunities for

social interactions and the performance of natural behaviours is limited (Hogan, Houpt and Sweeney, 1988; Cooper and Mason, 1998). Even in the presence of social companions, lack of foraging opportunities has been found by Benhajali *et al.* (2009) to increase levels of stress in horses as evidenced by increased time spent in locomotion, more time spent standing alert and reduced rates of conception compared with a control group where forage was provided.

Although many housing systems restrict the horse's ability to move around freely and limit their contact with conspecifics, there may still be a psychological need to respond to environmental factors even when the biological need to perform adaptive behavioural responses has been removed. It is these unfulfilled underlying mechanisms that may lead to negative affective states expressed as unnatural behavioural responses such as apathy, unresponsiveness, hyper-responsiveness, or stereotypic behaviour (Cooper and Albentosa, 2005). The behavioural deprivation experienced by horses housed under a domestic management regime could be detrimental to their welfare, with an imbalance of negative versus positive stimuli. Management practices such as housing, confinement and social contact along with factors that may affect the development of stereotypic behaviour are evaluated below.

1.3.1. Typical housing practices

Stabling represents the most common form of equine housing in the UK (Harris, 1999). Stable size and design is variable but is typically 13 m^2 in size (Henderson, 2007), usually with a small outward facing window alongside a front opening doorway (**Figure 1.1**) and full height walls between neighbouring stables (Evans and Borton, 1990). As a precaution against injury, more modern stable designs usually allow horses' visual but not tactile contact with neighbouring horses (**Figure 1.2**).

Figure 1.1. Stable design with solid full height walls between neighbouring stables.

Figure 1.2. Stable type offering visual contact with neighbouring horse via internal bars.

Seasonal variations in management practices occur mainly due to the weather in the UK and tend to reflect both the usage of the animal, the availability of pasture and the use of non-native horses that are not adapted for the environmental conditions they encounter. Generally, in the leisure and breeding industry animals will be turned out (time spent outside the stable in a paddock or pasture area) for longer during summer than winter when, weather permitting, they are usually allowed out to graze during the daytime and stabled at night.

Harris (1999) acknowledges that there is limited published information regarding the demographic characteristics and management of horses in Great Britain, but information collated from the following survey data gathered during the last decade presents at least a snapshot of modern stable management practices in the UK.

In a survey of management practices of 358 UK horse owners (with a median of 2 horses per owner) from the leisure and breeding sector Mellor *et al*. (2001) reported that during the summer months 14% of horses were stabled during the day for a median of 8 hours and 28% of horses were stabled at night for a median of 15 hours. During the winter months 2% of horses were stabled during the day for a median of 11 hours, 70% of horses were stabled at night for a median of 16 hours, with <1% of horses stabled both day and night all year round.

It was possible for Mellor *et al*. (2001) to divide the population of horses in this survey into two groups: a larger group kept in small numbers, belonging to one owner, kept at home in an area of low population density and spending more time turned out to grass; and a smaller group kept in large numbers belonging to multiple owners on shared premises, tending to spend shorter periods of time turned out to grass.

A mailed survey of 873 randomly selected horse owners in Great Britain by Hotchkiss, Reid and Christley (2007) reported that their horses spent a median of 23 hours turned out on pasture in summer and 8 hours in winter; during the summer 48.1% of horses spent 24 hours at pasture and 74.3% were stabled for part of the day in the winter. Furthermore, 67.2% of the horses included in Hotchkiss, Reid and Christley's (2007) survey were housed in a stable type building, 21.2% housed individually in indoor American barns, 5.6% in outdoor field shelters and 5.2% were not stabled at all. In the study conducted by Mellor *et al*. (2001) 58% of horses were turned out 24 h/day and 72% were stabled part of the time in winter, similar to the findings by Hotchkiss, Reid and Christley (2007).

Ideally, environments should be designed to fit both socially and physically with what is known of equine ethology and physiology. One aspect of social organisation of horses which remains constant in feral or domestic species is the preference for stable, prolonged associations between individuals in small groups (often related) (Klingel, 1975; Lee *et al*., 2011). Stable design characterised by isolation of individual horses accompanied by long periods of confinement where movement is restricted may therefore be far removed from the horse's physical and behavioural needs (Kiley-Worthington, 1990).

1.3.2. Social contact

Herd species are highly social animals and typically exhibit behavioural and physiological stress responses (separation anxiety) when separated from a familiar environment and/or visually isolated from herd mates and show strong responses to other group members, as has been

documented for cattle (Boissy and Le Neindre, 1997), sheep (Parrott, Houpt and Misson, 1988; Baldock and Sibly, 1990; Parrott, 1990) and horses (Mal *et al.*, 1991a; Mal *et al.*, 1991b; Jezierski and Górecka, 1999; Strand *et al.*, 2002).

Horses are gregarious grazing animals that exist in complex social groups and possess a strong drive to form social bonds with other horses. A social bond is defined by Newberry and Swanson (2001) as "a mutual, emotional attachment between two individuals that is relatively long-lasting and survives temporary separation". Given the option, horses will spend around half their time in contact with other horses (Houpt and Houpt, 1988). This intensely social nature is an important element of a horse's behavioural repertoire observed in both feral and domestic groups (Houpt and Houpt, 1988; Crowell-Davis, 1993; van Dierendonck *et al.*, 1996; Christensen *et al.* 2002a; Christensen *et al.*, 2002b; van Dierendonck, 2006).

The absence of social companions can have a major effect on physiological responses and the course of associated diseases in humans and animals. In human studies, Kaplan, Cassel and Gore (1977) assert that social support ameliorates stress, while Kiritz (1974) document the positive effects of social support, cohesion and affiliation on reducing recovery time from illness and were able to correlate depression with low peer cohesion. In animals, Conger, Sawrey and Turrell (1958) for example, found that isolated rats exposed to electric shocks were more prone to gastric ulceration than those shocked in the presence of other rats, which suggests that the presence of another animal of the same species may have a protective effect on an individual under some form of stress. Social isolation is in fact commonly used in order to experimentally induce a stress response in animals e.g. Mal *et al.* (1991a), Mal *et al.* (1991b), Boissy and Le Neindre (1997), Sandem and Braastad (2005), Reefmann *et al.* (2009b) and Reefmann, Wechsler and Gygax (2009). Separation-induced manifestations of stress are usually quantified in terms of heart rate (HR), adrenocorticotrophic hormone (ACTH), cortisol and β-endorphin responses or behavioural anomalies such as struggling (backward and forward movements, straining back, paddling with the back feet, kicking, kneeling and jumping) and

large increases in vocalisation as described in sheep by Baldock and Sibly (1990) and cattle by Boissy and Le Neindre (1997).

As horses usually maintain visual contact with others for social cohesion, social isolation can have a profound effect on many aspects of their behaviour i.e. frantic locomotory movement and vocalisation or the development of stereotypies (Mal *et al.*, 1991a; Nicol, 1999). Epidemiological research has revealed that lack of social contact may induce a chronic arousal state causing the horse to develop abnormal behavioural activities indicative of conflict (Luescher, McKeown and Halip, 1991) and has been associated with a higher reported prevalence of stereotypic behaviour in horses (Nicol, 1999; Cooper and Albentosa, 2005). The physiological response of mares to short term confinement and social isolation has been investigated by Mal *et al.* (1991b) who found a non-significant increase in leukocyte counts when horses were confined but allowed social contact with adjacent stalls and when in stalls preventing visual and tactile contact with others in comparison to free ranging conditions at pasture. Friend, Dellmeier and Gbur (1985) also discovered similar alterations in leukocyte counts in calves kept in close confinement and isolation.

The effect of stabling for the first time on the behaviour and welfare of young and naïve horses was studied by Visser, Ellis and Van Reenen (2008) who found that sudden isolated stabling is stressful as it resulted in a high prevalence of stereotypies and abnormal behaviours compared with paired housing. Similarly, Garey *et al.* (2009) also report that a change in housing from group to individual stalls significantly increased concentrations of stress-related compounds (corticosterone and dehydroepiandrosterone) in yearling horses to levels comparable with those found during transportation. Harewood and McGowan (2005) also reported significantly greater incidences of vocalisation and movement in young horses individually stabled for the first time than when housed in groups, but found no difference in salivary cortisol or HR measurements. Christensen *et al.* (2002a) recorded significantly more incidences of aggressive behaviour in stallions housed singly when later being mixed with other horses, than previously group housed

stallions. Prior exposure to newly introduced horses using neighbouring boxes that allow auditory and visual contact has been found to reduce 'contact aggression', especially biting, when horses were later turned out together (Hartmann, Christensen and Keeling, 2009).

Grooming is also an important interaction between group members in many social mammals (Schmied *et al*., 2008). Moehlman (1998) associates increased levels of mutual grooming in feral asses (*Equus africanus*) with greater group stability, as well as seasonal factors and it has been suggested by Feh and De Mazieres (1993) that the function of allogrooming in horses is related to reducing social tension between individuals. Feh and De Mazieres (1993) studied allogrooming sequences in a herd of Carmargue horses and identified their preferred grooming site as the base of the neck. Manual imitation of grooming at that preferred site on adult horses significantly reduced HR compared with grooming at a non-preferred area, indicating a calming effect for the recipient. McBride, Hemmings and Robinson (2004) found that massage of horses by human handlers induced a significant reduction in HR when directed at specific areas of the neck (withers, mid-neck and croup) that, judging by prior observation in the field, are the preferred grooming sites for the species. Similar positive effects have also been reported in sheep during grooming by a familiar handler by Reefmann, Wechsler and Gygax (2009). It is also relevant to note from a study by Flannigan and Stookey (2002) that despite being physically confined, horses in tie-stalls allowing tactile contact with neighbouring horses and readily available forage had similar time budgets to free-ranging horses. A modified stable design allowing visual or tactile contact with conspecifics may therefore facilitate the expression of specific activities such as environmental monitoring or social interaction denied by the conventional stable.

When visually isolated from herd mates, cattle show the characteristics of a fear response; tachycardia and behavioural signs of agitation. A study by Piller, Stookey and Watts (1999) tested whether exposure to a mirror reduced the stress of social isolation of heifers. It was found that the mirror image reduced the physiological and behavioural response to isolation

(lower HR and reduced movement). Studies using sheep have also recorded lower cortisol levels when isolated with a mirror, than isolated with no mirror, indicating that the sight of other sheep may have stress-reducing properties (Parrott, Houpt and Misson 1988; Parrott, 1990). Pictures of familiar sheep faces have also been reported to reduce behavioural, autonomic and endocrine indices of stress in isolated sheep (da Costa *et al.*, 2004), suggesting that sheep may find the sight of familiar faces comforting in times of stress, or at least serve as a positive distraction.

Presumably the sight of conspecifics or surrogate companions provides enrichment of the stable environment because the provision of mirrors (McAfee, Mills and Cooper, 2002) and life-size horse facial images have also been proven effective in decreasing weaving and nodding behaviour (Mills and Riezebos, 2005) and in reducing the behavioural and physiological signs of isolation stress in transported horses (Kay and Hall, 2009). Mills and Davenport (2002) investigated the effect of providing a neighbouring conspecific and the use of a mirror for the control of stereotypic weaving and nodding behaviour in the horse. It was found that, compared to the unmodified stable, incidences of stereotypic behaviour were significantly lower in the two modified stables. When the effect of using a stable mirror was compared to actual nose-to-nose contact with a horse in the neighbouring stable, the reduction in weaving and nodding was similar.

It appears that although increased opportunities for real social contact are preferable, the provision of pictures or a mirror may help to reduce social isolation in the stable as it is thought that by placing a mirror in a stable, the horse no longer feels isolated. There is however, no evidence to suggest that these animals are aware that their mirror reflections are not their own. Nor is it known if they view their reflection as a reasonable representation of a conspecific, since the mirror-image is devoid of appropriate olfactory, gustatory, vocal and tactile signals. It is possible that the mirror mimics visual contact with conspecifics (minimising social isolation) or at least provides environmental distraction and additional visual stimuli, altering the horses'

perception of the environment and their resultant responses to it (McAfee, Mills and Cooper, 2002).

There is evidence to suggest that housing systems offering opportunities for social interaction may prove beneficial in terms of the emotional response to frightening stimuli and increasing the trainability of young horses and other animals. For instance, when investigating social influences on learning and reactivity behaviour in heifers, Boissy and LeNeindre (1990) found that the presence of conspecifics positively affected learning ability and reduced reactions to a fear-inducing stimulus. Similarly, Christensen *et al*. (2008) report that horses paired with a (habituated) calm companion reacted less to a fear inducing stimulus than horses exposed with a non-habituated (control) companion. In separate studies, both Rivera *et al*. (2002) and Søndergaard and Ladewig (2004) also report that young horses housed in groups adapted more easily to training than stabled horses, with significantly lower total training times and less frequent undesired behaviour. In practice, older and experienced horses are sometimes used as companions when young horses are introduced to traffic and other fear-eliciting situations, the social influence of habituated companion horses when housing naïve horses may therefore prove useful.

Interestingly, Søndergaard and Halekoh (2003) found that during a handling test single-housed horses approached a person sooner, were more easily approached by a person and in a novel environment expressed less restless behaviour and vocalisation and more explorative behaviour than group housed horses. This indicates that single-housing might improve the horse-human relationship and highlights the importance of habituating horses to periods of isolation when housed in groups. In addition, although a combination of short-term confinement and social isolation resulted in higher activity patterns in horses when subsequently tested in an open field test, Mal *et al*. (1991a) suggest that more permanent individual stabling may result in depressed behaviour patterns such as apathy or lethargy. In some instances this could be a desired effect,

in that the horse may be easier to handle, offering an advantage in some situations such as riding schools.

In a report detailing the housing requirements of horses according to their species-specific behaviour, Zeeb and Schnitzer (1997) recommend loose housing in groups with access to paddocks as the most suitable management system and that if horses are individually housed, provision of auditory, visual and olfactory contact with animals of the same kind is essential. In environments where turn-out is limited for instance, management practices such as group housing which permits full social relationships may need to be considered.

In social housing, allowing horses to choose their social group and control their own personal space not only allows interactions with other horses but also provides the opportunity for the horse to express control over its own environment, a factor that has been shown to ameliorate chronic stress in animals (Wiepkema and Koolhaas, 1993; Winskill *et al*., 1995; Cooper and Albentosa, 2005). Horse ownership however, is an expensive past-time and owners may be understandably protective of their investment and fear that horses may injure themselves or others if allowed greater liberty. Increasing land prices may also mean that pasture acreage is a luxury few boarding facilities can provide (Henderson, 2007). As such, turnout is often restricted and even then, contact between horses may be prevented by double fencing or electric fencing between enclosures. Most domestic horses therefore live a life far removed from their wild ancestors, where they are physically pampered but, potentially, psychologically compromised (Henderson, 2007).

The overall psychological wellbeing of the modern horse could be substantially enhanced with management practices that include and acknowledge the strong relationship between domesticated horses and their ancestors (Henderson, 2007) and enable the performance of endogenously motivated behaviours whose chronic non-performance could result in diminished welfare (Dellmeier, 1989).

1.3.3. Physical confinement

To a naturally free-ranging animal like the horse, confinement is an unnatural situation that removes all possibility of escape or avoidance of aversive stimuli via the flight response. Horses are fearful of entering small, enclosed spaces with restricted visual horizons, as they are naturally both neophobic and claustrophobic (Miller, 1997). As highlighted in **Section 1.3.1**, confinement in a stable, sometimes for long periods of time, is a common management practice for domestic horses and influences the behaviour of the horse in that it limits the horse's ability to perform certain behaviours. When stabled, the horse's range of motion and amount of movement is severely restricted and contrasts with the normal living conditions of a horse kept in large paddocks or pastures. Confinement and isolation in a stable have been shown to be associated with physiological signs of mild stress in the horse (Mal *et al*., 1991a; Mal *et al*., 1991b) and in calves by Friend, Dellmeier and Gbur (1985) who found a significant relationship between physiological indicators of stress and increasing levels of confinement.

The recognition that animals have evolved endogenous motivation systems for many innate behaviour patterns is relevant to ethical questions regarding their prolonged close confinement. The topic of motivation is discussed further in **Section 1.3.4** in relation to stereotypic behaviours, in **Section 1.4** regarding the emotion of 'frustration' and also in **Section 1.5.2** in connection with behavioural indicators of emotion. As Lorenz (1981) comments, keeping a horse in close confinement for some time increases the accumulation of flight related locomotion behaviour, that is its motivation to bolt, buck, jump, stop and every other manoeuvre programmed into the phylogeny of flight animals to escape a predatory mammal.

This effect has also been observed in other animals, a study by Jensen (1999) testing the effect of different periods of confinement on the motivation of calves to perform locomotor behaviour found that calves confined for 4 weeks performed significantly more galloping and bucking behaviour during a short open-field test, than unconfined calves. Similar results were obtained by Veissier *et al*. (2008) who confirmed the presence of a rebound effect on locomotion (more

time walking and greater distance covered) in cows after one day housed in tie stalls compared with loose housing.

In a parallel study to Friend, Dellmeier and Gbur (1985), Dellmeier, Friend and Gbur (1985) reported a linear increase in locomotor behaviour (number of buck-kicks, rate of steps per minute at canter and trot) of calves during an open field test following 6.5 weeks of housing under increasing levels of confinement. Calves were also observed emitting a greater frequency of vocalisations associated with 'play' and engaged in a greater number of social encounters according to increasing levels of confinement during the open field test (Dellmeier, Friend and Gbur, 1985). Similar results were obtained from a later study by Dellmeier, Friend and Gbur (1990) where after 8 weeks under housing treatments allowing varying levels of confinement, open-field testing revealed that motivation for highly active locomotor behaviour escalated with increasing degree and duration of movement restraint and vice versa in calves.

It appears that the combination of confinement and social isolation causes greater post-inhibitory rebound behaviour in horses than confinement alone. Mal *et al*. (1991a) carried out an open field test in mares after 48 hours assigned in groups either at pasture, in confinement stalls that allowed social contact, or in complete isolation with no social contact. Isolated horses travelled further, trotted for longer and more often and spent less time standing still than horses at pasture or confined with company. This indicates that horses kept in confined *and* isolated environments show greater motivation for movement and perform more activities than those maintained on pasture or in confinement but with social contact.

In practical terms, Lesimple *et al*. (2011) associated individual stabling with more active locomotion patterns in riding school horses which could prove dangerous for novice riders yet advantageous for competitive riders where the horse's motivation for movement may be purposely harnessed in order to enhance expressive movements. It is also possible that the duration of physical exercise and/or distance travelled by a horse during training and

competition 'satisfies' the horse's internal motivation for movement to a greater extent in some disciplines than others. For example, in a study of 1750 horses used in different equestrian disciplines, McGreevy, French and Nicol (1995) report a higher prevalence of both weaving and crib-biting in dressage and event horses than horses used for endurance. This was in addition to the positive association between the performance of the stereotypy and the amount of time horses spent in the stable, both within and between disciplines.

Studies of various mammalian species have reported that play behaviour increases following periods of play or movement deprivation (Brownlee, 1954). Christensen *et al*. (2002a) discovered a significantly increased occurrence of both play behaviour *and* social grooming in individually stabled horses compared with group housed horses once social interaction was permitted. During a study by Jensen (2001) it was interesting to note that calves performed more walking and play behaviour when open-field tested in pairs after 2 weeks of confinement, than when tested individually. It is reasonable to concur with Lorenz (1981) therefore that these increased occurrences of play behaviour may relate to a build-up of motivation during periods when social interaction is prevented, which is expressed as a rebound effect when no longer socially deprived (Christensen *et al*., 2002a).

It has also been suggested that unrestricted exercise has a greater effect on post-inhibitory rebound behaviour than more restricted forms of exercise. Ethostasis is the concept of restricted behaviour as a stressor in animal husbandry as defined by Fraser (1974) and Fraser and Fox (1983). Because internal motivation for locomotion, including 'play' behaviour is particularly susceptible to "damming up" in horses as described by Lorenz (1981) it is thought that simply releasing confined animals into an exercise area would better prevent or alleviate ethostasis than would methods of forced exercise or training that do not permit animals to run, buck, kick, change directions abruptly, et cetera (Dellmeier, Friend and Gbur, 1985). Freire, Buckley and Cooper (2009) for example, found that although the provision of daily exercise for one hour either on a horse-walker, treadmill, free exercise in a paddock, or by being ridden significantly

reduced the amount of walking, trotting and cantering compared to un-exercised horses, only free exercise in a paddock significantly reduced bucking and rolling behaviour.

In the interests of improving equine welfare it is important to provide opportunities for positive emotional experiences such as pleasure and enjoyment, particularly at times when the horse is experiencing aversive events in other areas of life. Using physiological measurements and behavioural observations, the emotional response of horses to different types of housing allowing varying levels of social contact are investigated in **Chapter 6**. It is hoped that an optimal housing system can be identified that balances both the practical requirements of the horse owner and achieves the endogenous needs of the horse.

1.3.4. Stereotypic behaviour

In equine husbandry many endogenous motivations are potentially frustrated by different management practices or environmental factors. Ethologists have long recognised that the most probable general cause of behavioural abnormalities in animals is frustrated motivation e.g. confinement and/or social isolation (Dantzer, 1986; Odberg, 1987a). The definitive causes of stereotypic behaviour in horses are unknown, but research indicates that horses exhibiting these behaviours are, or have been, living in an inadequate environment with sensory deprivation and improper management being major contributory factors (Luescher, McKeown and Halip, 1991; Mason, 2004).

Stereotypies have been defined as repetitive, invariant, apparently functionless behavioural sequences such as crib-biting and wind sucking, box-walking and weaving, which are commonly seen in stabled horses (McGreevy *et al.*, 1995; McGreevy, French and Nicol, 1995; Cooper and Mason, 1998; Nicol, 1999) and rarely if ever reported in free-ranging feral horses. Less extreme examples such as mild box-walking, vertical head movement or nodding, tongue playing, licking or grasping of various stable fittings and even sham chewing or teeth-grinding (Cooper and McGreevy, 2002) may appear benign but still indicate some psychological stress

and have been associated with later development with more serious stereotypic behaviour (Nicol, 1999).

It is generally accepted that management practices have the greatest effect on the development of stereotypies in stabled horses (Luescher, McKeown and Halip, 1991; Houpt and McDonnell, 1993) and that implementing changes that allow a horse to perform actions within their natural behavioural repertoire will lead to a reduction in stereotypic expression (Cooper, McDonald and Mills, 2000).

In an internet survey investigating the risk factors associated with stable-related behavioural problems in over 1800 UK leisure horses, Hockenhull and Creighton (2010) reported an association between the time spent stabled and an increased risk of handling, oral/ingestive and aggressive problems. It has also been suggested that locomotor stereotypies such as weaving and box-walking may be derived from frustrated attempts to move or escape from the stable (Houpt and McDonnell, 1993; Cooper and Mason, 1998). This theory is substantiated by studies such as Mal *et al*. (1991) who reported that confined and isolated horses travelled further, exhibited a greater number of trotting bouts and spent less time standing still during an open field test than horses maintained on pasture with conspecifics. Nicol (1999) also proposed that weaving may be a response to the confinement of the stable as it is most easily triggered by removing a horse from a neighbouring stable.

Lack of full social contact in either the stable or field has been associated with behavioural and physical reactions such as frustration behaviour (Hockenhull and Creighton, 2010), redirected sexual behaviour (Luescher, McKeown and Halip, 1991) and increased HR and pawing (Jezierski and Górecka, 1999). The introduction of stable features that may reduce the aversive nature of this environment has been shown to reduce signs of stress in the horse. A survey of racehorse trainers by Redbo *et al*. (1998) identified that cribbing, weaving and box-walking were significantly less common in horses that had greater social contact with other horses and

were allowed more time to exercise freely. A similar investigation into the risk factors associated with behavioural disorders in horses by Bachmann, Audigé and Stauffacher (2003) identified that the development and expression of stereotypies may be prevented by adjusting husbandry conditions to allow daily free movement and tactile contact with other horses.

Increasing visual horizons to areas beyond the stable has been found to reduce stereotypic behaviour and frustration in stabled horses (Cooper, McDonald and Mills, 2000; Ninomiya *et al*., 2008). Cooper, McDonald and Mills (2000) investigated the importance of social factors by altering the stabled horse's visual horizons to provide either a view into a neighbouring horse's stable through an internal grille or allowing views from all four sides of the stable. A significant decrease in weaving behaviour was found under both regimes compared with the conventional stable design where only the front top-half of the door is open with a view towards the stable courtyard. As discussed in **Section 1.3.2**, providing surrogate companionship in the form of mirrors and pictures also seems to have achieved some success in mitigating the stress of social isolation and reducing the performance of stereotypic behaviours in horses and other animals.

A postal survey of racehorse trainers in England and Wales by McGreevy *et al*. (1995) found that box designs allowing either visual contact between horses via bars and grilles (**Section 1.3.1: Figure 1.2**), or both visual and tactile contact in group housing (**Figure 1.3**), rather than the traditional stable design (**Section 1.3.1: Figure 1.1**) were associated with a reduced risk of abnormal behaviour, however there was no additional benefit associated with visual and tactile contact over visual contact alone.

Figure 1.3. Group housing allowing visual, olfactory and tactile contact between horses.

The importance of social contact is further supported by the negligible rate of stereotypies in stall-tied horses such as cavalry mounts (Houpt and Ogilvie-Graham, 2002). A typical tie stall is long and narrow so the horse is unable to turn round, physical movement is therefore more restricted than in a stable but horses usually have visual, auditory and olfactory (but not tactile) contact with adjacent horses. In a study investigating the welfare of horses confined in a straight ('tie') stall for two weeks with no exercise, Houpt *et al*. (2001) reported that compared with control horses who were exercised daily, confined horses showed significant rebound locomotion when released from confinement (more trotting and galloping and less walking) indicating a response to exercise deprivation, but showed no signs of developing stereotypic behaviour. This suggests that although locomotor stereotypies may be related to exercise deprivation (Houpt and McDonnell, 1993), their prevalence may be mitigated by increasing opportunities to both forage *and* socialise.

The tenacity of stereotypic behaviours in stabled horses and conspicuous absence in free-ranging wild or semi-wild horses suggests that traditional stabling practices characterised by the isolation of individual horses, sensory deprivation and restriction of movement are at odds with the innate motivation of horses to spend time at grass and form social relationships with conspecifics. Increasing their opportunity to socialise using a modified stable design that allows visual contact with conspecifics may therefore improve welfare by encouraging the expression of specific activities, such as social interaction, denied by the conventional stable thereby increasing the frequency of positive experiences and reducing the incidence of equine stereotypic behaviour (Cooper and Mason, 1998). There is a clear need to develop a means of scientifically assessing the response of horses to these environmental conditions prior to the onset of stereotypic behaviour so that some aspects of management practices can be readdressed.

1.4. INTRODUCTION TO EMOTION

As discussed in **Section 1.3** the concept of animal welfare is not easily defined in scientific terms but it is reasonable to propose that a description of 'welfare' should include the animals' physical and psychological health, harmony of the animal with its environment and its ability to adapt to an artificial environment (Desire, Boissy and Veissier, 2002; Duncan, 2005). There is increasing acceptance among animal welfare scientists that subjective feelings should somehow be taken into account and that the assessment of emotion is an important factor when determining welfare (Broom, 1998; Bekoff, 2000; Duncan, 2005). If animal welfare is associated with the mental state of an animal then it is also reasonable to suggest that welfare is fulfilled when an animal does not feel any long lasting negative emotions and when they can experience positive ones.

An emotion can be defined as an intense but short-lived affective response to an event that is associated with specific body changes involving a subjective component and two expressive components; one behavioural and the other physiological (Dantzer, 1988; Desire, Boissy and Veissier, 2002; Veissier *et al*., 2009). 'Fear' for example, may involve fleeing behaviour, physiological stress responses, enhanced attention to the threatening stimuli and a subjective feeling of terror or panic. In animals, the subjective component, or emotional experience, can only be inferred from the behavioural and/or physiological components.

In order to study emotional states scientifically, the development of accurate measures is an essential first step. Some information can be obtained from the measurement of physiological changes including the brain activity, HR and adrenocortical activity changes that may coincide with or precede changes in emotion (Broom, 1998) and will be discussed further in **Section 1.5.1**. Generally, emotional responses in non-human animals are assessed by behavioural tests despite the measurement of subjective experience being fraught with difficulty. The whole concept, existence and expression of 'emotion' in animals is a controversial issue that has been

debated by naturalists and ethologists for well over a century (Darwin, 1872; Salzen, 1998; Desire, Boissy and Veissier, 2002; Mendl and Paul, 2004).

Most emotion researchers consider that emotions arise in situations that are important for the survival of an individual and that feelings play an important role within environmental coping mechanisms, so it is likely that emotions have evolved from basic mechanisms that gave animals the ability to avoid harm, punishment or noxious stimuli and to seek valuable resources, rewards and experience rewarding stimuli (Rolls, 1990; Rolls, 2000). The basic argument for animals' experience of pleasure is that they feel pain and emotions can therefore be generally categorised as either pleasant or unpleasant. For example, the unpleasant experience of pain helps 'steer' an animal away from behaviours that risk the evolutionary disaster of death whereas pleasure is beneficial by rewarding the individual for performing behaviours that promote survival and procreation such as feeding and mating (Broom, 1998; Balcombe, 2009). As several authors have referred to single unpleasant feelings or combinations of unpleasant feelings as suffering (Broom, 1991; Broom, 1998; Duncan, 2005) the behaviour or physiology of an individual in response to pleasant or unpleasant stimuli can therefore be used as reasonable evidence for the existence of affective state, thereby aiding welfare assessment (Dantzer, 2002).

As an example of a potential causal factor of negative emotion in animals, whenever a decision-making process exists, an individual might sometimes be unable to do what they most want to do. The Oxford English Dictionary (Soanes and Stevenson, 2008) defines frustration as feeling "dissatisfied or unfulfilled" or prevented from "doing or achieving something". An animal may be frustrated when motivation to perform a behaviour is high but circumstances beyond the animals' control, e.g. physical or social conditions, prevent the behaviour occurring (Desire, Boissy and Veissier, 2002). The feeling of frustration could lead to behavioural and physiological changes, such as the aggression expressed by pigs frustrated at feeding time (Carlstead, 1986) or manifested as stereotypies or withdrawal, that help the individual cope with

the frustrating situation. As animals are strongly motivated to perform certain important activities, chronic negative emotions such as anxiety or frustration are likely to occur in many domestic horses and could constitute suffering if the individual is not able to adapt or respond to their environment. The existence of motivational mechanisms in animals is discussed further in **Section 1.5.2** in relation to behavioural indicators of emotion in animals and has previously been considered in **Section 1.3.4** in terms of behavioural responses to stressors.

Another common example of a negative emotion is anxiety, defined by Blood, Studdert and Gay (2007) as "a feeling of uneasiness, apprehension or dread" which according to Broom (1998) depends on an individual's ability to predict future events based on previous stimuli and experience. In evolutionary terms, a genotype that promotes the ability to explore previous experiences in the brain and to feel anxiety when high levels of risk are detected would presumably be advantageous and therefore strongly favoured in natural selection. It is therefore possible that the tendency to feel anxiety may be greater in females because of the demands of maternal care when offspring are young and vulnerable (Broom, 1998). The selective advantage of high levels of anxiety during times of parental responsibility may outweigh any disadvantages associated with its persistence during other phases in life when it is unnecessary or harmful to the individual.

The assumption that animals can feel emotions has meant that international conventions as well as regional regulations have been adopted in order to protect animal welfare, on the grounds that they can suffer, the Council of the European Union (2004) for example, refers to animals as sentient beings. This assumption has also been utilised during the use of animal models to study human depression and to test anxiolytics. In fact, the analogy between human depressive chronic states and the effects of prolonged or repeated stress in animals provides evidence that animals experience emotional states that can have long term consequences, as observed by Seligman (1975) in laboratory animals. Schouten and Wiepkema (1991) also found that sows initially showing high levels of resistance to tethering were more likely to develop a state of

'helplessness' than the stereotypic behaviour expressed by low resisting sows. The effects of emotional experiences on health are discussed further in **Section 1.4.1.** It is clear though that developing mechanisms of assessing affective state in animals and identifying the factors that may affect emotions is a vital step towards improving welfare conditions by minimising the occurrence of negative emotional states such as anxiety and frustration.

In terms of positive emotional experiences, food is so indispensable to an animals' survival it is reasonable to assume that they are highly motivated to obtain it, that they anticipate it and that its consumption is rewarding. A variety of sensory systems are in operation when animals eat and it is likely that a general feeling of pleasure, resulting from the procedure of eating and the sensation of taste, will be experienced (Broom, 1998) and it has in fact been shown that animals produce opioids during both the search for food and its consumption (Berridge, 1996). Anticipation of the pleasure associated with eating would be a causal factor to promote eating and individuals would tend to work harder for food than they would if there was no such pleasure (Broom, 1998). The concept of anticipatory behaviour is discussed further in **Section 1.5.2** in relation to behavioural indicators of animal emotion.

It is thought that animals might not only get excited about the expectation of a reward but also about the realisation that they themselves have some level of control over the delivery of a reward. The ability to control something itself might be rewarding, studies by Hagen and Broom (2003, 2004) indicate that cattle show signs of increased behavioural activity associated with excitement and possible signs of pleasure during learning tests. Similarly, Ernst *et al*. (2005) report that positive emotions and, therefore, increased welfare of intensively housed pigs could be achieved by providing environmental enrichment in the form of successfully mastered challenges linked to a food based reward. Greiveldinger, Veissier and Boissy (2008) also demonstrated via measurements of cortisol, HR and behavioural activity that an aversive situation (an air blast and prevention of access to food) was perceived as less stressful by sheep when they could exert control over it by performing an operant task.

For social species, touch often plays a key role in interactions with other individuals. Given its importance in social contexts it can be assumed that touch is a source of pleasure in animals. Like many other mammalian species that form social groups, grooming is an important interaction between group members (Schmied *et al*., 2008) and horses regularly engage in mutual grooming (Feh and De Mazieres, 1993). Evidence from equine studies supports the theory that touch induces a pleasant, calming effect (Feh and De Mazieres, 1993; McBride, Hemmings and Robinson, 2004) and is associated with group stability (Moehlman, 1998). The importance of social contact has previously been considered in **Section 1.3.2.**

In the latter half of the 20th Century the primary focus of animal welfare science was understandably directed toward captive, exotic and endangered species (Jordan, 2005; Laule, 2005). More recently, optimising the output of economically productive species has been a major topic of interest in animal science, motivated by the detrimental effect aversive conditions or experiences can have on meat quality and therefore market value (Boissy *et al*., 2005). This area of research is of financial benefit to the livestock producer but has also driven forward animal welfare research, in particular the assessment of emotion in domestic (Beerda *et al*., 1997; Walker, Fisher and Neville, 1997; Bradshaw and Casey, 2007) and farm animals (Rushen, De Passille and Munksgaard, 1999; Rushen, Taylor and de Passillé, 1999; Desire, Boissy and Veissier, 2002; Paul, Harding and Mendl, 2005; Boissy *et al*., 2007a; Boissy *et al*., 2007b).

Discussions of animal sentience throughout this period have almost exclusively been in the negative realm of pain, fear, stress and distress to the extent that standard measures of negative emotions far outweigh those of positive ones (Boissy, 1995; Rushen, De Passille and Munksgaard, 1999; Rushen, Taylor and de Passillé, 1999; Mendl and Paul, 2004), resulting in an extensive list of indicators of behaviour and physiology that distinguish good from bad welfare. Whilst it is granted that some imbalance is to be expected, as pain has more gravity and moral urgency when compared to pleasure, in order to achieve good welfare the role of positive emotions such as pleasure in non-human animals deserves more attention. As horses are

primarily 'non-productive', they fall into the category of sport, leisure, or working animals and consequently fit into neither the farm nor companion animal remit. As such, scientific research into equine welfare issues tends to either follow several years after research in other species, or be overlooked altogether.

Research related to equine 'temperament' or 'personality' has begun to investigate the concept of 'emotionality' in horses via behavioural tests carried out to identify different characteristics such as gregariousness or fearfulness (Heird *et al.*, 1986; Wolff, Hausberger and Le Scolan, 1997; Christensen, Keeling and Nielsen 2005). More recently, behavioural indicators of satisfaction and anxiety in horses have been studied (Ninomiya *et al.*, 2007; Momozawa *et al.*, 2007) and Christensen and Rundgren (2008) investigated whether predator odour induced fear in horses. Research by Falewee *et al.* (2006) considered whether maternal pheromones had an appeasing effect on horses during a fear-eliciting situation and Leiner and Fendt (2011) measured behavioural and physiological fear responses in horses exposed to novel objects.

Studies such as these aim to identify 'reactivity' and indicate aspects of temperament especially coping styles (as shown in sows by Schouten and Wiepkema, 1991), emotionality, like fear and frustration, that may be relevant for horse owners and trainers on a practical level, particularly if they are able to predict horses' responses to environmental demands. However, these studies make no attempt to identify the underlying affective state of the animal when assessing the emotional response to different stimuli nor do they investigate the existence of positive affective states or factors that might influence positive emotions. As such, there is no strong scientific knowledge of what their emotional experiences are.

1.4.1. The effect of positive and negative emotional experiences on health.

The reactive response of an animal to a challenge in its immediate environment is determined by the duration and intensity of the situation, immediate environmental constraints, previous experiences and its physiological status. Several interacting mechanisms are incorporated in this

response including physiological, biochemical, immunological, anatomical and behavioural parameters (Stull, 1997; Leadon, 1994). In both humans and animals, physiological parameters that have been utilised in studying the response to aversive stimuli include measuring levels of hormones released from the brain or other organs, fluctuations in leukocyte parameters and changes in HR. Such physiological responses are relatively objective and easy to sample and measure.

The perception of an aversive situation normally initiates a neuroendocrine response via the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis plays a major role within the neuroendocrine system, having a primary function in the reaction to aversive stimuli by balancing hormonal release from the adrenal medulla (adrenaline) and adrenal cortex (corticosteroids). Upon activation of the HPA axis, corticotrophic-releasing factor (CRF) is produced by the hypothalamus, stimulating the anterior pituitary gland to produce ACTH and β–endorphin (**Figure 1.4**).
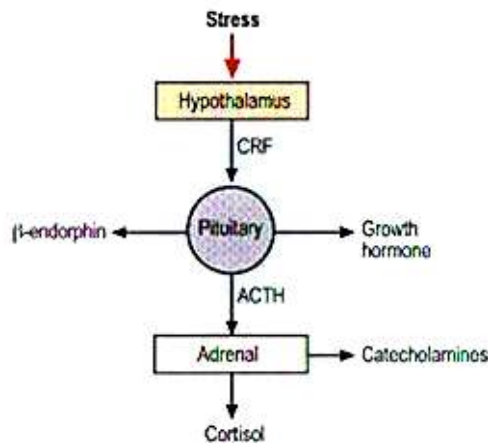
Figure 1.4. Hypothalamic-pituitary-adrenal axis (Horohov, 2004).

ACTH stimulates cortisol production by the adrenal glands, representing the chronic response to stress. Catecholemines such as adrenalin and noradrenalin are also produced by the adrenal glands and represent the acute stress response (Horohov, 2004).

Substances released into the bloodstream resulting from this response (catecholamine levels in the acute instance and glucocorticoids in both chronic and acute situations) can be measured and compared with normal parameters. Changes in cortisol or the reproductive hormones have been utilised in quantifying the response to aversive stimuli in sheep (Parrott, Houpt and Misson, 1988; Parrott, 1990; Parrott, Hall and Lloyd, 1998; Broom *et al*., 1996; Abdel-Rahman, 2000), pigs (Dalin *et al*., 1993), cattle (Villarroel *et al*., 2003) and horses (Smith *et al*., 1996; Stull *et al*., 2004). The ratio of neutrophils to lymphocytes can indicate the impact of negative events on the immune system (Cregier, 1987; Dalin *et al*., 1993; Stull, 1997; Stull *et al*,. 2004).

One feature of the stress response is that even a single acute stressor can lead to long-term neurochemical changes as suggested by Koolhaas *et al*. (1997) in rats exposed to social defeat, although little information currently exists as to how long after a stressor the effects remain in the body. A stressor that activates the HPA axis, particularly if it occurs over a long period of time or without time for recovery in between, could have detrimental effects on the individual. If anxious or fearful animals produce a stress response to many day-to-day stimuli such as housing or management practices, it follows that they may also live in a state of chronic physiological stress. The health implications associated with negative emotion include reduced life expectancy, impaired growth and/or reproduction, body damage, disease, immunosuppression, adrenal activity and behavioural anomalies according to a review of animal welfare by Broom (1991) and will contribute to lowering of the individual's quality of life.

Compelling evidence from psychoneuroimmunological studies in human medicine suggests that prolonged negative emotional circumstances can cause clinically relevant immunosuppression; pharmocologic doses of glucocorticoids are administered as treatment for various allergic, inflammatory and autoimmune disorders (Pruett, 2003). Similarly, psychological stressors accompanied by elevated plasma concentrations of noradrenaline have significant negative immuno-regulatory effects (Maes *et al*., 2000). Chronic stress associated with high levels of

cortisol in the blood stream has been related to a number of human disorders including obesity, insulin resistance, cardiovascular disease, immune disturbances, altered endocrine responses and nervous system disorders (Herbert, 1993; McEwen, 2005). Dreschel (2010) investigated the effects of fear and anxiety on the health and lifespan of pet dogs and found that animals with extreme fear and separation anxiety had an increased severity and frequency of skin disorders and a significantly shortened lifespan. Conversely, Rein, Atkinson and McCraty (1995) observed that five minutes of experiencing positive emotions, care and compassion, produced a significant increase in salivary immunoglobulin A levels in humans which lasted for an hour (S-IgA; used as a measure of secretory immunity) whereas negative emotions, in this case anger and frustration, had no effect on S-IgA levels.

Studies such as these provide evidence that the stress of living in fear or with anxiety can have negative effects on health due to the long term immunosuppressive effects of negative emotions and vice versa. Because the degree of environmental challenge can adversely affect the horse's capacity to grow, reproduce, train and maintain health, measuring these adaptive responses is vital in order to identify and ultimately minimise negative experiences and could protect the performance, economical potential and welfare of the horse (Stull, 1997; Baucus *et al*., 1990). It is reasonable to assume that equine welfare may be diminished by negative emotional states such as anxiety or frustration and may be improved by positive states such as pleasure or satisfaction. Lack of research in this area has meant that methods of detecting emotion in horses have not previously been conclusively determined. The information gained from this study will therefore form an important contribution to equine research and may offer a means of environmental enrichment by increasing the occurrence of positive experiences, potentially enhancing welfare.

1.5. METHODS OF ASSESSING EMOTION

As animals lack the ability to provide verbal information about their emotional state, the task of reliably establishing their affective state or emotional reaction is challenging. As discussed in **Section 1.4** it is suggested that anything which feels pleasant to an individual has survival value and is positively reinforcing so that actions made to obtain it are performed and that conversely, unpleasant feelings and negative reinforcement is associated with behaviour that does not have survival value (Rolls, 1990). Part of the assessment of welfare involves observations that indicate the extent to which the individual has positive or negative feelings. No single measure can be used to assess emotion so it is necessary to use a range of measurements in welfare assessment studies. There is scope in animal welfare science therefore to further develop understanding and measurement of animal emotions and the feelings that constitute suffering or pleasure.

Emotional states are recognised by human psychologists as comprising behavioural and physiological changes, in addition to subjective experience, involving parameters that can be assessed objectively without the need for linguistic report e.g. facial expression (Ekman and Friesen, 1971; Ekman *et al*., 1987; Ekman, 1992). In animals, behavioural activity such as fleeing, fighting or freezing behaviour and facial expressions along with alterations in physiology e.g. changes in HR parameters, blood pressure and circulating glucocorticoids can be measured to indicate an individuals' emotional state. The neurological substrates of fear and anxiety for example, have obvious effects on the HPA system and the sympathetic-adrenal-medullary (SAM) systems such as levels of glucocorticoids and alterations in HR or skin temperature (Boissy, 1995; LeDoux, 1995).

Until recently, the analysis of emotions in animals has focused on negative states, particularly responses to aversive situations that evoke negative affective states such as fear, frustration, anxiety and stress, resulting in standard measurements of negative emotions far outweighing those of positive ones (Dantzer and Mormede, 1983; Duncan, 2005; Forkman *et al*., 2007).

Indeed, most physiological measures tend to be interpreted as measuring negative affect and therefore offer limited assessment of positive affect. Measuring HPA axis activity often involves invasive procedures such as venepuncture which can contribute to the stress load of the individual, thus distorting results and, even where non-invasive methods are employed via salivary, faecal or urinary samples, the information gained can at best, only determine the presence or absence of 'stress' (Pell and McGreevy, 1999; Mormède *et al.*, 2007). Emotions of a positive nature, such as joy or pleasure, are rarely mentioned in animal welfare research and other than positive and negative contrast studies, very few tests use positive stimuli (Desire, Boissy and Veissier, 2002; Duncan, 2005). As a result, accurate interpretation of physiological measures in terms of affective state is not always straight forward. Situations likely to induce different affective states may evoke similar physiological responses that could simply be indicative of arousal. HR, for example, may rise in both fear inducing situations and in anticipation of rewarding stimuli (Baldock and Sibly, 1990). So while some physiological indicators offer useful information they are not devoid of interpretive problems (Paul, Harding and Mendl, 2005) in that they may relate to the intensity or arousal of emotional states rather than emotional valence.

Attempting to assess an individual's emotional state by measuring neurological substrates that are indicative of the processes occurring in the brain is also a popular area of animal science (LeDoux, 1995; Berridge, 1996; Damasio *et al.*, 2000; Rolls, 2000). However, many of these methods of investigation entail invasive procedures such as skin lesioning or electrical stimulation not suitable for this study. Alternatively, others involve the attachment of electrodes to the head that are vulnerable to disturbance by movement of the individual and are therefore impractical for 'remote' measures where the animal is unrestrained.

The visible percentage of 'white' in the eyes of cows exposed to frustrating situations (being denied access to food or their offspring) has been investigated by Sandem, Braastad and Boe (2002) and Sandem and Braastad (2005) who propose this as a dynamic indicator of emotion in

dairy cattle. Similar criteria has also been used to investigate the response to positive and negative stimuli in sheep by Reefmann *et al*. (2009a) and Reefmann, Wechsler and Gygax (2009). Unfortunately the ocular anatomy of the equine eye does not lend itself well to similar investigations as there is very little capacity for the upper and lower eyelid to be retracted far enough to expose the white of the eye.

While researchers that are interested in emotion might use *changes* in stress physiology as indicators of emotional states, physiological indicators studied in isolation offer limited precision in differentiating between highly arousing but differently valenced states. As demonstrated by Reefmann, Wechsler and Gygax (2009), physiological measures coupled with observations of behaviour may therefore more accurately determine the animal's affective state (Broom, 1991; Broom, 1998).

One major growth area is the study of heart rate variability alongside behavioural observations. It has long been recognised in human psychology that variations in inter-beat interval are influenced by an individual's emotional state. Heart rate variability (HRV) is a non-invasive electrocardiographic measure representing the sympatho-adrenomedullar axis and is an established parameter to quantify the state of the autonomic nervous system (Cerutti, Bianchi and Mainardi, 1995) reflecting the activity of the sympathetic and vagal components of the ANS on the sinus node of the heart (Tiller, McCraty and Atkinson, 1996). By presenting animals with stimuli that have specific positive or negative characteristics it may be possible to use both behavioural and physiological responses as an indicator of emotional state and will be the focus of analysis for this series of studies.

As discussed in **Section 1.5.2.1** for example, anticipatory behaviour in response to positive and negative stimuli has been identified as a potential indicator of emotion by Spruijt, van den Bos and Pijlman (2001) and Bos *et al*. (2003), the background to which is explained by Berridge (1996). Emotional responses are determined by the intensity of reward or punishment and also

by whether the stimuli are presented, omitted or terminated; presentation of rewards leads to positive emotions, while omission of rewards or the presentation of punishments may cause negative emotions. Avoidance behaviour, high levels of defecation, elevations in HR or stress hormones are all examples of responses indicative of 'fear' or 'anxiety' whereas increases in positional changes and level of activity along with facial expression and ear position have been found in response to positive stimuli. The resulting behavioural and physiological responses under these conditions could therefore be used as indicators of animal emotion (Mendl and Paul, 2004) and are discussed in **Sections 1.5.1** and **1.5.2** and investigated further in **Chapters 3** and **4.**

There are also many functional, behavioural, anatomical and physiological similarities between humans and animals that allow some exploration of affective state and some researchers have used techniques developed in human cognitive science in order to assess emotion in animals. Numerous cognitive changes occur in humans experiencing particular emotions or moods (Matthews, Wilkinson and Robbins, 1996) for instance, anxious people tend to have negative expectations of future events, a pessimistic outlook (MacLeod, 1996) with opposite biases observed in relaxed individuals (Nygren, 1996). Given the involvement of such cognitive processes in humans, methods of determining whether some animal species also exhibit similar biases according to their background emotional state have begun to be developed e.g. dogs (Burman *et al*., 2011), rats (Harding, 2004; Burman *et al*., 2008), sheep (Doyle *et al*., 2010; Sanger *et al*., 2011) and is reviewed by Paul, Harding and Mendl (2005) and Mendl *et al*. (2009). Such measures may indicate the presence of current emotions and predisposition to future emotions in animals as well as the effect of various environmental stimuli. Once preliminary studies have evaluated the efficacy of HRV measurements in determining emotional responses to different stimuli there is scope for this to be investigated in future studies.

1.5.1. Physiological assessment of emotion

Horses demonstrate extreme behavioural flexibility in adapting to a wide range of management practices over a lifetime. Housing, nutrition, social groupings, handling, training, breeding and transportation of horses are all widely diverse in terms of the natural behaviour of the horse (McDonnell, 2003) and have the potential to detrimentally influence affective state and reduce welfare. The relationship between emotions and health, particularly the effect on the immune system, receives much attention in the rapidly developing field of psychoneuroimmunology as discussed in **Section 1.4.1**.

As mentioned previously, exposure to either a negative or positive stimuli will induce a highly integrated behavioural and physiological response initiated and coordinated by the central nervous system (Wiepkema and Koolhaas, 1993). The autonomic nervous system (ANS) has two major sub-divisions; the sympathetic and the parasympathetic branch. An aversive stimulus that mobilises the sympathetic nervous system (SNS) will, via activation of the HPA axis, appear in a wide variety of physiological measures that are frequently used as indicators of reduced welfare.

In both humans and animals, physiological parameters utilised in studying health and welfare include measuring levels of hormones released from the brain or other organs, fluctuations in leukocyte parameters and changes in HR. Changes in cortisol levels for instance, are associated with the physiological response to chronic stress and are often utilised for quantifying the response to aversive stimuli in sheep (Parrott, Houpt and Misson, 1988; Broom *et al.,* 1996; Parrott, Hall and Lloyd, 1998), pigs (Dalin *et al.,* 1993), cattle (Villarroel *et al.,* 2003), dogs (Beerda *et al.,* 1996; Beerda *et al.,* 1998) and horses (Clark, Friend and Dellmeier, 1993; Smith *et al.,* 1996; Pell and McGreevy, 1999; Stull *et al.,* 2004).

The cardiovascular responses to negative stimuli include an increased HR, increased stroke volume, vasoconstriction of non-critical organs and skin, vasodilation in coronary vessels and in

working skeletal muscle and increased blood pressure which helps to ensure adequate blood flow for the critical organs (Bell *et al.,* 1983; Stephenson, 2002). In addition, an increase in plasma corticosteroid levels, elevated body temperature and changes in the immune system are also observed (Broom, 1991). In flight animals this response is consistent with the physiological preparation for physical activity; adrenaline and noradrenaline are involved in the mobilisation of energy stores, increased HR and blood-pressure and the redistribution of blood flow necessary for a sufficient energy supply to active muscles; corticosterone aids mobilisation of energy stores by stimulating gluconeogenesis in the liver and thereby rapid glucose mobilisation (Wiepkema and Koolhaas, 1993).

Clearly the HPA axis and the sympatho-adrenomedullar axis are important physiological systems responding to negatively valenced situations, enabling neurochemical and endocrine factors (e.g. catecholamines, adrenocorticotrophic hormone and glucocorticoids) to be measured during a stress response. For example, some of the physiological responses to horses exposed to social isolation have already been discussed in **Section 1.3.3.** Although these measurements can indicate the physiological reaction to either short or long term events and circumstances, investigation of physiological challenge normally involves invasive procedures that can contribute to the stress load, potentially distorting results and as such are not suitable for either the domestic or field setting. There is a need therefore to utilise non-invasive indices of autonomic nervous activity such as HR and HRV, alongside behavioural measurements, when assessing welfare in horses (Malik *et al.,* 1996).

A rapid heart rate is not, however, just a consequence of increased activity; the cardiovascular state of an animal does not necessarily signify physical activity alone and is determined by way of a mixture of reflex effects and psychogenic responses to external stimuli. Psychogenic responses are often triggered by sensory stimuli and involve neural pathways of the midbrain and forebrain, including the limbic system and cerebral cortex. For example, the defence-alarm reaction is an important psychogenic response for prey species and is an emotional response to a

threatening situation, physical injury or trauma involving increased sympathetic activity and decreased parasympathetic activity (Stephenson, 2002). Signs of increased sympathetic activity can therefore be observed as physiological responses indicative of negative stimuli in addition to behavioural responses (Wiepkema and Koolhaas, 1993) as reported by Friend, Dellmeier and Gbur (1985) and Mal *et al.* (1991).

Negatively valenced situations such as transportation have been found to be associated with tachycardia in farm animals (Baldock and Sibly, 1990; Stull, 1997) and with both tachycardia and hormonal disruption in the horse (Baucus *et al.,* 1990; Clark, Friend and Dellmeier, 1993; Stull *et al.,* 2004). Harlow *et al.* (1987) obtained a linear relationship between HR and blood cortisol when investigating methods of assessing responses to negative stimuli in sheep. This suggested that remote monitoring of cardiac frequency could be used as a predictor of adrenal function and, therefore, the possible immunological condition of an animal during exposure to negative stimuli.

When movement is not a factor, this means that increases in HR beyond resting values may indicate dominance of the SNS over parasympathetic activity or vice-versa, therefore reflecting an emotional response to a situation. Non-invasive measurements such as HR also have the obvious advantage of avoiding the methodological complications of obtaining stress-related blood parameters where the blood sampling procedure may cause significant stress to an animal. However, whilst HR is considered to be a valuable measure of welfare for short term aversive stimuli, one of the major limitations of interpreting HR measurements when attempting to determine the emotional response of an animal is the difficulty in differentiating between emotions where similar heart rates are recorded i.e. where high heart rates are detected whether this can be attributed to fear or excitement.

Physiological responses to aversive situations tend to be investigated more than behavioural profiles largely due to the relative ease of measurement and objectivity of physiological parameters. Such measurements are also useful as supporting evidence when attempting to interpret behaviour. Physiological measurements are often used during studies attempting to assess the effects of negatively valenced circumstances especially for economically productive species, but there is now a move towards recognising and promoting positive emotional circumstances (Boissy *et al.,* 2007b; Reefmann, Wechsler and Gygax, 2009; Doyle *et al.,* 2010; Burman *et al.,* 2011; Sanger *et al.,* 2011).

1.5.1.1. Heart rate variability

As previously discussed, short-term activation of the sympatho–adrenomedullary axis is widely used for monitoring acute stress responses in animals, but until recently the impact of the parasympathetic part of the ANS in modulating the stress response of both acute and chronic stress was largely neglected. Porges (1995) hypothesised that reduced activity of the vagus, one of the efferent parasympathetic nerves, would limit the physiological and behavioural capacity to cope with stressful events. Therefore, methods measuring vagal tone would provide more knowledge of both individual stress vulnerability and the magnitude of an actual stress response.

Heart rate is the net effect of the vagus that slows it down (thereby increasing the variability between the consecutive beats) and the sympathetic nerves that accelerate it by producing a more metronome-like heart beating (Pumprla *et al.,* 2002). Under resting conditions, both parts of the ANS are thought to be tonically active and the electrocardiogram of healthy individuals exhibits periodic variation in inter-beat intervals (Friedman and Thayer, 1998a, 1998b). Different stressors can induce a shift of the autonomic balance towards either a sympathetic or a parasympathetic dominance (Sgoifo *et al.,* 1997): a decreased HRV reflects a shift of the autonomic balance towards a more sympathetic dominance (Malik *et al.,* 1996; Sgoifo *et al.,* 1997).

Analysis of HRV therefore consists of a series of measurements of successive inter-beat interval variations of sinus origin which provide information about the individuals' autonomic tone (Pumprla *et al.,* 2002). Hence the beat-to-beat alterations in heart rate measured non-invasively can quantify the vagal as well as sympathetic activity; reflecting the dynamic response of the ANS to different physiological conditions.

As discussed throughout this section, evidence from clinical research strongly indicates that high variability in heart rate is a sign of good adaptability, implying a healthy individual with well-functioning autonomic control mechanisms. Conversely, lower variability is often an indicator of abnormal and insufficient adaptability of the ANS, implying the presence of a physiological malfunction in the individual; reduced HRV has thus been used as a marker of reduced vagal activity.

HRV analysis has been used as a research tool in farm and companion animal species to assess changes in sympathovagal balance associated to stress, pathology, behavioural dysfunction, training regimes and emotional states (Thayer *et al.,* 1997; Kuwahara *et al.,* 1999; Rietmann *et al.,* 2004b; Bachmann *et al.,* 2003; Kuwahara *et al.,* 2004; Visser *et al.,* 2002) and to determine how differences between sympathetic and parasympathetic contributions to HRV relate to disease, stress and coping strategies (von Borell *et al.,* 2007).

Unlike plasma cortisol (Murphy, 2009), no evidence of diurnal rhythm has been found in human (Hayano *et al.,* 1990) or equine HRV measurements over 12 hour recordings (Eager *et al.,* 2004). Although a circadian rhythm was reported in equine HRV by Gehrke, Baldwin and Schiltz (2011) and in miniature sows by Kuwahara *et al.* (2004) where night time measurements were found to be higher than daytime recordings over a 24hour period, this was expected due to the increased parasympathetic activity during sleep. All HRV data collected during this series of studies was obtained between the hours of 8am and 4pm and therefore not affected by time of day.

In addition, HRV analysis may have further advantages over salivary cortisol analysis; although a relatively non-invasive method is used for collection via an oral swab, catecholamine assay is complex, time consuming, expensive and of limited value in assessing autonomic modulation. Whilst it is useful in detecting the presence or absence of 'stress' hormones i.e. negative emotion, the absence of 'stress' hormones is not necessarily indicative of good welfare or positive emotion.

1.5.1.2. Research from human studies

Cardiac vagal tone assessed by HRV analysis has received considerable attention as a psychophysiological marker of emotion regulation and certain aspects of psychological adjustment in humans (Tiller, McCraty and Atkinson, 1996). There is a body of evidence to suggest that HRV analyses are useful for evaluating diseases (Bernasconi *et al.,* 1998), in particular cardiovascular (Carney, Freedland and Veith, 2005), psychiatric and psychological disorders (Karavidas *et al.,* 2007; Montano *et al.,* 2009). Indeed, the dynamics of HRV and the correlation of HRV to emotional states are well documented with different types of emotion being readily distinguished by changes in heart rhythm patterns that are independent of HR (McCraty *et al.,* 1995; Lane *et al.,* 2009). In addition, there is theoretical and empirical evidence for the emergence of HRV as an important marker of emotional regulatory ability (Tiller, McCraty and Atkinson, 1996; Appelhans and Luecken, 2006).

Several studies suggest that HRV analyses can be used to characterise a number of psychological illnesses, including major depression and panic disorders, providing a potential link between emotional states and HRV. Yeragani *et al.* (1990), Yeragani *et al.* (1991) and Yeragani *et al.* (1993) all reported significantly lower HRV in patients with these disorders compared with normal controls. Hanson *et al.* (2001) observed that adults suffered from symptoms of stress-related diseases with reduced HRV when living with an imbalance of effort and reward. Schwarz *et al.* (2003) studied HRV in participants during a chess game and

observed that periods of loss of optimism and control along with intensified helplessness and hopelessness correlated with a decrease in HRV, suggestive of decreasing vagal tone.

Low HRV has also been found in very anxious persons (Offerhaus, 1980; Kawachi *et al.,* 1995). In a study by Friedman and Thayer (1998a), it was shown that patients with chronic anxiety (panic disorder) exhibited lower levels of HRV compared to non-anxious controls. Frequent occurrences of severe panic attacks in humans have also been associated with lower HRV than control groups, indicating a shift towards sympathetic tone (Friedman and Thayer, 1998b).

As the parasympathetic branch of the ANS seems to be associated with adaptive responsivity to the environment (Friedman and Thayer, 1998b), individuals with high parasympathetic activity would be expected to show more exploratory and adaptive responses to environmental demands than individuals under sympathetic control. The fact that parasympathetic activity is suppressed during autonomic and behavioural responses to stress leads researchers to suggest that responses of the ANS to challenging situations might allow differentiation between individuals which could reflect differences in temperament (Porges, 1995).

Clinical research has focused mainly on negative emotions and emotional disorders such as anger-proneness, irritability, irritable distress and negative mood. However, cardiac vagal tone may also be an indicator of positive emotional states. McCraty *et al.* (1995) studied the effects of emotions on HRV and observed that feelings of anger and frustration significantly increased sympathetic activity, whereas feeling appreciation or a similar positive emotion increased parasympathetic activity. Recently, Lee *et al.* (2005a) found higher levels of HRV in healthy human participants after the intake of a traditional herbal remedy compared to a placebo control group. Similar findings were also reported by Lee *et al.* (2005b) in people undergoing Qi therapy, a Chinese external therapy similar to Reiki, the effect of which was correlated with more pleasant and calm emotions compared to a placebo group. Geisler *et al.* (2010) also found a positive association between HRV and the level of cheerfulness, calmness and satisfaction

with life via a self-report questionnaire by human participants. Positive self-esteem has also been associated with high levels of HRV in humans by Martens *et al.* (2010).

1.5.1.3. Research in animals

Evidence suggests that as well as in human research, measurement of vagal tone is a useful tool to assess affective states in animals (Porges, 1995). The use of HRV analysis in animal research is therefore of great relevance to determine the role of the ANS to disease, stress and coping strategies as well as their emotional response to different stimuli. Patterns of HRV reduction have already been used in numerous farm and companion animal studies to assess welfare and the reaction to stressors such as different management practices, human interaction, task learning, housing and environmental conditions e.g. in cows (Mohr, Langbein and Nurnberg, 2002; Hagen *et al.,* 2005), lambs (Greiveldinger, Veissier and Boissy, 2008) and goats (Langbein, Nürnberg and Manteuffel, 2004).

Studies on rodents (Sgoifo *et al.,* 1997) and quail (Valance *et al.,* 2007) have shown that stressful conditions, such as restraint, can result in a decrease in HRV and hence low levels of parasympathetic nerve activity. Reefmann, Wechsler and Gygax (2009) were able to relate high levels of HRV in sheep with low frequency of ear posture changes during grooming by a familiar handler (positive stimuli) compared with low HRV and high ear posture changes during social isolation (negative stimuli). A four-week period of social isolation in prairie voles was also reported by Grippo *et al.* (2007) to induce a significant reduction in HRV and led to anhedoia (reduced response to sucrose) and anxiety-related behaviours compared with a control group that were housed together in pairs. Prairie voles are a socially monogamous species, forming strong social bonds and a parallel study by Grippo *et al.* (2009) revealed that the administration of oxytocin mediated the behavioural and physiological consequences of long-term social isolation in prairie voles. This indicates that oxtocinergic systems may have a protective effect against negative social experiences in animals i.e. acting as an anti-depressant, a phenomenon reported in humans by Heinrichs *et al.* (2003) and may be relevant to the

veterinary profession for administration in horses where welfare conditions are compromised but are either practically or wilfully prevented from improvement.

HRV measurements have been found to be useful for determining the stress load in cows. Using a control group experiencing no stress, a second group kept in hot conditions and harassed by insects and a third group experiencing a strong physiological challenge (diarrhoea) Mohr, Langbein and Nurnberg (2002) discovered a relationship between declining HRV and increasing levels of physical (non-motor) stress. Gygax *et al.* (2008) investigated the physiological and behavioural response of dairy cows to different automated milking systems. Regardless of the type of milking system, the milking process itself generally increased stress levels as judged by restless behaviour and reduced HRV compared to resting levels. HRV measurements have also been used by Kuwahara *et al.* (2004) to evaluate porcine husbandry and management systems with a view to improving welfare and wellbeing in pigs and Nordmann *et al.* (2011) successfully identified the preferred feed barrier design using HRV and behavioural measurements in goats.

In a positive/negative contrast study using sheep conditioned to anticipate a standard feed Reefmann *et al.* (2009a) reported a decrease in HRV upon receipt of an unpalatable feed and an increase in HRV following the delivery of standard or enriched feed. Bergamasco *et al.* (2010) were able to demonstrate a positive effect of a handling regime on shelter dogs as HRV was positively correlated with the dogs' willingness to perform basic commands. Greiveldinger, Veissier and Boissy (2008) demonstrated that during an aversive situation (an air blast and prevention of access to food) lambs that could exert control over it by performing an operant task recorded significantly higher HRV than those who could not, indicating that the task was perceived as less stressful by controlling lambs. Evidence of positive emotion in pigs was also reported by Zebunke *et al.* (2011) who related significant increases in HRV measurements (compared with baseline values) with the successful learning of an operant task in order to gain

a food reward. In this study, HR also increased significantly alongside HRV which strongly indicates a state of arousal.

These studies reinforce the fact that HR and HRV are not necessarily negatively correlated and that high HR is not always indicative of a negative affective or 'stressful' state. Evidence from these studies also strongly indicate that presumed negative emotional states can be distinguished from presumed positive situations by using HRV and behavioural observations to determine the physiologic reaction of the ANS in animals. As Bergamasco *et al.* (2010) and Zebunke *et al.* (2011) were able to identify a relationship between positive handling regime and successful task solving with high levels of HRV and an increased willingness to perform there may be important implications for the management regimes and welfare of equine athletes that could lead to improvements in performance or willingness to perform. 'Happy' athletes may be more successful competitors.

1.5.1.4. Research in horses

Unlike HR, HRV is a potential measure free from the effect of physical activity (within certain parameters discussed later in this section) yet despite strong evidence linking modulation of the ANS with affective state in humans and other animals, relatively few studies have been conducted to investigate the relationship between affective state and HRV in horses. As horses are not typically a 'captive' or endangered species and are primarily 'non-productive' animals, they fall into the category of sport, leisure, or working animals and tend to circumvent the farm, companion and exotic animal remit. As such, scientific research into equine welfare issues tends to either follow several years after research in other species, or is overlooked altogether. Of the published work available, HRV has previously been used to gauge reactions to handling according to temperament (Visser *et al.,* 2002), to assess cardiovascular responses to pregnancy (Nagel, Aurich and Aurich, 2010, 2011; Nagel *et al.,* 2011), stress during transportation (Schmidt *et al.,* 2010a, 2010b, 2010d) and training (Schmidt *et al.,* 2010c) as well as responses

to pain, illness (Perkins *et al.,* 2000; Rietmann *et al.,* 2004a) and to investigate stress in stereotypic and non-stereotypic individuals (Nagy *et al.,* 2009).

Visser, Ellis and Van Reenen (2008) investigated whether individual or paired housing influenced the emotional response of young horses to a novel object test using a brightly coloured umbrella but found that housing group had no significant effect on HRV. Other management practices such as travel have been found to affect HRV. Schmidt *et al.* (2010a, 2010b, 2010d) have recently reported that short distance, long distance and repeated road transport leads to increased cortisol release and changes in HRV indicative of negative affective state. While investigating HRV in ridden and lunged horses at walk and trot, Schmidt *et al.* (2010c) discovered that HRV decreased to its lowest point whilst horses were being mounted by a rider which indicates an emotional response to a situation resembling an imminent and potentially lethal threat under natural conditions.

Equine stereotypies and stress sensitivity have been related to low levels of HRV and, in turn, negative emotion by Bachmann *et al.* (2003) who investigated whether stereotypies contributed to reducing chronic stress. Compared to crib-biters, control horses had a higher vagal tone and lower plasma cortisol levels at rest than crib-biting horses which indicated that crib-biters were not in a normal state of rest compared to controls. Conversely, Nagy *et al.* (2009) were unable to differentiate between baseline HRV in crib-biting versus control horses.

HRV has not been widely researched as a method of pain assessment and monitoring in animals despite evidence from human studies such as Franck *et al.* (2011) who related HRV measurements with pain responses in new born infants. Significantly lower HRV has been found in cattle suffering from diarrhoea by Mohr, Langbein and Nurnberg (2002) than in healthy controls, and lower HRV has been reported in horses suffering from grass sickness compared with double-matched healthy controls by Perkins *et al.* (2000). Similarly, Rietmann *et al.* (2004a) report HRV to be a reliable method of detecting pain and evaluating the

effectiveness of analgesic treatment in laminitic horses. In Rietmann and colleagues' study (2004a) non-steroidal anti-inflammatory treatment was found to increase HRV by way of a decrease in sympathetic activity and concurrent increase in parasympathetic activity. HRV also correlated negatively with weight-shifting, a behavioural indicator of physical discomfort in laminitics, whereas plasma cortisol levels did not. Therefore HRV could potentially provide a valuable alternative method of pain assessment to catecholamine measurement especially as continuous sampling of HRV is much more advantageous in determining the effect of analgesia compared with point sampling of blood or saliva.

Studies measuring equine HRV in relation to positive stimuli are scarce, but Kato *et al.* (2003) reported a significant increase in parasympathetic nervous system activity in horses during immersion in warm water compared with baseline values obtained at rest in the stable. It was possible to dissociate HRV levels from HR values as no significant difference in HR was obtained between the two conditions. This provides evidence that immersion in warm spring water, which in this case was up to the olecranon and with a shower of warm water across the back, may be a means of relaxation that induces positive emotion in horses.

Of the studies carried out to investigate different factors affecting HRV in horses, mixed results have been reported. Mohr, Witte and Voss (2000) found no difference between HRV at rest and during mental stress induced by a change in environment (hospitalisation). While breed has been reported to be of major importance in the personality of the horse by Lesimple *et al.* (2011), breed differences in HRV reported in cattle by Hagen *et al.* (2005) were thought to be related to temperament. Therefore it is not known whether breed might be a factor affecting HRV in horses and if so, whether such differences might actually be due to temperament rather than breed. No published studies have investigated the effect of age on HRV in animals but evidence of any such association has not been found in human studies (Reardon and Malik, 1996).

Visser *et al.* (2002) used HR and HRV measurements to assess temperament traits in response to a novel object (a brightly coloured open umbrella) and handling test (walking over a raised platform) in young horses aged between 9 and 22 months. Visser *et al.* (2002) were able to relate non-motor increases in HR with a reduction in HRV which revealed differences in emotional response (possibly fear) between handled and unhandled horses and indicated a link between mental stress and negative emotion. In a similar study using mature horses aged between 5 and 15 years, Visser *et al.* (2003a) found that a relationship existed between rider temperament ratings for traits of bravery and spookiness, high levels of resistance behaviour and low HRV during a handling test (walking over a raised platform). It was suggested by Visser *et al.* (2003a) that horses with low HRV and a high level of resistance behaviour were more agitated and less at ease than horses showing the opposite responses.

Eager *et al.* (2004) also reported HRV components to be significantly influenced by equine temperament. Visser *et al.* (2002) and Visser *et al.* (2003a) also suggest that individual characteristics such as temperament or reactivity to environmental changes may be responsible for the different individual modulation of the ANS that could be detected via HRV measurements and may provide useful information in attempting to quantify behavioural traits in horses.

Previous studies provide evidence to suggest that HRV in horses shows good stability across age and a high degree of repeatability when tested over subsequent days (Visser *et al.,* 2002; Rietmann *et al.,*2004b). The normal state of rest in horses is characterised predominantly by parasympathetic nervous activity (Kuwahara *et al.,* 1996) yet large inter-individual variations in basal values of HRV have been reported (Physick-Sheard *et al.,* 2000; von Borell *et al.,* 2007; Munsters *et al.,* 2011), which can contribute to the lack of significance found in some recorded HRV values. It has been proposed by von Borell *et al.* (2007) that several factors including the genotype, environment, temperament and nutritional status of the horse play a key role in the large inter-individual variations in basal HRV.

Evidence from human medicine documents the normal rhythm of the heart being controlled by direct autonomic innervation of the sinoatrial node by the sympathetic (adrenaline and noradrenaline) and parasympathetic systems (acetylcholine) via the vagus nerve (Malik *et al.,* 1996). In addition to its direct neural innervation of the heart, the sympathetic system can also modulate HR indirectly through the release of adrenomedullary catecholamines and via the activity of the renin-angiotensin system (Berntson *et al.,* 1997). In Ohmura and colleagues' study (2006) it was suggested that HRV could be a sensitive indicator of stress in transported horses, but the authors also noted that contributions to HRV by the SNS were not related to HR. Physick-Sheard *et al.* (2000) also investigated the relationship between HRV and HR in exercising horses and concluded that autonomic modulation is not reflected in HRV beyond moderate exercise intensities. Physick-Sheard *et al.* (2000) report that HRV is probably not a useful technique for the evaluation of autonomic activity above ~130bpm, which is around the average HR during trot (Rossdale and Young, 2002), beyond this humoral and other non-neural mechanisms become more important than autonomic modulation in influencing HR and HRV. Although the level of physical fitness has been to found correlate positively with HRV in human studies (Rossy and Thayer, 1998) this was not a factor that required control for the present series of studies as all equine participants followed a virtually identical workload and exercise regime as part of the Riding School.

Although anecdotal industry stereotypes are common, very little evidence exists to quantify differences in equine behavioural characteristics such as temperament and emotionality between sexes. Sex-related differences in trainability and personality have recently been reported in yearling horses in training by way of a questionnaire-based study. Duberstein and Gilkeson (2010) found that mares were more aggressive, tense, anxious, suspicious, excitable and panicked more easily than geldings who were rated as more affable and easily desensitised than mares during training. Factors such as these may account for the general preference for and dominance of male horses in equestrian sports (Whitaker, Olusola and Redwin, 2008). Momozawa *et al*. (2007) also report females to be more anxious than males in both a

behavioural test and handler questionnaire. As evidence from human research has identified females as having a greater vagal control of the heart than males who exhibit more sympathetic cardiac control (Rossy and Thayer, 1998) there is a potential benefit to the equine industry in categorising personality differences and isolating strengths and weaknesses of each sex using physiological measurements such as HRV alongside behavioural observations. This could potentially lead to modifications of training programmes, or selection for a particular niche based on sex-related differences

Whilst it has not been the direct focus of their research, equine HRV studies either report no effect of sex (Visser *et al.,* 2002; Rietmann *et al.,* 2004b; Visser, Ellis and Van Reenen, 2008), make no reference to the possible effects of sex when using mixed sex subjects (Minero *et al.,* 1999; Ohmura *et al.,* 2001; Quick and Warren-Smith, 2009; Schmidt *et al.,* 2010d), or lack detail as to the sex of experimental subjects (Nagy *et al.,* 2009; Kuwahara *et al.,* 1996; Physick-Sheard *et al.,* 2000).

These studies imply that individual characteristics related to factors such as temperament, personality, sex and underlying affective state may influence and be detected by HRV measurements. In a review of HRV as a tool to assess welfare in animals, von Borell *et al.* (2007) concur that HRV analysis appears to be a promising indicator of temperament and coping strategies in horses and therefore may prove to be a useful measure of their physical and emotional stress response. Monitoring activity of the ANS, through heart rate and its variability as suggested particularly by human studies, may deliver a suitable approach to assessing emotion and therefore welfare in horses when combined with behavioural records. Measuring behaviour and HRV together should provide a good indication of a horse's emotional reaction to their environment.

1.5.2. Behavioural indicators of animal emotion

It is generally accepted that along with initiating a physiological response, the autonomic system also plays a role in modulating the intensities of behavioural responses; in particular determining the nature of behaviour and of emotional states associated with behaviour (Fraser, 1983). The emotive state of an animal, or its motivation, can therefore often be deduced from its behaviour; motivation being the concept used to describe how behaviour is both initiated and directed either toward a goal or away from danger.

Motivation affects the welfare of enclosed animals via the physiological and psychological pathways that relate behaviour and welfare, particularly those involved in the body's maintenance of homeostasis and its response to acute and chronic stressors (Fraser, 1983). The concept of motivation is applicable to the welfare of domestic horses because behaviour, the manifestation of motivation, can function both as an indicator and as a determinant of wellbeing. It may be possible, therefore, to establish the emotional state of an individual via their behavioural expressions or activity levels in response to certain stimuli, or by identifying *changes* in behavioural patterns, which are among the first and sometimes the only detectable indications that an animal's perception of its environment is changing (Dellmeier, 1989).

Behaviours whose strength of motivation is directly proportional to the duration of elapsed time since their performance are often termed "drive-motivated" and are thought to feature prominently in the maintenance of homeostasis within an animal (Dellmeier, 1989). Other than eating, drinking, sleeping and copulation, behaviours for which endogenous motivation is relatively strong and increases in response to their prolonged non-performance include locomotion (Dellmeier, Friend and Gbur, 1985; Jensen, 1999; Jensen, 2001; Veissier *et al.,* 2008), play (Brownlee, 1954; Held and Špinka, 2011) and social interaction (Panksepp and Beatty, 1980; Dellmeier, Friend and Gbur, 1985; Christensen *et al.,* 2002a).

Behavioural expressions of emotion in response to exposure to prolonged aversive conditions featuring common elements of domestic horse management such as isolation, confinement or interrupted time budgets can be manifested in terms of aggression, reduced motivational activity, development of stereotypical behaviour, inappetence, nervousness, or a general alteration/suppression of normal behaviour (Christensen *et al.,* 2002a; Cooper and Albentosa, 2005; Morgan and Tromborg, 2007; Hall *et al.,* 2008).

For instance as highly social herd animals, visual contact with others is normally maintained for social cohesion and horses may exhibit behavioural and physiological stress responses during social isolation (Mal *et al.,* 1991a; Mal *et al.,* 1991b; Jezierski and Górecka, 1999; Strand *et al.,* 2002) i.e. frantic locomotor movement and vocalisation (**Figure 1.5**).

Figure 1.5. Behavioural expression of separation anxiety (McDonnell, 2003).

Alexander *et al.* (1988) report evidence of acute physiological and emotional stress within five minutes of isolation; horses exhibited signs of agitation (restless movement, calling to the herd, hyperventilation and sweating), whilst concentrations of adrenaline and noradrenaline increased dramatically. Similarly, Jezierski and Górecka (1999) recorded emotional excitement in the form of vocalisation, pawing, turning and trying to join other horses, along with significant increases in the HR of horses during isolation. A study by Sweeting, Houpt and Houpt (1985) found that isolation altered behavioural patterns with ponies spending more time standing non-alert and less time eating when visual contact was prevented. A subsequent study by Strand *et al.* (2002) reported similar results and Houpt and Houpt (1988) also discovered that isolated

horses spent 10% less time eating but were three times more active than horses that could make visual, auditory and physical contact with other horses.

Similarly, chronically unsatisfied motivation for locomotion and kinesis in response to deprivation of movement is a highly demonstrable effect of close confinement in animals where a situation requires but thwarts physical activity such as flight and may be manifested as stereotypic behaviour (Brownlee, 1954; Fraser and Fox, 1983; Dellmeier, Friend and Gbur, 1985) as discussed in **Section 1.3.4,** or other abnormal behaviours such as learned helplessness (Dellmeier, 1989). Although some horses are able to cope with their environment and show few behavioural abnormalities, for others much effort is required to adapt to the mental rigours of an aversive situation. Maintaining this effort for prolonged periods could increase the animal's susceptibility to disease; certainly in human medicine there is compelling evidence that prolonged negative emotional circumstances can cause clinically relevant immunosuppression (Herbert, 1993; Maes *et al.,* 2000; Pruett, 2003) as discussed in **Section 1.4.1**.

An important function of emotion is the ability of an individual to communicate their emotional state to others, particularly in social species (Rolls, 1990; Rolls, 2000). Communicating emotional states may have survival value by, for example, influencing the behaviour of other individuals, or enhancing the stability of social groups. As Equidae have a rich repertoire of facial and other expressive movements, body language, specifically ear movement, along with the position of the head and neck are used for non-vocal communication of mood and intention between horses (Schilder *et al.,* 1984; Weeks and Beck, 1996; Moehlman, 1998; Bennett and Hoffmann, 1999; McDonnell, 2003) and can also be directed toward human handlers (Aoyama *et al.,* 2001; Aoyama *et al.,* 2003). The ears can be moved independently or in unison around a lateral arc of 180° (Vallencien, 1963), enabling accurate sourcing of sounds and providing an indication of where the horse's attention is focused. For instance, a frontal 'threat' display with a gradation in intensity and posture may be observed in response to some acute stimuli, ranging from a subtle turning of the head by the sender to adding ears laid back, head down, approach at

fast walk, trot and/or gallop, drawing the corners of the lips up, mouth open, to actually biting the receiver (Schilder *et al.,* 1984; McDonnell, 2003).

Several studies indicate that it may be possible to replace some physiological measures with observations of ear postures. Reefmann *et al.* (2009b) for example, measured ear postures in sheep exposed to situations likely to induce states of negative, intermediate and positive emotional valence. It was reported that sheep separated from group members (negative stimulus) showed a high number of ear posture changes and a high proportion of forward ears, but whilst eating hay (positive stimuli) the ear position was passive. Reefmann *et al.* (2009b) also reported a high number of ear posture changes during anticipation of being fed but a low number of ear posture changes in response to grooming by a familiar handler (positive stimuli) (Reefmann, Wechsler and Gygax, 2009). Boissy *et al.* (2011) were also able to relate ear posture in sheep with specific emotional contexts; pointing the ears backward was associated with unfamiliar, unpleasant and uncontrollable situations likely to elicit fear whereas a horizontal ear posture corresponded with a neutral emotional state. Similar results have been recorded in horses by Ninomiya *et al.* (2007) who suggest that episodes of standing at rest with laterally rotated ears and a low neck position are behavioural indicators of satisfaction related to operant responses for a food reward. Along with other measures, ear position and the frequency of ear posture changes could therefore be a useful indicator of affective state and contribute to the assessment of welfare so will be investigated in this series of studies.

1.5.2.1. Anticipatory behaviour

One potential basis for studying animal emotion is by observing behavioural expressions and obtaining physiological data during anticipation of rewards (Spruijt, van den Bos and Pijlman, 2001). Psychopharmocological research has demonstrated that dopamine systems are involved in the processing of information related to rewards, meaning that positive rewards e.g. food have an emotional function by inducing subjective feelings of pleasure and hedonia (Schultz, 1997). Studies in humans have shown that the repeated pairing of the cue and reward shifts

activation of the dopamine neurons from just after the time of reward delivery to the time of cue onset (Blackburn *et al.,* 1989; Schultz, Dayan and Montague, 1992) so the expectation of a reward rather than the reward itself triggers dopamine release (de la Fuente-Fernández *et al.,* 2002).

Moe *et al.* (2006) investigated behavioural activity and emotional expressions in foxes during anticipation of either positive (food or toy) or negative rewards (capture with neck tong). Activity levels, time spent in the front of the cage and forward erect ear position were all found to increase when anticipating a positive reward compared with anticipating aversive stimuli. Similarly, Dudink *et al.* (2006) found that announcing the arrival of an enriched environment significantly increased play behaviour and reduced aggressive behaviour in piglets more than the unannounced enrichment. In a study investigating the emotional response of pigs anticipating either a positive or negative situation, Imfeld-Mueller *et al.* (2011) found that HRV was higher during the anticipatory phase regardless of whether the situation resulted in a positive or negative experience. It is therefore possible that in addition to the reward itself, reward expectancy may trigger dopamine release and serve to counteract stress (de la Fuente-Fernández *et al.,* 2002).

Social interaction, in addition to food and sexual behaviour, is considered by van der Harst and Spruijt (2007) to be rewarding to all animals. The reintroduction of a social partner has well known calming effects in mammals. For example, isolation-induced responses such as motor activity, vocalisations and HR decrease in heifers when a familiar partner is introduced after a short-isolation period (Boissy and Le Neindre, 1997) which indicates a positive emotional response to social recovery.

Anticipatory activity in preparation for consummatory behaviour when expecting a reward is also suggested to be a behavioural representation of the status of the underlying reward-related

neuro-circuitry (reward-sensitivity) and can be used as an indicator for the assessment of animal welfare (Von Frijtag *et al.,* 2000; Spruijt, van den Bos and Pijlman, 2001; van der Harst and Spruijt, 2007). Hyperactive behaviour when expecting food rewards has been shown in rats (van der Harst, Baars and Spruijt, 2003; van der Harst *et al.,* 2003) and mink (Vinke, Van den Bos and Spruijt, 2004; Hansen and Jeppesen, 2006). Anecdotal evidence suggests that when anticipating appetitive rewards horses also show anticipatory behaviour, notably vocalisation and hyperactivity. The amount an animal moves and the number of different activities that the animal performs during anticipation increases in number and kind compared with baseline activity (Spruijt, van den Bos and Pijlman, 2001). The amount of anticipatory activity is related to the needs of the animal; individuals that are lightly stressed show a higher anticipatory activity than "non-stressed" animals as they have become more sensitive to reward. This anticipatory increase in activity is quantifiable by the number of behavioural transitions performed and has been identified as a parameter indicative of the sensitivity to reward in various mammalian species (Bos *et al.,* 2003; Vinke, Van den Bos and Spruijt, 2004). Conversely, animals that are chronically stressed show stressor-induced insensitivity to rewards as chronic stress stimulates anhedonia which is a major symptom of human depression (van der Harst, Baars and Spruijt, 2005; van der Harst and Spruijt, 2007).

Social interaction, sexual behaviour and the provision of food are considered by van der Harst and Spruijt (2007) to be rewarding to all animals yet Bachmann *et al.* (2003) describe 'waiting to be fed' as a common "stress" situation known to any stabled horse and used the pre-feeding period as an aversive, but neither painful nor frightening stimulus in their study. In theory feeding time should be an excitable, positive situation, but in horses it has been associated with the initiation of bouts of stereotypic behaviour (Bachmann, Audigé and Stauffacher, 2003; Bachmann *et al.,* 2003; Cooper *et al.,* 2005; Nagy *et al.,* 2009).

Pre-feeding activities such as weaving, nodding and repetitive door kicking are anecdotally viewed as a negative activity akin to non-feed related stereotypic behaviour and are often

actively discouraged. Vocal dissuasion or physical measures are a common reaction via the use of physical restraint, pinning the door open to prevent kicking, spraying the horse with a jet of water or withholding food until the behaviour desists. More invasive measures are available for the prevention of crib-biting, from physical prevention using a collar (McBride, 2001; McGreevy and Nicol, 2010) to surgical neurectomy (Delacalle, 2002). Such behaviours may initially arise as anticipatory, appetitive activities and then become a conditioned response if they are repeatedly rewarded with food after their performance (Cooper and McGreevy, 2002).

Stereotypic behaviour in horses has been widely researched and can normally be attributed to sub-standard management regimes which prevent the exhibition of natural behaviours. Indeed, housing and management factors associated with chronic stress or with increased anticipatory behaviour have been found to have a strong influence on the prevalence of stereotypies (McGreevy *et al.,* 1995; McGreevy, French and Nicol, 1995; Bachmann, Audigé and Stauffacher, 2003). Equine stereotypies and stress sensitivity have also been related to low levels of HRV and therefore negative emotion by Bachmann *et al.* (2003) but it is not known whether there is a distinction between the underlying affective state during pre-feed stereotypic behaviour and non-feed related stereotypies when there is a difference in causation. If dopamine release is triggered by the expectation of being fed as well as on receipt of food, the exhibition of pre-feeding behaviours may be an indicator of positive affect and serve to enhance welfare rather than detract from it.

The physiology and behaviour of horses during anticipation or in receipt of a positive reward may therefore provide the opportunity to assess their emotional state (Spruijt, van den Bos and Pijlman, 2001; Boissy *et al.,* 2007b) and are investigated further in **Chapters 3** and **4.**

1.5.2.2. Avoidance behaviour and withdrawal

Avoidance behaviour along with high levels of vocalisation were identified by Imfeld-Mueller *et al.* (2011) as suitable parameters with which to assess the valence of a situation in pigs.

Aversive behaviour of sheep in response to handling, restraint and simulated shearing has also been used by Rushen (1996) to assess their mental state. In **Chapter 5,** the procedure of clipping is used as an aversive stimulus to elicit changes in HRV and behavioural activity in horses.

The clipping procedure is common practice in horse management and should not cause the horse any discomfort, the horse's size and temperament however, may make it potentially dangerous to deal with in practice. Some horses will not tolerate part or all of their body being clipped and may be unwilling to stand still, shake or twitch the skin and may kick with both the fore- and hind limbs. Such fractious behaviour during the clipping procedure is suggested to be due to the sound rather than the sight of the clippers according to Gough (1999), or may be associated with previous experience of discomfort caused by blunt or hot clipper blades according to Dyson and Carson (2002). It is not uncommon for some horses to require chemical restraint with a mild sedative as described by Dodman (1980), Geiser (1990) and Gough (1997) in order to ensure swift and safe completion of the process.

For prey species such as the horse, there is a survival value associated with the ability to mask pain or discomfort in order to minimise the risk of predation (O'Callaghan, 2002; Taylor, Pascoe and Mama, 2002; Mayer, 2007), therefore outward behavioural signs do not necessarily reflect the emotional state of the animal. Burn, Dennison and Whay (2010) assessed the behaviour of working equids in poor physical health and found that an unresponsive behavioural profile was consistent with exhaustion, chronic pain or depression-like states. Low HRV has also been reported by Schmidt *et al.* (2010) in horses being mounted by a rider during their initial training, a situation resembling a potentially lethal threat under natural conditions, yet the horses remained behaviourally compliant.

Unresponsiveness to the environment could be indicative of chronic pain, sickness, depression and exhaustion; but could equally be associated with neutral or even good welfare if an animal

perceives its situation to be secure enough to allow reduced vigilance (Paul, Harding and Mendl, 2005). Horses selected for use in a riding school environment tend to be chosen for their passive and tractable behaviour, but it is possible that this may mask physiological indicators of stress. The psychological condition of learned helplessness was described in ridden horses by Odberg, (1987b) and has been explored more recently by Hall *et al.* (2008). For horses that react passively and appear compliant during clipping, it is not known whether this is due to genuine relaxation during and habituation to the process, or if compliant horses are undergoing psychological distress masked by behavioural unresponsiveness indicative of withdrawal, apathy or learned helplessness.

1.5.2.3. Temperament assessment

The genetic diversity of a species gives rise to individual differences in behavioural tendency. Considering the presence of variations in equine temperament is important in order to optimise welfare when forming selective management and breeding programmes as well as when attempting to find the right match between the horses' temperament and the rider's personality, housing conditions, management practices and specific use. **Chapter 7** explores a wide range of factors that might affect HRV in horses and includes observer ratings of temperament for each of the horses used throughout this selection of studies.

It is likely that individual differences in behaviour may reflect the range of subjective feelings experienced by animals and are relevant to animal welfare. The importance of such individual differences to animal welfare issues has been highlighted by Schouten and Wiepkema (1991) and Manteca (1993) and is illustrated well by the inconsistent exhibition of stereotypic behaviour between individuals in the same environment (Bachmann, Audigé and Stauffacher, 2003). This raises questions over the welfare of those animals who do not exhibit such behaviours in that they might be more mentally robust and able to cope with the stress of the environment, or that they are unable to develop a coping strategy in which case the 'normal' behaving animals may be suffering more than their stereotyping counterparts.

Consistent behaviours (habits) form traits which describe the personality of an individual (Zuckerman, 1991). The tendency of a horse to evade being caught in the field may be considered a habit. If the horse consistently engages in other obstructive behaviours it may be described as having an 'uncooperative' trait. An individual's distinctiveness can be defined by its rating on a number of such traits. This variation can be assumed to be a result of interactions between the animal's inherited characteristics and environmental influences, which are generally perceived as governing an individual's 'temperament'. Temperament has been defined by Stur (1987) as the sum of all inherited and acquired behaviour patterns and by Kilgour (1975) as 'the behavioural characteristics resulting from the individual's physical, hormonal and nervous organisation'.

Temperament is one characteristic that is very difficult to assess objectively. In humans, the most widespread commonly employed method consists of using questionnaires where the subject or the subject's peers give an evaluation for each trait being considered (Tremblay, 1992). Commonly used methods for assessing temperament in animals include gathering observer ratings for behavioural traits (Hsu and Serpell, 2003), recording the reaction of an animal in a particular situation (Podberscek, Blackshaw and Beattie, 1991) or rating aspects of behaviour in their usual environment (Kimura, 1998).

Studies assessing equine temperament by behavioural test and/or a questionnaire survey have been increasing over the last two decades and multiple studies have been done to determine the best way to assess equine temperament. Some have presented horses with various situations in order to identify their personality based on their reaction (Visser *et al.,* 2003b). Visser *et al.* (2001) showed that when confronted with a challenging situation, horses exhibited individually different behavioural responses suggested to be linked with differences in temperament. Other studies compare behavioural responses with instructor ratings of temperament (Le Scolan, Hausberger and Wolff, 1997; Seaman, Davidson and Waran, 2002; Visser *et al.,* 2003a) or emotionality (McCall *et al.,* 2006).

Alternatively, questionnaires can be given to horse handlers, whereby persons familiar with the horses rate their personality traits (French, 1993; Momozawa *et al.,* 2003; Momozawa *et al.,* 2005; Lloyd *et al.,* 2007; Momozawa *et al.,* 2007; Lloyd *et al.,* 2008; McGrogan, Hutchison and King, 2008). Momozawa *et al.* (2003) demonstrated that personality surveys given to horse handlers could be reliably linked to the response of horses' to training situations. The authors reported a positive correlation between horses rated as highly anxious by their handler and HR during a balloon reactivity test and a negative relationship between anxiety level and their tendency to touch the balloon. Morris (2002) reported that inter-judge reliability was high when ranking a group of horses based on their anxiety trait. Later, Momozawa *et al.* (2005) developed and validated the use of a handler questionnaire survey to reliably assess the traits of anxiety, trainability and affability. Factor analysis indicated that anxiety traits had the highest internal consistency meaning that the anxiety trait can be assessed more accurately than other traits in horses.

Using assessor rating scales to distinguish individual differences in a single trait such as reactivity does not necessarily reflect the complex nature of personality and Mills (1998) report that inter-observer correlation based on subjective ratings with respect to familiar terms used to describe horse personality is generally low and is therefore unreliable for use in a scientific context. However, McCann *et al.* (1988) determined that horse handlers *could* reliably evaluate the emotionality level of yearlings. More recently Momozawa *et al.* (2007) were able to correlate questionnaire based anxiety scores (high score denoting greater anxiety) negatively with behavioural responses and positively with HR during transient social isolation in horses. Questionnaire based personality assessment studies by Lloyd *et al.* (2007) and Lloyd *et al.* (2008) identified anxiousness as one of six equine personality components which could be rated reliably by regular handlers of horses and discovered positive results as to the reliability of survey research in assessing equine behavioural traits associated with different breeds. Recent work done in horses using handler surveys has also shown that the personality traits of

neuroticism and emotionality represented by human personality adjectives could be identified in surveys given to horse handlers (McGrogan, Hutchison and King, 2008).

Boissy (1995) defines anxiety as an emotional state induced by the perception of potential danger that threatens the individual. At moderate levels anxiety leads to functional reactions that allow an individual to cope with and adapt to their environment, but at excessive levels becomes detrimental to welfare. Therefore it is important to understand the anxiety trait of individual horses. Studies such as Momozawa *et al.* (2005), Momozawa *et al.* (2007), Lloyd *et al.* (2007), Lloyd *et al.* (2008) and McGrogan, Hutchison and King (2008) propose that a questionnaire survey is an effective means of assessing equine anxiety traits. In addition, a questionnaire survey could be advantageous over a behaviour test in that data collected from the questionnaire is based on long-term observations of the respondents and therefore is not influenced by transient changes in physiological or behavioural status of the horse during a behavioural test.

McCall *et al.* (2006) report that, in isolation, behavioural tests and emotionality scores failed to predict reactivity in horses and recommend the use of both behavioural and physiological measures together. Studies that compare physiological measures directly to temperament are limited in number, especially in horses despite the fact that physiological responses in behavioural challenge tests are useful for strengthening the interpretation of behavioural responses to challenge or change. McCann *et al.* (1988) tested heart and respiration rates in yearling horses in addition to behaviour which involved assigning an emotionality score based on their behaviour in a pen, a chute and upon release from the chute. A correlation was found between HR and average emotionality score, indicating that a higher HR was noted with the more nervous yearlings. Anderson *et al.* (1999) report significantly higher plasma cortisol levels in horses rated by their instructor to spook easily and who demonstrated unpredictable and inconsistent behaviour than in horses with opposing traits.

The validity of physiological measures used during behavioural tests is questionable as HR in particular is largely influenced by physical activity. It is therefore difficult to determine whether observed changes are caused by anxiety, physical activity or stimulus-induced arousal in freely moving horses. Although the most commonly used variables to quantify temperamental traits are behavioural measurements, it has been shown that HRV is also useful to differentiate between individual animals (Sgoifo *et al.,* 1997). HRV therefore, seems a relevant variable to measure when quantifying individual differences in temperament.

In summary, there is a general lack of research into behavioural indicators of positive emotion in horses. It is likely that valuable information can be gained as to the affective state of horses by observing their behaviour under different conditions and in response to different stimuli. As this is a relatively new area of equine research very little published data is available, information gained from this study will therefore make a valuable contribution to equine welfare research.

1.6. OBJECTIVES

The overall aim of this project was to evaluate the use of heart rate variability alongside behavioural measurements as a non-invasive method of assessing affective state in horses. Ultimately it was anticipated that a method of assessing the emotional reaction in horses could be developed which may be used as a tool for enhancing equine welfare. The specific aims were two-fold; firstly to investigate whether an emotional response can be ascertained in horses from their heart rate variability and behaviour during exposure to housing and management conditions differing in their emotional valence and secondly to investigate factors that may affect underlying HRV. The ultimate aim of this research being the establishment of a preferable habitat and management regime for horses with a view to minimising negative experiences whilst promoting the occurrence of positive experiences.

This series of studies will therefore investigate physiological and/or behavioural signs of emotion in the horse. In order to achieve the study aims the objectives for this research were as follows:

- To determine whether differences in HRV and behavioural tendencies/activity can be identified when horses are exposed to management practices deemed positively valent (feeding) and negatively valent (sham-clipping).

- To establish whether differences in HRV and/or behaviour are evident before, during and after anticipation of a positive reward in the form of companionship.

- To investigate whether differences in HRV and/or behaviour are evident when horses are housed under four different management systems offering increasing levels of social contact and physical space: Farmhouse and Lodden stabling, Crew Yard and Paddock.

- To identify potential factors affecting HRV measurements in order to guide future application and the interpretation of findings.

Chapter Two

Standard Methodology and Pilot Study

2.1. COLLECTION AND ANALYSIS OF HEART RATE VARIABILITY MEASUREMENTS

In 1996 a Task Force comprising the European Society of Cardiology and the North American Society of Pacing and Electrophysiology defined and established standards of measurement and physiological interpretation of HRV in humans (Malik *et al.,* 1996). The Task Force recommend the use of time domain indices as one of the accepted clinical parameters used for analysis of HRV data. Time domain analyses measure the intervals between successive normal cardiac cycles; that is all intervals between adjacent R peaks (RR intervals) of the QRS complex resulting from sinus node depolarisations. Although a less sensitive technique for HRV analysis, time domain calculations are the simplest method of evaluating HRV and are much less susceptible than frequency domain analyses to errors in RR interval data (Malik *et al.,* 1996).

From a series of cardiac cycle intervals it is possible to detect how the cardiac signal changes over time. Statistical calculations can be derived from these time domain measures and are divided into two classes, a) those derived from direct measurements of the RR intervals, and b) those derived from the differences between RR intervals (Malik *et al.,* 1996; Berntson *et al.,* 1997; Sztajzel, 2004). The simplest variable to calculate from direct measurements is the standard deviation of RR intervals (SDNN) i.e. the square root of variance. The most commonly used measure derived from interval differences is the square root of the mean squared differences between adjacent RR intervals (RMSSD). The RMSSD value is obtained by calculating each successive time difference between the QRS complexes in milliseconds, each of these values is squared and the result is averaged before the square root of the total is obtained. For this series of studies SDNN and RMSSD were calculated from each set of RR interval data.

Generally speaking, reductions in the values of HRV indices (SDNN and RMSSD) reflect a shift of the autonomic balance towards a more sympathetic dominance, while increased values

of these indices indicate a shift towards a more parasympathetic dominance (Mohr, Langbein and Nurnberg, 2002; von Borell *et al.,* 2007). The SDNN has been shown to accurately predict overall HRV and includes the contribution of both branches of the autonomic nervous system to HR variations. SDNN is influenced by changes in both sympathetic and parasympathetic activity it is therefore a non-specific measure of sympathovagal balance as it reflects the total amount of variability (high and low frequency variations) during the recording period. SDNN is a useful tool for detecting changes in autonomic activity but cannot be used to quantify specific changes in sympathetic or parasympathetic activity. RMSSD provides measurement of parasympathetic activity as it reflects high-frequency, short-term variations of HR, which are mainly due to the presence or withdrawal of vagal activity by the parasympathetic nervous system (Malik *et al.,* 1996; Berntson *et al.,* 1997; Pumprla *et al.,* 2002; Sztajzel, 2004; von Borell *et al.,* 2007).

The Task Force recommends measuring more than one parameter when investigating HRV and both SDNN and RMSSD have been used as physiological indices in numerous equine HRV studies such as Mohr, Witte and Voss (2000), Visser *et al.* (2002), Visser *et al.* (2003a), Rietmann *et al.* (2004a), Schmidt *et al.* (2010a, 2010b, 2010c, 2010d), Gehrke, Baldwin and Schiltz (2011), Munsters *et al.* (2011) and Nagel, Aurich and Aurich (2010, 2011), Nagel *et al.* (2011) as well as in donkeys (McLean *et al.,* 2011), dogs (Bergamasco *et al.,* 2010), sheep (Greiveldinger, Veissier and Boissy, 2008; Reefmann *et al.,* 2009a), cows (Mohr, Langbein and Nurnberg, 2002; Hagen *et al.,* 2005; Gygax *et al.,* 2008) and pigs (Imfeld-Mueller *et al.,* 2011; Zebunke *et al.,* 2011).

Several models of Polar RR recording equipment have been used for bovine (Mohr, Langbein and Nurnberg, 2002; Hagen *et al.,* 2005), porcine (Imfeld-Mueller *et al.,* 2011; Zebunke *et al.,* 2011) and equine HRV studies (Visser *et al.,* 2002; Bachmann *et al.,* 2003; Visser *et al.,* 2003a; Rietmann *et al.,* 2004a; Rietmann *et al.,* 2004b; Nagy *et al.,* 2009; Schmidt *et al.,* 2010a, 2010b, 2010c, 2010d) and have previously been validated as an accurate alternative to standard 3-lead

and 12-lead ECG equipment by Nunan *et al.* (2008) and Marchant-Forde, Marlin and Marchant-Forde (2004). Equine HRV recording equipment has also been used to collect measurements from goats by Langbein, Nürnberg and Manteuffel (2004) and Nordmann *et al.* (2011). The Polar RS800 G3 model used throughout this series of studies has previously been used by Munsters *et al.* (2011) assessing horse and rider HRV and by Werhahn, Hessel and Van den Weghe (2011) measuring equine HRV at rest.

For this series of studies, heart rate - expressed as RR interval duration (m/sec) - was recorded using the Polar RS800G3 heart rate monitor. Upon activation the Polar device continually records changes in electrical potential to detect the R-peaks of the horses' electrocardiogram and stores RR intervals in digital form. In order to comply with the recommendations by Malik *et al.* (1996) and Berntson *et al.* (1997) the minimum length of recording duration was 5 minutes so where necessary recording lengths were standardised post-data collection in order to make accurate comparisons within each study.

The recording equipment consists of a girth belt with a built-in transmitter and a wrist-watch receiver. The positive electrode was located at the left dorsal thorax and negative electrode at the left ventral thorax as described in **Section 2.3.1**. Electrical conductivity was increased by the use of ultrasonic gel. The recording watch had a memory function that stored data from the transmitter, and was fastened to the built-in transmitter pocket on the girth belt. Following each study RR interval data were downloaded via a Polar Infrared Interface onto a PC with Polar Pro-Trainer 5 software installed.

Following initial pilot work, baseline HRV data were collected from all the resident horses at Brackenhurst Equestrian Centre (N=33) to be used for comparison with data collected in **Chapters 3, 4, 5** and **6** and was also used for the analysis of factors affecting HRV in **Chapter 7**. Prior to the horses being turned out to pasture for the University summer break in 2008, baseline HRV measurements were obtained whilst each horse was resting in its usual stable or

crew yard. Data collection took place during the morning over two consecutive days and each horse was fitted with HRV monitoring equipment (Polar Equine RS800G3) attached to the thorax with an elastic girth as described in **Section 2.3.1** (pilot study). Recording began as soon as the equipment had been fitted and heart rate signal obtained, and ceased after 15 minutes. Approximately two weeks later, once the horses had been turned out to pasture for the summer holidays, a second set of HRV measurements were obtained. Each horse was caught in the field, fitted with a head collar and lead rope and the heart rate monitor attached as previously described. Recording began once the equipment was successfully fitted and heart rate detected, then the lead rope was removed and the horse released to move around the field at liberty. After 15 minutes of recording the horse was caught, the lead rope reattached, the heart rate monitor was stopped and removed and the horse released into the field.

For analysis of all the HRV data collected, Kubios HRV software (version 2.0, Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was used. Kubios HRV is an advanced tool used for studying the variability of heart beat intervals in human subjects (Sollers *et al.,* 2007; Tarvainen *et al.,* 2008; Li *et al.,* 2009) and has previously been used for equine HRV research (Nagel, Aurich and Aurich, 2010; Schmidt *et al.,* 2010a, 2010b, 2010c, 2010d; Nagel *et al.,* 2011).

With the possible exception of anaesthetised or immobilised subjects, artefacts from a variety of sources are likely to contaminate RR interval recordings (Berntson *et al.,* 1997). Whilst artefacts can usually be visually identified by graphical displays of the RR interval series (missing or spurious R-waves), this method is tedious especially in long-term recordings and requires subjective judgements to be made. It is therefore preferable to apply an artefact-detection algorithm. As complex trends in the HRV signal can also cause distortion of HRV analysis, the Kubios HRV programme uses a de-trending procedure based on the widely documented smoothness priors approach as described by Tarvainen, Ranta-Aho and Karjalainen (2002) and in addition, an artefact correction was made following the method used in previous equine HRV

studies by Schmidt *et al.* (2010a, 2010b, 2010c, 2010d) and Nagel, Aurich and Aurich (2011). The smoothness parameter was set at 500ms. For artefact correction, the custom filter of the programme was set at 0.3, identifying RR intervals differing from the previous RR interval by more than 30% as artefacts. After abnormal interval removal, the programme's algorithm substitutes detected errors with interpolated intervals calculated from differences between previous and next accepted RR intervals. Mean RR interval was then determined, and its standard deviation (SDNN) and root mean square of successive RR differences (RMSSD) were calculated.

For all statistical comparisons, a p-value <0.05 was considered significant. Data are reported as mean±standard deviation of mean (SD). Where significant results were obtained, the magnitude of differences between means, or effect size, was calculated using either eta squared or partial eta squared and interpreted as small, medium or large according to Cohen's classifications (Cohen, 1988, Pallant, 2007). For all t-tests, Mann-Whitney U tests and Wilcoxon signed-rank tests Cohen's 'd' was utilised and the effect size was reported as small from 0.2 to 0.5, medium from 0.5 to 0.8 and large above 0.8. For one-way ANOVA tests Cohen's 'f' was utilised and the effect size was reported as small from 0.1 to 0.25, medium from 0.25 to 0.4 and large above 0.4.

2.2. ANALYSIS OF BEHAVIOURAL DATA

This series of studies involved the observation of both fine and whole animal behaviour in order to investigate the emotional states of horses. If behavioural responses can be associated with HRV according to the valence of a particular stimulus it may be possible to infer whether the subjective feelings experienced by the animal are positive or negative. Current knowledge of the emotional repertoire of horses is limited so it was hoped that this research could identify corresponding response patterns in order to describe emotion more objectively. This could then facilitate the identification of optimal equine husbandry systems that incorporate positive experiences and favour their welfare.

With the exception of **Chapter 5** (which assigned each horse a score based on their level of behavioural activity), an ad-libitum sampling regime was adopted throughout each study which enabled detailed analysis of the entire behavioural repertoire exhibited during each observation. Once data collection had ceased, preliminary analysis of video footage enabled an ethogram to be constructed for each study (**Appendices 1-5**) which provided an objective definition of each behaviour exhibited. For all behavioural footage obtained, analysis of the frequency of behavioural events and duration of behavioural states was carried out using a software package designed specifically for the analysis of behaviour (The Observer XT 8.0, Noldus Information Technology B.V., Wageningen, The Netherlands).

Analysis of behaviour in **Chapter 3** used an ad-libitum sampling regime to calculate descriptive statistics for the duration of behavioural states for each time-period (as described in **Appendix 2)** along with the frequency of interactive behaviour (**Appendix 1**). Durations of raw data for each horse in time format (seconds) were converted to a decimal number value using Microsoft Excel and imported into SPSS 15.0 for statistical analysis. Differences in behavioural measurements between each time-period were calculated for the whole group and according to management regime (horses stabled during the day versus horses living out at grass). Relationships between HRV and behaviour were also investigated.

For each of the six horses observed in **Chapter 4**, ten minutes of video footage was obtained prior to concentrate feed being placed in the stable. An ad-libitum sampling regime was adopted in order to calculate the duration of behavioural states for the observation period (**Appendix 3**). Durations of raw data for each horse in time format (seconds) were converted using Microsoft Excel to a decimal number value. The frequency of transitions between behavioural states was calculated in order to measure of the level of activity and descriptive statistics were obtained for the frequency of event behaviours (as described in **Appendix 4**). The relationship between anticipatory HRV and the duration of behavioural states, frequency of transitions between states and frequency of behavioural events were then investigated using SPSS 15.0.

From observation of the video footage obtained in **Chapter 5** individual horses were awarded a behavioural score based on their level of behavioural activity during each time-period (as described in **Section 5.5**). Behavioural scores were then entered into SPSS 15.0 for statistical analysis. Differences in behavioural score were calculated between each time-period for the whole group, per category (compliant/non-compliant) and according to sex. Relationships between HRV and behavioural score were also investigated.

In **Chapter 6**, behavioural data were analysed for each of the sixteen subjects using data collected on the fourth day (Thursday) of each week between the hours of 8.30am and 4pm. Thursday was chosen as an appropriate day from which to collect behavioural data as it allowed horses the longest length of time to become accustomed to each housing regime, and avoided potential disruptions that took place each Friday when horses were briefly involved in a 3rd year undergraduate project prior to being returned to their field for the weekend.

The duration of behavioural states observed for each horse (**Appendix 5**) was analysed via an ad-libitum sampling regime. Durations of raw data for each horse in time format (seconds) were converted to a decimal number value using Microsoft Excel and imported into SPSS 18.0 for statistical analysis. Differences in behavioural measurements according to housing system, week, sex and intra-horse were calculated. The relationship between HRV in the horses wearing monitoring equipment and their corresponding behavioural states was also explored.

For all statistical comparisons, a p-value <0.05 was considered significant. Data are reported as mean±standard deviation of mean (SD). Where significant results were obtained, the magnitude of differences between means, or effect size, was calculated using either eta squared or partial eta squared and interpreted as small, medium or large according to Cohen's classifications (Cohen, 1988, Pallant, 2007). For all t-tests, Mann-Whitney U tests and Wilcoxon signed-rank tests Cohen's 'd' was utilised and the effect size was reported as small from 0.2 to 0.5, medium

from 0.5 to 0.8 and large above 0.8. For one-way ANOVA tests Cohen's 'f' was utilised and the effect size was reported as small from 0.1 to 0.25, medium from 0.25 to 0.4 and large above 0.4.

2.3. PILOT STUDY

The aim of the pilot work was to test HRV recording equipment and assess its suitability as a measure to determine the underlying affective state of horses and their response to different emotion-eliciting conditions.

During this pilot study horses were placed in a familiar stable environment where they were exposed to different stimuli intended to generate a positive, negative or neutral affective state. As a 'neutral' condition, horses were left undisturbed in the stable for a period of time. As a negative stimulus, water was sprayed around the horse's head and neck area in a procedure similar to that used by Eager *et al.* (2004) when investigating factors affecting equine HRV. Desire, Boissy and Veissier (2002) relate the prevention of access to a substrate that allows the performance of a highly motivated behaviour to emotional responses believed to express frustration. A second negative stimulus was therefore used which involved placing a bowl of feed beyond the horse's reach but within their line of sight. This was intended to generate a negative emotional response akin to frustration which may be experienced at feed time when the horse can see others being fed prior to receiving their own feed. This stressor has previously been used as an arousal-inducing situation to elicit frustration in dairy cows by Sandem, Braastad and Boe (2002), and to assess HRV in crib-biting horses by Bachmann *et al.* (2003), later modified by Nagy *et al.* (2009) with horses eliciting a strong stress reaction in both studies. As a positive stimulus the horses were given access to the feed bowl previously left out of reach, and allowed to consume the contents.

2.3.1. Study protocol

Prior to the study commencing, the study protocol gained ethics approval and appropriate risk assessments were undertaken. Three mature horses (two geldings and one mare) were selected to participate according to their availability at the time the study commenced. The horses normally lived out at grass as members of a larger mixed-sex group and were accustomed to being stabled in isolation for periods up to four hours.

On the day of the study, each horse was brought in from the field and stabled individually out of sight of one another. Fifteen minutes prior to the start of the study each horse was fitted with HRV recording equipment. HRV measurements were recorded using a Polar Equine RS800 G3 heart rate monitor with RR interval recording capabilities as a non-invasive method of detecting inter-beat intervals and to support behavioural observations. The heart rate monitor consisted of two electrodes contained within an elasticated girth belt positioned proximally and distally at the left lateral thorax wall, with an attached sensor and watch receiver (**Figures 2.1** and **2.2**).

Figure 2.1. Polar Equine heart rate monitor girth belt and watch receiver

Figure 2.2. Polar Equine heart rate monitoring equipment in situ

The equipment was activated then the horse was left undisturbed in the stable for ten minutes (Rest 1). This was followed by a period of one minute when the handler entered the stable and gently sprayed water around the head and neck area, from an approximate distance of 20cm,

using a 500ml bottle spray (Spray). A second five minute period followed when the horse was left undisturbed in the stable (Rest 2). Then a portion of feed (chaff, pasture mix, garlic and sugar beet) contained in a feed bowl was placed in view beyond the stable door (Feed-out) for five minutes. The feed bowl was then placed inside the stable (Feed) and the horse was allowed to consume the contents. Recording ceased ten minutes after eating began. Once the feed had been consumed, recording equipment was removed and the horse returned to the field.

2.3.2. Data analysis and results

Using Kubios HRV analysis software, data were de-trended and an artefact correction was made as described previously. Time domain analyses were calculated from mean RR interval data for each time-period; Rest 1, Spray, Rest 2, Feed-out, Feed. For each time-period and individual subject SDNN and RMSSD were analysed using SPSS 15.0. The null hypotheses stated that there was no difference in HRV measurements between time-periods or between individual horses.

As a small sample size was used for the pilot study the non-parametric Friedman's ANOVA was used to determine whether differences in HRV existed between time-periods. Mean SDNN and RMSSD were found to be lowest during the Spray period (81.87±43.92ms, 78.36± 64.17ms) and highest during the Feed period (91.00±15.99ms, 129.20±30.93ms) closely followed by the Feed-out period (91.83±24.02ms, 119.30±50.57ms; **Figure 2.3**).

The Friedman's ANOVA indicated that mean HRV did not significantly change across the five time points, $X^2(4) =1.33$, $p=0.86$ for SDNN and $X^2(4) =2.40$, $p=0.66$ for RMSSD. It was therefore not possible to reject the null hypothesis.
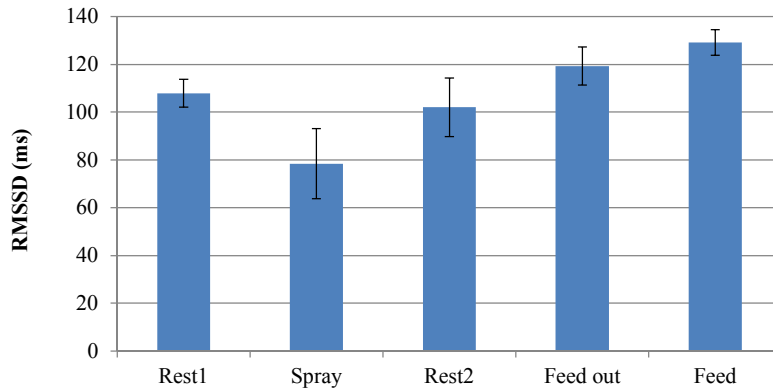
Figure 2.3. Mean RMSSD (square root of the mean squared differences between adjacent inter-beat intervals) for each time-period (±SE). Differences were not significant (Friedman's ANOVA).

Analysis of descriptive statistics indicated that inter-individual differences in HRV occurred between each time-period for both SDNN and RMSSD (**Figure 2.4**). Average HRV data from all time-periods revealed that Horse 1 recorded lower SDNN (66.92±24.33ms; 101.88±24.89ms, respectively) and RMSSD than Horse 3 (73.82±35.08ms; 134.48±30.73ms, respectively).
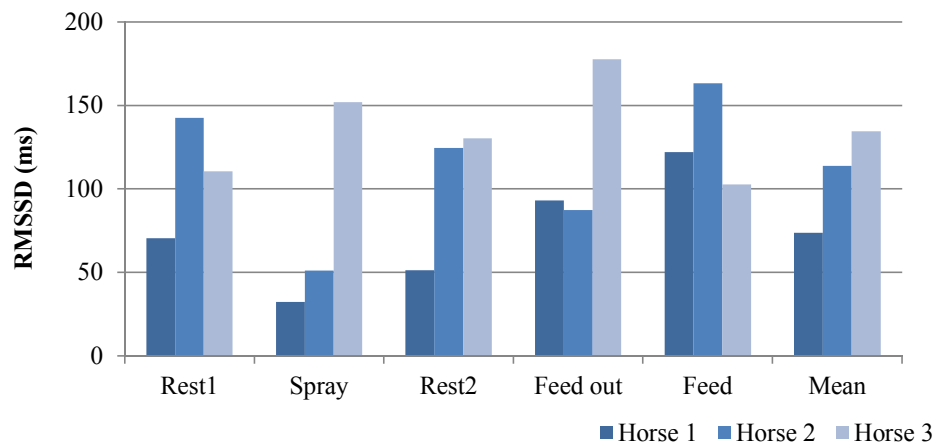
Figure 2.4. Inter-individual RMSSD (square root of the mean squared differences between adjacent inter-beat intervals) per time-period and over all mean. Differences were not significant (Kruskal-Wallis).

A non-parametric Kruskal-Wallis test was carried out to compare HRV for individual horses using the average data from all time-periods. Individual differences in HRV were not significant, H(2) =4.94, *p*=0.85 for SDNN and H(2) =4.94, *p*=0.85 for RMSSD. It was not possible to reject the null hypothesis.

2.3.3. Discussion

This pilot study indicated that variations in HRV occurred in response to external situations differing in their emotional valence and that numerical differences in HRV existed between individual horses.

HRV for Horse 2 followed a similar pattern to that of Horse 1 particularly during the Spray period when both horses recorded their lowest levels of HRV. Horse 1 recorded substantially lower HRV than Horse 3 during both 'Rest' periods, and the 'Spray' and 'Feed out' time-periods. As previously reported, low HRV has been associated with negative emotions in both human and animal studies and it is therefore possible that Horse 1 interpreted these periods more negatively than Horse 3. In contrast, it was interesting to note that during the 'Feed' period when the food was consumed, which it is reasonable to assume was a pleasant experience, HRV for Horse 1 increased beyond that of Horse 3.

Behaviour during the study was observed but not recorded for analysis. All three horses were observed to be highly behaviourally active during the 'Feed-out' period, more so than at any other time. Horses were observed sniffing, snorting, nickering, whinnying, circling the stable, leaning over the stable door towards the feed bucket, head tossing, licking and chewing, kicking the stable door and attempting to undo the stable door latch with the lips. Such behaviour has been previously reported under similar arousal-inducing conditions by Bachmann *et al.* (2003) and Nagy *et al.* (2009). Interestingly, the level of behavioural activity corresponded positively with the high levels of HRV recorded for all three horses during this period, and were exceeded only by HRV levels during the period of food consumption. Placing the food within visual and

olfactory range but beyond physical reach was intended as a negative stimulus to provoke a 'frustration' response. Observing this level of behavioural activity in isolation may be interpreted as such and it is anecdotally viewed as 'negative' behaviour that is often discouraged at feeding times. The high levels of HRV obtained at this time however, would suggest that this behaviour is indicative of a positive experience, such as anticipation of a positive reward which enhances and enriches rather than diminishes the horses' experience prior to meal-times.

2.3.4. Conclusion

Whilst these data were from a very small sample set it is possible to conclude that individual horses have numerically differing levels of HRV at rest which could reflect their underlying affective state, and that differences in emotional response may occur during positive and negative experiences according to an individual's interpretation of external events. This study also highlighted the importance of recording behavioural footage for accurate data analysis, and raised the question as to whether horses express outward signs of 'emotion' in their behaviour which are a true reflection of their internal physiological function. Measuring HRV data alongside behavioural observations to assess emotional responses to different situations was therefore considered worthy of further investigation.

Chapter Three

HRV and Behavioural Responses to a Positive Reward in the

Form of Companionship

3.1. INTRODUCTION

Research has demonstrated that positive rewards have an emotional function by inducing subjective feelings of pleasure and hedonia (Schultz, 1997). Repeated pairing of the cue and reward followed by an increased time interval between the two can result in the development of anticipatory behaviour and is thought to bring forward activation of the dopamine neurons to the time of cue onset (Blackburn *et al*., 1989; Schultz, Dayan and Montague, 1992). Therefore, in addition to the reward itself, reward expectancy may trigger dopamine release and serve to counteract stress (de la Fuente-Fernández *et al*., 2002). Anticipatory behaviour in other species has included increased activity levels, time spent in the front of the cage and forward, erect ear position in foxes (Moe *et al*., 2006), increased play behaviour and reduced aggression in piglets (Dudink *et al*., 2006). Studies measuring HRV during anticipatory behaviour are scarce but Imfeld-Mueller *et al.* (2011) found HRV in pigs to be higher during the anticipatory phase regardless of whether the situation resulted in a positive or negative experience. In addition to the amount an animal moves and the number of different activities performed during anticipation, this indicates that a physiological response also occurs during the anticipatory phase, which taken together may be indicative of affective state.

Social interactions, in addition to food and sexual behaviour are considered by Van der Harst and Spruijt (2007) to be rewarding to all animals. The reintroduction of a social partner has previously been observed in heifers; motor activity, vocalisations and HR decreased when a familiar partner was introduced after a short-isolation period (Boissy and Le Neindre, 1997). As horses are a highly social species (McCort, 1984; Goodwin, 1999), routinely subjected to long periods of physical isolation (Mellor *et al*., 2001; Hotchkiss, Reid and Christley, 2007), social companionship was used as a positive reward in this study. Baseline HRV data collected prior to the study was compared with that obtained during anticipation of and in receipt of a positive reward. The horses' behaviour was recorded throughout the study to examine the relationship between behaviour and HRV.

3.2. SUBJECTS

Twelve adult geldings were selected from Brackenhurst Equestrian Centre. Horses were chosen in pairs based on their preference for a particular associate according to prior observation of their proximity to neighbouring horses in the field. Over a period of one hour the horses were observed at liberty in the field and selected in pairs based on their nearest neighbour whilst grazing and/or their preference for a particular partner to engage in mutual grooming behaviour (for definition see **Appendix 1**). From these observations it was possible to identify three pairs from a group living out at grass 24 hours per day and a further three pairs of horses that were housed in neighbouring stables during the day (to limit grazing) and turned out in a field with other horses overnight. The intention being that the existence of a pair-bond would be advantageous when allowing access to the preferred partner as a reward.

3.3. TRAINING PROCESS

Prior to the study commencing, the study protocol gained ethics approval and appropriate risk assessments were undertaken. A crew yard, open on three sides and measuring 9.4m x 10m with a wood shaving bed over concrete flooring was used for both the training process and main study (**Figures 3.1** and **3.2**). One horse from each pair (**Table 3.1**) underwent training to associate a conditioned stimulus (CS) in the form of an audible cue (doorbell) with entry of the 'paired' horse as the unconditioned stimulus (US, reward) into the crew yard.

Table 3.1. Profile of subjects

Horse	Age (years)	Height (hands)	Breed
Conan	11	17	Irish Draught x Thoroughbred
Rufus	14	16	Warmblood
Beamish	13	16.1	Irish Draught x Thoroughbred
Dixon	11	17.2	Irish Draught
Hovis	9	16	Thoroughbred
Desmond	8	16.1	Irish Draught x Thoroughbred
Mean	11		
St. Dev.	2.3		

The horse acting as US was temporarily stabled out of visual and olfactory contact and was led using a head collar and lead rope into the crew yard by the handler. The doorbell was activated by the handler using a wireless remote control hidden from the horses' view and the chiming unit was connected to mains electricity on the central pillar at the front of the crew yard (**Figure 3.1**) next to the gateway where the paired horse would enter.

Figure 3.1. Detail of crew yard, showing gateway and position of chiming unit (circled).

A delayed conditioning process took place in order to gradually increase the time lag between the CS and US from 0 to 5 minutes. Day one of the conditioning process involved the CS being activated as the US entered the crew yard (10 trials). Subsequently, the interval between activation of the CS and arrival of the US was increased (trace conditioning, Lieberman, 1993): 10 trials of a 60s interval and 10 trials of a 120s interval. Day two consisted of 10 trials of a 180s interval, 10 trials of a 240s and finally 10 trials of a 300s interval (**Table 3.2**). This procedure was repeated for each pair of horses. As described in **Chapter 2: Section 2.1**, baseline HRV measurements were obtained from each of the participants prior to the commencement of the study whilst in their usual stable or crew yard and when turned out to pasture.

3.4. TEST AREA AND APPARATUS

The conditioned horse was fitted with HRV monitoring equipment attached to the thorax with an elastic girth throughout both the main study and the training process. The positive electrode was located at the left shoulder and the negative electrode at the lower left thorax as described in **Chapter 2: Section 2.3.1** (pilot study). Behavioural footage was captured via a continuous recording schedule using a Hitachi DVD/HDD digital video camera which was situated opposite the crew yard gate (**Figure 3.2**). The camera was mounted on a table and tripod with the head at a height of 2 metres. A 'Fast Time' digital stopwatch was used to synchronise activation of HRV and video recording equipment, activation of the audible cue and entry/removal of the 'paired' horse. Horses had access to water, but not food during the conditioning and main study periods.
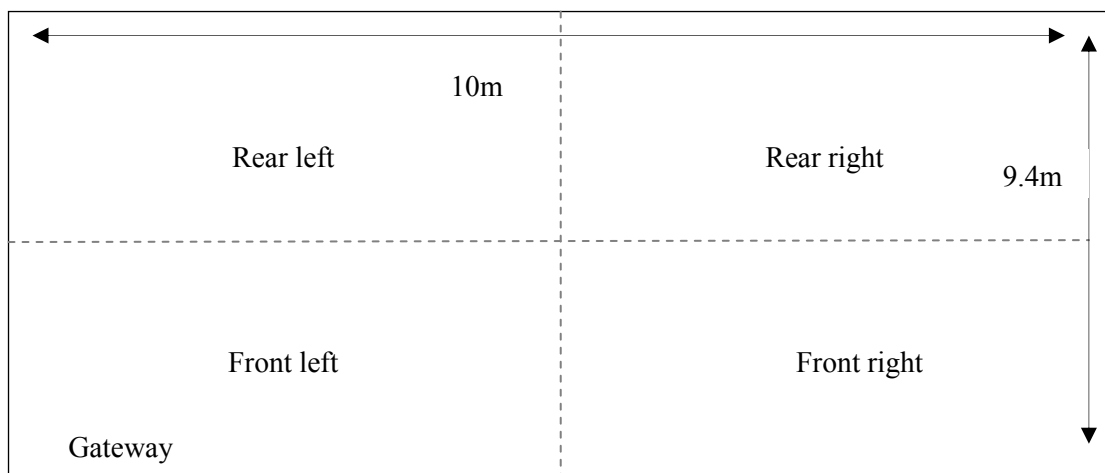
Figure 3.2. Diagram of crew yard showing point of entry at the gateway, segments used for behavioural analysis and position of video recording equipment.

3.5. STUDY PROTOCOL

Following the conditioning sessions on Days 1 and 2, the study for each horse took place at the end of Day 3 (**Table 3.2**). The conditioned horse was brought from its respective stable/field, placed alone in to the crew yard and fitted with HRV recording equipment. Out of sight of the conditioned horse, the companion horse was then taken to a holding stable elsewhere on the

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yard, out of visual and olfactory contact. After the conditioned horse had been left alone in the

crew yard for 1hr 45 minutes the handler activated the video camera for recording then entered

the crew yard and activated the HRV recording equipment which was synchronised with the

stopwatch. At 1hr 55 minutes the five minute pre-cue period began (PC) and at 2hrs the audible

cue (doorbell) was activated which initiated the five minute post-cue (PoC) period. At 2hrs 5

minutes the companion horse was brought into the crew yard and released to interact freely with

the conditioned horse which initiated the five minute post-reward (PR) period. The horses were

allowed to remain in the crew yard together until 2hrs 20 minutes when the handler ceased

video recording and removed the HRV recording equipment. The horses were then returned to

their respective stables/field.

Table 3.2. Detail of trials carried out during delayed conditioning process (Days 1 and 2), timing of study procedure and data collection (Day 3).

Day 1	Day 2	Day 3	
Delayed conditioning process Time lag between CS & US increased from 0 to 5 minutes		*Data collection*	
10 trials 0s	10 trials 180s	0 mins:	Conditioned horse placed alone in crew yard
10 trials 60s	10 trials 240s	1hr 45 mins:	Video and HRV recording activated
10 trials 120s	10 trials 300s	1hr 55 mins:	Pre-cue (PC) period begins
		2hrs:	Audible cue (doorbell) activated, post-cue (PoC) period begins
		2hr 5 mins:	Companion horse arrives in crew yard, post-reward (PR) period begins
		2hr 10 mins:	End of PR period
		2hr 20 mins:	Video and HRV recording ceased, horses returned to respective stable/fields.

3.6. BEHAVIOURAL DATA ANALYSIS

Using The Observer behavioural analysis software, an ad-libitum sampling regime was adopted

in order to calculate the behaviour of each of the six conditioned horses during the three time-

periods (PC, PoC and PR). The duration of behavioural states for each time-period (position in

and movement around the crew yard and ear position; **Appendix 2)** and frequency of

behavioural events during the PR period (interactivity behaviour; **Appendix 1**) were obtained. Single incidences of vocalisation (whinny and nicker) were observed but excluded from data analysis. Durations of raw data for each horse in time format (seconds) were then converted to a decimal number value using Microsoft Excel and imported into SPSS 15.0 for statistical analysis.

Kolmogorov-Smirnov tests for normality were carried out on each data set and statistical tests chosen accordingly. Differences in behavioural measurements between each time-period (PC, PoC and PR) were tested using the non-parametric Friedman's ANOVA followed by a Wilcoxon signed ranks test where appropriate.

Preliminary viewing of video footage indicated that differences in behaviour may occur between horses from different management regimes (horses stabled during the day versus horses living out at grass). These data were not normally distributed so were analysed on the basis of management regime using a Mann-Whitney U test. The frequency of bouts of interactive behaviour observed during the PR period were also analysed according to management regime. These data were normally distributed and were analysed using an independent samples t-test and all analyses were two-tailed.

3.7. HRV DATA ANALYSIS

R-R interval data were analysed using Kubios HRV analysis software. To remove trend components, data were de-trended and an artefact correction was made as described in **Chapter 2: Section 2.1.** Time domain analyses were then calculated from mean R-R interval data for each horse and for each time-period; stable baseline (SB), field baseline (FB), PC, PoC and PR measurements. For each data set SDNN and RMSSD were analysed using SPSS 15.0. Kolmogorov-Smirnov tests for normality were carried out on each data set and statistical tests chosen accordingly.

Differences in HRV measurements between time-period and in comparison with baseline measures were tested using a one-way repeated measures ANOVA. Differences in HRV between individual horses were investigated using a one-way between-groups ANOVA. Relationships between HRV and behaviour were investigated using Spearman's rank order correlation. Since preliminary analysis of video footage had indicated that differences in behaviour may exist related to management regime, an additional null hypothesis was proposed stating that there was no difference in HRV according to management regime. This was investigated using a mixed between-within subjects ANOVA to establish whether there was an effect of management regime or time-period and if there was an interaction between management regime and time-period.

3.8. RESULTS

3.8.1. Heart rate variability analysis per time-period

Mean SDNN values (**Figure 3.3**) were highest during the PR period (62.63±22.75ms) with the lowest values recorded during the PC period (54.08±17.61ms). A one-way repeated measures ANOVA was conducted to compare HRV between each time-period; stable baseline (SB), field baseline (FB), pre-cue (PC), post-cue (PoC) and post-reward (PR) measures. SDNN was not found to differ significantly between each period, Wilks' Lambda = 0.67, $F_{(4, 2)}$ =0.25, p=0.89.

Mean RMSSD values (**Figure 3.3**) did not vary greatly, ranging from 54.93±19.10ms during the PC period to 58.40±21.87ms for FB measurements. RMSSD was not found to differ significantly between each period, Wilks' Lambda = 0.62, $F_{(4, 2)}$ = 0.31, p=0.85. It was therefore not possible to reject the null hypothesis.
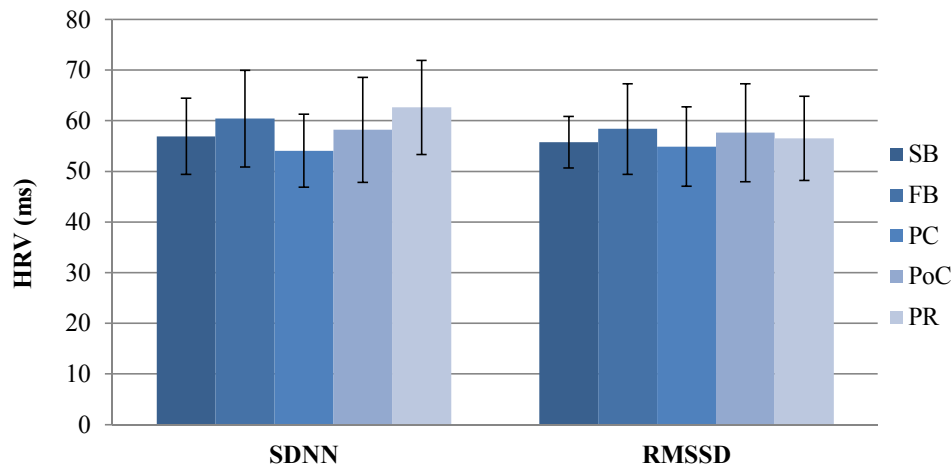
Figure 3.3. Mean heart rate variability (HRV) values (±SE) for baseline measures taken in the stable (SB) and field (FB) and for pre-cue (PC), post-cue (PoC) and post-reward (PR) time-periods. HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (one-way repeated measures ANOVA).

3.8.2. Inter-individual heart rate variability

Considerable inter-individual variations were observed in SDNN and RMSSD for baseline HRV measurements and throughout the study. Using mean HRV data from each time-period and baseline values, a one-way between-groups ANOVA was conducted to explore the differences in HRV between individual horses. There was a significant difference in HRV between horses: SDNN F (5, 24) =7.3, $p<0.001$; RMSSD F (5, 24) =14.1, $p<0.001$. The difference in mean HRV measurements between individual horses was large. Using eta squared, large effect sizes were found for SDNN (0.60) and RMSSD (0.75), respectively.

Post-hoc comparisons using the Bonferroni test revealed that SDNN for Horse 1 (31.90±9.67ms) was significantly lower than for Horse 2 (81.16±11.91ms, $p<0.001$), Horse 4 (66.58±5.48ms, $p=0.011$) and Horse 6 (66.02±18.68ms, $p=0.012$). SDNN for Horse 3 (48.38±11.73ms) was also significantly lower than Horse 2 ($p=0.018$; **Figure 3.4**).

Post-hoc analysis of RMSSD values using the Bonferroni test values revealed that significantly lower RMSSD was obtained for Horse 1 (29.96±5.96ms) than for Horse 2 (68.72±13.58ms, $p<0.001$), Horse 4 (77.52±11.12ms, $p<0.001$), Horse 5 (64.80±13.48ms, $p<0.001$) and Horse 6 (52.22±7.50ms, $p=0.032$). Horses 3 and 6 also had lower RMSSD than Horse 4 ($p=0.001$; $p=0.010$; **Figure 3.4**). It was therefore possible to reject the null hypothesis.
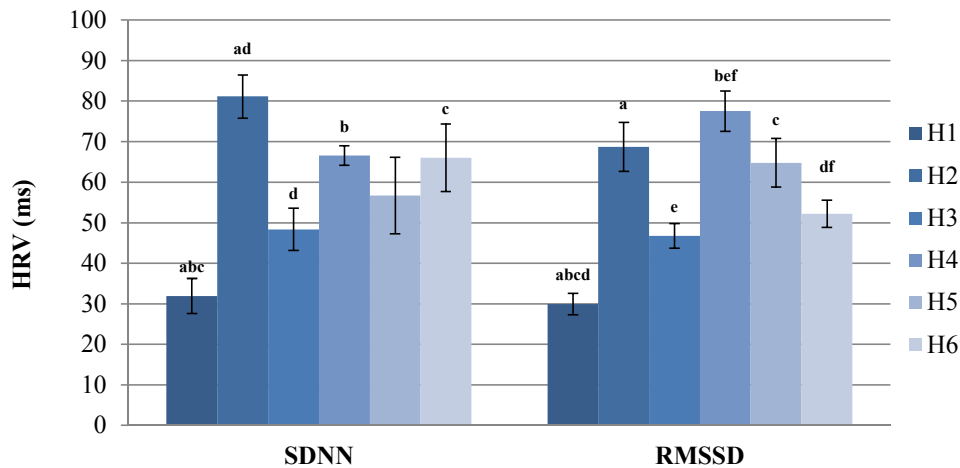
Figure 3.4. Inter-individual differences in mean heart rate variability (HRV) using the entire set of HRV data (±SE). HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Paired superscript letters denote significant inter-individual differences for each variable (one-way ANOVA and Bonferroni post-hoc comparison; p≤0.05).

3.8.3. Effect of management regime on HRV

As preliminary analysis of video footage indicated that differences in behaviour may occur according to management regime, HRV data were also explored on this basis (i.e. stabled (S) versus grass-kept horses (G)). Analysis of descriptive statistics revealed that HRV for S horses was higher than G horses for all time-periods (e.g. for SDNN **Figure 3.5**).
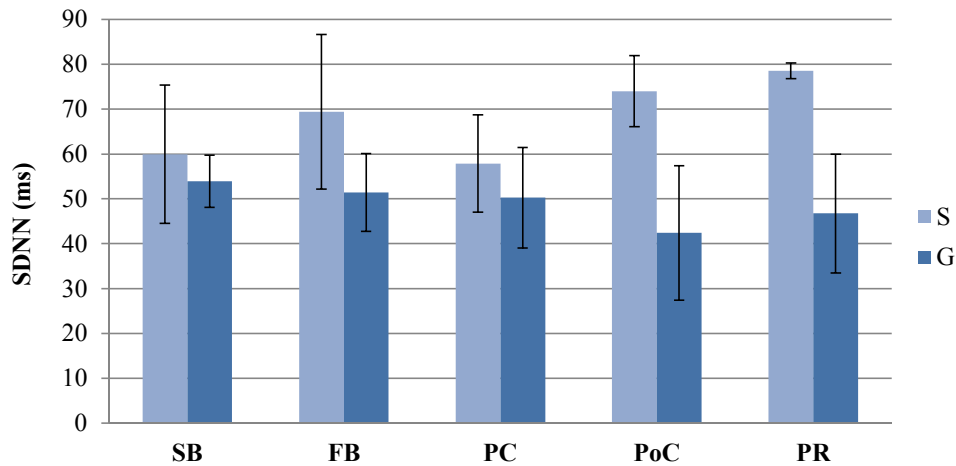
Figure 3.5. Descriptive statistics showing mean SDNN (standard deviation of inter-beat intervals; ±SE) comparing stable (S) and grass-kept horses (G) according to time-period; baseline measures taken in the stable (SB) and field (FB) and for pre-cue (PC), post-cue (PoC) and post-reward (PR) time-periods.

Data were normally distributed so a mixed between-within subjects ANOVA was conducted to assess the effect of management regime on HRV for each time-period (SB, FB, PC, PoC and PR).

For SDNN, a significant interaction was present between management regime and time-period, Wilks' Lambda <0.001, F (4, 1) = 657.93, p=0.029, partial eta squared = 0.99. This indicates that the HRV response over time differed according to management regime. There was a trend for the main effect of time-period, Wilks' Lambda = 0.002, F (4, 1) = 140.24, p=0.063, partial eta squared = 0.99. The main effect of management regime was not significant F (1, 4) = 2.39, p=0.20, partial eta squared = 0.37.

For RMSSD, there was no significant interaction between management regime and time-period, Wilks' Lambda = 0.54, F (4, 1) = 0.21, p=0.90, partial eta squared = 0.46 and no significant main effect of time-period, Wilks' Lambda = 0.60, F (4, 1) = 0.17, p=0.93, partial eta squared

= 0.40. The main effect of management regime was also non-significant F (1, 4) = 0.505, p=0.52, partial eta squared = 0.112.

3.8.4. Behavioural results per time-period

During the PC period the predominant position in the crew yard was standing in the front left quadrant facing the gateway (171±84sec) and the least amount of time was spent in the rear right quadrant (0sec). During the PoC period horses spent most time in the front right quadrant of the crew yard (152±153sec) and the least in the rear right quadrant (0sec). In the PR period horses were predominantly positioned in the front left quadrant facing away from the gateway (133±119sec) and spent the least amount of time in the rear right quadrant (2±5sec).

A Friedman's ANOVA was carried out to analyse behavioural data according to time-period; PC, PoC and PR. The results indicated that there was a significant difference in the duration of time horses were positioned in the front left section of the crew yard, facing towards the gate, across the three time-periods, X^2 (2) =8.10, p=0.017 (**Figure 3.6**).
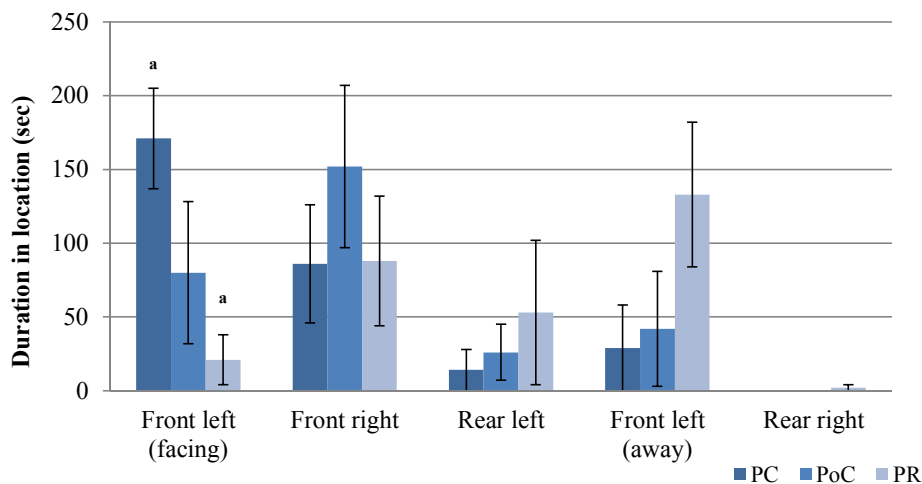
Figure 3.6. Mean duration of location in crew yard for each time-period (±SE); pre-cue (PC), post-cue (PoC) and post-reward (PR). For analysis, the crew yard was divided into quadrants and when positioned in the front left quadrant, orientation towards or away from the entrance gateway was noted. Paired superscript letters denote significant differences between time-periods (Friedman's ANOVA and Wilcoxon; p≤0.05).

Post-hoc analysis via a Wilcoxon signed-rank test revealed that horses spent significantly longer in this position during the PC period (171±84sec) than the PR period (21±41sec), $z = -2.21$, $p=0.027$, with a medium effect size ($r = 0.64$).

Whilst positioned in the front left quadrant facing towards the entrance gateway, the horses' head position was analysed according to their position over or behind the gate and whether they were orientated left, right or centre (**Table 3.3**). A Friedman's ANOVA indicated that there was a significant difference in the duration of time horses stood with the head held over the gate and orientated to their right (towards the direction the horses were led to/from the crew yard) across the three time-periods, X^2 (2) =6.13, $p=0.047$. A Wilcoxon signed-rank test revealed a greater duration of this behaviour during the PC period (40±37sec) than PR period (0sec), $z = -2.02$, $p=0.043$, with a medium effect size ($r = 0.58$). As significant differences in location and position were evident between time-periods it was possible to reject the null hypothesis.

Table 3.3. Duration (seconds) of head orientation in relation to the entrance gateway when located in the front left quadrant of crew yard (mean±SD). Paired superscript letters denote significant differences between time-periods (Friedman's ANOVA and Wilcoxon; p≤0.05).

	PC	PoC	PR
Over left	8±10	9±22	0
Over right	40±37[a]	14±29	0[a]
Over centre	30±32	25±56	0
Behind left	24±29	2±4	5±12
Behind right	16±27	13±20	7±10
Behind centre	52±122	17±43	9±22

During the PC period, ear position was predominantly to the front for both left and right ears (83±32sec and 65±55sec, respectively). The left ear was positioned predominantly to the side (43±74sec) and right ear to the front (67±73sec) during the PoC period. During the PR period both ears were mainly positioned to the side (75±85sec and 76±91, respectively). Friedman's ANOVA tests revealed that ear positions were not significantly affected by time-period.

Horses carried out more standing behaviour during the PC period (265±33sec) and walked most during the PoC period (37±67sec) compared with the other time-periods. Friedman's ANOVA tests confirmed that the duration of standing and walking behaviours were not significantly affected by time-period.

3.8.5. Effect of management regime on behaviour

As preliminary observation of video footage had indicated that behaviour may differ according to management regime (i.e. stabled (S) versus grass (G)), data were analysed to examine behavioural results on this basis. Data were not normally distributed so Mann-Whitney U tests were conducted to compare behavioural observations between groups using the total data from all three time-periods.

A significant difference in position within the crew yard was revealed between groups, with S horses (184±272sec) spending longer in the rear left portion of the crew yard than G horses (0sec), $U = 18.0$, $z = -2.52$, $p=0.012$, with a medium effect size ($r = 0.59$).

A Mann-Whitney U test revealed that, over the 15 minutes of data collection, S horses spent significantly less time standing still (721±145sec) than G horses (860±25sec), $U = 21.0$, $z = -2.057$, $p=0.04$, with a small effect size ($r = 0.48$). S horses also spent significantly more time walking (176±141sec) than G horses (43±30sec), $U = 7.5$, $z = -3.181$, $p=0.001$, with a medium effect size ($r = 0.75$; **Figure 3.7**).
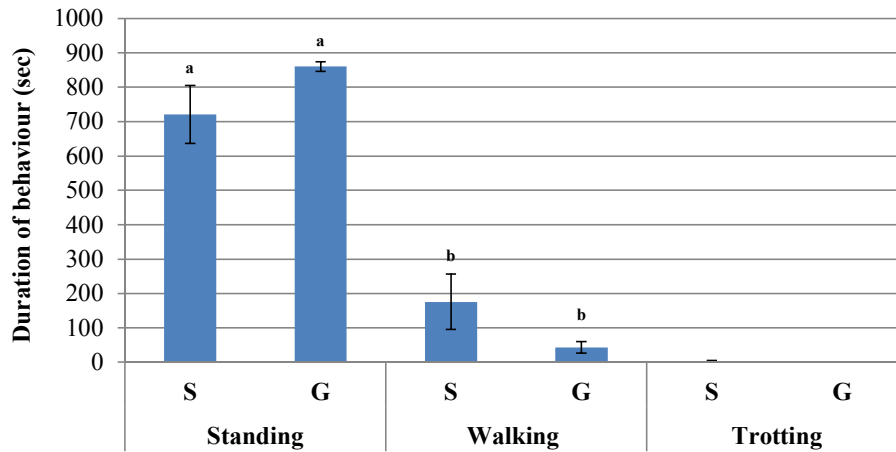
Figure 3.7. Mean duration of movement around crew yard according to group for the entire study period (±SE); Stabled (S) and Grass (G). Paired superscript letters denote significant differences between groups (Mann-Whitney U; p≤0.05).

Pair-interaction activity data were also obtained for the post-reward period (**Table 3.4**). Data were analysed according to management regime using an independent samples t-test. S horses performed a significantly greater frequency of interactive behavioural events (5.88±3.46) with their paired companion than G horses (0.75±0.61), t (10) =3.59, p=0.014. The magnitude of the difference in the means (mean difference = 5.14, 95% CI: 1.52 to 8.76) was medium (eta squared 0.6). It was therefore possible to reject the null hypothesis, as differences in behaviour according to management regime were observed.

Table 3.4. Mean frequency of bouts of interactive behaviour between pairs of horses during the post-reward period according to group; Stabled (S) and Grass (G). Paired superscript letters denote significant differences between groups (independent samples t-test; p≤0.05).

Interactive behaviour	S	G
Play fight	8.0	1.0
Mutual groom	4.0	0.0
Chase	11.0	1.0
Close proximity	7.0	1.5
Sniff nose	1.3	0.0
Sniff body	4.0	1.0
Mean frequency	**5.88[a]**	**0.75[a]**

3.8.6. Relationship between behaviour and HRV

Kolmogorov-Smirnov tests for normality carried out on each data set revealed that 11 out of 28 behavioural measurements were non-normally distributed. Non-parametric Spearman's rank order correlations were therefore performed to determine whether a statistically significant relationship existed between HRV (SDNN and RMSSD) and behaviour during each time-period (PC, PoC and PR).

During the PC period, low SDNN was associated with a longer duration of the left ear being orientated to the rear, but not at statistically significant levels (r_s=-0.77, p=0.72; **Figure 3.8**).
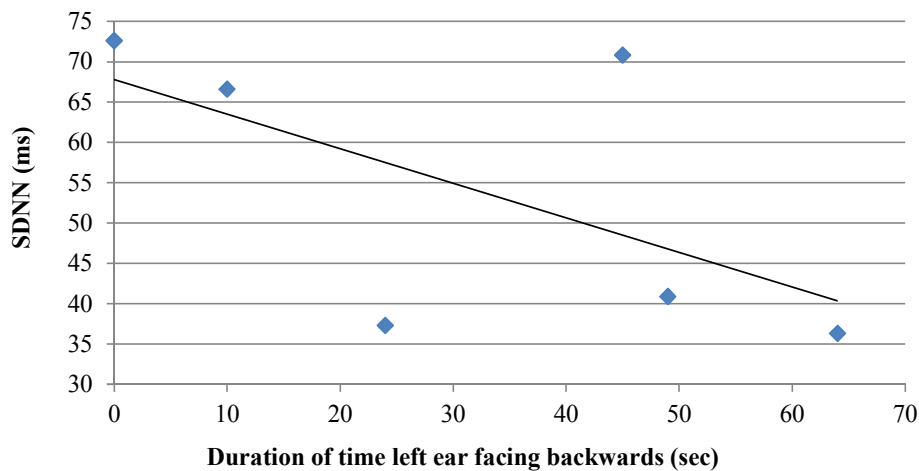
Figure 3.8. An inverse relationship was found between ear position and SDNN (standard deviation of inter-beat intervals) during the pre-cue period. The relationship was not significant (Spearman's rank order correlation).

There were no significant correlations present between HRV and behaviour during the PoC period. During the PR period high RMSSD was associated with a longer duration of play fighting behaviour which enabled the null hypothesis to be rejected (r_s=0.89, p=0.041; **Figure 3.9**). During this period higher HRV was also associated with a longer duration of time spent in the front left quadrant of the crew yard facing away from the gate, but not at statistically significant levels (r_s=0.75, p=0.84; **Figure 3.10**).
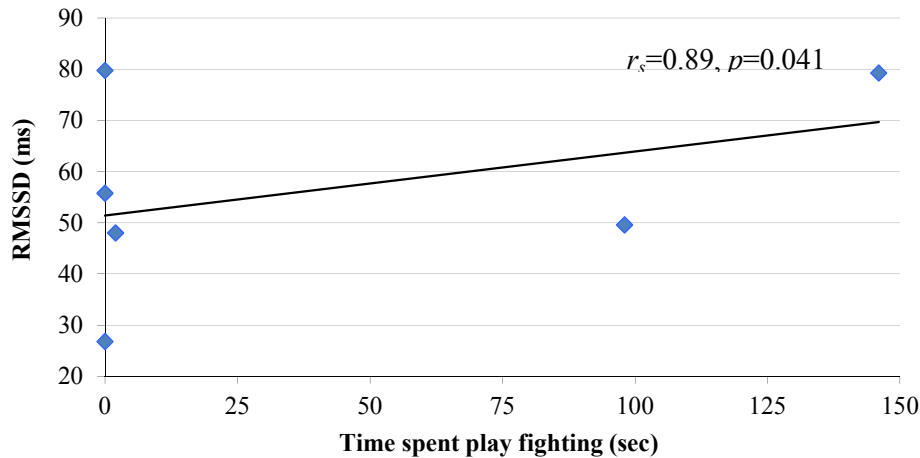
Figure 3.9. A significant positive relationship was found between time spent play fighting and RMSSD (square root of the mean squared differences between adjacent inter-beat intervals) during the post-reward period (Spearman's rank order correlation; p≤0.05).
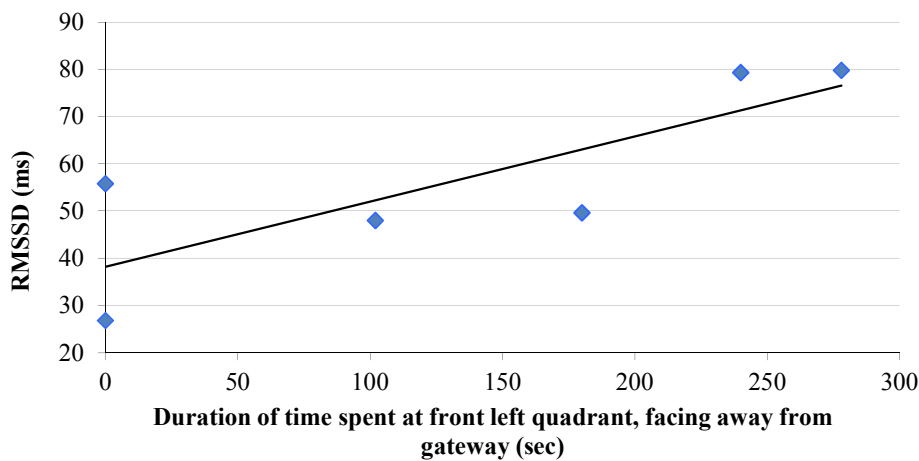
Figure 3.10. A positive relationship was present between the horses' position in the crew yard and RMSSD (square root of the mean squared differences between adjacent inter-beat intervals) during the post-reward period. The relationship was not significant (Spearman's rank order correlation).

3.9. DISCUSSION

Analysis of behavioural data revealed that the horses spent significantly longer positioned in the

front left portion of the crew yard, facing towards the gate and with their head over the gate

orientated to their right during the pre-cue period than the post-reward period. This suggests that their attention was focussed in the direction the companion horse arrived from which was the right hand side of the passageway. These behaviours may also be analogous to anticipatory behaviour observed in other species; Moe *et al*. (2006) for example, report an erect ear position and more time spent at the front of the cage in foxes expecting a positive reward. Pond *et al.* (2010) reported a similar behavioural stance prior to receiving an appetitive reward where horses typically had their ears forward, stood by the stall door watching the aisle for the grain and hay cart to come to their stall. In a study of pre-feeding activity in horses, Cooper *et al.* (2005) also reported that the predominant activity was standing alert (32.3% of time observed) during the hour before feeding. Although Van Dierendonck *et al.* (2010) also cite increased activity, in the form of locomotion and investigation, as being indicative of anticipatory behaviour in horses expecting an appetitive reward, this behaviour was not evident in the present study.

It was interesting to note that the horses' attention appeared to be orientated in the direction the companion horse arrived from *prior* to activation of the audible cue. Likely explanations for this behaviour include the possibility that the horses maintained this position, which could be described as expectant or vigilant, when looking for other horses as a direct response to being isolated. Then upon activation of the bell signifying the arrival of their companion, they were able to relax and abandon or lessen this stance. Alternatively, although highly unlikely, it could indicate that instead of reacting to the audible stimulus, the horses identified an inadvertent 'cue' from the handler that acted as a trigger for early anticipatory behaviour prior to the activation of the auditory stimulus.

Analysis of behavioural data according to management regime revealed that throughout the study, stabled horses performed significantly less standing behaviour and spent longer walking around the crew yard than horses living at grass. These findings are in agreement with the observations of Mal *et al.* (1991a) who reported that horses kept in confined and isolated

environments travelled further, exhibited a greater number of trotting bouts and spent less time standing still during an open field test than horses maintained on pasture with conspecifics.

During confinement in the stable, horses may experience a rise in internal motivation for exercise which is reflected as rebound activity when given the space to move around freely. In the present study, analysis of HRV data revealed that the impact of time-period was significantly influenced by management regime and stable-kept horses tended to record greater variability in SDNN throughout the study. Previous mammalian studies such as Grippo *et al.* (2007) provide evidence of social isolation inducing reduced HRV along with depression and anxiety-like behaviours in animals. In addition, cognitive bias studies have shown that sheep released from restraint (Doyle *et al.,* 2010) and shearing (Sanger *et al.,* 2011) display a positive emotional state compared with control animals. Although sample numbers in the current study were low it is cautiously proposed that stabled horses might benefit from the space and freedom granted by the crew yard as a contrast to their normal daytime stabled regime and that the benefit may be greater for stabled than grass-kept horses.

During the post-reward period, stabled horses were observed carrying out significantly more bouts of interactive behaviour with their companion than grass-kept horses, including simply being in close proximity to each other, mutual grooming and engaging in chasing or play-fighting behaviour. An increased occurrence of specific behaviours after a period of prevention has been shown in other species (e.g. play and locomotion behaviour in cattle by Dellmeier, Friend and Gbur ,1985 and Jensen, 1999). Incidences of play similar to the current study were also reported by Christensen *et al.* (2002a) who observed greater levels of mutual grooming and play behaviour in singly housed horses than those housed in groups, once free interaction was allowed.

Play behaviour in animals has long been identified as a potential welfare indicator (Boissy *et al.,* 2007; Held and Špinka, 2011) and has been associated with positive feelings (Jensen, 1999).

Held and Špinka (2011) propose that play indicates the absence of fitness threats, acts as a reward, brings immediate psychological benefits and long-term fitness and health benefits thus improving current and future welfare and is socially contagious and therefore capable of spreading good welfare in groups. However, according to Held and Špinka (2011) play can also increase in stressful situations, in response to reduced parental care, or as a rebound after a period of deprivation and therefore does not consistently reflect favourable environmental conditions. In horses, play behaviour is often associated with foals and yearlings as a developmental activity but also occurs in adults as a means of strengthening bonds between individuals (McDonnell and Poulin, 2002). In the present study, correlation analysis of HRV and behavioural data for all horses during the post-reward period, irrespective of management regime, revealed a significant positive relationship between RMSSD and the duration of play fighting behaviour. This provides evidence that social interaction, particularly engaging in play, can be associated with a positive emotional experience. As a welfare indicator, play may signal both the absence of bad welfare and the presence of good welfare, thus covering both extremes of the welfare spectrum and acting as a tool to improve it.

Social grooming in horses is believed to play a role in social affiliation (Crowell-Davis, Houpt and Carini, 1986). It has also been proposed as a means of reducing stress as Hogan, Houpt and Sweeney (1988) identified a negative correlation between incidences of mutual grooming and enclosure size in Przewalski horses. The effect of management regime on the level of interactive behaviour between pairs in this study may therefore relate to a build-up of motivation (a rebound effect) for socially deprived horses, which did not exist for grass-kept horses. A similar observation has been documented in rats by Panksepp and Beatty (1980) who reported an increase in play behaviours following social deprivation and a reduction upon social satiation.

Differences in HRV between time-periods were not statistically significant. Numerically, the lowest SDNN and RMSSD measurements were both recorded during the pre-cue period. During

this time there was a strong inverse relationship between SDNN and the length of time the left ear was orientated backwards, which approached statistical significance. Ear position is considered to be a good outward indicator of emotional state in horses (Houpt, 2005) and may be useful in assessing emotional valence in other animals. Boissy *et al.* (2011) were able to relate ear posture in sheep with specific emotional contexts; pointing the ears backward was associated with unfamiliar, unpleasant and uncontrollable situations likely to elicit fear. On this occasion however, ear position could not be related physiologically to an overall emotional response to a particular time-period, but may be indicative of a trend towards a negative affective state concomitant with other significant behaviours observed during this period.

The fact that there was no significant difference in HRV between time-periods infers that the interpretation of events during the pre-cue and post-cue periods had no greater or lesser emotional valence than post-reward or baseline periods. There are several possible explanations as to why HRV was not significantly affected by time-period. Although the horses were emotionally responsive to each time-period, as riding school horses they may have become accustomed to long periods of isolation, with the removal and return of companions throughout the day being a regular and familiar occurrence. In addition, the inter-individual variation in HRV recorded during different study conditions and for baseline values may mean that using average HRV data from the group as a whole neutralised any effect of time-period. Most studies using HRV measurements in horses omit detail as to the presence of inter-individual variation in between-subjects measurements. It would appear from this study however, that individual variation is normal.

There was no evidence that the subjects associated the bell with the arrival of the companion horse. It is possible that more conditioning sessions and associative training may be required in order for this to be achieved. Reward-based operant conditioning studies are often used in equine learning and cognition research (e.g. Ninomiya *et al.*, 2007), but the majority of anticipatory behaviour studies are carried out using smaller mammals and incorporate hundreds

of conditioning sessions per animal (van der Harst, Baars and Spruijt, 2003; van der Harst *et al.*, 2003; Vinke, Van Den Bos and Spruijt, 2004; van der Harst, Baars and Spruijt, 2005; Dudink *et al.*, 2006). In future studies, increasing the number of conditioning sessions may strengthen the association between the cue and reward.

As mentioned previously, considerable inter-individual variations in HRV were found for baseline measurements and throughout the study. When HRV data for individual horses were collated for the entire study period significant individual differences were noted which may be related to underlying emotional states; Horses 1 and 3, for example, had significantly lower SDNN and RMSSD than the other subjects. It is interesting to note that Horse 1 was occasionally observed crib-biting during the study, a common stereotypical behaviour in horses. Previous studies have linked high levels of sympathetic nervous system activity via HR (Minero *et al.*, 1999) and low levels of parasympathetic nervous system activity via HRV activity (Bachmann *et al.*, 2003) in horses with stereotypic behaviours. Such studies are scarce, however, and further research is necessary to establish a link between physiological measures and affective state.

Previous studies and observation of Horse 3 reveal a high degree of sensitivity, as evidenced by behavioural reactivity to aversive stimuli. This observation is in accordance with previous studies such as Eager *et al.* (2004) who related HRV components to equine temperament and Visser *et al.* (2002) who suggest that HRV could be a useful measure for quantifying behavioural traits in horses. In addition, Visser *et al.* (2003a) associated low levels of HRV with aspects of handling such as restlessness and resistance behaviour and reluctance to approach a novel object. Conversely, horses with high levels of HRV were found by Visser *et al.* (2003a) to be associated with aspects of handling such as 'bravery' when passing frightening objects and events, low levels of 'spooky' behaviour (the frequency and intensity of shying away from something) and low levels of attentiveness to their surroundings. Although not accounted for in the selection of subjects for the present study, breed differences in HRV have previously been

reported in cattle which were thought to be related to temperament (Hagen *et al.*, 2005). These findings imply that individual characteristics related to factors such as temperament, personality and underlying affective state may influence and be detected by HRV measurements. Further research is therefore necessary to determine whether breed and temperament differences in HRV do exist in horses. These features are explored in **Chapter 7** which investigates factors affecting HRV.

Whilst human studies have suggested a link between positive emotions and increased parasympathetic activity (McCraty *et al.*, 1995), the findings of this study imply that horses that are housed individually indoors – and, therefore, prevented from both physical movement and interaction with conspecifics - may experience a positive emotion when allowed to carry out these activities. As horses are a naturally social and free ranging species this result is unsurprising, but it may indicate that semi-isolated horses gain more 'pleasure' from social interaction and could benefit more from a contrast between isolation and social interaction than horses in permanent social contact with others. Modification of existing management regimes to include the presentation of companionship as a reward for (or in contrast to) social isolation may compensate negative experiences with positive ones and could serve as a tool to counteract the chronic stress associated with individual housing in horses.

3.10. CONCLUSION

Equine welfare science strongly needs additional objective parameters for the assessment of welfare. This study investigated the relationship between HRV and behaviour in horses conditioned to expect a positive reward in the form of social companionship.

HRV was not affected by time-period indicating that for these horses, anticipating the arrival of a companion was no more or less emotionally valenced than spending time in their stable or the field. Interactive behaviour between pairs, particularly 'play' behaviour may be indicative of

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positive emotion after a period of social deprivation. Individual differences in HRV were evident which may be associated with underlying affective state, temperament or breed as has been purported by other researchers. These results indicate that HRV measurements may be a useful non-invasive method of relating outward signs of behaviour with underlying affective states in horses and could prove a valuable way of assessing their reaction to different management practices.

In order to further examine the relationship between HRV and behaviour during anticipation of a positive reward, **Chapter 4** investigates the response of stabled horses to routine feeding, a procedure to which domestic horses appear to become naturally conditioned. **Chapter 5** investigates behavioural and HRV responses to a potentially negatively valenced management practice. The effect of visual horizons and level of social contact offered by different housing designs and management regimes are investigated in **Chapter 6** and some of the potential factors that might affect HRV are investigated in **Chapter 7**.

Chapter Four

HRV and Behavioural Responses of Horses Anticipating an

Appetitive Reward.

4.1. INTRODUCTION

As previously discussed in **Chapter 1: Section 1.5.2.1** and **Chapter 3: Section 3.1**, research has demonstrated that positive rewards have an emotional function by inducing subjective feelings of pleasure and hedonia (Schultz, 1997). In addition to the reward itself, reward expectancy may trigger dopamine release and serve to counteract stress (Blackburn *et al.,* 1989; Schultz, Dayan and Montague, 1992; de la Fuente-Fernández *et al.*, 2002). The expression of anticipatory behaviour such as increased activity levels and play behaviour as well as positioning of the body and facial expressions reveal whether the animal perceives a reward as positive or aversive (Dudink *et al.*, 2006; Moe *et al.,* 2006).

The amount an animal moves and the number of behavioural transitions that the animal performs during anticipation of consummatory behaviour have been identified in various mammalian species as indicators for the assessment of animal welfare (Von Frijtag *et al.*, 2000; Spruijt, van den Bos and Pijlman, 2001; Bos et al, 2003; Van der Harst and Spruijt, 2007) such as rats (van der Harst, Baars and Spruijt, 2003; van der Harst *et al.*, 2003) and mink (Vinke, Van den Bos and Spruijt, 2004; Hansen and Jeppesen, 2006).

As discussed in **Chapter 1: Sections 1.4 and 1.5.2.1,** food is so indispensable to an animals' survival it is reasonable to assume that they are highly motivated to obtain it, that they anticipate it and that its consumption is rewarding. Feeding time, therefore, should be an excitable, apparently positive or eustress situation. However, Bachmann *et al.* (2003) describe 'waiting to be fed' as a common 'stress' situation known to any stabled horse and use the pre-feeding period as an aversive, but neither painful nor frightening stimulus in their studies. Similarly, Desire, Boissy and Veissier (2002) relate the prevention of access to a substrate that allows the performance of a highly motivated behaviour to emotional responses believed to express frustration. It is unsurprising then that feeding time in horses has been associated with the initiation of bouts of stereotypic behaviour (Bachmann *et al.*, 2003; Cooper *et al.*, 2005; Nagy *et*

al., 2009) that are often actively discouraged, as discussed in **Chapter 1: Section 1.5.2.1**. Such behaviours may initially arise as anticipatory, appetitive activities and then become a conditioned response if they are repeatedly rewarded with food after their performance (Cooper and McGreevy, 2002). Stereotypic behaviour in horses can normally be attributed to sub-standard management regimes that prevent the exhibition of natural behaviours and have been related to low levels of HRV and therefore negative emotion by Bachmann *et al.* (2003), but are not necessarily associated with elevated cortisol levels indicative of chronic stress (Pell and McGreevy, 1999). It is not known whether there is a distinction between the underlying affective state during pre-feed stereotypic behaviour and non-feed related stereotypies when there is a difference in causation. If dopamine release is triggered by the expectation of being fed as well as on receipt of food, the exhibition of pre-feeding behaviours may be an indicator of positive affect and serve to enhance welfare rather than detract from it.

Most anticipatory behaviour research lacks physiological data to support behavioural observations, so at best it is only possible to infer the underlying affective state. This study was conducted to investigate both HRV and behavioural responses in horses anticipating a positive reward in the form of food. Horses at Brackenhurst Equestrian Centre are routinely given concentrate feed daily between approximately 5.05pm and 5.10pm. Observation of behaviour prior to the study identified that a period of anticipatory behaviour existed before feeding in the form of vocalisation, orientation and movement of the head and ears, aggression directed towards neighbouring horses and locomotion around the stable. The study aimed to compare baseline HRV data obtained prior to the commencement of the study with that obtained during anticipation of and during consumption of a positive reward. In addition, the horses' behaviour was recorded during the anticipation of feeding in order to investigate whether a relationship existed between behaviour and HRV.

4.2. SUBJECTS

Six mature horses (**Table 4.1**) were selected from Brackenhurst Equestrian Centre. The horses were chosen according to their location which optimised the capture of behavioural footage: horses were housed indoors in a row of open fronted stables (**Figure 4.1**) and, as the study took place after dark, lighting levels in this location were superior to other locations on the premises.

Figure 4.1. Detail of stable design used for maximal capture of behavioural footage.

Table 4.1. Profile of subjects

Horse	Age (years)	Height (hands)	Breed	Sex (Gelding/Mare)
Benson	8	16.2	Irish Draught	G
Blackberry	13	16.2	Irish Draught x Thoroughbred	M
Conan	12	17	Irish Draught x Thoroughbred	G
Dixon	11	17.2	Irish Draught	G
Pye	8	15.2	Cob	M
Tosca	14	15.2	Cob	M
Mean	**11**			
St.Dev.	**2.5**			

Prior to the study, horses were discreetly observed for a period of one hour and were found to be highly behaviourally active for a prolonged period of time prior to receiving their concentrate feed. Horses were observed sniffing, snorting, nickering, box-walking, stretching the head and

neck over the stable door, nodding, licking, sham chewing and kicking the stable door. Pilot testing of HRV and video recording was carried out in order to determine the final protocol.

4.3. STUDY PROTOCOL

Prior to the study commencing, the study protocol gained ethics approval and appropriate risk assessments were undertaken. As described in **Chapter 2: Section 2.1**, baseline HRV measurements were obtained from each of the participants prior to the commencement of the study whilst in their usual stable or crew yard and when turned out to pasture.

Data collection took place over a six day period during December 2008. All horses were bedded on straw and remained in their own stable (measuring 4m x 4m) throughout the process. They had access to water and a hay net attached to a tie-ring positioned at head height on the front (n=1), rear (n=2) or right hand wall (n=3) of the stable.

Approximately 30 minutes prior to feeding each day one horse was fitted with HRV monitoring equipment (Polar Equine RS800G3) attached to the thorax with an elastic girth, which remained in-situ beneath the horse's rug throughout the study. The positive electrode was located at the left shoulder and the negative electrode at the lower left thorax as described in **Chapter 2, Section 2.3.1** (pilot study). Behavioural footage was captured via a continuous recording schedule using a Hitachi DVD/HDD digital video camera mounted on a tripod, positioned opposite the horses stable and was synchronised with HRV recording. As the exact feeding time varied slightly each day HRV and video recording began approximately 30 minutes prior to feeding, of this the last ten minutes of data collected prior to feeding was used for analysis. Once the concentrate feed had been placed in the stable, HRV and video recording continued for a further ten minutes. Video recording was then stopped and the HRV equipment removed.

4.4. HRV DATA ANALYSIS

RR interval data were analysed using Kubios HRV analysis software. To remove trend components, data were de-trended and an artefact correction was made as described in **Chapter 2: Section 2.1.** Time domain analyses were then calculated from mean RR interval data for each horse and for each time-period; stable baseline (SB), field baseline (FB), during anticipation of feed arrival (ANT) and whilst consuming the feed (CONS). For each data set SDNN and RMSSD were analysed using SPSS 15.0. Kolmogorov-Smirnov tests for normality were carried out on each data set and statistical tests chosen accordingly. Differences in HRV measurements between time-period and in comparison with baseline measures were tested using a one-way repeated measures ANOVA. Differences in HRV between individual horses were investigated using a one-way between-groups ANOVA followed by Bonferroni and Fisher's LSD post-hoc comparison tests. Differences in HRV according to sex were tested using a mixed between-within subjects ANOVA to establish whether there was an effect of sex or time-period and if there was an interaction between sex and time-period.

4.5. BEHAVIOURAL DATA ANALYSIS

For each of the six horses, ten minutes of video footage was obtained prior to concentrate feed being placed in the stable. The main characteristic of pre-feeding anticipation in horses has previously been identified as either increased activity (VanDierendonck *et al.,* 2010) or time spent standing alert (Cooper *et al.,* 2005). Using The Observer behavioural analysis software, an ad-libitum sampling regime was adopted in order to calculate the duration of behavioural states for the observation period; time spent in each position in stable, ear and head position (**Appendix 3**). Durations of raw data for each horse in time format (sec) were then converted using Microsoft Excel to a decimal number value and imported into SPSS 15.0 for statistical analysis.

The frequency of behavioural transitions is also a common measure of anticipatory behaviour in other mammalian species such as mink (Vinke, Van den Bos and Spruijt, 2004) so the frequency of transitions between behavioural states was calculated in order to measure of the level of activity. Descriptive statistics were also obtained for the frequency of event behaviours; weaving, nodding, aggression towards neighbouring horses, oral stereotypy, vocalisations and box-walking (**Appendix 4**).

Spearman's rank order correlations were used to investigate the relationship between anticipatory HRV and the duration of behavioural states, frequency of transitions between states and frequency of behavioural events.

4.6. RESULTS

4.6.1. Heart rate variability analysis per time-period

SDNN values were highest during the CONS period (81.95±21.42ms) and lowest during the ANT period (68.72±7.97ms). RMSSD was similar during the FB and CONS periods (85.38±34.08 and 83.30±21.10, respectively) and lowest during the ANT period (69.65±16.63; **Figure 4.2**).

Using a one-way repeated measures ANOVA, HRV for baseline measures (SB and FB) and each time-period (ANT, CONS) were compared. SDNN was not found to differ significantly between each period, Wilks' Lambda =0.44, $F_{(3, 3)}$ =1.28, p=0.42. RMSSD was not found to differ significantly between each period, Wilks' Lambda = 0.48, $F_{(3, 3)}$ = 1.07, p=0.48. It was therefore not possible to reject the null hypothesis.
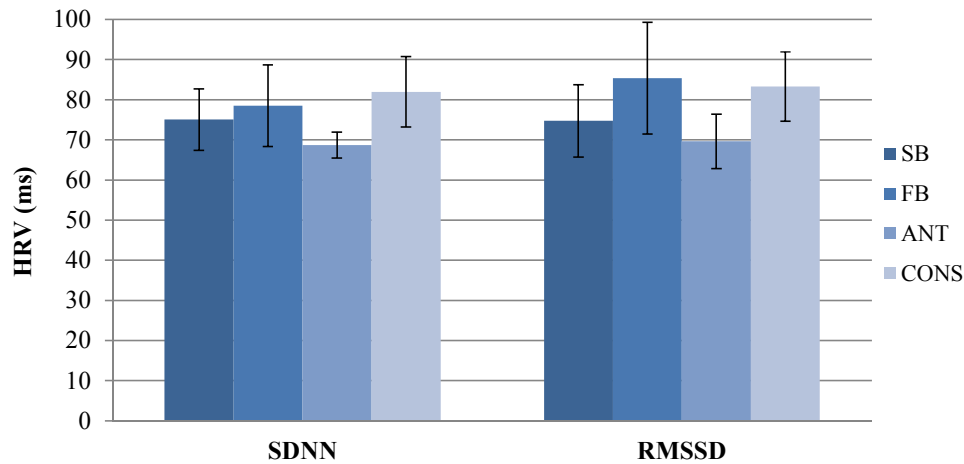
Figure 4.2. Mean heart rate variability (HRV) values (±SE) for baseline measures taken in the stable (SB) and field (FB), during anticipation of feed arrival (ANT) and whilst consuming the feed (CONS). HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (one-way repeated measures ANOVA).

4.6.2. Inter-individual heart rate variability

Considerable inter-individual variations were observed in SDNN and RMSSD for baseline HRV measurements and throughout the study (**Figure 4.3**). Using mean HRV data from each time-period and baseline values a one-way between-groups ANOVA was conducted to explore inter-individual differences in HRV.

Differences in SDNN between individuals approached significance, $F_{(5, 23)} = 2.487$, $p=0.07$. RMSSD values differed significantly between horses, $F_{(5, 23)} = 3.374$, $p=0.025$, which enabled the null hypothesis to be rejected. The difference in mean RMSSD measurements between individual horses was large. The effect size, calculated using eta squared, was 0.48. Despite reaching statistical significance, post-hoc comparisons of RMSSD between individuals using the Bonferroni test failed to identify where the differences occurred at the $p \leq 0.05$ level. Application of Fisher's LSD post-hoc test identified Horses 2 and 6 as having higher RMSSD

than the other subjects but only at the p≤0.05 level and not the revised alpha level of p≤0.01 required to control for type 1 errors with this test.
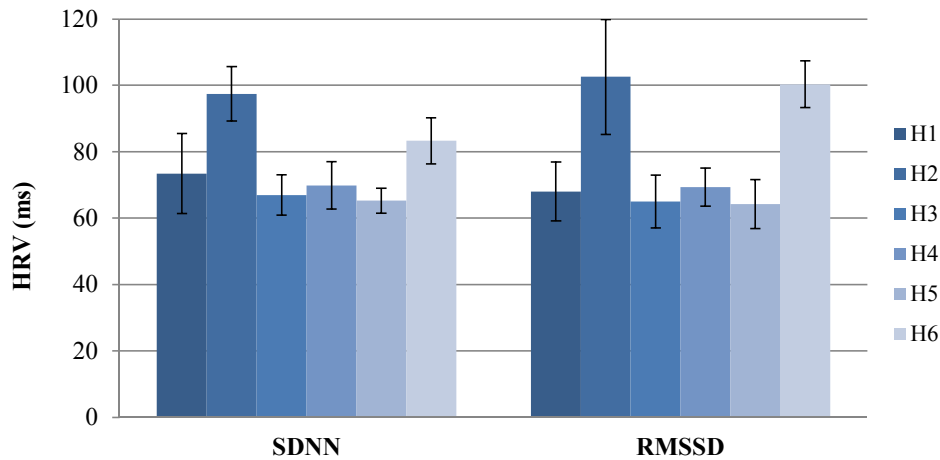
Figure 4.3. Inter-individual differences in mean heart rate variability (HRV) using the entire set of HRV data (±SE). HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Significant inter-individual differences were present for RMSSD (one-way ANOVA; p≤0.05) but could not be accurately identified using Bonferroni post-hoc comparisons at the p≤0.05 level. Fisher's LSD post-hoc test identified Horses 2 and 6 as having higher RMSSD than the other subjects but only at the p≤0.05 level and not the revised alpha level of p≤0.01.

4.6.3. Heart rate variability analysis according to sex

Differences in HRV were explored according to sex. Analysis of descriptive statistics revealed that HRV for mares was higher than that of geldings for all time-periods (e.g. for RMSSD **Figure 4.4**).

Data were normally distributed so a mixed between-within subjects ANOVA was conducted to assess the effect of sex on HRV for each time-period (SB, FB, ANT and CONS).

For SDNN, there was no significant interaction between group and time-period, Wilks' Lambda = 0.65, $F_{(3, 2)} = 0.36$, $p=0.79$ and no significant main effect for time-period, Wilks' Lambda =

0.35, F (3, 2) = 1.23, p=0.48. The main effect comparing the two groups was also not significant F (1, 4) = 1.56, p=0.28.
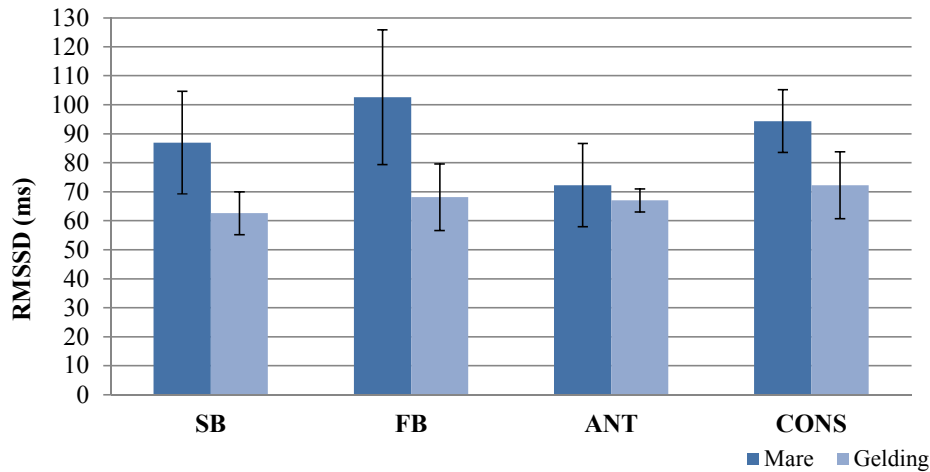
Figure 4.4. Descriptive statistics showing mean RMSSD (square root of the mean squared differences between adjacent inter-beat intervals; ±SE) comparing mares and geldings according to time-period; baseline measures taken in the stable (SB) and field (FB) during anticipation of feed arrival (ANT) and whilst consuming the feed (CONS). Differences were not significant (mixed between-within ANOVA).

For RMSSD, there was no significant interaction between group and time-period, Wilks' Lambda = 0.64, F (3, 2) = 0.38, p=0.78 and no significant main effect for time-period, Wilks' Lambda = 0.42, F (3, 2) = 0.92, p=0.56. The main effect comparing the two groups was also not significant F (1, 4) = 3.0, p=0.16. It was not possible to reject the null hypothesis.

4.6.4. Analysis of behavioural states

The predominant position was at the front of the stable with the head positioned to the right (40% of time observed), in the direction from which feed arrived (**Figures 4.5, 4.6 & 4.7**). Spearman's rank order correlations confirmed that within this posture, laterally placed ears were associated with a high head level (r_s=0.84, p=0.036) and that a forwards ear position was associated with both a high (r_s=0.81, p=0.05) and mid head level (r_s=0.94, p=0.005).

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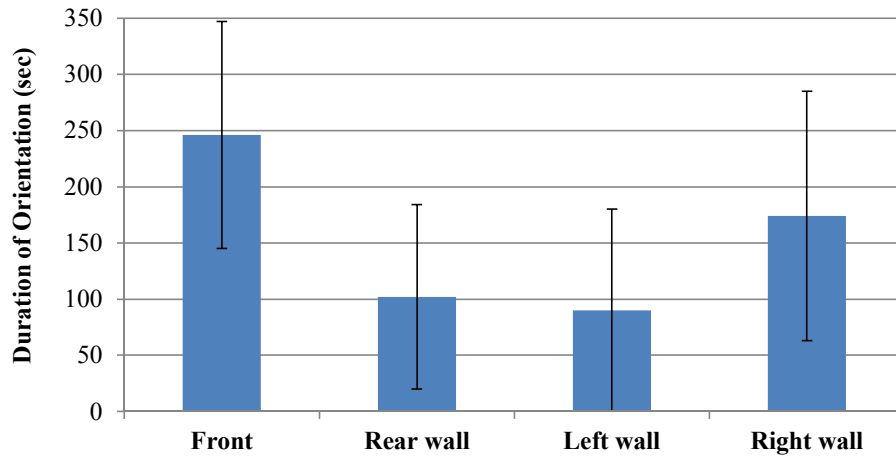
Figure 4.5. Descriptive statistics showing the mean duration (±SE) of horses' orientation in the stable during the 10 minutes prior to the arrival of feed.
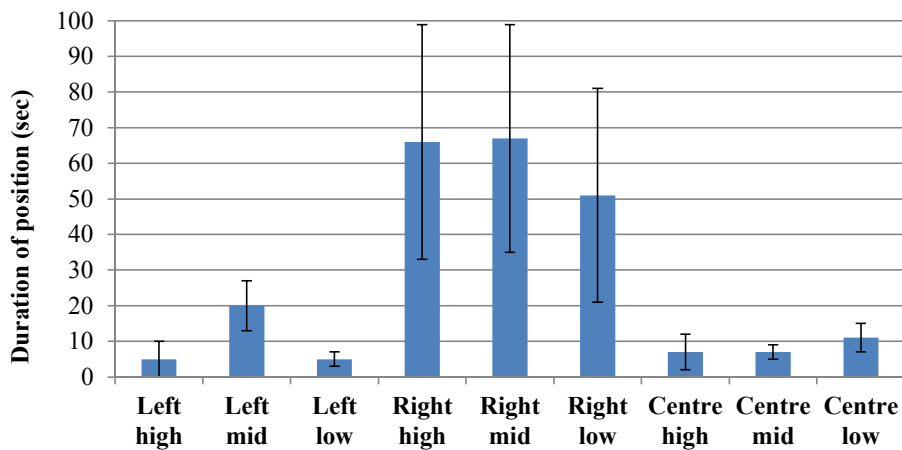
Figure 4.6. Descriptive statistics showing the mean duration (±SE) of horses' head position when positioned at the front of the stable during the 10 minutes prior to feed arrival, as shown in Figure 4.5. Orientation was predominantly to the right, in the direction from which the food was due to arrive.
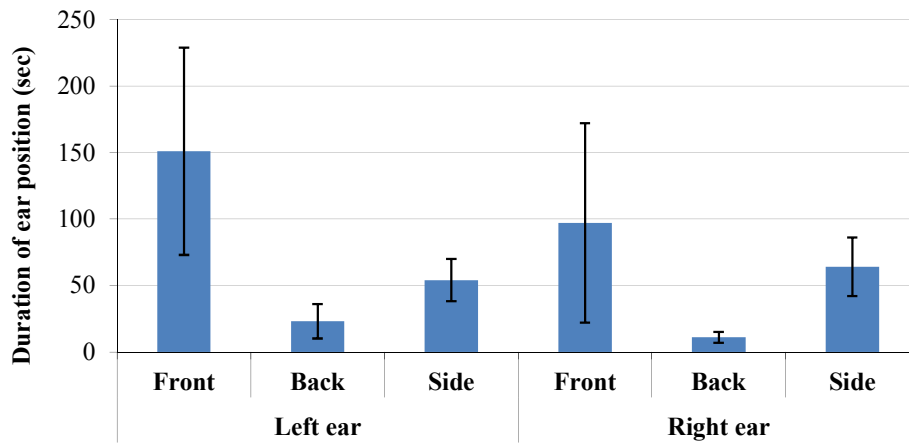
Figure 4.7. Descriptive statistics showing the mean duration (±SE) of horses' ear position when positioned at the front of the stable during the 10 minutes prior to feed arrival, as shown in Figure 4.5. Ears were predominantly positioned forwards.

4.6.5. Relationship between HRV and behavioural states

The relationship between anticipatory HRV and the duration of each position in the stable, and each head and ear position was investigated using Spearman's rank order correlations. A strong positive relationship was found between SDNN and the duration of time spent orientated towards the right wall, r_s=0.84, p=0.034. A strong inverse relationship was also present between RMSSD and time spent facing the rear wall which approached significance, r_s=-0.78, p=0.069. No other significant relationships were present between variables.

4.6.6. Relationship between HRV and behavioural activity

During anticipation of feed arrival, large individual variations in the level of behavioural activity (calculated from the number of transitions between the different positions in the stable, head and ear positions) were evident (**Figure 4.8**). The number of transitions between the nine different head positions ranged from 7 for Horse 6, to 91 for Horse 5 (37±31). The number of transitions between the three left ear positions ranged from 13 to 129 (50±42) and for the right ear ranged from 11 to 121 for Horses 6 and 5, respectively (50±39). The number of transitions between positions inside the stable ranged from 1 for Horse 2, to 18 for Horse 5 (6±6).
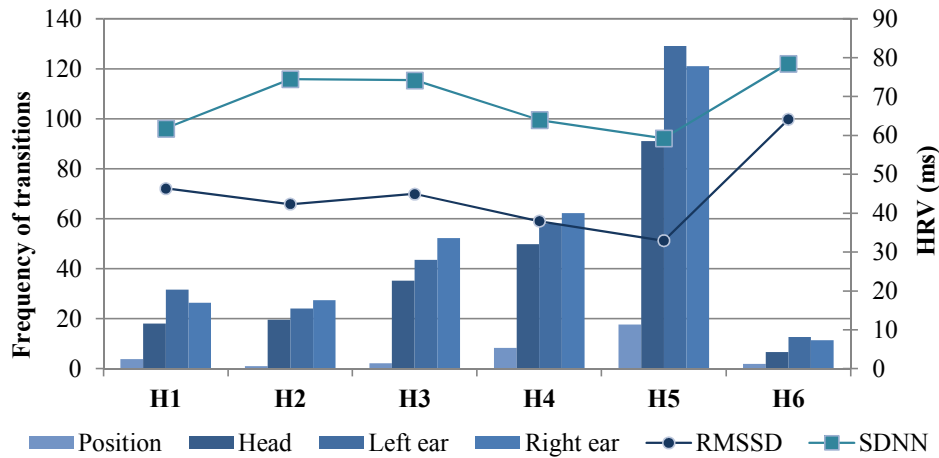
Figure 4.8. Descriptive statistics showing total behavioural activity for changes in position in the stable, movement of the head and ears as each horse anticipated feed arrival. Mean HRV during this period is also shown for each horse; standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD).

Preliminary analysis of anticipatory HRV data indicated that low levels of behavioural transitions were associated with high HRV and vice-versa (**Figure 4.8**). The relationship between the total number of transitions of position in the stable, head position, ear position and HRV was investigated using Spearman's rank order correlations (**Table 4.2**).

Table 4.2. Relationship between the total frequency of head and ear movements, positional changes within the stable and heart rate variability (HRV) during anticipation of feed arrival (Spearman's rank order correlations; p≤0.05). HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD).

	Head	Left ear	Right ear	Position	SDNN	RMSSD
Head	-	r_s=0.94 p=0.005	r_s=1.00 p<0.001	n/s	n/s	r_s=-0.94 p=0.005
Left ear		-	r_s=0.94 p=0.005	r_s=0.81 p=0.05	r_s=-0.83 p=0.042	r_s=-0.83 p=0.042
Right ear			-	n/s	n/s	r_s=-0.94 p=0.005
Position				-	r_s=-0.84 p=0.036	n/s

Low levels of RMSSD were associated with frequent head and ear movements, which were positively correlated with each other. Low levels of SDNN were associated with frequent changes in orientation and movement of the left ear, which were also positively correlated with each other. It was therefore possible to reject the null hypothesis.

4.6.7. Relationship between behavioural events, behavioural activity, behavioural states and HRV

Analysis of descriptive statistics revealed that oral stereotypy was the most frequent observed behaviour, followed by vocalisation, weaving, aggressive behaviour and nodding. Box-walking was the least frequent behaviour (**Figure 4.9**).
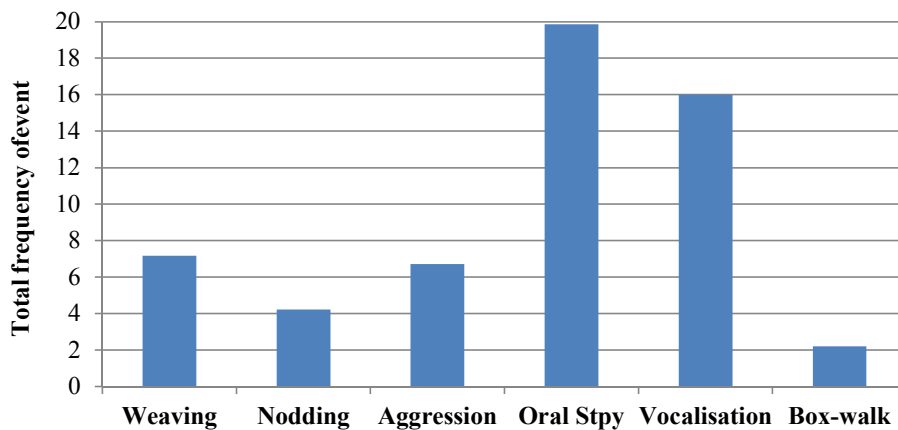
Figure 4.9. Descriptive statistics showing the total frequency of behavioural events from observation of all six horses during anticipation of feed arrival.

Spearman's rank order correlations confirmed that higher incidences of vocalisation were strongly associated with time spent orientated towards the rear of the stable (r_s=0.90, p=0.015), a higher frequency of changes in orientation within the stable (r_s=0.89, p=0.016) and higher incidences of weaving (r_s=0.94, p=0.005), which was itself strongly correlated with time spent at the rear of the stable (r_s=0.84, p=0.034). A strong inverse relationship was also found between SDNN and the frequency of nodding behaviour, r_s=-0.85, p=0.034 (**Figure 4.10**)

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which enabled the null hypothesis to be rejected. No other significant correlations were found between variables.
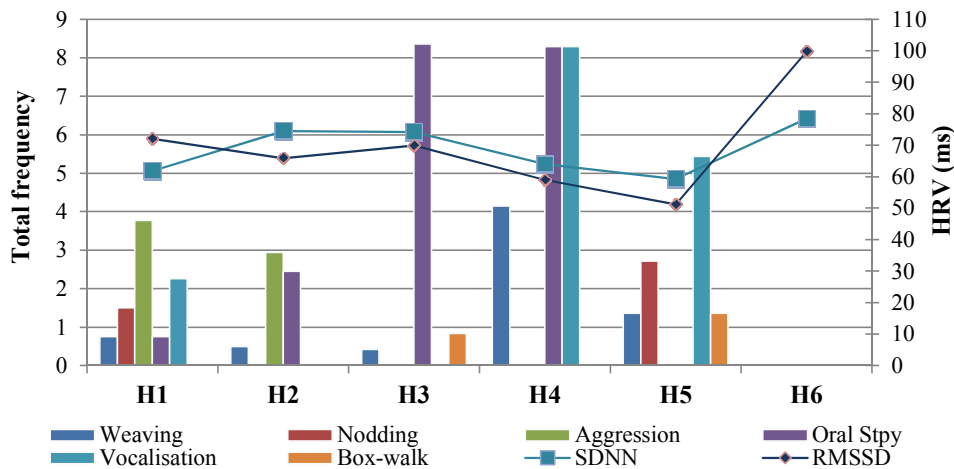
Figure 4.10. Descriptive statistics showing the total frequency of behavioural events and heart rate variability (HRV) measures for each horse during anticipation of feed arrival. HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Nodding behaviour was positively correlated with weaving and negatively correlated with SDNN (Spearman's rank order correlation; p≤0.05).

4.7. DISCUSSION

No significant differences in HRV values were found between baseline measures, during anticipation or consumption of feed. Numerically, SDNN and RMSSD were both lowest during the anticipatory period and the highest levels recorded during consumption for SDNN and in field baseline measures for RMSSD. Research in this area is scarce but Reefmann *et al.* (2009a) reported a similar non-significant pattern of HRV change in sheep when anticipating and in receipt of a standard feed. In human studies Hayano *et al.* (1990) and Lu *et al.* (1999) both report that vagal activity diminishes post-prandially indicating dominance of the sympathetic nervous system at this time but, unlike the current study, did not measure HRV during consumption of the food itself. No published equine studies in this area of research exist for direct comparison.

It would have been useful to directly compare anticipatory HRV measurements from this study with baseline data from stereotypic horses. Unfortunately this was not possible as both published studies (Bachmann *et al.*, 2003; Nagy *et al.*, 2009) measuring HRV in stereotypic horses used a power spectral analysis method to quantify the activity of the autonomic nervous system, rather than the time domain analysis carried out currently, rendering the specific figures incomparable. Bachmann *et al.* (2003) established that control horses had a higher vagal tone at rest than crib-biting horses which suggests that crib-biters were not in a normal state of rest. Conversely, Nagy *et al.* (2009) were unable to differentiate between baseline HRV in crib-biting versus control horses. The fact that there was no significant difference in HRV between time-periods in the present study infers that despite the expression of stereotypic behaviours, the interpretation of events during the anticipatory period has no more or less emotional valence than during consumption or baseline periods.

When HRV data for individual horses were analysed using data collected during both time-periods and baseline values, significant individual differences were noted; Horses 2 and 6 recorded higher RMSSD than the other subjects. Horses with high levels of HRV have previously been associated with aspects of handling such as 'bravery' when encountering frightening objects and events, low levels of 'spooky' behaviour (the frequency and intensity of shying away from something) and low levels of attentiveness to their surroundings (Visser *et al.*, 2003a). Eager *et al.* (2004) have also previously related HRV components to equine temperament and Visser *et al.* (2001, 2002) suggest that individual characteristics such as temperament or reactivity to environmental changes may be responsible for the different individual modulation of the ANS which can be detected via HRV measurements. HRV may therefore be useful in differentiating between individuals and quantifying aspects of temperament with important implications for breeding, housing and management of the horse. Breed and temperament are features included in **Chapter 7** which investigates factors affecting HRV.

Mares recorded numerically higher HRV than geldings during all time-periods. These differences were not statistically significant but show the same inclination as found in human research, where sex-related differences in HRV have identified females having a greater vagal control of the heart than males who exhibit more sympathetic cardiac control (Rossy and Thayer, 1998). Information is lacking as to the effect of sex on equine HRV. Existing equine HRV research either report no effect of sex (Visser *et al.*, 2002; Rietmann *et al.*, 2004b; Visser, Ellis and Van Reenen, 2008), make no reference to the possible effects of sex when using mixed sex subjects (Minero *et al.*, 1999; Ohmura *et al.*, 2001; Quick and Warren-Smith, 2009; Schmidt *et al.*, 2010d), or lack detail as to the sex of participating subjects (Kuwahara *et al.*, 1996; Nagy *et al.*, 2009; Physick-Sheard *et al.*, 2000). Differences in HRV between sexes are a factor investigated in **Chapter 7**.

During the anticipatory period horses were predominantly positioned at the front of the stable with the head held either at a high or mid-level and with the head and both ears orientated towards the direction from which feed was due to arrive. Pond *et al.* (2010) reported a similar behavioural stance prior to feeding where horses typically orientated their ears forward and stood by the stall door watching the aisle for the grain and hay cart to approach their stall. In a study of pre-feeding activity in horses, Cooper *et al.* (2005) also reported that the predominant activity was standing alert (32.3% of time observed) during the hour before feeding.

A relationship was found between anticipatory HRV and the horses' positions in the stable; higher HRV was associated with more time orientated to the right hand wall of the stable, while HRV correlated negatively with time spent facing the rear wall. This may be related to hay net position, as three horses had their hay net tied on the right hand wall and two horses on the rear wall. It has recently been shown in goats that feeding stations allowing greater all round vision of the surrounding environment significantly reduce HRV and faecal cortisol levels compared with designs that impair visual fields (Nordmann *et al.,* 2011). It is therefore possible that horses preferred to eat from a hay net positioned on the right hand wall so they were better able

to visually and acoustically monitor the surrounding environment compared to when they faced directly away from the open stable doorway. Two of the three horses with hay nets on the right hand wall were identified as the individuals with higher HRV than other subjects, but it is possible that hay net position could be a contributory factor as no other significant relationships were identified for these horses between anticipatory HRV and other behavioural states.

Analysis of behavioural activity, calculated as the frequency of transitions between behavioural states (changes in orientation, head and ear movement), revealed large individual variations; Horse 5 was most active, Horse 6 changed head and ear position the least and Horse 2 recorded the fewest positional changes. It was interesting to note that Horse 5 had the lowest HRV and Horse 6 the highest suggesting that frequent head and ear position changes may be indicative of reduced HRV and vice versa. Analysis of anticipatory HRV and behavioural activity for the group as a whole confirmed that there was a significant relationship between low RMSSD and frequent head and ear movements as well as between low SDNN and frequent positional changes. The relationship between HRV and behaviour can be dissociated from motor-induced sympathetic nervous system activation and attributed to an emotional response because Horse 2, with the lowest number of positional changes i.e. movement of the whole body, did not record the highest HRV.

There are indications that ear postures may be useful in assessing emotional valence in animals; in cattle, for example, a high occurrence of pendulous ear postures was considered indicative of animals' positive ratings of their favourite grooming sites by Schmied *et al.* (2008). The frequency of ear posture changes has also been identified as a method of assessing emotional reactions in sheep (Reefmann *et al.*, 2009b; Reefmann, Wechsler and Gygax, 2009; Boissy *et al.,* 2011). In Reefmann and colleagues' study (2009b), negatively valenced situations (social separation) induced a high number of ear posture changes and forward ear posture. In contrast, positive situations (feeding) were characterised by few posture changes and a high proportion of passive ear postures. Boissy *et al*. (2011) investigated how ear postures in sheep were affected

by specific emotional states: horizontal ear postures were found to correspond with a neutral state; ears pointing backward were associated with fear during unfamiliar, unpleasant and uncontrollable situations and ears were pointed up when facing a negative but controllable situation. Reefmann *et al.* (2009b) and Reefmann, Wechsler and Gygax (2009) hypothesise that because ears are essential for gathering, integrating and appraising information from the environment, negative situations may require increased attention to environmental surroundings to allow for problem-solving, whilst ear activity decreases once attention to external stimuli is no longer necessary. Fridlund (1991) speculated that the erectors of the ear auricle in non-human mammals are homologous to the muscles involved in lowering eyebrows in humans. The tension in these eyebrow muscles is higher during negative than during positive emotional states (Witvliet and Vrana, 1995) which would indicate that negative emotional states might coincide with erect ears (in the current study forward or active ears) and positive emotional states might coincide with non-erect (i.e. passive or laterally placed) ears.

In the present study, laterally placed ears were associated with a high head position but were observed infrequently compared with forward facing ears, which were associated with both high and mid-level head position. As there was no significant relationship between ear position and anticipatory HRV however, ear position, whilst considered to be a good outward indicator of emotional state (Houpt, 2005) could not be related physiologically to an emotional response on this occasion.

Stereotypical behaviours, described as repetitive, invariant movements fixed in orientation and place, without an obvious goal or function (Mason 1991) are often described in relation to the same underlying neurophysiological mechanisms as anticipatory activity (opioid and dopaminergic related systems; Cabib, 1993). As research investigating anticipatory behaviour normally lacks physiological data it is not known whether a relationship exists between anticipatory activity and stereotypical behaviour where anticipatory activity may even be a

predictor of the development of stereotypical behaviour, or if behaviours expressed during the anticipation of a positive reward are indicative of a 'pleasant' experience.

In this study, activity during anticipation of food arrival consisted of both locomotor and oral stereotypical behaviours. Analysis of behavioural events identified oral stereotypy (licking stable fittings, tongue rolling and sham-chewing) as the most frequent behaviour, closely followed by vocalisation, weaving and nodding. Feeding behaviours are perhaps the most highly motivated of any behaviour pattern and frustrated feeding motivation is often linked to stereotypies resembling feeding behaviours (Carlstead, 1998). The extensive research on domestic ungulates (Redbo, 1990) supports the association between feeding motivation and the prevalence of oral stereotypies (including tongue-playing, bar biting and licking of non-food objects in grazers) postulated by Mason (1997). Redbo (1990) observed that these behaviours were associated with behaviours related to food searching and only occurred in conditions where the animals (heifers) were not at pasture. Oral stereotypies, particularly sham-chewing have a high prevalence (75% of farms visited) in sows and piglets (Scott *et al.,* 2009) and have been linked with feeding schedules by Rushen (1985). The relationship between oral stereotypies and type or amount of food provided (Redbo *et al.*, 1998) or feeding duration has also been established in horses (Willard, 1977), pigs (Terlouw, 1991; Redbo, 1997) and broiler breeders (Savory, 1993). Bashaw (2001) also reports non-food licking to be a prevalent stereotypic behaviour associated with feeding motivation which represented 72.4% of all stereotypies observed in captive giraffe and okapi. These could be directly related to management strategies such as the number of hours the individuals spent indoors, access at night to conspecifics, feeding frequency, method of feeding and type of food provided were all predictors of stereotypic licking.

The licking behaviour observed in the current study fits the description of a stereotypy (Kennedy, Schwabe and Broom, 1993; Cooper and McGreevy, 2002), has a prevalence of 10% in stereotypic horses (Casey, 2010) and has previously been reported in horses prior to being fed

(Kiley-Worthington, 1997). The expression of this behaviour in horses is not necessarily indicative of negative affective state as there was no association with HRV in the present study. It does however confirm the link between confinement in a stable and the incidence of oral stereotypies which in addition to restricted resources (e.g. the type and amount of food provided (Redbo *et al.*, 1998) or feeding duration (Willard, 1977)) could also be due to the prevention of natural appetitive behaviour as described in heifers by Redbo (1990).

The head can be used to communicate a number of emotional states, when irritated or frustrated, for instance, the animal may toss and jerk the head (Weeks and Beck, 1996). Cooper *et al.* (2005) reported infrequent occurrences of nodding and weaving (1.5% and 0.82% of time observed, respectively) whilst studying pre-feeding activity in horses. Yet when meal frequency increased, weaving and nodding behaviour also increased in both treatment horses and in horses elsewhere on the yard. This suggests that weaving and nodding are anticipatory responses to feeding time. In the current study, nodding behaviour was strongly associated with low HRV so may be indicative of negative affective state. Operant foraging devices provide feed over a large number of discreet meals and may reduce pre-feeding stereotypies due to the removal of cues that predict feeding, such as activity in the feed room. In practical terms though, the removal of pre-feeding cues may be difficult on working yards.

The specific calls of animals have long been used as markers of emotion. Darwin believed that over time the production of some sounds came to be associated with specific emotions, such as pain, pleasure, or rage and as a result, these vocalisations had come to serve a communication function (Darwin, 1872). More recently, analysis of anticipatory behaviour and correlated vocalisations have been investigated as a non-invasive method of assessing animal welfare (Knutson, 2002; Manteuffel, 2004) and are of particular relevance when providing evidence of positive experiences (Knutson, 1998; Burgdorf, 2000; Boissy *et al.*, 2007).

Although knowledge of the social behaviour of horses is increasing, the range of equine vocal communications has not yet been widely researched. Vocalisation in the form of the 'nicker' in horses is thought to indicate positive anticipation of a pleasant event such as feeding (Waran, 2001) and is suggested by Kiley (1972) and Waring (2003) to announce the horse's presence and anticipation. Proops, McComb and Reby (2008) also provide evidence that horses use vocalisation (whinny) as part of the individual recognition of conspecifics. Recent research by Pond *et al.* (2010) has identified the different acoustic signals between equine vocalisations emitted during pre-feeding (a eustress situation) and those emitted during social separation (a distress situation).

As a potential indicator of affective state, these vocalisations might be useful in future as a tool to assess animal welfare but on this occasion vocalisations were not found to be related to a particular affective state according to HRV measures. High incidences of vocalisation were however, strongly associated with more time spent facing the rear of the stable, a greater frequency of changes in orientation and higher incidences of weaving. As previously noted, low HRV was associated with time spent at the rear of the stable; hay nets positioned on the rear wall may have caused those horses to change position more frequently, vocalise more and perform more weaving behaviour than other horses. As a group-living prey species, horses normally communicate via non-verbal means such as facial expressions and body language. The level of vocalisation observed in anticipation of feeding in this and other studies (Waran, 2001; Pond *et al.,* 2010) may indicate that domestic horses, safe from the risk of predation, are more willing to rely on vocalisations and auditory signals than their free-ranging counterparts.

4.8. CONCLUSION

This study investigated the relationship between HRV and pre-feeding anticipatory behaviour as observed in horses during the initial pilot study (**Chapter 2: Section 2.3**). HRV was not affected by time-period indicating that for these horses, the anticipation and consumption of

feed was no more or less emotionally valenced than spending time in their stable or the field. As previously identified in **Chapter 3: Section 3.8.2** and purported by other researchers; individual differences in HRV were evident which may be associated with underlying affective state, temperament or breed. These findings highlighted the need for the identification of factors affecting individual HRV, which are investigated in **Chapter 7**.

High levels of behavioural transitions, ear movement and incidences of nodding behaviour were associated with low HRV and vice-versa. An association was also made between HRV (and therefore affective state) and the positions of the horse's hay net; rear placed nets were linked with low HRV, high levels of vocalisation, frequent orientational changes and weaving. This suggests that hay net position can affect the horse's capacity for environmental monitoring and seemed to affect their emotional experience; repositioning the location of the hay net closer to the doorway may therefore provide a simple improvement to their environment.

In summary, this study identified some elements of pre-feeding anticipatory behaviour that were indicative of negative affective state, but on the whole the pre-feeding period appeared to be a neutrally valenced experience. As the behavioural and HRV response in horses anticipating positive rewards has been explored in the present and previous chapter, **Chapter 5** investigates the effect of sham-clipping, a common but potentially negatively valenced management practice.

Chapter Five

HRV and Behavioural Responses to an Aversive Stimulus

(Sham-Clipping)

5.1 INTRODUCTION

A study was conducted to investigate whether differences in HRV and behavioural responses were evident in horses undergoing a potentially negatively valenced procedure in the form of sham clipping. Clipping is a common practice in the management of domestic ridden horses to ensure partial or full coat removal, usually on a bi-monthly basis through the winter months. Horses from Brackenhurst Equestrian Centre were selected to participate in this study as they are ridden for up to three hours per day and are routinely clipped during the winter months to facilitate management.

The clipping process is a non-surgical husbandry practice that involves removing some or all of the hair, an insulating layer, using an electric clipping machine during a procedure normally lasting around 30 minutes. This can be advantageous for heat dissipation enabling the horse to work comfortably and without undue sweating during the winter months when the coat grows naturally thicker (Gough, 1997; Morgan, 2002). Anecdotal evidence suggests that riders and trainers believe clipping enhances the performance of the horse by minimising the rise in body temperature thought to be a limiting factor for maximal exercise. Evidence of this being the case is provided by Morgan, Funkquist and Nyman (2002) who reported that clipping reduces strain on the thermoregulatory system by enhancing heat loss during intense exercise compared with non-clipped horses.

Clipping involves close contact with the handler during the procedure and exposes the animal to persistent noise, coat cutting, potentially minor discomfort from heated clipper blades and possible minor skin injury. Some horses will not tolerate part or all of their body being clipped and may exhibit fractious non-compliant behaviour such as reluctance to stand still, shaking or twitching the skin and kicking with both the fore- and hind limbs. This type of behaviour has been linked to the sound of the clippers by Gough (1999) and associated with previous experience of discomfort caused by blunt or hot clipper blades by Dyson and Carson (2002). It

is the combination of these components that makes clipping a significant short-term stressor that may compromise welfare as indicated by the need to chemically restrain some horses with a mild sedative as described by Dodman (1980), Geiser (1990) and Gough (1997) in order to ensure swift and safe completion of the process. The clipping process has also been used experimentally in order to induce acute stress by Sanger *et al.* (2011) when studying cognitive bias in sheep.

According to Dantzer (2002) the affective component of an emotional state can be simply assessed by determining whether an animal approaches or avoids the eliciting situation, i.e. if the stimulus is aversive the animal exposed to it is assumed to experience a negative emotion if the animal makes an effort to avoid it. For prey species such as the horse however, there is a survival value associated with the ability to mask pain or discomfort in order to minimise the risk of predation (O'Callaghan, 2002; Taylor, Pascoe and Mama, 2002; Mayer, 2007), therefore outward behavioural signs do not necessarily reflect the emotional state of the animal. Horses selected for use in a riding school environment tend to be chosen for their passive and tractable behaviour, but it is possible that this may mask physiological indicators of stress. The psychological condition of learned helplessness was described in ridden horses by Odberg (1987b) and has been explored more recently by Hall *et al*. (2008). For horses that react passively and appear compliant during clipping, it is not known whether this is due to genuine relaxation during and habituation to the process, or if compliant horses are undergoing psychological distress masked by behavioural unresponsiveness indicative of withdrawal, apathy or learned helplessness.

This study aimed to a) investigate whether differences in behaviour exist prior to, during and after sham-clipping, b) compare baseline HRV data with that obtained prior to, during and after sham-clipping, c) investigate whether a relationship exists between HRV and behaviour and d) determine whether the behavioural and physiological responses differ in horses selected for compliant or non-compliant behaviour during clipping.

5.2 SUBJECTS

Eight mature horses (**Table 5.1**) were chosen from Brackenhurst Equestrian Centre; four horses (three geldings and one mare) were reported by the Yard Manager to show non-compliant behaviour during clipping as described in **Section 5.1** i.e. movement away from the clippers, unwillingness to stand still and one horse which required sedation to complete the process (non-compliant; NC); four horses (three mares and one gelding) who tolerated the process and could be clipped quickly and easily without showing any outward signs of non-compliance (compliant; C).

Table 5.1. Profile of subjects.

Horse	Compliant/ Non-compliant	Age (years)	Height (hands)	Breed	Sex
Angus	NC	12	16.3	Danish Warmblood x Thoroughbred	Gelding
Beau	C	15	16.0	Irish Draught x Thoroughbred	Mare
Conan	NC	12	17.0	Irish Draught x Thoroughbred	Gelding
Desmond	NC	9	16.1	Irish Draught x Thoroughbred	Gelding
Harriett	NC	14	16.0	Irish Draught x Thoroughbred	Mare
Pye	C	8	15.2	Cob	Mare
Tosca	C	14	15.2	Cob	Mare
Visi	C	19	16.0	Thoroughbred	Gelding
	Mean	13			
	St. Dev.	3.5			

5.3. STUDY PROTOCOL

Prior to the study commencing, the study protocol gained ethics approval and appropriate risk assessments were undertaken. As described in **Chapter 2: Section 2.1**, baseline HRV measurements were obtained from each of the participants prior to the commencement of the study whilst in their usual stable or crew yard and when turned out to pasture. Data collection took place over a two-day period during December 2008. One horse at a time was led from the stable and loosely tied up in a large indoor barn close to the power supply. The horse was fitted with HRV monitoring equipment (Polar Equine RS800G3) attached to the thorax with an elastic girth, which remained in-situ beneath the horse's rug throughout the study. The positive

electrode was located at the left shoulder and the negative electrode at the lower left thorax as described in **Chapter 2: Section 2.3.1**.

Behavioural footage was captured via a continuous recording schedule using a hand held Hitachi DVD/HDD digital video camera which was synchronised with HRV recording. Ten minutes of data were obtained prior to clipping (PC). The clippers (Wolseley Swift) were then switched on and held touching the horses' skin without actually clipping the hair for 10 minutes (Clp). The clippers were then turned off and HRV and video recording continued for a further ten minutes (PoC). The HRV recording equipment was then removed, video recording ceased and the horse was led back to the stable.

5.4. HRV DATA ANALYSIS

RR interval data were analysed using Kubios HRV analysis software. To remove trend components, data were de-trended and an artefact correction was made as described in **Chapter 2: Section 2.1.** Time domain analyses were then calculated from mean RR interval data for each horse and for each time-period; stable baseline (SB), field baseline (FB), PC, Clp and PoC measurements. For each data set SDNN and RMSSD were analysed using SPSS 15.0. Kolmogorov-Smirnov tests for normality were carried out on each data set and statistical tests chosen accordingly.

Differences in HRV measurements between time-period and in comparison with baseline measures were tested using a one-way repeated measures ANOVA. Differences in HRV between individual horses were investigated using a one-way between-groups ANOVA. Differences in HRV according to compliance during clipping (compliant and non-compliant) were tested via a mixed between-within subjects ANOVA to establish whether there was an effect of group or time-period and if there was an interaction between group and time-period. Differences in HRV according to sex were tested via a mixed between-within subjects ANOVA

to establish whether there was an effect of sex or time-period and if there was an interaction between sex and time-period. All analyses were two-tailed.

5.5. BEHAVIOURAL DATA ANALYSIS

After the study, video footage was observed and individual horses were awarded a behavioural score for each time-period, based on the level of behavioural activity predominantly observed during each time-period; PC, Clp and PoC (**Table 5.2**).

Table 5.2. Behavioural score criteria

Behavioural score	Criteria
1	Head low, nose below the withers, loose/drooping lower lip, eyes half closed, one or both ears turned to side. Very little movement.
2	Head low, nose below the withers, eyes open, lips closed. Some movement of head and ears, minimal neck movement.
3	Head high, nose above the withers, vigilant head, neck and ear movement. Absence of whole body movement.
4	Head high, nose above the withers, active movement of the head, neck and ears, snorting, eyes wide open. Absence of whole body movement.
5	High degree of whole body movement including feet.

The scoring criteria was adapted from a similar method used by Christensen, Rundgren and Olsson (2006) during the habituation of horses to a frightening stimulus and measures of alertness in working horses used by Burn, Dennison and Whay (2010). Rietmann *et al.* (2004b) attribute a low head position as a behavioural indicator of a calm horse whilst investigating stress related behaviour in horses during a walking task. Vigilance posture described as 'the horse standing still, with elevated neck, intently oriented head and ears' has also been incorporated into assessment of emotionality in horses by Wolff, Hausberger and Le Scolan (1997). Leiner and Fendt (2011) also associate an elevated neck and evasive movement (steps) to the side or back with avoidance behaviour in horses during threatening situations. In addition

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to the level of behavioural activity, head position and vigilance posture were included in the behavioural categorisation for this study as these have previously been linked with emotionality in horses.

Behavioural scores were entered into SPSS 15.0 for statistical analysis. Kolmogorov-Smirnov tests for normality were carried out on each data set and statistical tests chosen accordingly. Differences in behavioural score between each time-period (PC, Clp and PoC) for the group as a whole were tested using the non-parametric Friedman's ANOVA. Differences in behavioural score according to group (compliant and non-compliant during clipping) were tested via the non-parametric Mann-Whitney U test for each time-period. Differences in behavioural score between mares (M) and geldings (G) were also tested via a Mann-Whitney U test for each time-period. Relationships between HRV and behavioural score were investigated using Spearman's rank order correlations.

5.6. RESULTS

5.6.1. Heart rate variability analysis per time-period

SDNN values were highest during the Clp period (83.65±21.19ms) with the lowest values recorded during the FB period (70.86±13.21ms). Data were normally distributed so a one-way repeated measures ANOVA was conducted to compare HRV between baseline measures (SB and FB) and each time-period (PC, Clp and PoC). SDNN was not found to differ significantly between each period, Wilks' Lambda = 0.64, F (4, 4) =0.56, p=0.70 (**Figure 5.1**).

Mean RMSSD values did not vary greatly, ranging from 79.76±23.36ms during the PC period to 91.98±27.53ms during clipping. RMSSD was not found to differ significantly between each period, Wilks' Lambda = 0.64, F (4, 4) = 0.56, p=0.70. It was therefore not possible to reject the null hypothesis.
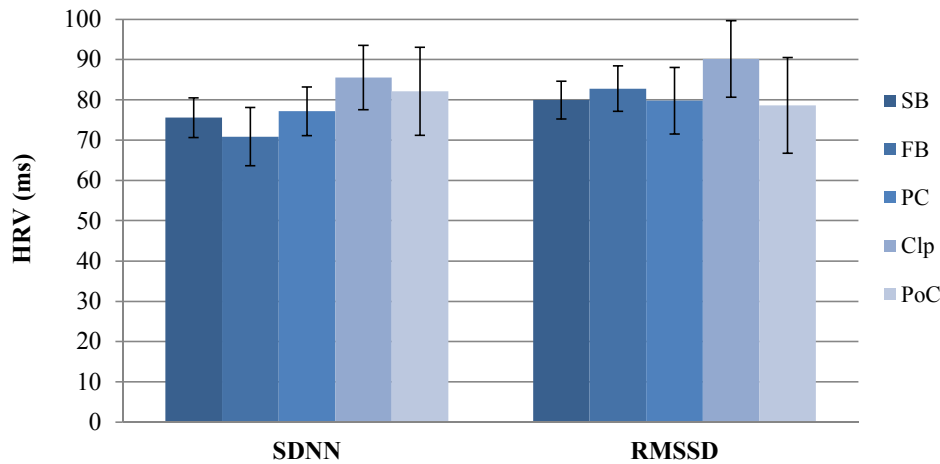
Figure 5.1. Mean heart rate variability (HRV) values (±SE) for baseline measures taken in the stable (SB) and field (FB), prior to clipping (PC), during clipping (Clp) and post-clipping (PoC). HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (one-way repeated measures ANOVA).

5.6.2. Inter-individual heart rate variability

Considerable inter-individual variations were observed in SDNN and RMSSD for baseline HRV measurements and throughout the study. Using mean HRV data from each time-period and baseline values a one-way between-groups ANOVA was conducted to explore the differences in HRV between individual horses. There was a significant difference in HRV between horses: SDNN $F_{(7, 32)} = 4.264$, $p=0.002$; RMSSD $F_{(7, 32)} = 6.281$, $p<0.001$, which enabled the null hypothesis to be rejected. The difference in mean HRV measurements between individual horses was large. The effect sizes, calculated using eta squared, were 0.48 for SDNN and 0.58 for RMSSD, respectively.

Post-hoc comparisons using the Bonferroni test revealed that SDNN for Horse 5 (104.26±15.31ms) was significantly higher than for Horse 1 (64.32±13.73ms, $p=0.012$), Horse 2 (65.36±22.66ms, $p=0.016$) and Horse 3 (63.80±6.73ms, $p=0.010$; **Figure 5.2**).
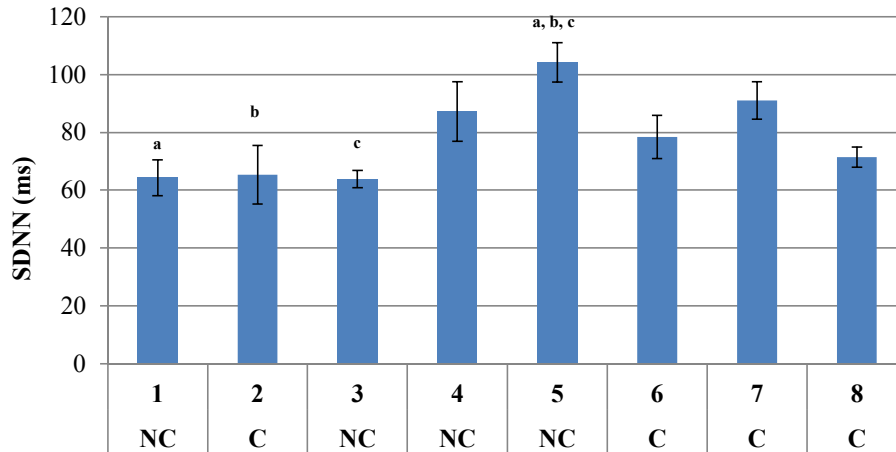
Figure 5.2. Inter-individual differences in mean heart rate variability (HRV) using the entire set of HRV data (±SE). HRV variables were standard deviation of inter-beat intervals (SDNN) and horses are labelled as compliant (C) or non-compliant (NC) during clipping. Paired superscript letters denote significant inter-individual differences (one-way ANOVA and Bonferroni post-hoc comparison; p≤0.05).

Post-hoc analysis of RMSSD values using the Bonferroni test revealed that significantly higher RMSSD was recorded for Horse 7 (117.38 ±23.98ms) than Horse 1 (76.82±14.25ms, *p=0.019*), Horse 2 (78.40±25.14ms, *p=0.029*), Horse 3 (47.98±4.83ms, *p<0.001*) and Horse 6 (77.76±20.23ms, *p=0.024*). Horse 3 also recorded significantly lower RMSSD than Horse 5 (90.50±13.61ms, *p=0.011*) and Horse 8 (86.00±13.01ms, *p=0.036*; **Figure 5.3**).
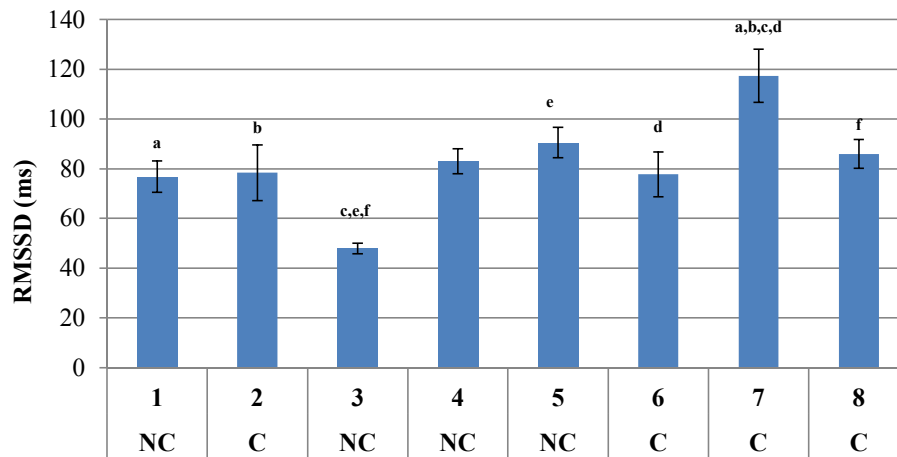
Figure 5.3. Inter-individual differences in mean heart rate variability (HRV) using the entire set of HRV data (±SE). HRV variables were square root of the mean squared differences between adjacent inter-beat intervals (RMSSD) and horses are labelled as compliant (C) or non-compliant (NC) during clipping. Paired superscript letters denote significant inter-individual differences (one-way ANOVA and Bonferroni post-hoc comparison; p≤0.05).

5.6.3. Heart rate variability analysis according to group

Differences in HRV were explored according to group (i.e. compliant versus non-compliant). Analysis of descriptive statistics revealed that C horses recorded consistently higher HRV than NC horses for all time-periods (e.g. for RMSSD **Figure 5.4**).

Data were normally distributed so a mixed between-within subjects ANOVA was conducted to assess the effect of group on HRV for each time-period (SB, FB, PC, Clp and PoC).

For SDNN, there was no significant interaction between group and time-period, Wilks' Lambda = 0.32, $F\,(4, 3) = 1.56$, $p=0.37$ and no significant main effect for time-period, Wilks' Lambda = 0.60, $F\,(4, 3) = 0.50$, $p=0.74$. The main effect comparing the two groups was also not significant $F\,(1, 6) = 0.05$, $p=0.82$.
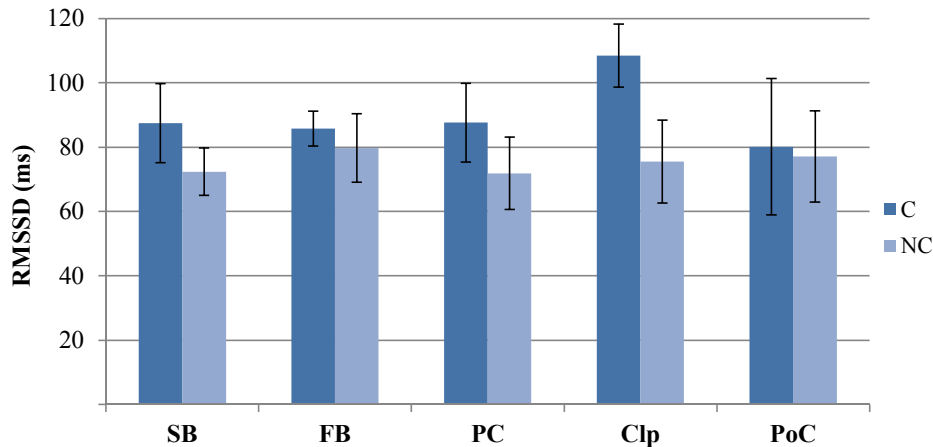
Figure 5.4. Mean heart rate variability (HRV) values (±SE) for baseline measures taken in the stable (SB) and field (FB), prior to clipping (PC), during clipping (Clp) and post-clipping (PoC). Horses are labelled as compliant (C) or non-compliant (NC) during clipping and the HRV variable is square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (mixed between-within ANOVA).

For RMSSD, there was no significant interaction between group and time-period, Wilks' Lambda = 0.45, F (4, 3) = 0.90, p=0.56 and no significant main effect for time-period, Wilks' Lambda = 0.52, F (4, 3) = 0.70, p=0.64. The main effect comparing the two groups was also not significant F (1, 6) = 1.18, p=0.32. It was not possible to reject the null hypothesis.

5.6.4. Heart rate variability analysis according to sex

Differences in HRV were explored according to sex. Mares tended to record higher HRV than geldings before, during and after clipping as well as for baseline measurements (e.g. for SDNN, **Figure 5.5**).

Data were normally distributed so a mixed between-within subjects ANOVA was conducted to determine the effect of sex on HRV for each time-period (SB, FB, PC, Clp and PoC).
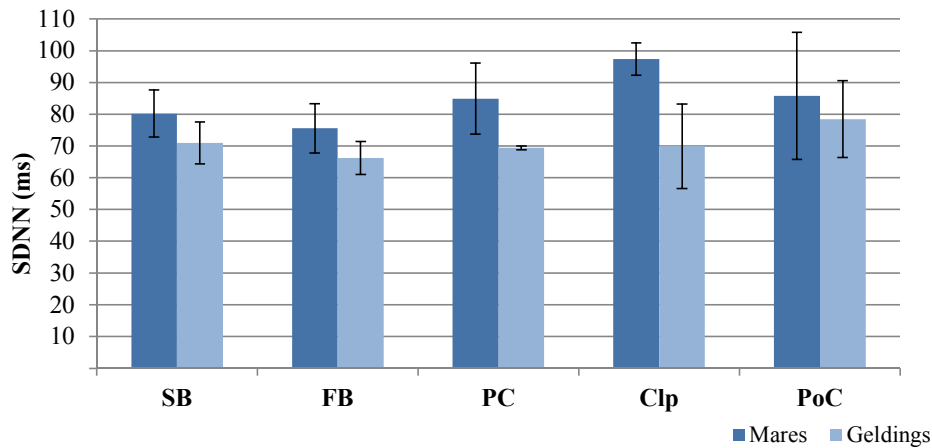
Figure 5.5. Mean heart rate variability (HRV) values (±SE) for baseline measures taken in the stable (SB) and field (FB), prior to clipping (PC), during clipping (Clp) and post-clipping (PoC). Horses are labelled as compliant (C) or non-compliant (NC) during clipping and the HRV variable is standard deviation of inter-beat intervals (SDNN). Differences were not significant (mixed between-within ANOVA).

For SDNN, there was no significant interaction between group and time-period, Wilks' Lambda = 0.51, $F(4, 3) = 0.73$, $p=0.63$ and no significant main effect for time-period, Wilks' Lambda = 0.54, $F(4, 3) = 0.63$, $p=0.68$. The main effect comparing the two groups was also not significant $F(1, 6) = 2.07$, $p=0.20$.

For RMSSD, there was no significant interaction between group and time-period, Wilks' Lambda = 0.59, $F(4, 3) = 0.52$, $p=0.73$ and no significant main effect for time-period, Wilks' Lambda = 0.57, $F(4, 3) = 0.57$, $p=0.71$. The main effect comparing the two groups was also not significant $F(1, 6) = 1.7$, $p=0.24$. It was not possible to reject the null hypothesis.

5.6.5. Behavioural score analysis per time-period

Horses tended to score higher during clipping, with post-clip scores returning to pre-clip levels (**Figure 5.6**).
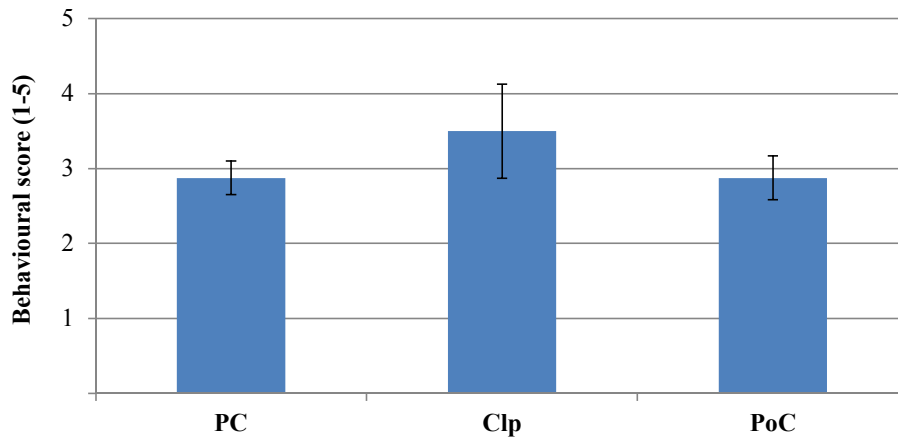
Figure 5.6. Mean behavioural scores (±SE) according to time-period; prior to clipping (PC), during clipping (Clp) and post-clipping (PoC). Behavioural scores were awarded on a scale of 1 (very little movement) to 5 (unwillingness to stand still). Differences were not significant (Friedman's ANOVA).

Data were non-normally distributed so were tested using the non-parametric Friedman's ANOVA to analyse behavioural data for the group as a whole according to time-period; PC, Clp and PoC. The results indicated that there was no significant difference in behavioural score across the three time-periods, X^2 (2) =2.16, $p=0.34$. It was not possible to reject the null hypothesis.

5.6.6. Behavioural score analysis according to group

Data were not normally distributed so a Mann-Whitney U test was carried out to compare behavioural scores between C and NC horses for each time-period (PC, Clp and PoC). The test revealed that during the pre-clipping period, no significant difference in behavioural score was present between compliant (*Md* = 2.5) and non-compliant horses (*Md* = 3.0), *U* = 3.0, *z* = -1.67, *p*=0.096 (**Figure 5.7**).
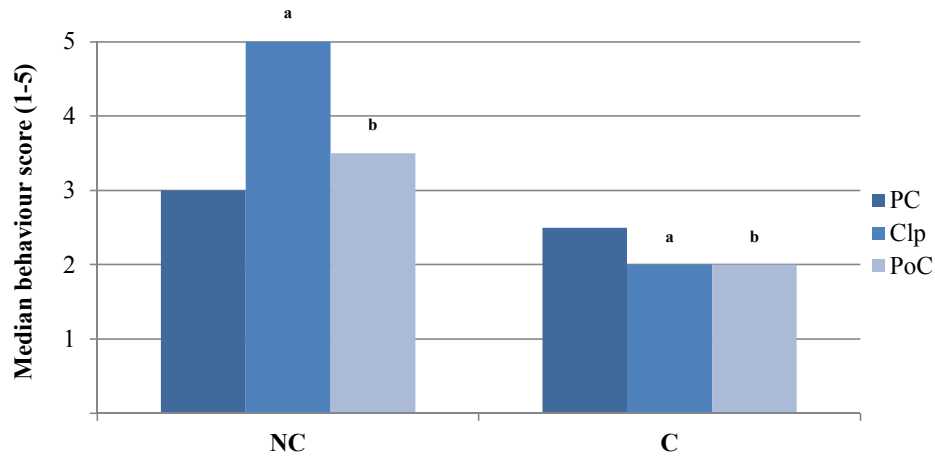
Figure 5.7. Mean behaviour scores for compliant (C) and non-compliant (NC) horses according to time period; prior to clipping (PC), during clipping (Clp) and post-clipping (PoC). Behavioural scores were awarded on a scale of 1 (very little movement) to 5 (unwillingness to stand still). Paired superscript letters denote significant differences between groups (Mann-Whitney U; p≤0.05).

There was a significant difference in behavioural score between compliant ($Md = 2.0$) and non-compliant horses ($Md = 5.0$) during the clipping period, $U = 0.0$, $z = -2.50$, $p=0.013$, with a large effect size ($r=0.88$). A significant difference was also present in behavioural scores between compliant ($Md = 2.0$) and non-compliant horses ($Md = 3.5$) during the post-clipping period, $U = 1.0$, $z = -2.14$, $p=0.032$, with a large effect size ($r=0.76$). It was therefore possible to reject the null hypothesis.

5.6.7. Behavioural score analysis according to sex

As analysis of HRV data had indicated the presence of significant sex-related differences, a comparison was also made between the behavioural scores for mares (M) and geldings (G). Data were not normally distributed so a Mann-Whitney U test was carried out to compare behavioural scores between M and G horses for each time-period (PC, Clp and PoC).

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The test revealed that behavioural scores did not differ significantly for M (*Md* = 3.0) and G (*Md* = 3.0) during the pre-clipping period, *U* = 6.5, *z* = -0.50, *p*=0.62 (**Figure 5.8**). During the clipping period there was no significant difference in behavioural scores between M (*Md* = 2.0) and G (*Md* = 5.0), *U* = 5.0, *z* = -0.93, *p*=0.35 or during the post-clip period (M: *Md* = 2.5; G: *Md* = 3.5; *U* = 4.0, *z* = -1.22, *p*=0.22). It was therefore not possible to reject the null hypothesis.
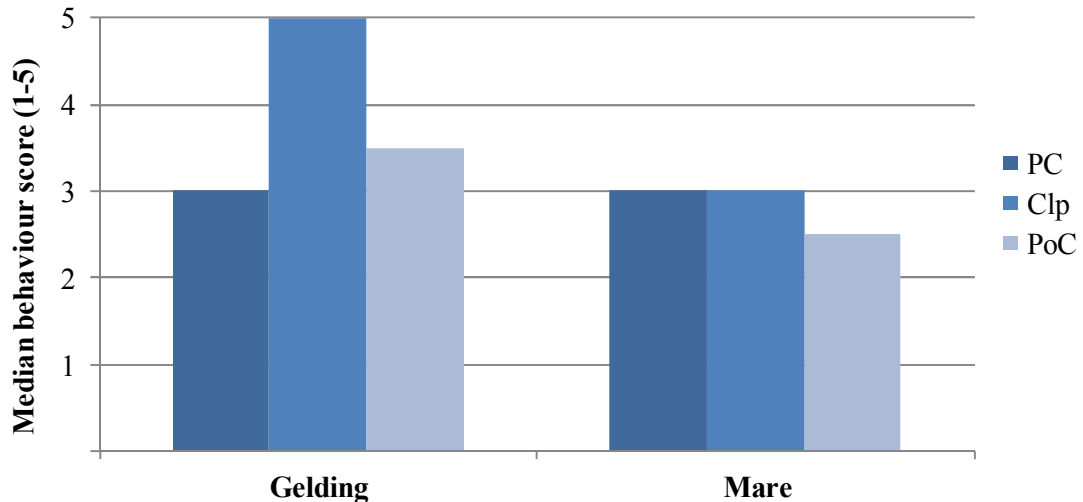
Figure 5.8. Median behaviour scores for mares and geldings according to time period; prior to clipping (PC), during clipping (Clp) and post-clipping (PoC). Behavioural scores were awarded on a scale of 1 (very little movement) to 5 (unwillingness to stand still). Differences were not significant (Mann-Whitney U).

5.6.8. Relationship between HRV and behavioural score

Non-parametric Spearman's rank order correlations were carried out to determine whether a statistically significant relationship existed between HRV (SDNN and RMSSD) and behavioural score during each time-period (PC, Clp and PoC).

During the PC period, a positive relationship was present in non-compliant horses between behavioural score and RMSSD where higher HRV was associated with more behavioural activity, but not at statistically significant levels (r_s=0.77, *p*=0.22). During the Clp period a positive relationship was found between compliant horses and HRV but this was also not at

statistically significant levels (RMSSD: r_s=0.89, p=0.11; SDNN: r_s=0.89, p=0.11). A positive relationship was also found between compliant horses and HRV in the PoC period, but again this did not reach significance (RMSSD: r_s=0.77, p=0.22; SDNN: r_s=0.77, p=0.22). No other significant relationships were present and it was not possible to reject the null hypothesis.

5.7. DISCUSSION

For the group as a whole, no significant differences in HRV values were found between time-periods or baseline values. Numerically, SDNN and RMSSD were both highest during the clipping period and the lowest levels recorded during the pre-clip period for RMSSD and in field baseline measures for SDNN. Although the highest behavioural scores were found during the clipping period there was no significant effect of time-period on behavioural score. The absence of significant differences in either behaviour or HRV between time-periods infers that, for the group as a whole, the interpretation of events during the clipping period has no more or less emotional valence than pre-clip, post-clip or baseline periods.

When HRV data for individual horses were analysed using data collected during all time-periods and baseline values, significant inter-subject variability existed in SDNN and RMSSD. Horse 5 recorded significantly higher SDNN than Horses 1, 2 and 3. Horse 7 recorded significantly higher RMSSD than Horses 1, 2, 3 and 6. While Horse 3 recorded significantly lower RMSSD than Horses 5 and 8.

Horse 3 was a non-compliant horse that is routinely chemically sedated in order for clipping to be successfully performed. Previous studies and general observation of this horse reveal a high degree of sensitivity, as evidenced by behavioural reactivity to aversive stimuli and unfamiliar handlers. Low levels of HRV have previously been associated with aspects of handling such as restlessness, resistance behaviour and reluctance to approach a novel object (Visser *et al.*, 2003a). The current observations are also in accordance with studies such as Eager *et al.* (2004) who related HRV components to equine temperament and both Visser *et al.* (2002) and Visser

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et al. (2003a) who suggest that HRV could be a useful measure for quantifying behavioural traits in horses. These findings imply that individual characteristics related to factors such as temperament, personality and underlying affective state may influence and be detected by HRV measurements.

A novel object and handling test carried out in horses by Visser *et al*. (2002) also suggested that measurement of HRV may be useful in differentiating between individuals and quantifying aspects of temperament. Objective methods of temperament assessment can have important implications for breeding, housing and management of the horse. They may contribute to breeding or selecting the winning athlete in sports and could prove useful in selecting the right horse to match with a recreational rider. Although not accounted for in the selection of subjects for the present study, breed is reported to be of major importance in the personality of the horse (Lesimple *et al.,* 2011) and breed differences in HRV have previously been reported in cattle which were thought to be related to temperament (Hagen *et al*., 2005). Further research is therefore necessary to determine whether breed and temperament differences in HRV do exist in horses. If so, HRV measurement could prove to be a useful objective criterion of temperament assessment particularly during bloodstock grading and futurity evaluations. These events run by equine breed associations and national equestrian federations, aim to augment selective breeding programmes by assessing horses for their performance potential as dressage horses, showjumpers, eventers or endurance horses and publicising their ratings. One of the strategic objectives of the British Equestrian Federation Equine Development Portfolio is to support 'the breeding of horses and ponies of suitable type and temperament to safely meet the needs of a growing base of riders, drivers and vaulters' (British Equestrian Federation, 2012).

Analysis of HRV data for each group according to time-period revealed that although compliant horses tended to record numerically higher HRV than non-compliant horses, the differences were not significant.

Behavioural score data can be interpreted as the level of behavioural activity on a scale of one (very little movement) to five (unwillingness to stand still). Compliant horses were relatively inactive throughout the study with mean behavioural scores of 2.5, 2.0 and 2.3 (out of a maximum score of 5.0) for Pre-clip, Clip and Post-clip periods, respectively. Analysis of behavioural score data revealed that non-compliant horses were significantly more behaviourally active than compliant horses during both the clipping and post-clipping periods, but not during the pre-clipping period. Avoidance behaviour in response to an aversive stimulus has been identified by Dantzer (2002) as evidence of negative emotion and Sanger *et al.* (2011) used the shearing procedure as a short-term stressor when studying cognitive bias in sheep. The behavioural results of the current study confirm that sham-clipping can be classed as an aversive stimulus for some horses.

The largest magnitude of differences in HRV between groups was found during clipping which provides evidence that the underlying psychophysiological state in these horses might be linked to the level of behavioural activity displayed during a negatively valenced event. It was not possible to confirm this statistically however, as correlation analysis did not reveal a relationship between HRV and behaviour score. Schmidt *et al.* (2010c) have previously reported low HRV in behaviourally compliant horses in response to being mounted by a rider, a situation resembling a potentially lethal threat under natural conditions. In the present study, compliant horses recorded numerically higher HRV throughout the study than the non-compliant group which provides evidence of behavioural tolerance of the procedure being due to genuine relaxation and not emotional depression, withdrawal or learned helplessness.

Mares recorded numerically higher SDNN and RMSSD than geldings throughout each time-period and in comparison with baseline measurements, with the largest magnitude of differences occurring between the two groups during clipping. Although geldings were more behaviourally active during and after clipping, no significant differences in behaviour score were found according to sex.

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It is relevant to note that the compliant group was in fact comprised of three mares and one gelding. As groups were not selected according to sex but solely for their tolerance/intolerance towards the clipping process there was an unbalanced ratio of mares:geldings in both groups which would undoubtedly skew sex-based analysis. However, it is possible that females have a naturally higher tolerance to aversive stimuli than males which might explain why there was a greater proportion of mares in the compliant group upon selection. Whilst small sample sizes may have limited statistical analysis in the current study, the pattern of differences in HRV between sexes follows the same sex-related differences in HRV that have previously been reported in human research; females have a greater vagal control of the heart than males who exhibit more sympathetic cardiac control (Rossy and Thayer, 1998). One of the limitations of this study was that the majority of horses at Brackenhurst Equestrian Centre, from which subjects were selected, fell into the compliant category. This meant that only a small number of non-compliant horses were available for testing from which it was not feasible to block for any effects of sex.

Although anecdotal industry stereotypes are common, very little evidence exists to quantify differences in equine behavioural characteristics such as temperament and emotionality between sexes. Sex-related differences in trainability and personality have recently been reported in yearling horses in training via a questionnaire-based study. Duberstein and Gilkeson (2010) found that mares were more aggressive, tense, excitable and panicked more easily than geldings, who were rated as more easily desensitised than mares during training. Although not directly comparable, both the present study and Duberstein and Gilkeson's work (2010) highlight the potential benefit to the horse industry in categorising personality differences and isolating strengths and weaknesses of each sex using physiological measurements such as HRV alongside behavioural observations. This could potentially lead to modifications of training programmes, or selection for a particular niche based on sex differences.

As previously stated by Porges (1995), reduced activity of the vagus, one of the efferent parasympathetic nerves, limits physiological and behavioural capacity to cope with stressful events; measuring vagal tone therefore provides knowledge of individual stress vulnerability. In the current study, analysis of HRV data according to group revealed that, although not significant, non-compliant horses had numerically lower HRV measurements than compliant horses over all. This may be indicative of an underlying negative affective state present in non-compliant horses, where the variability of inter beat intervals is under greater sympathetic, rather than parasympathetic, control than the compliant horses. Although none of the horses in this study exhibit stereotypic behaviour and the sample size in each group is small, these findings are akin to those of Bachmann *et al*. (2003) and Nagy *et al*. (2009) who reported lower basal parasympathetic activity in crib-biting horses than healthy controls. Non-compliant horses chosen for this study, based solely on their previous behavioural response to clipping, may therefore experience a more negative affective state at rest and be more susceptible to stress than the compliant horses.

5.8. CONCLUSION

This study investigated the relationship between HRV and behaviour during a negatively valenced management procedure. Horses chosen for their non-compliant behaviour had numerically lower HRV than compliant horses which may be indicative of their underlying affective state, although on this occasion it was not possible to rule out an association with sex based differences. On the whole, time-period seemed to affect behaviour but not HRV, suggesting that for these horses, the sham-clipping period was no more or less emotionally valenced than spending time in their stable or the field despite the fractious behaviour of some individuals. It was also useful to establish that the compliant horses were not concealing a negative affective state beneath a calm exterior.

As previously identified in **Chapter 3: Section 3.8.2** and **Chapter 4: Section 4.6.2** individual differences in HRV were also evident in the present study which may be associated with underlying affective state or temperament, as has been purported by other researchers. Factors affecting individual HRV, including temperament, are therefore investigated in **Chapter 7.**

In summary, the results of this study suggest that alongside behavioural observations, HRV measurements could provide useful reinforcement a) for detecting underlying affective states in horses, b) determining the relationship between externally manifested behaviour and internal affective state in response to different management practices, and c) assessing the welfare of horses, particularly how they are coping with their environment and the challenges with which they are presented.

Chapter Six

The Effect of Different Housing Systems on Equine HRV

and Behaviour.

6.1. INTRODUCTION

As reviewed throughout **Chapter 1**, horses have evolved to exist in complex social groups and possess a strong drive to form social bonds with other horses. This intensely social nature is an important element of a horse's behavioural repertoire observed in both feral and domestic groups (Houpt and Houpt, 1988; Crowell-Davis, 1993; van Dierendonck *et al*., 1996; Christensen *et al*. 2002a; Christensen *et al*., 2002b; van Dierendonck, 2006). Given the option, horses usually maintain visual contact with others for social cohesion and will spend around half their time in contact with other horses (Houpt and Houpt, 1988; Lee *et al.,* 2011).

Chapter 1: Section 1.3.1 identified the confinement of horses in solitary housing for long periods of time as a common management practice despite social isolation being highlighted as a major factor affecting physiological responses and the course of associated diseases in humans and animals. The potential for social isolation to detrimentally affect welfare is further emphasised by its frequent use as a stimulus to induce a stress response in animals in studies undertaken by Mal *et al.* (1991a), Mal *et al.* (1991b), Boissy and Le Neindre (1997), Sandem and Braastad (2005), Reefmann, Wechsler and Gygax (2009) and Reefmann *et al.* (2009b).

The effect of domestic management practices on equine health and welfare has been evaluated in **Chapter 1: Section 1.3**. In summary, equine research to date has examined the effects of different housing systems from a variety of perspectives. Lesimple *et al.* (2011) related housing conditions with behavioural measures of emotionality in horses. Hartmann, Christensen and Keeling (2009) reported the benefits of pre-exposing unfamiliar horses to each other in neighbouring boxes prior to turn out. Visser, Ellis and Van Reenen (2008) studied the effect of individual versus paired housing on the behaviour, physiology and temperament of young horses. Harewood and McGowan (2005) measured the physiological and behavioural effects of confinement and isolation in naïve horses. Christensen *et al.* (2002a) investigated the effects of

individual versus group stabling on social behaviour in stallions. Rivera *et al.* (2002) and Søndergaard and Halekoh (2003) considered the effects of group housing and training on the horse-human relationship. Heleski (2002) also studied the effect of solitary confined housing versus group paddock housing on weanling behaviour and physiology. In addition, numerous investigations have been made into the effects of stable design and modifications on stereotypic behaviour (e.g. Cooper, McDonald and Mills, 2000, McAfee, Mills and Cooper, 2002, Mills and Davenport, 2002, Mills and Riezebos, 2005 and Ninomiya *et al.,* 2008), yet studies investigating the effect of different housing systems on affective state in horses are scarce.

The majority of these studies collate behavioural data to either analyse time-budgets or note the presence or absence of certain behaviours. When using behavioural evidence alone, however, it is only possible to infer the underlying affective state. Where physiological data is also gathered to support behavioural observations, these studies tend to rely on HR data which, as considered in **Chapter 1: Section 1.5.1** is difficult to interpret, or take invasive physiological measurements from blood/plasma samples in an attempt to establish the presence or absence of stress hormones. Of the equine housing studies cited above, only Visser, Ellis and Van Reenen (2008) incorporate HRV measurements in their investigation into whether individual or paired housing influenced the emotional response of young horses to a novel object test using a brightly coloured umbrella; it was found that housing group had no significant effect on HRV.

During autumn and winter, horses at Brackenhurst Equestrian Centre are housed either individually in one of two types of indoor stables (each offering a different level of visual contact with neighbouring horses), or are loose housed in pairs within an indoor crew yard. While the horses are exercised every day, due to the adverse weather and ground conditions, they are not routinely turned out for unrestricted social interaction in a paddock. During the spring and summer months, horses are housed singly or in pairs as described previously, but spend evenings and weekends turned out to graze in paddocks in small groups. **Chapter 3**

investigated the response of horses to a positive reward in the form of companionship and identified behavioural and HRV responses that appeared to be related to management regime and the presence or absence of social contact. The current study was therefore conducted to investigate these responses in horses kept in four different housing systems offering increasing levels of social contact and physical space.

The study aimed to compare HRV and behavioural data from each housing system in order to investigate the effects of social isolation and companionship on affective state and establish whether a relationship existed between behaviour and HRV. In the interests of improving equine welfare it is important to provide opportunities for positive emotional experiences and it was hoped that the findings of this study could identify an optimum housing system that balances the practical requirements of the horse owner whilst achieving the endogenous needs of the horse.

6.2. SUBJECTS

Sixteen mature horses (age 14.1±2.8 years) consisting of eight mares and eight geldings were selected from Brackenhurst Equestrian Centre (**Table 6.1**). Subjects were allocated by the Yard Manager according to their availability for the duration of the study and their suitability for being housed in close proximity to the rest of their group during the study. The subjects were familiar with each other as they had resided at the Equestrian Centre for at least twelve months prior to the study, some of whom would have previously been stabled or turned out to pasture within sight of each other and regularly took part in riding lessons and hacking out together, although not necessarily in the same segregated groups as used currently. Two weeks prior to the study commencing, the horses were separated by sex into groups of four and turned out to pasture and left to graze in their respective groups to allow the group members to become accustomed to each other.

Table 6.1. Profile of subjects

Group	Horse	Age	Height (hands)	Breed	Sex
1	Conan	12	17.0	Irish Draught x Thoroughbred	Gelding
	Kitkat	8	15.2	Cob	Gelding
	Visi	19	16.0	Thoroughbred	Gelding
	Woody	15	17.2	Warmblood	Gelding
2	Angus	12	16.3	Warmblood	Gelding
	Del	14	16.0	Shire	Gelding
	Herbert	11	17.1	Thoroughbred	Gelding
	Ernie	16	16.0	Thoroughbred	Gelding
3	Libby	12	15.2	Irish Draught x Thoroughbred	Mare
	Ellie	13	16.1	Irish Draught x Thoroughbred	Mare
	Blackberry	15	16.2	Irish Draught x Thoroughbred	Mare
	Liesle	15	15.3	Warmblood	Mare
4	Tosca	14	15.2	Cob	Mare
	Fanta	16	15.2	Cob	Mare
	Beau	15	16.0	Irish Draught x Thoroughbred	Mare
	Puzzle	19	15.3	Cob	Mare
	Mean	14.1			
	St.Dev.	2.8			

6.3. STUDY PROTOCOL

Prior to the study commencing, the study protocol gained ethics approval and appropriate risk assessments were undertaken. As described in **Chapter 2: Section 2.1**, baseline HRV measurements were obtained from each of the participants prior to the commencement of the study whilst in their usual stable or crew yard and when turned out to pasture. Over a period of four weeks each group was rotated through four different housing systems in a latin square design (**Table 6.2**).

Table 6.2. Rotation of housing design according to group.

	Week 1	Week 2	Week 3	Week 4
Paddock	1	3	2	4
Crew yard	2	1	4	3
Farmhouse	3	4	1	2
Lodden	4	2	3	1

Each group of horses was brought in from the field and placed in their respective housing between 8.30am and 4.30pm Monday to Friday of each week, returning to their respective paddocks at pasture overnight and at the weekends. Housing systems changed on the Monday of each of the four weeks. Whilst kept in each of the housing systems, all subjects had access to water on an ad-lib basis, no supplementary feed was provided.

Each housing system allowed the horses a different amount of space and level of social contact. The paddock was 80m long and measured approximately 20m at the widest point, tapering to approximately 10m at the narrowest point. All four horses in each group were turned out together and allowed to roam freely whilst housed in the paddock (**Figures 6.1** and **6.2**).

Figure 6.1 (l). View of the paddock housing system from one of two CCTV video cameras used to capture behavioural footage, showing the full extent of enclosure.
Figure 6.2 (r). View from within the paddock housing system showing a horse fitted with HRV recording equipment.

The two crew yards used were adjacent to each other situated indoors within a barn area. Each crew yard measured 9.4m x 10m and had a wood shaving bed over concrete flooring. Two horses from each group were housed in each crew yard, separated by 1.8m high metal fencing (**Figures 6.3** and **6.4**).

Figure 6.3. (l) View of the crew yard housing system from one of the CCTV video cameras used to capture behavioural footage.
Figure 6.4. (r) Detail of within the crew yard showing a pair of horses used in the study, one of which is fitted with HRV recording equipment.

A row of four adjacent concrete-block built stables were used to house horses individually (Farmhouse). These were situated indoors within a barn, measured 3.5 m x 3.5 m and comprised an open fronted design where horses could see adjacent horses only when looking out of the front of the stable. Solid internal walls at the rear and sides of the stable prevented visual contact with neighbouring horses from within the stable (**Figures 6.5** and **6.6**).

Figure 6.5. (l) Detail of farmhouse housing system showing height of internal walls and proximity of neighbouring stable.
Figure 6.6. (r) View of a farmhouse stable from one of the CCTV video cameras used to record behavioural activity.

The final housing system was comprised of four Lodden Ascot design stables used to house horses individually. These were situated indoors within a barn, measured 3.5 m×3.5 m and were constructed of timber panel and galvanised steel vertical bar fronts and sides with a sliding door

(Lodden Livestock Equipment, Lodden). This type of housing allowed visual and olfactory contact between neighbouring horses (**Figures 6.7** and **6.8**).

Figure 6.7. (l) View of a Lodden stable from one of the CCTV video cameras used to capture behavioural footage.
Figure 6.8. (r) Detail from within a Lodden stable showing view into adjacent stable.

6.4. DATA COLLECTION

Behavioural data were captured on high definition wide-angled cameras permanently fixed above each of the housing systems (one above each of the four Farmhouse and Lodden stables, one above each crew yard and two overlooking the paddock). The cameras continually recorded 24 hours per day and data were stored on a hard drive situated on-site inside the yard manager's office. At the end of every day data from each camera recorded between the hours of 8.30am and 4pm were downloaded from the hard drive onto DVD disc format for analysis.

One horse from each group was selected using a simple random sampling procedure (names drawn from a pot) to be fitted with HRV monitoring equipment (one horse for each available Polar Equine RS800G3 heart rate monitor) which remained in situ between the hours of 8.30am and 4pm, Monday to Friday for the duration of the study. The equipment was attached to the thorax with an elastic girth with the positive electrode located at the left shoulder and the negative electrode at the lower left thorax as described in **Chapter 2: Section 2.3.1**. The equipment was fitted as soon as the horse was brought in from the field and placed in the respective housing system. Electrical conductivity was increased by the use of ultrasonic gel and recording began as soon as the heart rate signal was obtained. Recording stopped and the

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equipment was removed prior to the horse being returned to their field overnight which was much larger and contained lush grass in comparison to the paddock used as a housing type for the study. At the end of each day data from the four Polar recording devices were downloaded via a Polar Infrared Interface using Polar Pro-Trainer 5 software and stored on a PC for analysis.

6.5. HRV DATA ANALYSIS

RR interval data were analysed using Kubios HRV analysis software. To remove trend components, data were de-trended and an artefact correction was made as described in **Chapter 2: Section 2.1.** Time domain analyses were then calculated from mean RR interval data for each horse, per day, per week and for each housing system; Paddock, Crew yard, Farmhouse and Lodden. Baseline data were not included in statistical analysis due to the recording durations being considerably shorter (15mins) than data collected during the study.

For each data set SDNN and RMSSD were analysed using SPSS 18.0. Kolmogorov-Smirnov tests for normality were carried out on each data set and statistical tests chosen accordingly. Differences in HRV measurements between housing systems, effect of day and week were tested using the non-parametric Friedman's ANOVA. Differences in HRV between individual horses were investigated using non-parametric Kruskal-Wallis tests, followed by post-hoc Mann-Whitney U tests where significant differences occurred.

6.6. BEHAVIOURAL DATA ANALYSIS

Behavioural data were analysed for the sixteen subjects using data collected during the fourth day (Thursday) from each week of the study. The duration of behavioural states observed for each horse between the hours of 8.30am and 4pm on the fourth day each week were analysed via an ad-libitum sampling regime. Using an ethogram (**Appendix 5**) the duration of browsing, standing, lying, locomotion, social interaction (positive and negative) stereotypic behaviour and auto-grooming behaviours were recorded. Durations of raw data for each horse in time format

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(seconds) were converted to a decimal number value using Microsoft Excel and imported into SPSS 18.0 for statistical analysis.

Kolmogorov-Smirnov tests for normality were carried out on each data set and statistical tests chosen accordingly. Differences in behavioural measurements between each housing system (Lodden, Farmhouse, Crew Yard and Paddock) and between each week of the study were investigated using the non-parametric Friedman's ANOVA followed by individual Wilcoxon signed ranks tests where appropriate (using Bonferroni adjusted alpha values to control for Type 1 error; Pallant, 2007). Differences in behavioural measurements between sexes were measured using a non-parametric Mann-Whitney U test. Differences in behaviour between individual horses were investigated using a non-parametric Kruskal-Wallis test.

From the four horses wearing HRV monitoring equipment, mean SDNN and RMSSD data from days 1-5 of each week were calculated and used to compare the relationship between HRV and corresponding behavioural states in those horses using Spearman's rank order correlations.

6.7. RESULTS

6.7.1. Heart rate variability analysis per housing system

Mean SDNN values were highest when housed in the Crew Yard (106.65±21.40ms) and Paddock (104.13±23.99ms) and lowest when housed in the Farmhouse stables (76.61±10.90ms). Mean RMSSD values did not vary greatly, ranging from 87.94±15.31ms whilst housed in the Lodden stables, to 98.41±10.08ms when housed in the Crew Yard (**Figure 6.9**).
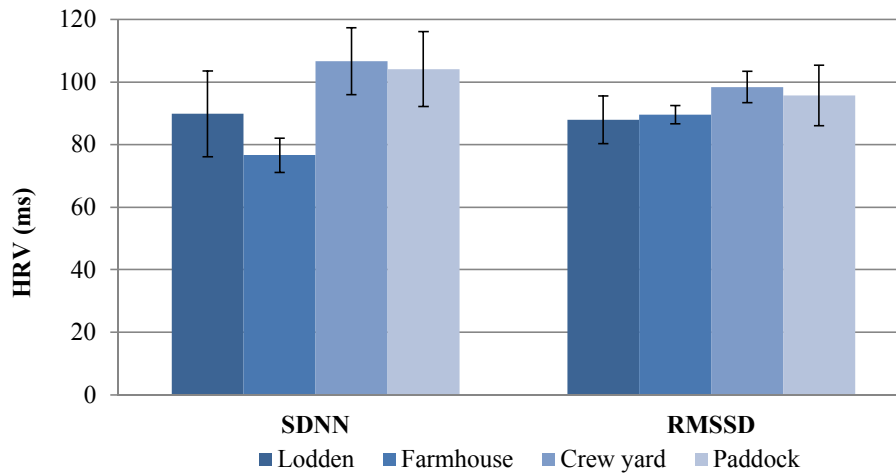
Figure 6.9. Mean heart rate variability (HRV) values for each housing type (±SE). HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (Friedman's ANOVA).

A Friedman's ANOVA test confirmed that differences in SDNN were not significant, X^2 (3) = 5.70, p=0.13. Differences in RMSSD measurements were also not significant, X^2 (3) = 0.30, p=0.96. It was not possible to reject the null hypothesis.

6.7.2. Inter-individual heart rate variability

Considerable inter-individual variations were observed in mean SDNN values throughout the study (**Figure 6.10**). A Kruskal-Wallis test identified that significant differences in SDNN existed between individual horses, X^2 (3) = 17.13, p=0.001. A Mann-Whitney U test confirmed that significantly lower SDNN was recorded for Horse 1 (*Md* = 68.6ms) than for Horse 2 (*Md* = 96.7ms), U = 64.0, z = -3.4, p=0.001, r = 0.6), Horse 3 (*Md* = 88.4ms) U = 85.0, z = -2.95, p=0.003, r = 0.5) and Horse 4 (*Md* = 96.65ms), U = 76.0, z = -3.2, p=0.001, r = 0.5). It was therefore possible to reject the null hypothesis.

Differences in RMSSD between individual horses were not significant, X^2 (3) = 2.87, p=0.41.
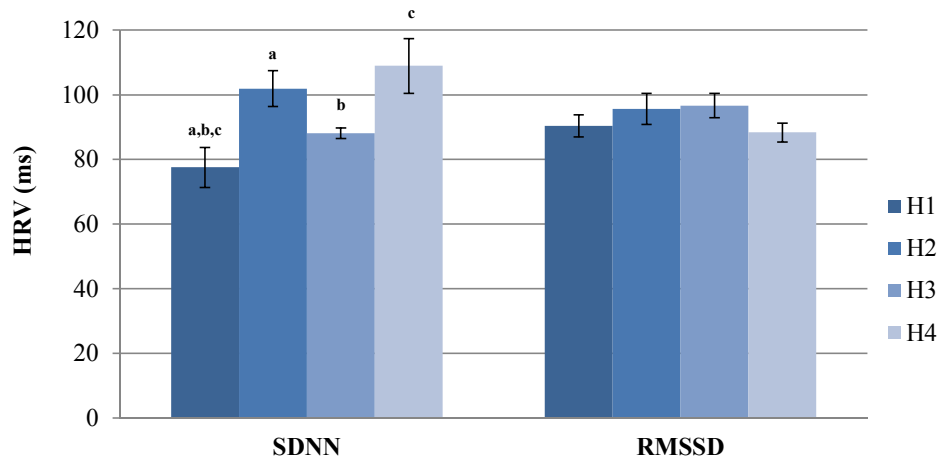
Figure 6.10. Inter-individual differences in mean heart rate variability (HRV) using data collected throughout the study (±SE). HRV variables were standard deviation of inter-beat intervals (SDNN). Paired superscript letters denote significant inter-individual differences (Kruskal-Wallis and Mann-Whitney U post-hoc comparisons; p≤0.05).

6.7.3. Heart rate variability analysis per day of study

Mean daily SDNN values throughout the study ranged from 86.60±19.01ms on Tuesdays to 101.33±15.38ms on Fridays. Mean RMSSD values did not vary greatly, ranging from 89.26±5.44ms on Tuesdays to 95.67±3.68ms on Thursdays (**Figure 6.11**).
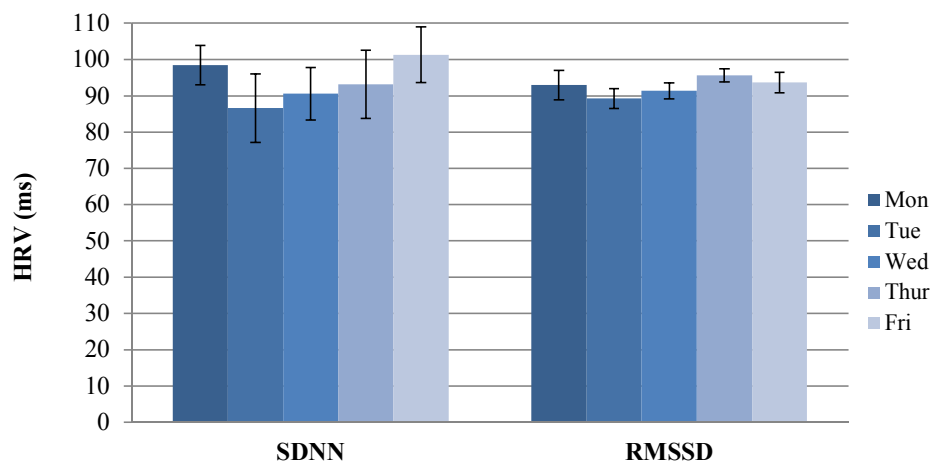
Figure 6.11. Mean heart rate variability (HRV) values for each day of the study (±SE). HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (Friedman's ANOVA).

The results of the Friedman's ANOVA indicated that SDNN measures did not differ significantly according to the day of the week, X^2 (4) = 7.40, p=0.12. There was also no significant effect of week day on RMSSD, X^2 (4) = 2.80, p=0.59. It was therefore not possible to reject the null hypothesis.

6.7.4. Heart rate variability analysis per week of study

Mean weekly SDNN values throughout the study ranged from 86.69±2.69ms for Week 4 to 98.36±22.43ms in Week 3. Mean RMSSD values ranged from 86.45±14.83ms during Week 1 to 100.84±15.98ms for Week 2 (**Figure 6.12**).
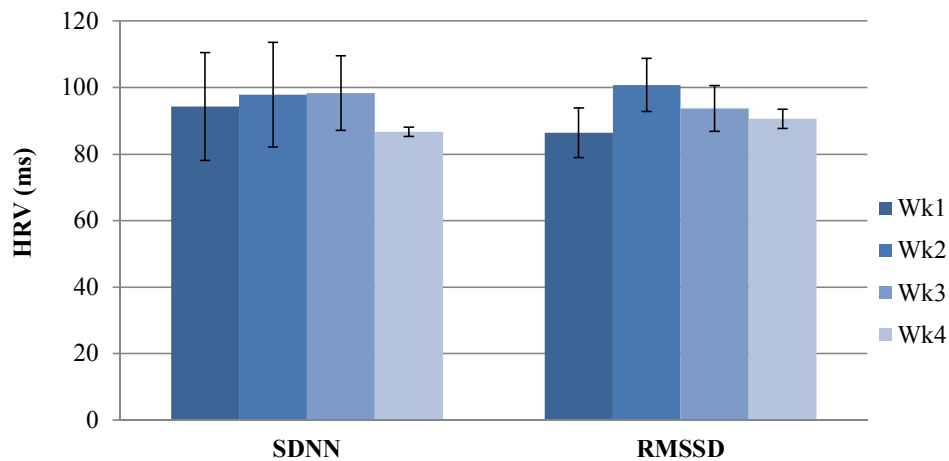
Figure 6.12. Mean heart rate variability (HRV) values for each week of the study (±SE). HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (Friedman's ANOVA).

The results of the Friedman's ANOVA indicated that SDNN measures did not differ significantly each week, X^2 (3) = 0.90, p=0.83. There was also no significant difference in RMSSD according to week, X^2 (3) = 1.20, p=0.75. It was not possible to reject the null hypothesis.

6.7.5. Behavioural results per housing system

A Friedman's ANOVA was carried out to investigate whether differences in behaviour occurred according to housing system; Lodden, Farmhouse, Crew Yard and Paddock.

Horses spent the longest time browsing in the Paddock (36.2% of time observed), followed by the Crew Yard (4.4%), the Lodden stables (4.3%) and the shortest time in the Farmhouse stables (0.7%, **Table 6.3**). The results of the Friedman's ANOVA confirmed that housing type significantly affected the duration of time the horses spent browsing X^2 (3) =32.58, $p<0.001$. Post-hoc analysis via a Wilcoxon signed-rank test (using an adjusted alpha value of 0.01) revealed that horses spent significantly longer browsing whilst housed in the paddock than when housed in either the Lodden stables (z = -3.52 $p<0.001$, r = 0.6), the Farmhouse stables (z = -3.52 $p<0.001$, r = 0.6) or the Crew Yard (z = -3.52 $p<0.001$, r = 0.6). There was also significantly more browsing behaviour observed when housed in the Crew yard than in the Farmhouse stables (z = -2.99 $p=0.003$, r = 0.5) and in the Lodden stables compared with the Farmhouse stables (z = -2.07 $p=0.038$) although this was not significant at the revised alpha value of 0.01. It was therefore possible to reject the null hypothesis.

Table 6.3. Mean (sec) and standard deviation (SD) of the duration of behavioural states according to housing system. Paired superscript letters denote significant differences between housing types (Friedman's ANOVA; p≤0.05 and Wilcoxon signed-rank tests using Bonferroni adjusted alpha values).

	Lodden		Farmhouse		Crew Yard		Paddock	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Browsing	1098[a]	1123	186[bd]	222	1055[cd]	1487	8955[abc]	2940
Standing	23106[ad]	2191	23287[be]	2248	19593[cde]	1897	14369[abc]	2950
Lying	490[b]	1532	587	1675	1431[ab]	1951	28[a]	41
Locomotion	677[a]	366	562[bc]	321	1279[c]	1083	1648[ab]	689
Social +ve	69	155	0[ab]	0	355[a]	410	338[b]	466
Social -ve	3	9	0	0	228	447	3	7
Stereotypy	37[a]	108	957[abc]	1493	11[b]	35	0[c]	0
Auto-groom	268[a]	278	329[abc]	353	254[b]	268	4[c]	11

Horses spent the longest time standing in the Farmhouse stables (89.9% of time observed) with similar levels observed in the Lodden stables (89.7%), followed by 80.9% in the Crew Yard and 55.6% in the Paddock (**Table 6.3**). Housing system had a significant effect on the duration of 'standing' behaviour, X^2 (3) =38.85, $p<0.001$. Post-hoc analysis via a Wilcoxon signed-rank test (using an adjusted alpha value of 0.01) identified that significantly less standing behaviour occurred in the Paddock than in either the Lodden stables (z = -3.52 $p<0.001$, r = 0.6), the Farmhouse stables (z = -3.52 $p<0.001$, r = 0.6) or the Crew Yard (z = -3.36 $p<0.001$, r = 0.6). Significantly less standing behaviour was also observed in the Crew Yard than in either the Lodden stables (z = -3.26 $p=0.001$, r = 0.6) or the Farmhouse stables (z = -3.46 $p=0.001$, r = 0.6). It was therefore possible to reject the null hypothesis.

Horses spent the longest time lying down or rolling in the Crew Yard (5.9% of time observed), followed by 2.3% in the Farmhouse stables, 1.9% in Lodden stables and was rarely seen in the Paddock (0.1%, **Table 6.3**). The duration of time horses were observed lying down or rolling was significantly affected by housing system, X^2 (3) =7.81, $p=0.050$. Post-hoc analysis via a Wilcoxon signed-rank test (using an adjusted alpha value of 0.025) identified that this behaviour occurred significantly more in the Crew Yard than in the Paddock (z = -2.52 $p=0.012$, r = 0.4) and the Lodden stables (z = -1.96 $p=0.050$, r = 0.3) although this was not significant at the revised alpha level of 0.025. It was therefore possible to reject the null hypothesis.

Horses performed slightly more locomotory behaviour in the Paddock (6.7% of time observed) than in the Crew Yard (5.3%), followed by 2.6% in the Lodden stables and 2.2% in the Farmhouse stabled (**Table 6.3**). There was a significant effect of housing system on the duration of locomotory behaviour X^2 (3) =22.26, $p<0.001$ which enabled the null hypothesis to be rejected. Post-hoc analysis via a Wilcoxon signed-rank test (using an adjusted alpha value of 0.0125) revealed that significantly more locomotory behaviour was observed in the Paddock than in either the Lodden stables (z = -3.26 $p=0.001$, r = 0.6) or the Farmhouse stables (z = -3.52 $p<0.001$, r = 0.6). Significantly more locomotory behaviour was also found in the Crew

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Yard than in the Farmhouse stables (z = -2.5 p=0.012, r = 0.4) and the Lodden stables (z = -2.01 p=0.045) although this was not significant at the revised alpha level of 0.0125. It must be noted however that physical confinement rendered it difficult for horses housed in the Lodden or Farmhouse stables to carry out much locomotory behaviour and any incidences of repetitive box-walking were classified as 'stereotypic' as defined in **Appendix 5** rather than 'locomotory' behaviour.

The performance of positive social interactions was relatively infrequent regardless of housing type (**Table 6.3**). Similar durations were seen in the Crew Yard (1.5% of time observed) and the Paddock (1.4%) but the behaviour was rarely seen in the Lodden stables (0.3%), which allowed olfactory contact between the bars, and was physically prevented in the Farmhouse stables. Significant differences in the duration of positive social interactions were found according to housing type, X^2 (3) =18.59, p<0.001, which enabled the null hypothesis to be rejected. Post-hoc analysis via a Wilcoxon signed-rank test (using an adjusted alpha value of 0.016) highlighted the lack of opportunity for social interaction in the Farmhouse stables as this behaviour was significantly lower here than in either the Crew Yard (z = -2.805 p=0.005, r = 0.5) or the Paddock (z = -2.81 p=0.005, r = 0.5).

Negative social interactions were rarely observed (**Table 6.3**) and tended to be carried out by the male horses whilst in the Crew Yard. The duration of this behaviour was significantly affected by housing type X^2 (3) =8.43, p=0.038 which enabled the null hypothesis to be rejected. Post-hoc analysis via a Wilcoxon signed-rank test (using an adjusted alpha value of 0.016) compared this behaviour in the Crew Yard with the Lodden stables (z = -1.89 p=0.058), Paddock (z = -1.89 p=0.058) and Farmhouse stables (z = -2.02 p=0.043) but differences in the duration of this behaviour did not reach significance at the revised alpha level of 0.016.

Bouts of stereotypic behaviour were mainly observed in the Farmhouse stables (3.7% of time observed), rarely in the Lodden stables (0.15%) and Crew Yard (0.05%) and did not occur in the

Paddock (**Table 6.3**). The Friedman's ANOVA confirmed that there was a significant effect of housing system on the duration of stereotypic behaviour X^2 (3) =20.53, $p<0.001$ which enabled the null hypothesis to be rejected. Post-hoc analysis via a Wilcoxon signed-rank test (using an adjusted alpha value of 0.016) identified that horses carried out significantly more stereotypic behaviour whilst housed in the Farmhouse stables than in the Paddock (z = -2.52 $p=0.012$, r = 0.4), Crew Yard (z = -2.52 $p=0.012$, r = 0.4) or Lodden stables (z = -2.52 $p=0.012$, r = 0.4).

Significant differences in the amount of auto-grooming were observed according to housing type, X^2 (3) =23.65, $p<0.001$. This behaviour was very rarely observed in the Paddock (0.02% of time observed), but ranged from 1.04% to 1.27% in the other housing types (**Table 6.3**). Post-hoc analysis via a Wilcoxon signed-rank test (using an adjusted alpha value of 0.016) confirmed that horses carried out significantly less auto-grooming behaviour whilst housed in the Paddock than in the Farmhouse stables (z = -3.30 $p=0.001$, r = 0.6), Crew Yard (z = -3.18 $p=0.001$, r = 0.6) and Lodden stables (z = -3.30 $p=0.001$, r = 0.6). It was therefore possible to reject the null hypothesis.

6.7.6. Behavioural results according to sex and inter-individual differences

A Mann-Whitney U test revealed that there were no significant differences in behaviour according to sex which meant it was not possible to reject the null hypothesis. However, there was a trend for mares performing less stereotypic behaviour (198±302sec) than geldings (1813±2032sec) overall (U = 413.5, z = -1.94, $p=0.052$, r =0.5). It was evident from analysis of descriptive statistics that the effect of sex on stereotypic behaviour was mainly due to differences when housed in the Farmhouse stables (**Figure 6.13**).
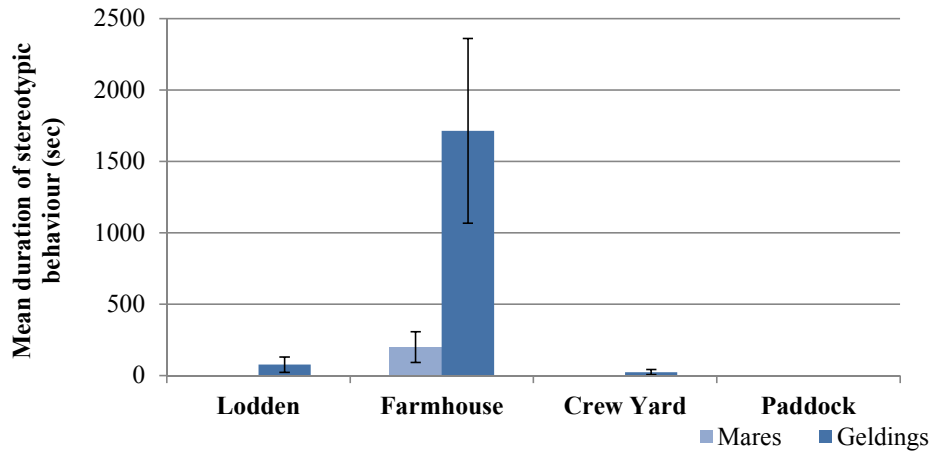
Figure 6.13. Mean duration of stereotypic behaviour (sec) per housing type according to sex (±SE). A trend was present for mares performing less stereotypic behaviour than geldings (Mann Whitney U; p≤0.05).

No significant differences in the duration of behavioural states were found between individual horses. It was therefore not possible to reject the null hypotheses relating to these criteria.

6.7.7. Behavioural results per week of study

A Friedman's ANOVA was carried out to investigate whether differences in behaviour also occurred over the duration of the study. The duration of positive social interactions between horses was higher during Week 1 than Weeks 2 to 4 of the study (**Table 6.4**) and the results of the Friedman's ANOVA indicated that there was a significant difference in the duration of positive social interaction between weeks X^2 (3) =8.55, p=0.036 which enabled the null hypothesis to be rejected.

Table 6.4. Mean duration (sec) and standard deviation (SD) of positive social interactions for the duration of the study. Differences between weeks were significant (Friedman's ANOVA; p≤0.05) but not at the post-hoc revised alpha level (Wilcoxon signed-ranks; p≤0.016).

Week	1	2	3	4
Mean	482	172	11	96
SD	511	289	31	186

It was possible to confirm via post-hoc analysis using a Wilcoxon signed-rank test (using an adjusted alpha value of 0.016) that horses spent significantly longer engaging in positive social interactions during Week 1 of the study than during Week 3 ($z = -2.68$ $p=0.007$, $r = 0.5$) and longer in Week 1 than Week 4 although this did not reach significance at the revised alpha level of 0.016 ($z = -2.06$ $p=0.039$). Horses also spent longer engaging in this behaviour during Week 2 than Week 3 but again this did not reach significance at the revised alpha level ($z = -2.05$ $p=0.040$).

Significant differences in the time spent auto-grooming were also observed between weeks of the study, $X^2 (3) =8.65$, $p=0.034$ (**Table 6.5**). Post-hoc analysis via a Wilcoxon signed-rank test (using an adjusted alpha value of 0.025) confirmed that horses spent significantly longer performing auto-grooming behaviour during Week 4 of the study than during Week 2 ($z = -2.67$ $p=0.008$, $r = 0.5$). It was therefore possible to reject the null hypothesis. No significant differences were found in any of the other behavioural states according to week.

Table 6.5. Mean duration (sec) and standard deviation (SD) of auto-grooming behaviour for the duration of the study. Paired superscript letters denote significant differences between weeks (Friedman's ANOVA; p≤0.05 and Wilcoxon signed-rank; p≤0.025).

Week	1	2	3	4
Mean	168	99[a]	281	306[a]
SD	206	138	371	333

6.7.8. Relationship between HRV and behavioural states

The relationship between mean SDNN and RMSSD and the duration of each behavioural state was investigated using Spearman's rank order correlation. A significant positive relationship was found between mean SDNN and the duration of time spent carrying out browsing behaviour, $r_s=0.53$, $p=0.036$ (**Figure 6.14**).
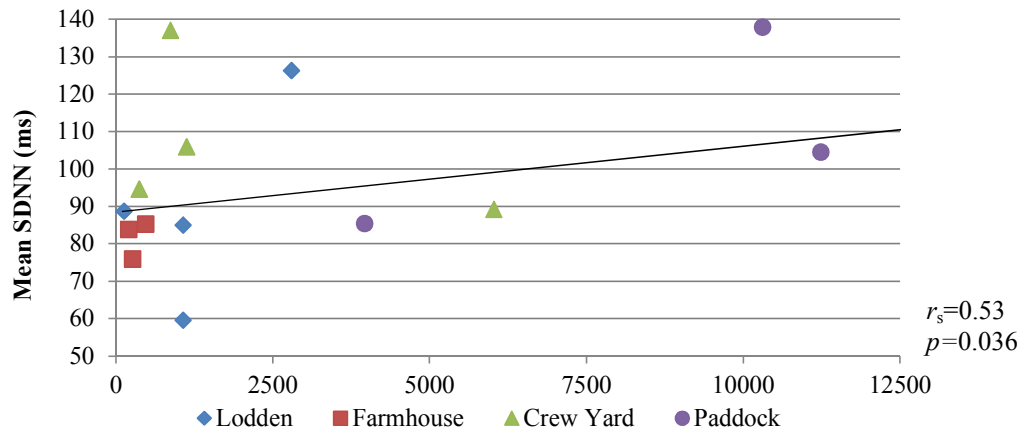
Figure 6.14. Relationship between mean duration of browsing behaviour (sec) and heart rate variability (HRV), shown according to housing system. HRV variable was standard deviation of inter-beat intervals (SDNN). SDNN correlated positively with browsing behaviour (Spearman's rank order correlation; p≤0.05).

A significant inverse relationship was found between mean SDNN and the duration of 'standing' behaviour, r_s=-0.65, p=0.007 (**Figure 6.15**).
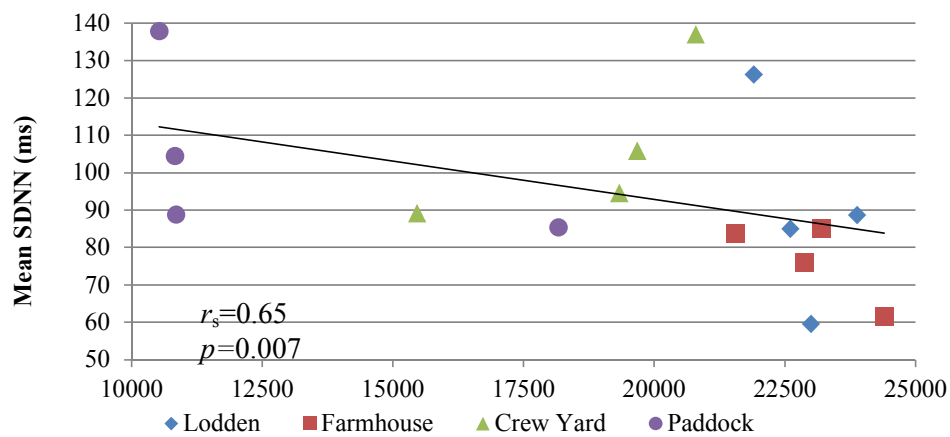
Figure 6.15. Relationship between mean duration of standing behaviour (sec) and heart rate variability (HRV), shown according to housing system. HRV variable was standard deviation of inter-beat intervals (SDNN). SDNN correlated negatively with standing behaviour (Spearman's rank order correlation; p≤0.05).

A significant inverse relationship was also found between mean SDNN and the duration of auto-grooming behaviour, r_s=-0.53, p=0.036 (**Figure 6.16**).
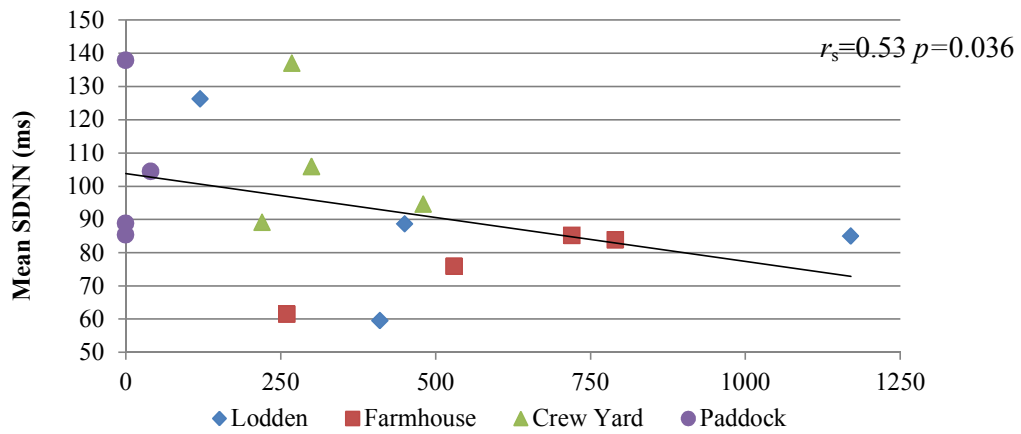
Figure 6.16. Relationship between mean duration of auto-grooming behaviour (sec) and heart rate variability (HRV), shown according to housing system. HRV variable was standard deviation of inter-beat intervals (SDNN). SDNN negatively correlated with auto-grooming behaviour (Spearman's rank order correlation; p≤0.05).

A strong positive relationship was found between mean RMSSD and the duration of time spent carrying out negative social behaviour, r_s=0.63, p=0.009 (**Figure 6.17**). Raw data was examined as an outlier was apparent following Spearman's rank order correlation analysis. This was found to be legitimate and was therefore retained within the data set.
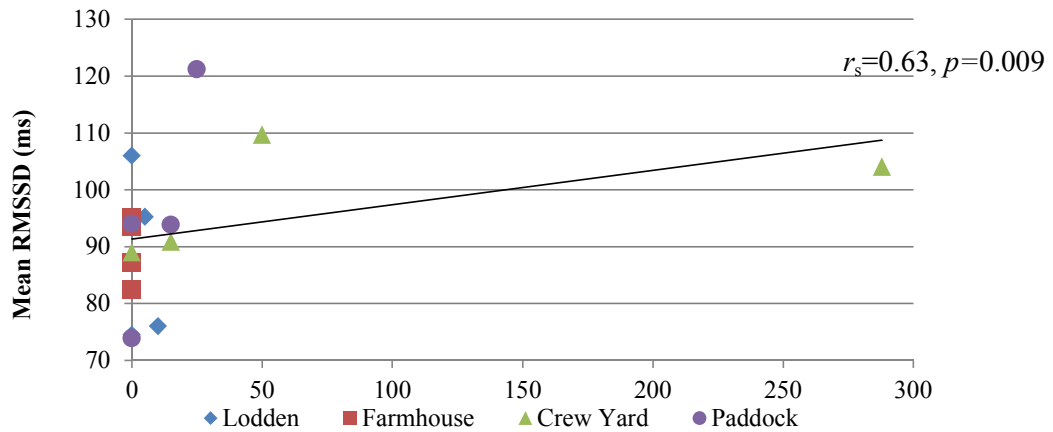
Figure 6.17. Relationship between mean duration of negative social behaviour (sec) and heart rate variability (HRV), shown according to housing system. HRV variable was square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). RMSSD correlated positively with negative social behaviour (Spearman's rank order correlation; p≤0.05).

An inverse relationship was found between mean SDNN and the duration of stereotypic behaviour, r_s=-0.51, p=0.046 (**Figure 6.18**). It was therefore possible to reject the null hypotheses for these variables. No other significant relationships were present.
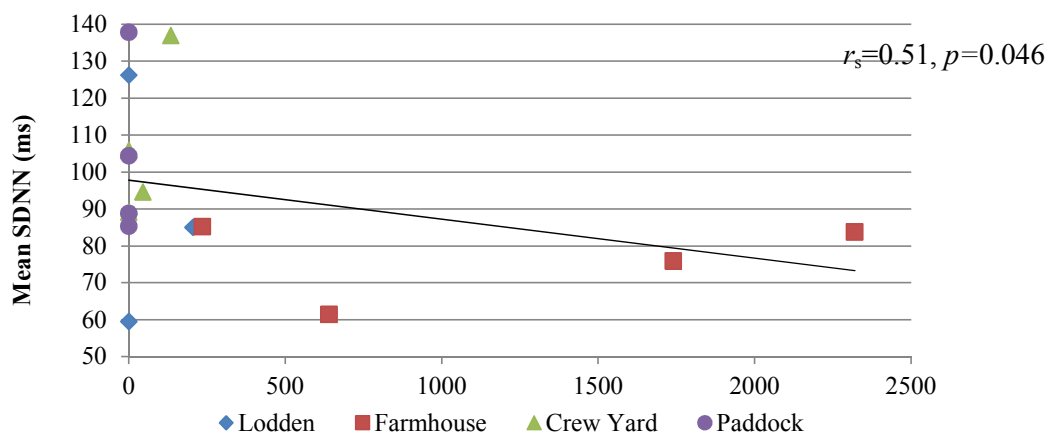
Figure 6.18. Relationship between mean duration of stereotypic behaviour (sec) and heart rate variability (HRV), shown according to housing system. HRV variable was standard deviation of inter-beat intervals (SDNN). SDNN negatively correlated with stereotypic behaviour (Spearman's rank order correlation; p≤0.05).

6.8. DISCUSSION

Analysis of behavioural data revealed that there was a significant effect of housing on 'browsing' behaviour - defined as the ingestion of either vegetation, bedding material or oral investigation of bedding material. Horses were observed engaging in significantly more browsing behaviour when housed in the paddock than in the crew yard, lodden and farmhouse stables and also in the crew yard and lodden stables compared with the farmhouse stables. During the study horses were given access to water in each housing type but not food as they had access to lush grass in their own paddocks which they returned to overnight and at weekends. At the time of the study, the grass in the paddock was very short but horses were observed attempting to graze and also browsing the Blackthorn (*Prunus spinosa*) hedgerow which bordered one side of the paddock for 36.2% of the time observed. The sparse grazing accounts for this figure being below summer grazing levels of 55% observed over 24 hours in free ranging horses by Duncan (1980) but is similar to the 35% reported between the hours of 8am and 4pm by Boyd, Carbonaro and Houpt (1988).

Horses were also observed directing their browsing behaviour to the wood shavings bed in the crew yard and lodden stables, either ingesting or investigating the bedding with the muzzle for around 4% of the time observed. This is considered a redirected behaviour compensating for inadequate grazing by Mills, Eckley and Cooper (2000) and is indicative of frustration according to Ninomiya *et al.* (2004), Ninomiya, Sato and Sugawara (2007) and Ninomiya *et al.* (2008) yet was rarely observed in the farmhouse stables which also contained wood shavings bedding. This suggests that rather than being an indicator of frustration, having space to move around in the crew yard or the increased visual horizons in the lodden stables towards neighbouring horses may facilitate the motivation to perform and express some browsing behaviour irrespective of the nutritional sparseness associated with these two housing types. Benhajali *et al.* (2009) report that even in the presence of social companions, lack of foraging opportunities increased the time spent in locomotion and led to reduced rates of conception (indicative of increased stress levels) compared with a control group where forage was

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provided. Preference testing of bedding substrates by Mills, Eckley and Cooper (2000) saw bedding directed behaviour reach 15% of the time observed when bedded on straw and horses demonstrated a preference for straw over paper and shavings bedding substrates. This implies that in housing systems or management regimes where forage is limited, there is a benefit to horse welfare in providing a bedding substrate that allows the horse to express motivationally significant activities such as foraging. As a significant positive relationship was found between HRV and the duration of browsing behaviour, the current study provides further evidence that the expression of browsing behaviour is strongly associated with a positive affective state.

A significant effect of housing was found for 'standing' behaviour. Horses stood less in both the paddock and the crew yard than in the lodden or farmhouse stables and stood less in the paddock compared with the crew yard. Standing behaviour far exceeded that observed in feral herds even in housing systems where horses had the space to move around. Duncan (1980) reported that in warm weather feral horses spent an average of 24% of their overall time budget standing. This figure was slightly greater in pasture-kept ponies observed by Crowell-Davis (1994) at 32.7% and averaged 47% during daytime observations in warm weather as reported by Boyd, Carbonaro and Houpt (1988). In the current study horses stood on average for 56% of the time in the paddock, 81% of the time in the crew yard, rising to 90% in the farmhouse and lodden stables. This result suggests that standing behaviour is not simply a result of confinement in the stable but even in environments where movement is possible such as a crew yard, the absence of suitable foraging opportunities leaves the horse standing for long periods of time. As a significant negative relationship was found between HRV and the duration of standing behaviour the present study provides strong physiological evidence that the duration of standing behaviour is associated with a negative affective state.

Locomotory activity in the paddock was, on average, 6.7% of the time observed which is approaching that noted by Boyd, Carbonaro and Houpt (1988) during the daytime of 9.7% and 10% by Duncan (1980). In the Crew yard horses spent 5.3% of the observed time in forward

movement, compared with 2.6% in lodden and 2.2% in farmhouse stables, although this activity was somewhat restricted by physical confinement in both stable types. The duration of locomotory behaviour was similar in the paddock (mean 1648sec) and crew yard (mean 1279sec). Analysis of HRV data confirmed that numerically, the highest measurements were found in the crew yard and paddock, with mean differences between the two housing systems being within 2-4ms of each other, suggesting that these two housing types were similar in their emotional valence.

Horses spent little time engaging in positive social interactions such as sniffing and mutual grooming whilst in the paddock (1.4% of time observed) and crew yard (1.5%). However, this was not too dissimilar to levels of mutual grooming observed by Boyd, Carbonaro and Houpt (1988) of 1.75% between the hours of 8am and 4pm. As mutual grooming has been identified as a comforting behaviour (Feh, 2005) with an appeasing effect (Kolter and Zimmermann, 1988) the relative absence of this behaviour may be indicative of the social stability achieved by familiarising the horses with each other prior to the study and turning the horses out in pairs and small groups.

Social interaction was physically prevented in the farmhouse stables but was also rarely observed in the lodden stables despite there being increased opportunities for visual, auditory and olfactory contact through the open barred walls. It is possible however, that subtle interactions occurred which were not evident during observation as horses sometimes rested adjacent to the neighbouring horse and occasionally engaged in nose to nose sniffing through the bars of the lodden stables. Previous research has identified that visual access to adjacent stables is associated with a reduced risk of abnormal behaviour (McGreevy *et al.*, 1995; Cooper, McDonald and Mills, 2000) which may imply that stabled horses do not necessarily require physical access to a companion in order to benefit from their presence.

Although relatively infrequent, the level of auto-grooming was also significantly affected by housing type. Horses engaged in this behaviour less often whilst in the paddock (0.02%) than in the crew yard (1.05%), lodden (1.04%) or farmhouse stables (1.27%). In the crew yard and both types of stable these results are comparable to the levels of auto-grooming observed in stabled ponies of 1.3% by Sweeting, Houpt and Houpt (1985). In the paddock however, levels were lower than the 1.5% recorded in free ranging horses by Boyd, Carbonaro and Houpt (1988). Physiological evidence gained from the present study suggests that the exhibition of auto-grooming behaviour is associated with a negative affective state as a significant negative relationship was found between HRV and the duration of auto-grooming behaviour. Although little information exists regarding the function of auto-grooming this suggests that horses do not necessarily resort to self-grooming in the absence of a social companion with whom to engage in mutual grooming, but that the behaviour may simply occur due to lack of other stimuli.

Negative social behaviour was rarely observed but was mainly carried out in the crew yard by the geldings (mean <240sec). The nature of aggressive behaviours observed were predominantly threat postures which form part of normal play fighting behaviour in male horses (McDonnell and Haviland, 1995; McDonnell and Poulin, 2002). The physiological evidence gained from the present study supports the findings of **Chapter 3**, that the expression of play fighting behaviour is strongly associated with a positive affective state as a significant positive relationship was found between HRV and the duration of negative social interaction between horses. This provides further evidence as reported by Christensen *et al.* (2002b) that the natural behavioural repertoire of male horses persists in domestic individuals, the expression of which is indicative of good welfare. The fact that play fighting behaviour was observed in the crew yard and not the paddock may be due to the absence of other activities available to occupy their time; mares tended to browse the shavings bed for longer than the geldings (mean 1582sec versus 527sec, respectively) rather than engaging in play fighting. This difference is consistent with observations by Duncan (1980) who found that free ranging male horses spent more time standing alert and in rapid movements (trot, gallop) than females at the expense of less time

spent foraging. As Duncan (1980) proposed that males may require extra time for herding and territorial defence activities which are obtained from their foraging time, this suggests that male behaviour patterns persist even in gelded horses.

Significantly more stereotypic behaviour was observed whilst in the farmhouse stables (mean 957sec) than any other housing type (lodden 37sec; crew yard 11sec; paddock zero) and was predominantly performed by the geldings than mares, although the difference did not reach significance (mean 1816sec and 196sec, respectively). There is strong physiological evidence gained from the present study that the presence of stereotypic behaviour is associated with a negative affective state as a significant negative relationship was found between the duration of stereotypic behaviour and HRV which, numerically, was lower in the farmhouse stables than the other housing types.

This type of stabling was the most socially isolating as the occupants' visual horizons were restricted to one side only (the front) so visual contact with neighbouring horses was only accessible whilst the head was held out over the stable door, although auditory and presumably a degree of olfactory communication were still possible. There was virtually no stereotypic behaviour observed in the lodden stabling, which was equally as confining in terms of floor space, prevented physical interaction between horses and had the same bedding substrate. This suggests that rather than the restriction of movement or absence of forage it is the lack of visual contact with conspecifics that plays a major role in the expression of stereotypic and frustration behaviour in horses and is in agreement with the findings of McGreevy *et al.* (1995), Cooper, McDonald and Mills (2000) and Ninomiya *et al.* (2008). Analysis of physiological data substantiates this theory as HRV in the lodden stables was slightly lower than, but not statistically different to that of the crew yard and paddock. This infers that these three housing types were not greatly dissimilar in their emotional valence. Research has also shown that even the provision of surrogate companionship via a picture or mirror seems to successfully mimic the presence of social contact so sufficiently that it alleviates social deprivation and positively

influences behaviour (McAfee, Mills and Cooper, 2002; Mills and Davenport, 2002; Mills and Riezebos, 2005; Kay and Hall, 2009).

There were no significant differences in HRV between each day of the study or between weeks. Horses carried out significantly more positive social interactions in Week 1 than during the rest of the study and although not significant, it was interesting to note that RMSSD for Week 1 was numerically lower than Weeks 2-4. It is possible to speculate that regardless of which housing type they were placed in, the disruption to their routine by being brought in from their paddocks might have negatively influenced the horses' affective state during the first week and initiated the expression of comforting behaviour in the form of social interaction.

When HRV data for individual horses were analysed regardless of housing system, significant individual differences were noted; Horses 2, 3 and 4 recorded higher SDNN than Horse 1. As previously discussed, horses with high levels of HRV have been associated with traits such as 'bravery', low incidences of 'shying' behaviour and low levels of attentiveness to their surroundings (Visser *et al.*, 2003a). Previous studies such as Eager *et al.* (2004) also relate HRV components to equine temperament; Visser *et al.* (2002) and Visser *et al.* (2003a) suggest that individual characteristics such as temperament or reactivity to environmental changes may be detected via HRV measurements. As this has important implications for breeding, housing and management of the horse further research is necessary and is discussed in **Chapter 7**.

6.9. CONCLUSION

This study identified that behaviour (but not HRV) was significantly affected by housing system. As previously identified in **Chapters 3, 4** and **5** individual differences in HRV were evident and factors affecting individual HRV are investigated in **Chapter 7**.

The presence of significant relationships between HRV and behaviour strongly link the lack of visual contact with conspecifics with both stereotypic behaviour and negative affective state and reinforce previous research such as that by McGreevy *et al*. (1995) and Redbo *et al*. (1998) that highlight the positive benefits of social housing.

Play fighting in male horses (as previously observed in **Chapter 3)** along with browsing behaviour in all horses was associated with positive affective state while long periods of standing were associated with a negative affective state. Research investigating the effect of housing system on affective state in horses is scarce, yet solitary housing is commonplace. In terms of improving equine welfare, these findings reinforce the importance of facilitating play behaviour via social housing and the provision of adequate forage in maintaining innate time budgets regardless of housing type. Additionally, in the absence of physical companionship a stable design with visual access to conspecifics seems a preferable alternative to stabling where such contact is severely restricted.

Chapter Seven

Factors affecting Equine Heart Rate Variability

7.1 INTRODUCTION

Large inter-individual variations in basal values of HRV have previously been reported in horses (Physick-Sheard *et al*., 2000; Munsters *et al*., 2001) and other animals (von Borell *et al*., 2007) which can contribute to the lack of significance found in some HRV values. Several causal factors including genotype, environment, temperament and nutritional status of the horse have been proposed by von Borell *et al.* (2007) to play a key role in such inter-individual variations. **Chapters 3-6** investigated HRV and behavioural responses as a method of detecting emotion in horses when anticipating positive rewards (companionship and food), during an aversive management procedure and when exposed to housing regimes offering different levels of social contact. These studies identified differences in HRV according to management regime, the presence or absence of social contact, according to sex and individual differences that may be associated with underlying affective state, temperament or breed.

Research in this area is scarce but as discussed in **Chapter 1: Section 1.5.1.4**, studies relating to the different factors that might affect HRV in horses has yielded mixed results. In summary, low levels of HRV (and therefore negative emotion) have been related to equine stereotypies by Bachmann *et al*. (2003), yet Nagy *et al*. (2009) were unable to differentiate between HRV in stereotypic and non-stereotypic horses. Strong evidence links modulation of the ANS with affective state in humans (**Chapter 1: Section 1.5.1.2**) but HRV has not been widely researched as a method of pain assessment in animals despite significantly lower HRV being associated with grass sickness (Perkins *et al*., 2000) and laminitis (Rietmann *et al*., 2004a). Although significant differences between sexes were obtained in **Chapters 4** and **5** and evidence from human research identifies females as having a greater vagal control of the heart than males (Rossy and Thayer, 1998), no other equine studies using mixed sex subjects have reported an effect of sex on HRV (Visser *et al.,* 2002; Rietmann *et al.,* 2004b; Visser, Ellis and Van Reenen, 2008). The effect of age on HRV has not yet been investigated in animals although there is no evidence of any such association in humans (Reardon and Malik, 1996). Breed has previously

been related to equine personality and temperament by Lesimple *et al*. (2011) and Keeler (1947) documents numerous anecdotal statements of temperament differences according to coat colour. Chestnuts, for example are described as having an unruly temperament, blacks as docile and slow, greys being gentle and easy to handle and palominos being tame and tractable (Keeler, 1947). These features are therefore of interest when determining factors affecting HRV.

As reviewed in **Chapter 1: Section 1.5.2.3**, the genetic diversity of a species gives rise to individual differences in behavioural tendency which has important welfare implications for appropriately matching the horse's temperament with rider personality, sporting discipline, housing conditions and management practices. Individual differences in behaviour may reflect the range of subjective feelings experienced by animals that are relevant to animal welfare and is illustrated well by the inconsistent exhibition of stereotypic behaviour between individuals in the same environment (Bachmann, Audigé and Stauffacher, 2003). This raises questions over the welfare of those animals who do not exhibit such behaviours in that they might be more mentally robust and able to cope with the stress of the environment, or that they are unable to develop a coping strategy in which case the 'normal' behaving animals may be suffering more than their stereotyping counterparts.

Temperament, defined by Stur (1987) as 'the sum of all inherited and acquired behaviour patterns', is one characteristic that is very difficult to assess objectively. Commonly used methods for assessing temperament in animals include gathering observer ratings for behavioural traits (Hsu and Serpell, 2003), recording the reaction of an animal in a particular situation (Podberscek, Blackshaw and Beattie, 1991) or rating aspects of behaviour in the usual environment (Kimura, 1998). Methods for studying equine temperament include presenting horses with various situations to gauge their personality based on their reaction (Visser *et al*., 2001; Visser *et al*., 2003b), comparing behavioural responses with instructor ratings of temperament (Le Scolan, Hausberger and Wolff, 1997; Seaman, Davidson and Waran, 2002; Visser *et al*., 2003a) and emotionality (McCall *et al*., 2006) or via questionnaires given to horse

handlers, whereby persons familiar with the horses rate their personality traits (French, 1993; Momozawa *et al.*, 2003; Momozawa *et al.*, 2005; Momozawa *et al.*, 2007; Lloyd *et al.*, 2007; Lloyd *et al.*, 2008; McGrogan, Hutchison and King, 2008).

Studies that compare physiological measures directly to temperament are limited in number despite their usefulness in strengthening the interpretation of behavioural responses. As discussed in **Chapter 1: Section 1.5.1**, the validity of some physiological measures used during behavioural tests is questionable, fluctuations in HR for instance are influenced by anxiety, physical activity or stimulus-induced arousal. The use of HRV however, has been used successfully to differentiate between individual animals in stressful conditions by Sgoifo *et al.* (1997) and seems a relevant variable to reflect differences in temperament (Porges, 1995). As reviewed in **Chapter 1: Section 1.5.1.4**, findings from existing research into the relationship between temperament and HRV in horses suggest that individual characteristics such as temperament or reactivity to environmental changes may be responsible for the different individual modulation of the ANS which can be detected via HRV measurements and propose that HRV could be a useful measure for quantifying behavioural traits in horses (Visser *et al.*, 2002; Visser *et al.*, 2003a; Eager *et al.*, 2004).

In order to further explore these findings, the aim of the current study was to investigate whether sex, age, breed and other management factors were attributable to differences in HRV measurements taken at rest both in the stable and at liberty in the field. In addition, as responses of the autonomic nervous system may also be useful in differentiating between individual horses in terms of temperament, a questionnaire was designed in order to collect observer ratings of temperament to investigate whether this was a factor affecting HRV in horses.

7.2. DATA COLLECTION

7.2.1. Temperament survey data collection

A temperament survey was carried out for the entire population of riding school horses belonging to the Brackenhurst Equestrian Centre (N=33). The survey was completed by the respective Yard Manager who was considered to be the individual who was most knowledgeable about each horse, as they were familiar with the behaviour of the subjects in the course of stable management and under saddle in a variety of circumstances. The survey was in the form of a single page Microsoft Excel spread sheet that was sent via email to the Yard Manager and completed during August 2010.

The survey consisted of ten questions related to personality traits used to quantify temperament (**Table 7.1**) and was based on those used in a previous equine temperament study by Seaman, Davidson and Waran (2002). The horses' attitude to other horses and people was recorded, along with events commonly encountered by domestic horses including social isolation, loud noises, clipping, shoeing and willingness to be caught in the field. Podberscek, Blackshaw and Beattie's (1991) study testing feline behaviour and French's (1993) assessment of donkey temperament both report that temperament is an influential factor on an animal's attitude towards familiar and unfamiliar humans. This differentiation was therefore included in the survey rather than simply determining the reaction of horses to people and other horses generally. Other elements of the survey related to previous research by Visser *et al*. (2003a) who reported that horses with high levels of HRV could be associated with aspects of handling such as 'bravery' when passing frightening objects and events, low levels of 'spooky' behaviour (the frequency and intensity of shying away from something) whereas low levels of HRV were associated with restlessness, resistance behaviour and reluctance to approach a novel object.

Table 7.1. List of questions comprising the temperament survey. Score 0=none, 1=mild, 2=moderate, 3=severe.

Tendency to respond with:

1. Aggression* or fear** towards familiar horses
2. Aggression or fear towards unfamiliar horses
3. Aggression or fear towards familiar humans
4. Aggression or fear towards unfamiliar humans
5. Negative reaction to isolation (restlessness, agitation, vocalisation, pacing)
6. Fear to sudden/loud noises
7. Fear to unfamiliar objects/situations
8. Non-compliance during clipping
9. Non-compliance during shoeing/trimming
10. Non-compliance when being caught (from field)

*ears back, rump swing, kick threat, kick, bite threat, bite, barging
**avoidance, evasion, movement away

As the responses to these behaviours were most readily evaluated in terms of intensity, the evaluator was asked to score each horse using a 4-point qualitative (semantic differential, Osgood, Suci and Tannenbaum, 1967) rating scale similar to that used in a questionnaire to assess aggression, fear, anxiety and excitability in dogs by Hsu and Serpell (2003). The temperament scoring was therefore based on a numerical scale where the evaluator rated each horse according to the intensity of their reaction to potential anxiety-inducing situations on a scale of zero (no reaction) to three (severe reaction). This was based on overall behavioural tendencies during the horse's tenure at the Equestrian Centre.

As discussed in **Chapter 1: Section 1.5.1.2**, low HRV has been found in very anxious persons (Offerhaus, 1980; Kawachi *et al.,* 1995; Friedman and Thayer, 1998a). As anxiety has also been identified as a component that could be rated reliably by regular horse handlers (Morris, 2002; Momozawa *et al*., 2005; Momozawa *et al*., 2007; Lloyd *et al*., 2007; Lloyd *et al*., 2008; McGrogan, Hutchison and King, 2008), the survey was devised in an attempt to identify and measure the level of the temperament trait 'anxiety' from which a total temperament score could be used for comparison with baseline HRV measurements. The wording of the temperament scoring criteria used in the current study was regarded as appropriate for assessing equine anxiety as factor analysis by Momozawa *et al*. (2005) has previously categorised nervousness,

excitability, panic, vigilance, skittishness and timidity within the collective term 'anxiety'. In the case of aggression and fear responses the survey included a definition of the sorts of behavioural signs that the evaluator could use when scoring the horses. For example, in the case of aggression typical signs might include flattened ears, swinging the rump, threatening to kick, kicking, threatening to bite, biting or barging.

7.2.2. Horse profile and management data collection

Background data and management information was collated for each horse residing at Brackenhurst Equestrian Centre during July 2010. Information was gathered from paper record files kept for each horse that contain information on the animals' sex, breed, height, coat colour, health, previous history (origin), date of birth and date of arrival at the Equestrian Centre. In addition, supplementary management information was also obtained directly from the Yard Manager as follows: presence of stereotypic behaviour, type of housing design each horse was normally kept in, behavioural response during clipping, type of forage and concentrate diet fed and bedding used whilst stabled. Data were entered onto a Microsoft Excel spread sheet for analysis. Date of birth and date of arrival at the Equestrian Centre were used to calculate the age of each horse at the time of the study and their length of tenure at the Centre. The height of each horse in hands was converted into centimetres. All other data were non-numeric so were coded numerically prior to analysis.

Data from the 33 horses residing at Brackenhurst Equestrian Centre comprised both mares (n=9) and geldings (n=24; **Table 7.2**). The age of the subjects ranged from 7 to 20 (mean = 14±4 years) and the group comprised Irish Draught x Thoroughbred horses (n=11) known as Irish Sport horses, Cob type horses (n=7), Warmbloods (n=6), Thoroughbreds (n=5), Irish Draught (n=2) and Shire horses (n=2). Of these, the majority (n=25) did not exhibit any form of stereotypic behaviour, 3 horses were known to perform oral stereotypies (chewing and windsucking) and 5 horses performed locomotor stereotypies (box-walking).

Table 7.2. Profile of subjects.

Horse	Age	Height	Breed	Sex
Angus	13	16.3	Warmblood	Gelding
Arthur	12	17.3	Irish Draught	Gelding
Beamish	15	16.1	Irish Sport	Gelding
Beau	16	16	Irish Sport	Mare
Benson	10	16.2	Irish Sport	Gelding
Blackberry	16	16.2	Irish Sport	Mare
Conan	13	17	Irish Sport	Gelding
Del	15	16	Shire	Gelding
Dicken	8	17.2	Shire	Gelding
Dixon	13	17.2	Irish Draught	Gelding
Dylan	8	16.1	Thoroughbred	Gelding
Ellie	14	16.1	Irish Sport	Mare
Ernie	17	16	Thoroughbred	Gelding
Fletcher	7	16.2	Irish Sport	Gelding
Harriet	15	16	Irish Sport	Mare
Harvey	10	15.3	Cob	Gelding
Herbert	12	17.1	Thoroughbred	Gelding
Hovis	20	15.3	Thoroughbred	Gelding
Jonty	17	16.1	Irish Sport	Gelding
Kitkat	9	15.2	Cob	Gelding
Libby	13	15.2	Irish Sport	Mare
Liesle	16	15.3	Warmblood	Mare
Orca	10	15.2	Cob	Gelding
Paddy	17	15.3	Warmblood	Gelding
Pluto	20	15.1	Cob	Gelding
Puzzle	20	15.3	Cob	Mare
Pye	7	15.1	Cob	Mare
Rufus	16	16	Warmblood	Gelding
Rupert	20	17	Warmblood	Gelding
Sparky	12	16.2	Irish Sport	Gelding
Tosca	15	15.2	Cob	Mare
Visi	20	16	Thoroughbred	Gelding
Woody	16	17.2	Warmblood	Gelding
Mean	**14**	**16.1**		
St. Dev.	**4**	**0.3**		

The horses were from a variety of backgrounds and disciplines ranging from Riding Clubs (n=14), local charity rescue centre (n=7) and the Army (n=4) to dressage (n=5) and racing (n=3). The length of time the horses had resided at the Equestrian Centre ranged from one year to 16 years (mean = 5 years ±4 SD). The bedding substrate was almost equally split between wood shavings (n=16) and straw (n=17). Twenty horses were fed hay in their stable as forage, the remainder were fed haylage (n=13).

The housing system horses were normally kept in was included in the analysis of profile data. As each system provides a different level of floor space and social contact with neighbouring horses the potential effect each system may have on HRV was investigated.

Six horses were housed in pairs in a crew yard. The crew yards were situated indoors within a barn area which measured 9.4m x 10m and had a wood shaving bed over concrete flooring (**Figure 7.1**). Ten horses were housed individually in adjacent concrete-block built stables (Farmhouse). These were situated indoors within a barn (8 faced inward onto the crew yard, 2 faced outward onto the yard), measured 3.5 m x 3.5 m and comprised an open fronted design where horses could see adjacent horses only when looking out of the front of the stable. Solid internal walls at the rear and sides of the stable prevented visual contact with neighbouring horses from within the stable (**Figure 7.2**).

Figure 7.1. Detail of crew yard housing system showing one horse fitted with HRV monitoring equipment

Figure 7.2. Detail of farmhouse housing system

The remaining horses (n=17) were housed individually in Lodden Ascot design stables. These were situated indoors within a barn (12 faced indoors towards other Lodden stables, 5 faced outward towards the yard), measured 3.5 m×3.5 m and were constructed of timber panel and galvanised steel vertical bar fronts and sides with a sliding door (Lodden Livestock Equipment, Lodden). This type of housing allowed visual and olfactory contact between neighbouring horses (**Figures 7.3** and **7.4**).

Figures 7.3. and 7.4. Detail from above and within the Lodden housing system.

The previous health history of each horse since their arrival at the Equestrian Centre was obtained from individual record files. From this it was possible to identify five categories of previous illness or injury which the horses had previously experienced. Lower limb bone problems such as inflammation of the splint bone (n=4), lower limb soft tissue injuries e.g. flesh wounds and tendon sprains (n=6). Problems relating to the foot or hoof capsule such as laminitis (n=2), any other health related problems e.g. colic or dental problems (n=3) or none (n=18). At the time that HRV data were collected, all horses were free from injury and illness.

The evaluator was also required to classify horses into one of four groups according to their degree of behavioural compliance during clipping:

- Horses that could not be clipped without being either chemically sedated or physically restrained with a nose twitch (n=4).

- Horses with an acceptable response to clipping - considerable movement of feet and body but able to follow the horse's movement to complete the task without resorting to chemical/physical restraint (n=5).

- Horses that were good to clip – little movement of the feet, moderate amount of body movement, task completed with relative ease (n=13)

- Horses that were very good to clip - very little movement, obvious signs of relaxation, resting one leg, head and bottom lip held low, task completed quickly (n=11).

Whilst stabled, horses were routinely fed concentrate feed at around 5pm each day. Five different concentrate feed diets were provided depending on the nutritional and energy requirements of each horse and was also based on their body condition. Horses were provided either Pasture cubes (n=7), Competition cubes (n=3), High-fibre nuts (n=15), High-energy mix (n=5) or Chaff (n=3). The nutritional content of each feed type is detailed in **Appendix 6**.

7.2.3. Heart rate variability data collection

Prior to the horses being turned out to pasture for the University summer break, HRV measurements were obtained whilst each horse was resting in their own stable or crew yard. Each horse was fitted with HRV monitoring equipment attached to the thorax with an elastic girth. The positive electrode was located at the left shoulder and the negative electrode at the lower left thorax as described in **Chapter 2: Section 2.3.1**. Electrical conductivity was increased by the use of ultrasonic gel. Recording began as soon as the equipment had been fitted and heart rate signal obtained and ceased after 15 minutes. Approximately two weeks later, once the horses had been turned out to pasture for the summer holidays, a second set of HRV measurements were obtained. Each horse was caught in the field, fitted with a head collar and lead rope and the heart rate monitor attached as previously described. Recording began once the equipment was successfully fitted and heart rate detected, then the lead rope was removed and the horse released to move around the field at liberty. After 15 minutes of recording, the horse was caught, the lead rope reattached, the heart rate monitor was stopped and removed and the horse released into the field.

7.3. DATA ANALYSIS

7.3.1. Factors affecting heart rate variability

Data from the Polar recording device were downloaded via a Polar Infrared Interface using Polar Pro-Trainer 5 software and stored on a PC. RR interval data were then analysed using Kubios HRV analysis software. To remove trend components, data were de-trended and an

artefact correction was made as described in **Chapter 2: Section 2.1**. Time domain analyses were then calculated from mean RR interval data for each horse from HRV measurements collected whilst at rest in their own stable and when at liberty in the field.

For each data set SDNN and RMSSD were analysed using SPSS 18.0. Kolmogorov-Smirnov tests for normality were carried out on each data set and statistical tests chosen accordingly. All HRV data were normally distributed except SDNN collected from horses in the field. These data were therefore transformed using square root to enable the use of parametric statistical tests (Pallant, 2007).

To examine differences in HRV measurements between stable and field data were tested using a paired-samples t-test. Differences in HRV between sexes, forage and bedding types were investigated using an independent-samples t-test and all analyses were two-tailed. HRV variables were compared between groups via a one-way ANOVA for stereotypy, housing type, breed, coat colour, medical history, background, response to clipping and type of concentrate feed. As group sizes were unequal, homogeneity of variances was checked using Levene's test. If the homogeneity of variance assumption was broken the Brown-Forsythe F statistic was used (Field, 2009).

Data for height, age, length of tenure at the Equestrian Centre and total temperament score for each horse were tested using Kolmogorov-Smirnov tests for normality (SPSS 18.0). The relationship between these data and HRV were then investigated via either Spearman's rank order or Pearson product-moment correlations.

7.3.2. Factors affecting temperament score

Total temperament score data for each horse were not normally distributed. Differences in temperament score between sexes and according to the type of forage and bedding provided were investigated using a Mann-Whitney U test. Temperament scores were also compared

between groups via a Kruskal Wallis test for stereotypy, housing type, breed, coat colour, medical history, background, response to clipping and type of concentrate feed. The relationship between height, age, length of tenure at the Equestrian Centre and total temperament score for each horse was tested using Spearman's rank order correlations.

7.4. RESULTS

7.4.1. Analysis of baseline heart rate variability measurements.

Using a paired-samples t-test, analysis of log transformed SDNN data revealed that HRV was significantly higher when stabled (75.78±19.73ms) than in the field (64.78±19.87ms), t (32) = -2.76, p=0.009. RMSSD was also significantly higher when stabled (83.87±26.55ms) than in the field (69.45±27.74ms), t (32) = 2.34, p=0.025 (**Figure 7.5**). The eta squared statistic (0.19 SDNN, 0.15 RMSSD) indicated a small effect size for both measurements. It was therefore possible to reject the null hypothesis.
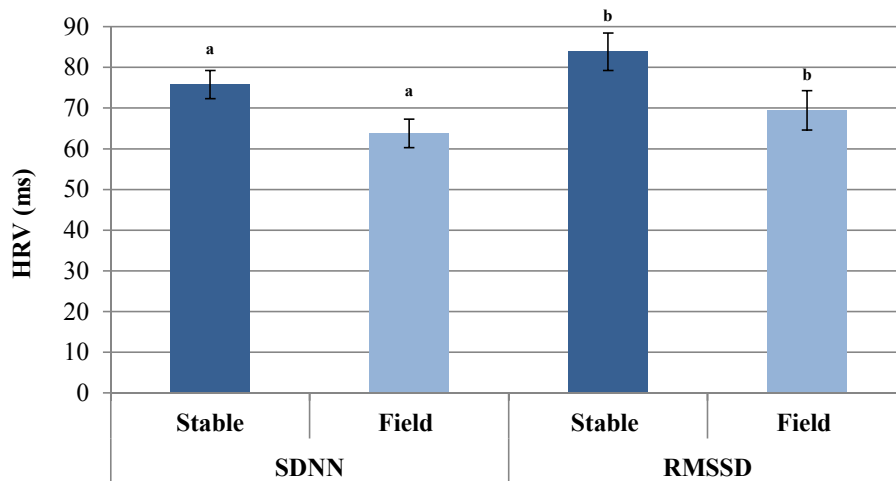
Figure 7.5. Mean baseline heart rate variability (HRV) values (±SE). HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Paired superscript letters denote significant differences for each variable (paired-samples t-test; p≤0.05).

7.4.2. The effect of stereotypic behaviour on HRV

A one-way ANOVA was carried out to identify whether the presence of stereotypic behaviour was a factor affecting HRV. Horses were placed into three groups according to whether they displayed oral (n=3), locomotor (n=5) or no stereotypic behaviour (n=25).

The highest HRV was found in horses that had no stereotypic behaviour when measured in the stable (SDNN 78.56±18.54ms, RMSSD 87.35±25.90ms). In the field, HRV in horses with no stereotypy (SDNN 65.78±19.68ms, RMSSD 71.03±28.04ms) was similar to that in horses with locomotor stereotypies (SDNN 65.36±21.85ms, RMSSD 74.20±30.80ms), both of which were higher than that of horses with oral stereotypies (SDNN 44.53±10.16ms, RMSSD 48.37±15.09ms; **Figure 7.6**).
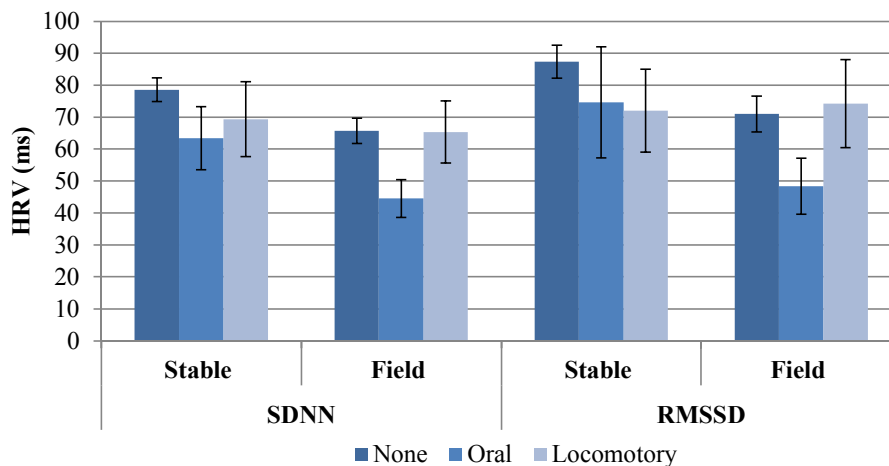
Figure 7.6. Mean baseline heart rate variability (HRV) values (±SE) according to the presence or absence of stereotypic behaviour. HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (one-way ANOVA).

None of the differences between HRV according to the presence or absence of stereotypic behaviour were statistically significant; Stable SDNN, $F_{(2, 30)} = 1.11$, $p=0.34$, Field SDNN, F

(2, 30) = 1.90, *p*=0.17, Stable RMSSD, *F* (2, 30) = 0.89, *p*=0.42, Field RMSSD, *F* (2, 30) = 0.99, *p*=0.39. It was therefore not possible to reject the null hypothesis.

7.4.3. The effect of housing type on HRV

A one-way ANOVA was carried out to identify whether housing type was a factor affecting HRV. Horses were placed into five groups according to whether they were housed in a crew yard (n=6), farmhouse (indoor=8, outdoor=2), or Lodden stable (indoor=12, outdoor=5).

The highest HRV was found in horses kept in a crew yard from measurements taken whilst the horses were in the crew yard (SDNN 84.93±16.91ms, RMSSD 105.60±23.23ms). The lowest HRV taken whilst in the stable was found in horses kept in Farmhouse stables that faced outward towards the yard (SDNN 59.05±35.57ms, RMSSD 66.55±24.11ms). Of the field measurements, the highest HRV measurements were found in horses from the indoor Farmhouse stables (SDNN 76.23 ±25.31ms, RMSSD 83.05±33.19ms) and the lowest HRV was recorded in horses from the indoor Lodden stables (SDNN 56.03±16.17ms, RMSSD 58.93±23.86ms; **Figure 7.7**).
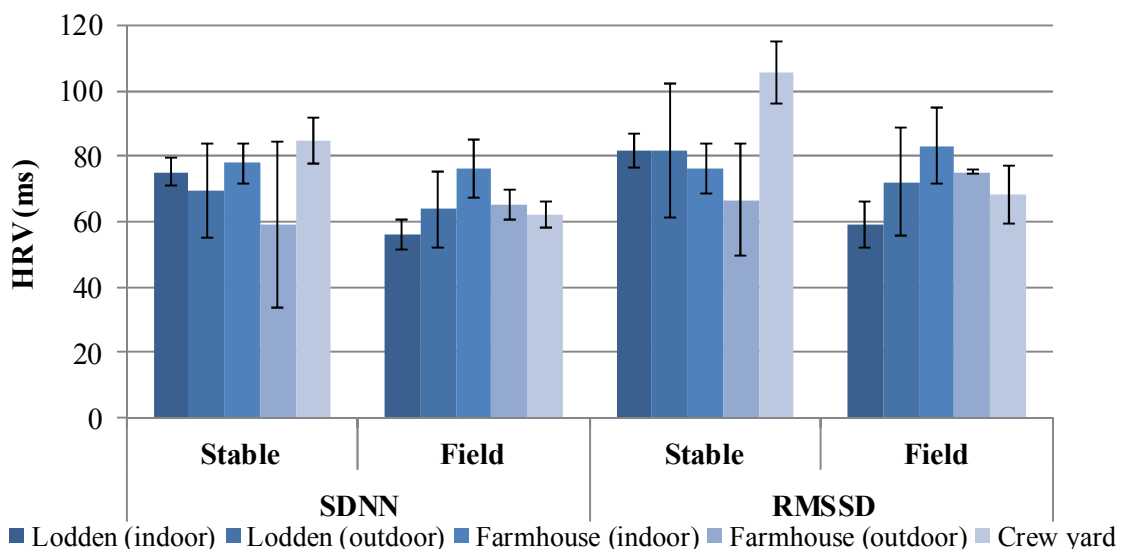
Figure 7.7. Mean baseline heart rate variability (HRV) values (±SE) according to the housing type. HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (one-way ANOVA).

None of the differences between HRV according to housing type were statistically significant; Stable SDNN, $F_{(4, 28)} = 0.81$, $p=0.53$, Field SDNN, $F_{(4, 28)} = 1.24$, $p=0.32$, Stable RMSSD, $F_{(4, 28)} = 1.49$, $p=0.23$, Field RMSSD, $F_{(4, 28)} = 0.94$, $p=0.46$. It was therefore not possible to reject the null hypothesis.

7.4.4. The effect of sex on HRV

An independent-samples t-test was carried out to identify whether sex (mares n=9, geldings n=24) was a factor affecting HRV, and this was higher in mares than geldings for all measurements (**Figure 7.8**).

Statistical analysis revealed that there was no significant difference in stable SDNN for mares (83.11±17.47ms) and geldings 73.04±20.17ms; $t_{(31)} = 1.32$, $p=0.20$. There was also no significant difference in stable RMSSD for mares (91.19±27.28ms) and geldings 81.12±26.32ms; $t_{(31)} = 0.97$, $p=0.34$.
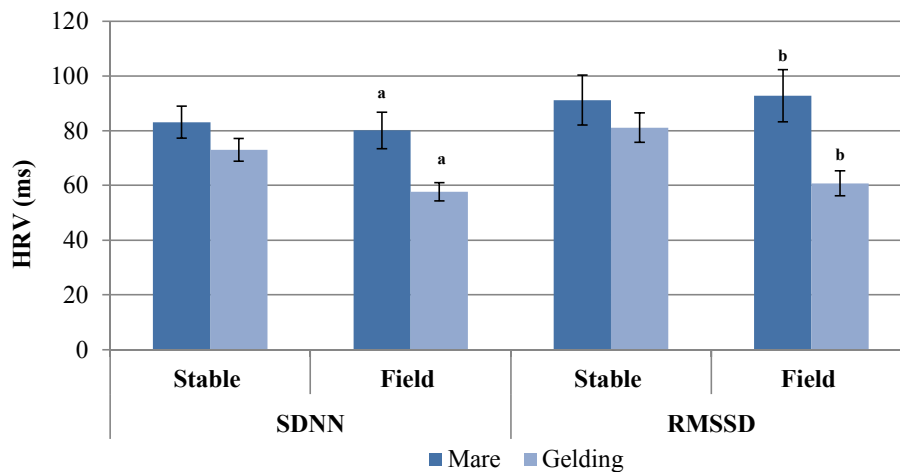
Figure 7.8. Mean baseline heart rate variability (HRV) values (±SE) according to sex. HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Paired superscript letters denote significant differences for each variable (independent-samples t-test; p≤0.05).

Mares recorded significantly higher field SDNN than geldings (80.14±19.88ms, 57.65±16.36ms, respectively), t (31) = 3.35, p=0.002. Field RMSSD was also significantly higher in mares (92.77±28.58ms) than geldings 60.70±22.23ms; t (31) = 3.41, p=0.002. The eta squared statistic was 0.27 for both variables which indicates a small effect size. It was possible to reject the null hypothesis.

7.4.5. The effect of breed on HRV

A one-way ANOVA was carried out to identify whether breed was a factor affecting HRV. Horses were placed into six groups according to breed; Irish Draught (n=2), Irish Sport (n=11), Cob (n=7), Shire (n=2), Thoroughbred (n=5) and Warmblood (n=6).

The highest HRV measured in the stable was found in Shire horses (SDNN 87.65±21.14ms, RMSSD 95.45±23.12ms) and lowest in Irish Draught horses (SDNN 60.15±5.73ms, RMSSD 63.60±18.24ms). Conversely, of the field measurements, Irish Draught horses recorded the highest HRV (SDNN 78.40±25.88ms, RMSSD 88.55±40.23ms) and Shire horses recorded the lowest (SDNN 49.55±5.44ms, RMSSD 44.75±1.34ms; **Figure 7.9**).
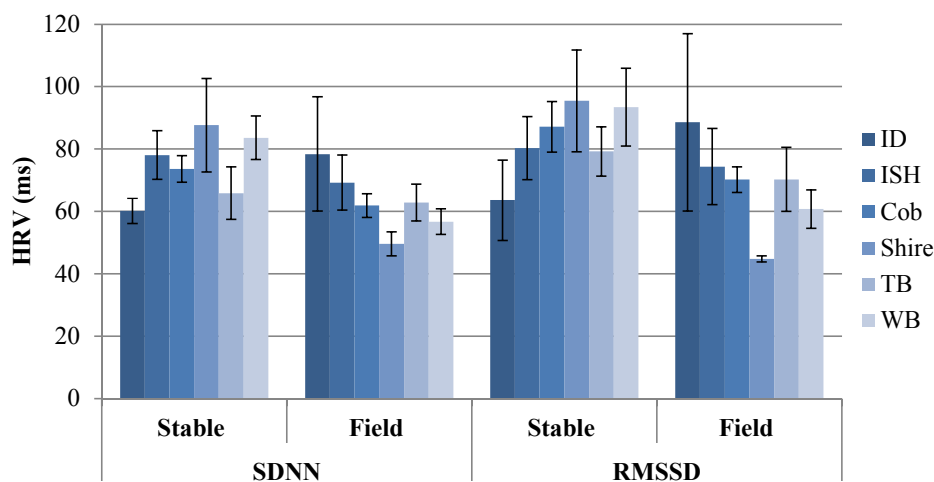
Figure 7.9. Mean baseline heart rate variability (HRV) values (±SE) according to breed; Irish Draught (ID), Irish Sport (ISH), Cob, Shire, Thoroughbred (TB) and Warmblood (WB). HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (one-way ANOVA).

None of the differences between HRV according to breed were statistically significant; Stable SDNN, $F (5, 27) = 0.86$, $p=0.52$, Field SDNN, $F (5, 27) = 0.85$, $p=0.69$, Stable RMSSD, $F (5, 27) = 0.51$, $p=0.76$, Field RMSSD, $F (5, 27) = 0.80$, $p=0.66$. It was therefore not possible to reject the null hypothesis.

7.4.6. The effect of coat colour on HRV

A one-way ANOVA was carried out to identify whether coat colour was a factor affecting HRV. Horses were placed into seven groups according to coat colour; Brown (n=6), Grey (n=4), Black (n=3), Coloured (n=5), Bay (n=10), Chestnut (n=4) and Palomino (n=1).

The highest HRV measured in the stable was found in the Palomino horse (SDNN 87.90ms, RMSSD 116.10ms) and lowest in the Chestnut horses for SDNN (67.65±29.76ms) and in Grey horses for RMSSD (62.63±16.88ms). Of the field measurements, Black horses recorded the highest HRV (SDNN 79.07±29.12ms, RMSSD 94.97±48.97ms) and Bay horses recorded the lowest (SDNN 54.79±19.43ms, RMSSD 56.09±28.66ms; **Figure 7.10**).
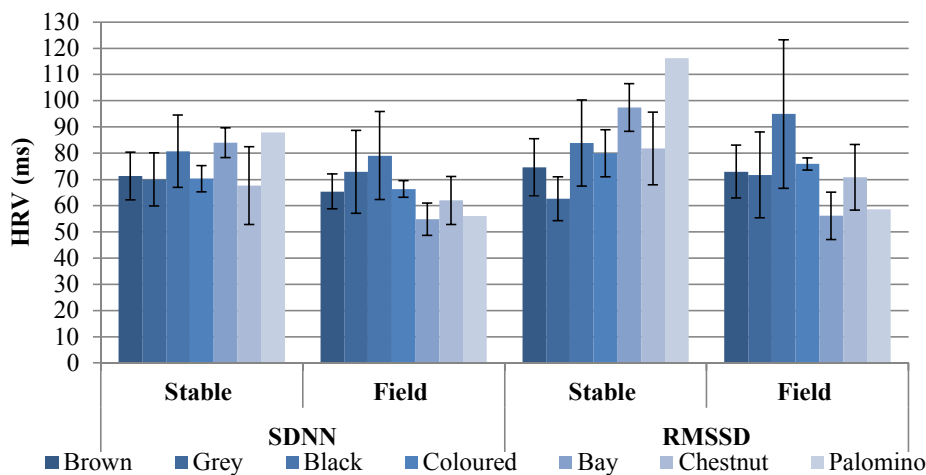
Figure 7.10. Mean baseline heart rate variability (HRV) values (±SE) according to coat colour. HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (one-way ANOVA).

None of the differences between HRV according to coat colour were statistically significant; Stable SDNN, $F_{(6, 26)} = 0.62$, $p=0.71$, Field SDNN, $F_{(6, 26)} = 0.81$, $p=0.57$, Stable RMSSD, $F_{(6, 26)} = 1.32$, $p=0.28$, Field RMSSD, $F_{(6, 26)} = 0.88$, $p=0.52$. It was therefore not possible to reject the null hypothesis.

7.4.7. The effect of medical history on HRV

A one-way ANOVA was carried out to identify whether the previous health record of the horses during their tenure at the Equestrian Centre was a factor affecting HRV. Horses were placed into five groups according to previous health problems; None (n=18), Lower limb bone (n=4), Lower limb soft tissue (n=6), Foot (n=2), Other (n=3).

The highest HRV measured in the stable was found in the horses that previously had problems affecting the lower limb bone (SDNN 87.75±15.32ms; RMSSD 92.98 ±16.87ms) and lowest in the horses with previous problems affecting the lower limb soft tissues (SDNN 66.28±30.34ms; RMSSD 78.87±38.38ms; **Figure 7.11**).
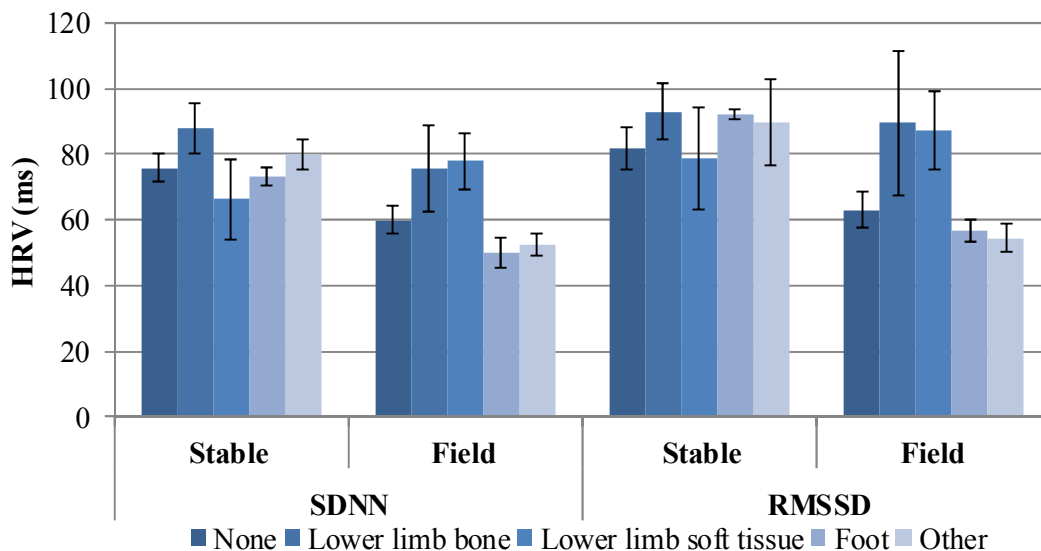
Figure 7.11. Mean baseline heart rate variability (HRV) values (±SE) according to previous medical history. HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (one-way ANOVA).

Of the field measurements, horses with previous lower limb soft tissue problems recorded the highest SDNN (77.93±20.99ms) and horses that previously had lower limb bone problems recorded the highest RMSSD (89.50±43.74ms). Lowest SDNN in the field was found in horses with previous foot problems (49.75±6.58ms) and lowest RMSSD in horses with 'other' ailments (54.43±7.57ms).

None of the differences between HRV according to their medical history were statistically significant; Stable SDNN, F (4, 28) = 0.72, $p=0.58$, Field SDNN, F (4, 28) = 2.02, $p=0.12$, Stable RMSSD, F (4, 28) = 0.26, $p=0.90$, Field RMSSD, F (4, 28) = 1.89, $p=0.14$. It was therefore not possible to reject the null hypothesis.

7.4.8. The effect of background history on HRV

A one-way ANOVA was carried out to identify whether the background of the horses was a factor affecting HRV. Horses were placed into five groups according to their previous discipline or origin; Army (n=4), Riding club (n=14), Rescue (n=7), Dressage (n=5), Racing (n=3).

The highest HRV measured in the stable was found in the horses that originated from a dressage background (SDNN 85.92±20.40ms, RMSSD 97.78±32.69ms) and lowest in the horses from a racing background for SDNN (57.67±20.61ms) and from the army for RMSSD (74.05±28.88ms).

Of the field measurements, horses from a racing background recorded the highest HRV (SDNN 68.63±13.60ms, RMSSD 78.80±21.05ms). Lowest SDNN in the field was found in horses originating from a dressage background (SDNN 53.76±13.99ms, RMSSD 56.76±19.14ms; **Figure 7.12**).
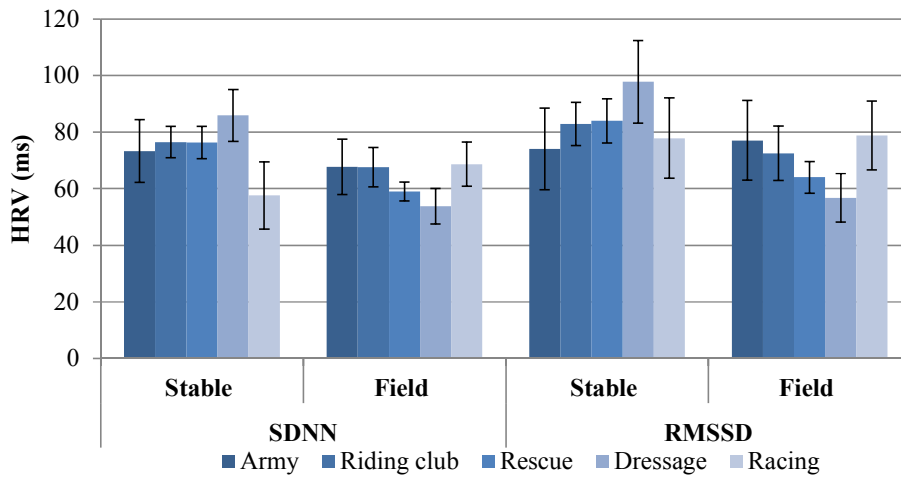
Figure 7.12. Mean baseline heart rate variability (HRV) values (±SE) according to background history. HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (one-way ANOVA).

None of the differences between HRV according to background history were statistically significant; Stable SDNN, F (4, 28) = 0.98, p=0.43, Field SDNN, F (4, 28) = 0.57, p=0.69, Stable RMSSD, F (4, 28) = 0.49, p=0.74, Field RMSSD, F (4, 28) = 0.50, p=0.74. It was therefore not possible to reject the null hypothesis.

7.4.9. Level of compliance during clipping and its effect on HRV

A one-way ANOVA was carried out to identify whether the level of compliance displayed by the horses during clipping was a factor affecting HRV. Horses were placed into four groups according to their level of compliance; Twitch/sedate (n=4), Acceptable (n=14), Good (n=7), Very good (n=5).

The highest HRV measured in the stable was found in the horses classed as 'acceptable' in response to clipping (SDNN 81.78±11.01ms, RMSSD 90.20±13.07ms) and lowest in the horses who required chemical or physical restraint (SDNN 68.30±12.93ms, RMSSD 73.08±16.24ms; **Figure 7.13**).

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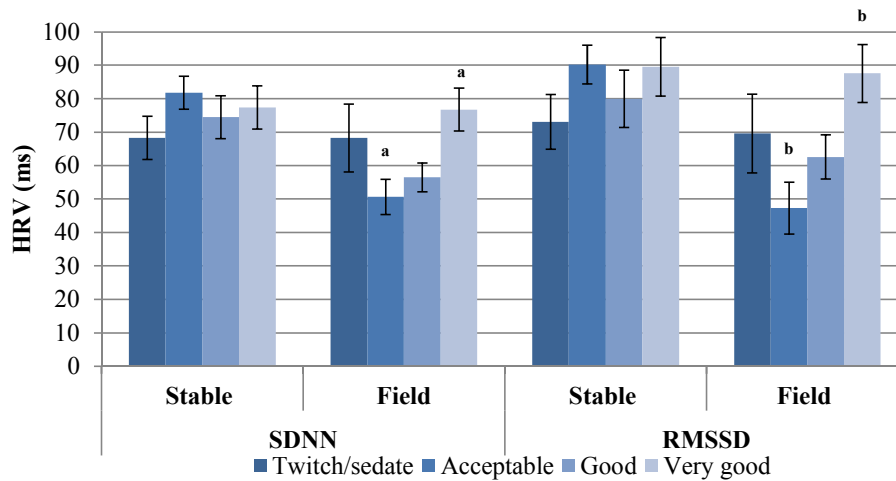
Figure 7.13. Mean baseline heart rate variability (HRV) values (±SE) according to level of compliance during clipping. HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Paired superscript letters denote significant differences for each variable (one-way ANOVA; p≤0.05).

Of the field measurements, horses classed as 'very good' recorded the highest HRV (SDNN 76.74±21.16ms, RMSSD 87.55±28.83ms). Lowest SDNN in the field was found in horses with an 'acceptable' response to clipping (SDNN 50.62±11.85ms, RMSSD 47.30±17.41ms).

Differences in stable HRV measurements were not significant; SDNN, $F\,(3, 29) = 0.36$, $p=0.78$, RMSSD, $F\,(3, 29) = 0.55$, $p=0.65$. There were significant differences between field HRV measurements; SDNN, $F\,(3, 29) = 3.82$, $p=0.020$, RMSSD, $F\,(3, 29) = 3.58$, $p=0.026$. The eta squared statistic (0.28 SDNN, 0.27 RMSSD) indicated a medium effect size for both measurements. Post-hoc comparisons using the Bonferroni test revealed that RMSSD for the horses classed as 'very good' was significantly higher than those classed as 'acceptable' ($p=0.033$). SDNN was also higher in horses classed as 'very good' than 'acceptable' ($p=0.056$) and 'good' horses ($p=0.053$), which can be considered a trend.

As horses were grouped on a scale of 1-4 according to their level of compliance and data were normally distributed a Pearson product-moment correlation coefficient was used to investigate

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the relationship between compliance and length of tenure at the Equestrian Centre. There was a significant positive relationship between the two variables (r=0.54, p=0.001) where level of compliance increased with length of tenure and vice-versa.

7.4.10. The effect of forage type on HRV

An independent-samples t-test was carried out to identify whether the horses' normal forage diet fed when housed (hay n=20, haylage n=13) was a factor affecting HRV, and for all measurements this was higher in horses on a haylage diet than for horses fed hay.

Horses fed a haylage diet recorded significantly higher SDNN in the stable than horses fed hay (86.18±18.43ms, 69.03±17.87ms, respectively), t (31) = -2.66, p=0.014 (**Figure 7.14**). The eta squared statistic was 0.19 which indicates a small effect size and it was possible to reject the null hypothesis.
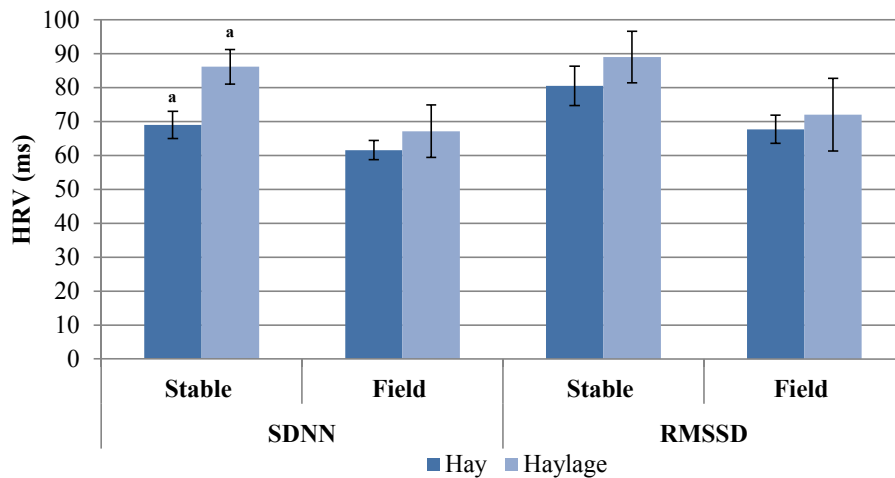
F igure 7.14. Mean baseline heart rate variability (HRV) values (±SE) according to the type of forage diet fed when stabled. HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Paired superscript letters denote significant differences for each variable (independent samples t-test; p≤0.05).

There was no significant difference in field SDNN between hay (61.60±12.78ms) and haylage diets 67.15±27.82ms; t (31) = -0.52, p=0.61. There was also no significant difference in stable

RMSSD for hay (80.49±26.04ms) or haylage 89.06±27.52ms; t (31) = -0.89, $p=0.38$. Differences in field RMSSD were also not significant between hay (67.77±18.77ms) or haylage diets 72.04±38.51ms; t (31) = -0.43, $p=0.67$.

7.4.11. The effect of bedding type on HRV

An independent-samples t-test was carried out to identify whether bedding substrate when housed (shavings n=16, straw n=17) was a factor affecting HRV, and for all measurements this was found to be higher for horses bedded on shavings than for horses bedded on straw (**Figure 7.15**).
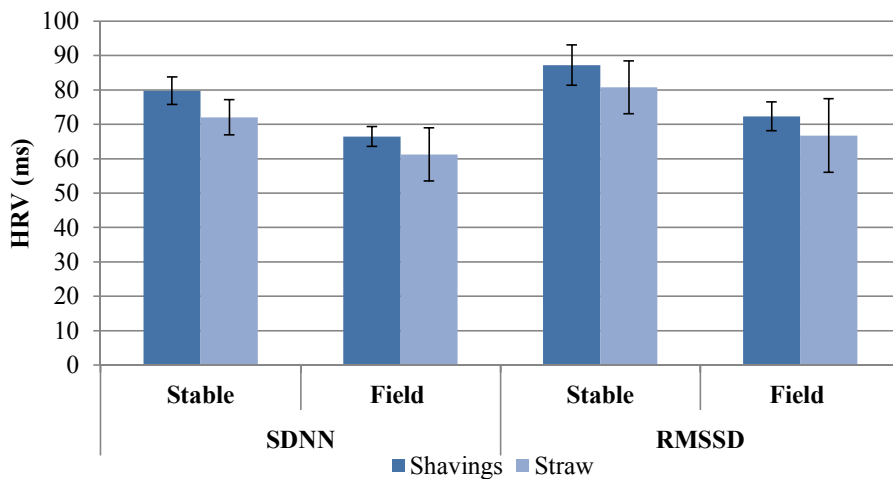
Figure 7.15. Mean baseline heart rate variability (HRV) values (±SE) according to bedding substrate used whilst stabled. HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (independent-samples t-test).

Differences in stable SDNN between bedding substrates were not significant (shavings 79.74±18.05ms, straw 72.06±21.05ms), t (31) = 1.12, $p=0.27$. There was no significant difference in field SDNN between bedding substrates (shavings 66.48±23.58ms, straw 61.25±15.94ms), t (31) = 0.61, $p=0.54$. No significant difference in stable RMSSD was found between bedding substrates (shavings 87.18±27.01ms, straw 80.75±26.54ms), t (31) = -0.69, $p=0.50$. Differences in field RMSSD were also not significant between bedding substrates

(shavings 72.34±30.63ms, straw 66.72±25.37ms), *t* (31) = 0.57, *p*=0.57. It was not possible to reject the null hypothesis.

7.4.12. The effect of concentrate feed type on HRV

A one-way ANOVA was carried out to identify whether the type of concentrate feed provided whilst stabled was a factor affecting HRV. Horses were placed into five groups according to their concentrate feed diet; Pasture cubes (n=7), Competition cubes (n=3), High-fibre nuts (n=15), High-energy mix (n=5) and Chaff (n=3).

The highest HRV measured in the stable was found in the horses fed pasture cubes for SDNN (82.11±17.06ms) and in horses fed high-fibre nuts for RMSSD (87.71 ±30.25ms) and lowest in the horses fed chaff (SDNN 69.87±15.40ms, RMSSD 62.70±12.67ms). Of the field measurements, horses fed chaff recorded the highest SDNN (77.13±19.90ms) and horses fed competition cubes recorded the highest RMSSD (94.03±59.17ms). Lowest SDNN in the field was found in horses fed pasture cubes (SDNN 59.69±26.47ms, RMSSD 61.77±35.52ms; **Figure 7.16**).
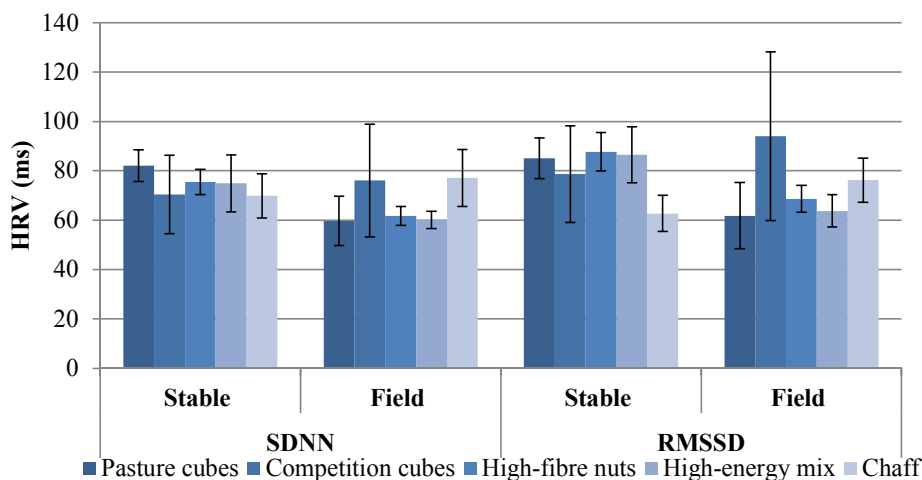
Figure 7.16. Mean baseline heart rate variability (HRV) values (±SE) according to type of concentrate feed provided when stabled. HRV variables were standard deviation of inter-beat intervals (SDNN) and square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). Differences were not significant (one-way ANOVA).

None of the differences between HRV according to concentrate feed diet were statistically significant; Stable SDNN, F (4, 28) = 0.28, p=0.89, Field SDNN, F (4, 28) = 0.68, p=0.61, Stable RMSSD, F (4, 28) = 0.57, p=0.69, Field RMSSD, F (4, 28) = 0.80, p=0.53. It was therefore not possible to reject the null hypothesis.

7.4.13. The effect of height on HRV

The height of each horse ranged from 15.1 hands to 17.3 hands (16.1 ±0.3 hands). Height in hands was converted to inches (one hand equating to four inches) and then into centimetres. Height data were not normally distributed and remained as such after being transformed using square root. Using untransformed data the relationship between horse height and HRV was investigated using a non-parametric Spearman's rank order correlation. There was no significant relationship between height and stable SDNN, r_s=-0.09, p=0.62, field SDNN, r_s=-0.11, p=0.52, stable RMSSD, r_s=-0.29, p=0.11 and field RMSSD, r_s=-0.16, p=0.37. It was not possible to reject the null hypothesis.

7.4.14. The effect of age on HRV

The age of horses (at the time of data collection) ranged from 7 to 20 years (14 ±3.9 years). Age data were normally distributed so a parametric Pearson product-moment correlation coefficient was used to investigate the relationship between age and HRV. There was no significant relationship between age and stable SDNN, r=0.11, p=0.53, field SDNN, r=0.13, p=0.48, stable RMSSD, r=0.22, p=0.22 and field RMSSD, r=0.16, p=0.38. It was not possible to reject the null hypothesis.

7.4.15. Length of tenure at the Equestrian Centre and its effect on HRV

The duration of tenure at the Equestrian Centre (at the time of data collection) ranged from 1 to 16 years (5±3.8 years). Data were not normally distributed and remained as such after log transformation. Using untransformed data the relationship between length of tenure and HRV was investigated using a non-parametric Spearman's rank order correlation. A moderately

positive relationship approaching significance was present between tenure and field SDNN data (**Figure 7.17**), with longer tenure being associated with higher HRV, r_s=0.34, p=0.053.

There was no significant relationship between tenure and other HRV measurements; stable SDNN, r_s=0.10, p=0.56, stable RMSSD, r_s=0.17, p=0.34 and field RMSSD, r_s=0.22, p=0.22. It was therefore not possible to reject the null hypothesis.
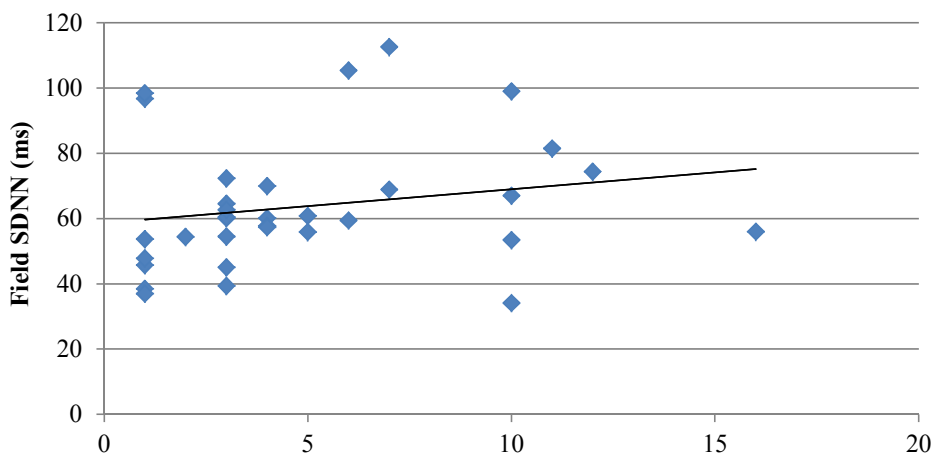
Figure 7.17. Relationship between length of tenure at Brackenhurst Equestrian Centre (years) and mean heart rate variability (HRV). HRV variable was square root of the mean squared differences between adjacent inter-beat intervals (RMSSD). A trend for a positive relationship between the two variables was present (Spearman's rank order correlation; p≤0.05).

7.4.16. Relationship between temperament score and HRV

Out of a potential maximum of 30 points (3 points per question), the temperament score results for each horse ranged from 1 to 14 points (4.4±2.9 points). Data were not normally distributed and remained as such after transformation using square root. Using untransformed data the relationship between temperament score and HRV was investigated using a non-parametric Spearman's rank order correlation.

There was no significant relationship between temperament score and stable SDNN, r_s=0.04, p=0.82, field SDNN, r_s=0.20, p=0.27, stable RMSSD, r_s=-0.18, p=0.32 and field RMSSD, r_s=0.11, p=0.53. It was not possible to reject the null hypothesis.

7.4.17. Analysis of temperament score data

For each of the ten questions comprising the temperament questionnaire, horses were awarded a numeric score according to whether their behavioural response to each scenario was none (0), mild (1), moderate (2) or severe (3). Of horses demonstrating *some* reaction to each scenario (i.e. scoring between 1-3), the highest score for the severity of their reaction was in response to social isolation (**Table 7.3**), scoring on average 1.7 points per horse and seen in 79% of subjects.

Table 7.3. Summary of temperament survey results. Total score for the whole sample group (n=33), number and percentage of sample group scoring between 1-3 for each question (i.e. that demonstrate *some* reaction to each stimuli), and mean score awarded for each of these respondents are shown. For example, the highest number of points were awarded for responses to isolation; 26 horses (79% of study group) had moderate responses to this stimuli, scoring 1.7 on average.

Tendency to respond with:			Subjects	
	Total	Mean	N°	%
Aggression*or fear** towards familiar horses	7	1.4	5	15
Aggression or fear towards unfamiliar horses	12	1.3	9	27
Aggression or fear towards familiar humans	5	1.7	3	9
Aggression or fear towards unfamiliar humans	6	1.5	4	12
Response to isolation (restlessness, agitation, vocalisation, pacing)	43	1.7	26	79
Fear to sudden/loud noises	27	1.0	26	79
Fear to unfamiliar objects/situations	25	1.0	24	73
Non-compliance during clipping	7	1.4	5	15
Non-compliance during shoeing/trimming	1	1.0	1	3
Non-compliance when being caught (from field)	12	1.3	9	27

*ears back, rump swing, kick threat, kick, bite threat, bite, barging
**avoidance, evasion, movement away

The lowest score was awarded for non-compliance to shoeing or trimming, seen in just one horse which indicated general compliance to this procedure. Three horses were awarded a mean

score of 1.7 for responding to familiar humans with aggression. Most horses showed some response to sudden or loud noises (79%) and fear to unfamiliar objects or situations (73%). Twenty seven per cent of horses reacted with aggression or fear towards unfamiliar horses and were non-compliant when attempting to be caught from the field.

7.4.18. Factors affecting temperament score

A Kruskal-Wallis test was carried out to investigate whether the presence or absence of stereotypic behaviour was a factor affecting temperament score. Horses with no stereotypy were awarded a lower median score (*Md*=3) than horses with oral (*Md*=4) or locomotory (*Md*=4) stereotypies. There was no significant difference between groups, X^2 (2) = 1.52, *p*=0.47, so it was not possible to reject the null hypothesis.

A Kruskal-Wallis test was carried out to investigate whether housing type was a factor affecting temperament score. Horses normally housed in Farmhouse stables facing inwards towards the Crew Yard had a higher median temperament score (*Md*=5) than horses in other housing types; Lodden indoor or outdoor stables (*Md*=4), crew yard or outward facing Farmhouse stables (*Md*=3). There was no significant difference between groups, X^2 (4) = 4.67, *p*=0.32, it was not possible to reject the null hypothesis.

Differences in temperament score between sexes were investigated using a Mann-Whitney U test. Mares scored higher (*Md*=5) than Geldings (*Md*=4) but this difference was not significant, *U* = 72.5, *z* = -1.46, *p*=0.14 and it was not possible to reject the null hypothesis.

A Kruskal-Wallis test revealed a statistically significant difference in temperament score according to breed, X^2 (5) = 14.91, *p*=0.011. Irish Sport horses had the highest median temperament score (*Md*=6), followed by Warmblood horses (*Md*=4), Irish Draught (*Md*=3), Thoroughbred (*Md*=3), Cob (*Md*=3) and Shire horses (*Md*=2). A follow up Mann-Whitney U test revealed that Irish Sport horses scored significantly higher than Shire horses, *U* =0.00, *z* =-

2.20, *p*=0.028, *r*=0.61 which indicated a medium effect size. It was therefore possible to reject the null hypothesis.

A Kruskal-Wallis test was carried out to investigate whether coat colour was a factor affecting temperament score. Black horses had a higher median temperament score (*Md*=6) than grey (*Md*=5), brown (*Md*=4), bay (*Md*=4), coloured (*Md*=3), chestnut (*Md*=3) and palomino horses (*Md*=2). There was no significant difference between groups, X^2 (6) = 8.05, *p*=0.23, it was not possible to reject the null hypothesis.

The possible effect of medical history on temperament score was investigated using a Kruskal-Wallis test. Horses that had previously experienced lower limb soft tissue injuries had a higher median temperament score (*Md*=5) than horses with no previous health problems (*Md*=4), lower limb bone problems (*Md*=4), foot problems (*Md*=3) and 'other' health problems (*Md*=2). There was no significant difference between groups, X^2 (4) = 2.30, *p*=0.68, it was not possible to reject the null hypothesis.

A Kruskal-Wallis test was carried out to investigate the effect of background history on temperament score. Horses from a dressage background had a higher median temperament score (*Md*=5) than Army (*Md*=4), riding club (*Md*=4), rescue centre (*Md*=3) and ex race horses (*Md*=2). There was no significant difference between groups, X^2 (4) = 8.83, *p*=0.065, which although indicates a trend did not enable the null hypothesis to be rejected.

Horses that required either chemical or physical restraint during clipping had a higher medial temperament score (*Md*=6) than horses that were good (*Md*=4), acceptable (*Md*=3) and very good (*Md*=3) during clipping. A Kruskal-Wallis test revealed that these differences were not significantly different, X^2 (3) = 2.87, *p*=0.41 and did not enable the null hypothesis to be rejected.

Differences in temperament score according to the type of forage provided were investigated using a Mann-Whitney U test. Horses receiving a haylage diet scored higher (*Md*=4) than horses provided with hay (*Md*=3) but this difference was not significant, $U = 96.5$, $z = -1.26$, *p*=0.21 and it was not possible to reject the null hypothesis.

A Mann-Whitney U test found no significant difference in temperament score according to bedding substrate. The median temperament score for horses bedded on shavings was the same for horses bedded on straw (*Md*=4), $U = 132.5$, $z = -0.128$, *p*=0.90 so it was not possible to reject the null hypothesis.

Horses receiving pasture cubes, competition cubes and high-fibre nuts all had a median temperament score (*Md*=4) higher than horses fed chop (*Md*=3) and high-energy mix (*Md*=2). A Kruskal-Wallis test revealed that these differences were not significantly different, $X^2 (4) = 4.69$, *p*=0.32 and did not enable the null hypothesis to be rejected.

The relationship between temperament score and height, age and length of tenure at Brackenhurst Equestrian was investigated using Spearman's rank order correlation. No significant relationships existed between variables; height, r_s=0.14, *p*=0.43, age, r_s=0.04, *p*=0.83, length of tenure, r_s=-.0.54, *p*=0.77. It was not possible to reject the null hypotheses.

7.5. DISCUSSION

HRV data collection took place during the summer months towards the end of the academic year. At that time of year, the horses at Brackenhurst Equestrian Centre were housed singly in indoor stables or loose housed in pairs during the daytime which is when the 'stable' data were collected, then spent evenings and weekends turned out to graze in paddocks in small groups. 'Field' data were collected around two weeks later once the academic term had finished and horses were turned out to pasture 24hrs per day. Analysis of HRV data revealed that

measurements taken whilst the animals were housed in their usual stabling or crew yard were significantly higher than those taken whilst at liberty in the field. This suggests that, on this occasion, their affective state when stabled was more positively valenced than whilst out at pasture.

One of the possible reasons for this is that horses at the Equestrian Centre are accustomed to being used for a variety of under-graduate and post-graduate research projects that incorporate the collection of behavioural and non-invasive physiological data during term time. All the horses are therefore familiar with the fitting and removal of HRV monitoring equipment whilst stabled, but due to the risk of equipment being lost or damaged it is very rarely fitted while the horses are at liberty in the field. Therefore, the horses were either very accustomed to their stable environment, including the variety of additional stimuli presented to them during their normal routine, and are able to maintain a positive affective state or, since they were unaccustomed to being fitted with equipment whilst at liberty, they would not expect it to be fitted when turned out to pasture and may have associated the procedure with being caught and taken in from the field to be ridden which resulted in lower HRV measurements. Further studies over a longer time scale would therefore be necessary to establish whether differences in HRV persist in stabled versus field conditions and if this might be attributable to measurements being taken 'in' and 'out' of term-time.

In the present study, none of the differences in HRV from different housing types were significant so the variance between stabled and field measurements could not be attributed to the influence of a particular housing type. However, it was interesting to note that the results whilst stabled were generally in agreement with data obtained in **Chapter 6: Section 6.7.1**; the highest HRV was found in horses living in paired housing in a crew yard and the lowest measurements were from horses housed individually in farmhouse stables that faced outward over the yard. Although not significant, HRV from the outward-facing farmhouse stables was numerically lower than the identically constructed inward-facing farmhouse stables. As they directly

overlook conspecifics in the crew yard it is possible that the inward facing stables provide slightly more positive stimuli and enriched environment than the stables overlooking the yard, especially when there is little activity outside. Conversely, when measured in the field, horses from outward-facing farmhouse stables recorded the highest HRV which may be interpreted cautiously as a rebound effect to confinement in that type of stabling. These findings support those of Cooper, McDonald and Mills (2000) and Ninomiya *et al*. (2008) who document the benefits of increasing visual horizons to areas beyond the stable.

Equine stereotypies and stress sensitivity have been related to low levels of HRV and therefore negative emotion by Bachmann *et al*. (2003), but Nagy *et al*. (2009) were unable to differentiate HRV in crib-biting horses from control horses. However, the presence of sex-based differences in HRV found in this series of studies casts some doubt over the validity of Bachmann and colleagues study (2003) as their protocol was based on a comparison between matched pairs of crib-biting and control horses that were not matched for sex. In the present study, sample numbers for horses with stereotypies were small and, although accounted for during statistical analysis, differences in HRV did not reach significance. This indicates that overall affective state did not differ between subjects. Whilst stabled however, horses with no stereotypic behaviour recorded numerically higher HRV than horses with either oral or locomotor stereotypies. In the field, HRV for horses without stereotypies was similar to that of horses with locomotor stereotypies which could be attributed to the fulfilment of their motivation to move around. Field measurements of HRV for horses with oral stereotypies were considerably lower than all other measurements. This may indicate that despite the fulfilment of their foraging requirements these horses experience a slightly more positive affective state in a stable environment, possibly because the expression of stereotypic behaviour is facilitated via the range of surfaces available for grasping in order to wind suck or chew. The presence of either oral or locomotor stereotypies could not be attributed to a particular breed or housing type, although low cell frequencies prevented this being analysed statistically using a Chi-squared test.

Results from **Chapters 3, 4, 5** and **6** identified individual differences in HRV and it was speculated that the causal factor was either underlying affective state, temperament or breed. As Lesimple *et al*. (2011) related breed with personality of the horse, the present study investigated the association between HRV and six different breeds of horse but obtained non-significant results. It was therefore not possible to identify breed as a factor that affected HRV and infers that affective state was similar in different breeds of horse. Similarly, a recent study by Nagel *et al*. (2011) also found no difference in HRV measurements between pony and horse breeds. The results of the present study suggest that differences in HRV between individual horses remain attributable to either temperament or affective state.

Despite anecdotal evidence suggesting that coat colour may be associated with personality or temperament in horses (Keeler, 1947) there was no evidence in the present study to suggest that coat colour was a factor that influenced HRV. Horses in the present study ranged in age from 7 to 20 but as no significant relationship was found it was possible to eliminate age as a factor affecting HRV and is in agreement with the findings from human studies (Reardon and Malik, 1996). Height differences between horses were also not found to be a significant factor.

Although age was not found to be a significant factor, the duration of tenure ranged from one to 16 years and there was a trend for horses that had spent longer at the Equestrian Centre to have higher HRV in the field than those with shorter lengths of tenure. It is possible to speculate that whilst these horses had similar affective states whilst stabled, those with longer time served had a slightly more positive affective state once turned out to pasture than horses with shorter durations of stay. These results are similar to the findings of **Chapter 3: Section 3.8.6** where a positive affective state was observed during bouts of free exercise and interaction with a conspecific particularly after a period of social deprivation. Horses with more experience of the environment and familiarity with the routine of the Equestrian Centre may therefore find their 'free-time' slightly more rewarding than horses of shorter tenure but as the results fell short of significance this must be interpreted with caution.

HRV did not differ significantly between horses from different backgrounds so there was no evidence to suggest that this was a factor that affected HRV. It was interesting to note that whilst stabled, horses from a dressage discipline recorded the highest numerical HRV and horses from racing recorded the lowest, whereas in the field the opposite was found. These differences could not be attributed to a particular breed or housing type, although low cell frequencies prevented this being analysed statistically using a Chi-squared test. Further work is necessary to compare these results with horses that are currently active in different disciplines and may provide an important insight into the affective state of horses from a variety of competitive sporting industries.

For prey species such as the horse, there is a survival value associated with the ability to mask pain or discomfort in order to minimise the risk of predation (O'Callaghan, 2002; Taylor, Pascoe and Mama, 2002; Mayer, 2007), therefore outward behavioural signs do not necessarily reflect the emotional state of the animal. In the present study all participating subjects were reported by the Yard Manager to be clinically 'sound' when HRV measurements were taken. Differences in HRV between horses grouped according to the presence or absence of previous ailments were not significant. In the stable however, horses with previous lower limb bone ailments had the highest numerical HRV and the lowest was found in horses previously suffering from lower limb soft tissue problems, whereas in the field the opposite results were obtained. It was not possible to associate different bedding types with these findings and low cell frequencies prevented statistical analysis using a Chi-squared test. These results may infer that even in clinically sound horses, those that have previously experienced bone or soft tissue related ailments may find comfort in different surface properties. As previously reported by Perkins *et al*. (2000) and Rietmann *et al*. (2004a) HRV seems to be a reliable method of detecting pain, or in this case the absence of pain in horses. Further research would be very useful to establish whether HRV can provide physiological evidence of pain or discomfort in horses with sub-clinical ailments and would also have important implications for the management and monitoring of horses undergoing rehabilitative treatment.

Approximately half the horses at the Equestrian Centre are bedded on straw and half on shavings. Bedding substrate is chosen according to various factors including the cleanliness of the individual horse, whether thermal insulation is required due to the location and design of the stable, if the horse has dust allergies or a tendency to eat bedding, cost, availability and ease of disposal. HRV for horses bedded on wood shavings was always numerically higher than for those bedded on straw but the differences were not significant. This either implies that bedding was not a factor influencing HRV or that all the horses tested were bedded on a substrate that 'suited' their requirements. As the present study was undertaken to investigate potential factors that may influence HRV and did not involve the experimental manipulation of conditions, further study would be necessary using a crossover design in order to fully investigate these findings.

Horses have evolved as grazing herbivores but due to climatic constraints in the UK pasture grass cannot always be provided all year round. The grass therefore has to be conserved and traditionally hay-making has been the most common form of conservation. In recent years however, hay has increasingly been replaced by haylage in equine diets (Harris, 1999; Mellor, Gettinby and Reid, 1999; Hotchkiss, Reid and Christley, 2007). Haylage is forage made for horses from grass that is cut earlier than hay, turned until the correct dry matter is achieved and then sealed in plastic. A natural fermentation process takes place, which preserves the grass and its nutrients and results in dust-free forage that is low in mould spores and highly palatable (Clements, 2007a). The increasing popularity of haylage can be explained in practical terms, as unlike hay which needs to be kept dry, storage of wrapped haylage is weather resistant. Also, feeding haylage removes the requirement to soak hay in order to reduce airborne particle levels in the stable for horses suffering from respiratory ailments such as recurrent airway obstruction (Clements, 2007a; Clements, 2007b).

In the present study, horses were grouped according to the type of forage provided and when measured in the stable, HRV was found to be significantly higher in horses fed haylage than

those fed hay. Field measurements were similar but did not reach significance. The results of the present study infer that a haylage diet can be associated with positive affective state in stabled horses and that this trend persists when horses are turned out to pasture where they are able to consume forage in its natural form. Previous research by Goodwin, Davidson and Harris (2002) and Muller and Uden (2007) found through preference testing that given the choice, horses selected haylage over hay but were unable to elucidate why this might be. Although every batch varies, haylage and hay differ in their chemical composition (Muller and Uden, 2007; Muller, von Rosen and Uden, 2008) which might affect their gustatory or olfactory qualities. Studies of emotion in animals routinely use foods containing high levels of sucrose (Spruijt, van den Bos and Pijlman, 2001; Van der Harst, J. E. and Spruijt, 2007) to investigate motivation for a reward of high hedonic value (Chandrashekar, 2006). Haylage has been found to contain higher levels of sucrose than hay (although not at significant levels) and contains a significantly higher quantity of fructans (g/kg DM; Muller, von Rosen and Uden, 2008) which may account for its palatability. Horses have also been found to express a preference for haylage harvested at different times of year; haylage cut in June was chosen in preference over later cuts in July and August, it was also eaten, chewed and swallowed faster than later batches and contained significantly more free glucose and fructose than the later cut batches (Muller, 2011). Human studies that have investigated the interaction of emotion, mood and food choice also suggest that palatable foods can improve mood and mitigate effects of stress via brain opioidergic and dopaminergic neurotransmission (Gibson, 2006). Results from the present study indicate that the type of forage provided could influence affective state. It may therefore be possible to use forage type as a form of reward or environmental enrichment if providing haylage (either regularly or irregularly) can be associated with positive emotion. As the present study did not involve the experimental manipulation of conditions, further measurements using a crossover design would be necessary in order to verify these findings.

HRV did not differ significantly between horses grouped according to their type of concentrate feed diet and can be discounted as a factor affecting HRV. Selection of the appropriate

concentrate feed diet is dependent on the nutritional and energy requirements of each horse was based on their body condition. HRV in the stable was found to be numerically highest in the horses fed concentrates with a low digestible energy content (high-fibre nuts and pasture cubes) but this did not appear to be associated with different breeds of horse and low cell frequencies prevented statistical analysis using a Chi-squared test.

As discussed in **Chapter 1: Section 1.5.1.4**, none of the existing published equine HRV studies using mixed sex subjects report an effect of sex on HRV. Despite sex not being the main focus of investigation, hence the relatively small sample sizes, **Chapters 4, 5** and **6** involved subjects of both sexes. All three studies consistently identified that mares recorded numerically higher HRV than geldings, but that the differences were not significant. In order to investigate further, the present study used a larger sample size to establish whether sex was a potential factor affecting HRV. Mares were found to have significantly higher HRV than geldings when measured in the field and recorded higher HRV in the stable which was not significant. These findings are in accordance with human research where sex-related differences in HRV have identified females as having a greater vagal control of the heart than males who exhibit more sympathetic cardiac control (Rossy and Thayer, 1998). This suggests that sex-based differences in HRV are not necessarily indicative of affective state but occur naturally and are a factor requiring control when designing HRV studies using mixed sex subjects.

Horses selected for use in a riding school environment tend to be chosen for their passive and tractable behaviour, but it is possible that this may mask physiological indicators of stress. For example, whilst behaviourally compliant, Schmidt *et al.* (2010c) reported low HRV in young horses undergoing initial training when they were mounted by a rider, a situation resembling a potentially lethal threat under natural conditions. **Chapter 5** investigated whether HRV differed in horses identified by the Yard Manager to show either compliant or non-compliant behaviour during clipping, i.e. movement away from the clippers, unwillingness to stand still or requiring sedation. Non-compliant horses were found to have numerically lower HRV than compliant

horses, which infers a) that behavioural compliance was *not* masking physiological signs of distress and b) that the procedure was a potentially negative experience for the non-compliant horses. Using a larger sample size the present study explored whether differences in HRV between compliant and non-compliant horses persisted at rest, as if so this might indicate an underlying level of negative affective state or anxiety. Horses were grouped according to their level of compliance during clipping. When measured in the stable, HRV between groups did not differ significantly. However, when measured in the field, HRV for horses classed as 'good' or 'acceptable' were significantly lower than those with 'very good' responses during clipping. The horses' level of compliance during clipping also significantly increased with length of tenure at the Equestrian Centre (and vice-versa) indicating that a habituation process occurs over time. Although sample numbers were low and statistical significance was not reached, it appears that while it was possible in **Chapter 5**, to identify fractious and calm horses by their HRV during clipping, these differences were not maintained at rest in the stable, indicating that changes in affective state are transient and occur during exposure to the stimuli but do not persist afterwards. Further measurements would be necessary to elucidate why HRV in the two central groups ('good' and 'acceptable') reduced when measured in the field in comparison to HRV in the stable.

The relationship between HRV and overall temperament score awarded for each horse from the temperament survey did not yield significant results. As mentioned previously in **Chapter 1: Section 1.5.2.3**, there is much debate as to the efficacy of temperament surveys used in isolation (McCall *et al*., 2006) but anxiety had been identified as a component that could be rated reliably by regular horse handlers (Lloyd *et al*., 2008; McGrogan, Hutchison and King, 2008). The categorisation of horses according to their behaviour during clipping was related numerically (but not statistically) to HRV in **Chapter 5** which identified an association between behavioural tendencies and HRV values amongst this study group. As previously mentioned however, it appears these differences are only apparent *during* exposure to the particular stimuli which might explain why the survey failed to detect this relationship using resting measurements even

though 'compliance during clipping' and other similar factors were included for rating. Alternatively, inconsistencies may have occurred during completion of the survey as horses that required sedating for clipping did not necessarily gain a high score in the survey. Although previous equine temperament studies have successfully used information from a single evaluator e.g. Seaman, Davidson and Waran (2002), increasing the number of contributors in future studies may improve objectivity and therefore accuracy in future studies.

A novel object and handling test carried out in horses by Visser *et al*. (2002) suggested that measurement of HRV may be useful in differentiating between individuals and quantifying aspects of temperament with important implications for breeding, housing and management of the horse. HRV may therefore be more useful in determining equine temperament when measured alongside behavioural observations during exposure to an environmental challenge than by relying on a questionnaire alone. Further research using HRV to assess differences in temperament is recommended as it is a difficult characteristic to assess objectively.

In addition to horse profile and management factors being investigated as possible influences on HRV, the potential relationship between these data and temperament score were also analysed. Of all the variables tested, only 'breed' was identified as a factor affecting temperament score with Irish Sport horses scoring significantly higher than Shire horses. Some similarities were present between the two; neither breed reacted with aggression to familiar humans, both were reported as compliant during shoeing and on average, both breeds responded with mild fear towards unfamiliar objects or situations. However, where Shire horses showed no response, Irish Sport horses showed mild non-compliance during shoeing and when being caught from the field and were rated as responding with mild aggression towards familiar or unfamiliar horses and unfamiliar humans. Irish Sport horses were also rated higher than Shire horses in response to isolation and fearfulness of sudden or loud noises. Anecdotal evidence from horse owners and handlers as well as behavioural research suggests the existence of breed-typical behaviour and personality in horses (Lloyd *et al*., 2008). Breeds are often described as having breed

typical behaviours and such claims are supported by anecdotal evidence from breed enthusiasts, with breed societies often promoting a breed by describing its typical temperament and personality. For example the Shire horse is described as 'placid' (The Shire Horse Society, 2011) and the Irish Sport horse as being of 'good temperament, intelligent and willing to perform' (Horse Sport Ireland, 2011). Results of the temperament survey infer that, of the breeds tested, the Irish Sport horse scored higher than the Shire horses for anxiety yet results from HRV measurements were mixed. In the stable, HRV for Shires was higher than Irish Sport horses while the opposite was recorded in the field and, as results were not significant, breed was discounted as a factor affecting HRV. As previously stated, it seems that practical experience of the horses' existing response to an aversive management practice (clipping) was a more accurate indicator of equine anxiety than the temperament survey.

7.6. CONCLUSION

Previous equine studies suggest that individual characteristics related to factors such as temperament, personality and underlying affective state may influence and be determined by HRV measurements. Despite HRV appearing to be a promising indicator of affective state in horses, factors affecting HRV have not been satisfactorily explored. **Chapters 3 – 6** investigated HRV as a method of detecting emotion in horses under a variety of circumstances. Differences in HRV were discovered according to management regime and the presence or absence of social contact. Individual differences were also noted that may be associated with underlying affective state, temperament or breed. In order to explore some of these issues, the present study provided a comprehensive investigation of multiple variables in order to examine which factors might affect HRV.

HRV was found to be significantly higher in the stable than in the field, inferring that affective state was not negatively valenced during confinement, although the results showed a similar pattern to those of **Chapter 6**, that the provision of physical or at least visual contact with

conspecifics appeared to be beneficial. There was a trend for length of tenure at the Equestrian Centre to affect HRV in the field which may indicate a rebound effect occurring in response to on-going confinement or participation in riding school activities. Horses also appeared to habituate to clipping over time as individuals that had resided at the Equestrian Centre for longer were rated as more compliant (and vice-versa). Horses with oral and locomotor stereotypies showed slight differences in affective state between field and stable measurements but further measurements using similarly sized groups is recommended. Differences in HRV were identified relating to the type of forage diet provided and are strongly recommended for further investigation as this could be relevant to the promotion of positive experiences in stabled horses. Numerical differences in HRV present during clipping in **Chapter 5** were not evident at rest suggesting that non-compliant horses do not maintain a persistent negative affective state. Affective state appeared to be more accurately identified during exposure to a routine management practice (clipping) than by means of a temperament survey. Sex differences in HRV were confirmed to occur and should be taken into account when selecting participants for research projects. The remaining factors did not affect HRV and can be disregarded when selecting individuals for future HRV studies.

Chapter Eight

General Discussion

8.1. SUMMARY OF FINDINGS

The aim of this research was to investigate the use of heart rate variability alongside behavioural measurements as a non-invasive method of assessing affective state in horses. High HRV was evident during rewarding situations - i.e. during social interaction with a companion particularly when engaging in 'play' behaviour. Provision of a forage diet of haylage (rather than hay) was also associated with high HRV. Conversely, disruption to the horses' seasonal routine was associated with low HRV and negative affective state. HRV provided an additional objective means of interpreting behaviour and assessing underlying affective state; specific aspects of pre-feeding anticipatory behaviour (behavioural transitions, ear movement and head nodding) were associated with low HRV. Certain social and spatial factors were also related to affective state; high HRV was associated with management regimes where tactile contact with neighbouring horses was permitted and where internal stable fixtures were positioned to optimise environmental monitoring. A natural variation in HRV was present between equine sexes and between individuals, changes in HRV were found to be situation-specific.

It is advantageous to be able to identify both positive and negative emotional states in horses. In a media-rich world, public perception regarding the ethics of using horses in sport in general and ethical equitation is regularly debated in equestrian culture (McGreevy, 2007; McGreevy *et al.*, 2011) and there is growing awareness that the way horses are managed and housed is suboptimal for this species. The FEI has recently introduced the concept of the "happy equine athlete" following high profile criticism of certain training methods used in warming-up elite dressage horses (van Breda, 2006, von Borstel *et al.,* 2009; FEI, 2011) and their Code of Conduct for international dressage competitions declares that the welfare of the horse must be paramount. In addition, the Animal Welfare Act (2006) specifies that the person responsible for an animal should take into account the animal's need for a suitable environment, to be able to exhibit normal behaviour patterns and its need to be housed with, or apart from, other animals.

Accurate identification of their emotional reaction is a vital tool for enhancing welfare as it provides valuable information as to whether certain management practices can be deemed either positive experiences (to be promoted), or negative experiences (to be minimised). Existing physiological measures are often invasive, tend to be interpreted as measuring negative affect and offer limited assessment of positive affect. Knowledge of what horses perceive as pleasant or unpleasant is therefore limited to the interpretation of behavioural observations yet it has important implications for stable management systems and management practices. From a practical perspective, management factors may require modification in order to improve the welfare of domestic horses. Given these issues there is clearly an urgent need for new methods for assessing emotion in horses to be developed.

Research in humans and other animals indicates that valuable information may be gained as to the affective state of horses by observing their physiology and behaviour under different conditions and in response to different stimuli. With this in mind, the objective of this investigation was firstly to establish whether an emotional response could be ascertained in horses from their physiology and/or behaviour during exposure to housing and management conditions differing in their emotional valence, and secondly to identify factors that might affect underlying HRV in order to guide future application and the interpretation of findings.

Specifically, this research investigated whether differences in HRV and behaviour were evident before, during and after anticipation of a positive reward in the form of companionship (Chapter 3), when horses were exposed to management practices deemed positively valent (feeding; Chapter 4), negatively valent (sham-clipping; Chapter 5), when housed under different management systems allowing varying levels of social contact (Chapter 6) and provided a thorough examination of potential factors that might affect HRV measurements (Chapter 7).

8.1.1. Rebound, reward and the effect of contrast

Chapter 3 examined HRV and behaviour in horses conditioned to expect a positive reward in the form of social companionship. Although inter-individual variation in HRV could account for the absence of significant differences in mean HRV between time-periods, the fact that anticipatory behaviour was not evident prior to the arrival of the companion indicates that the anticipation of social interaction was neutrally valenced i.e. no more or less emotionally valenced than being in the stable or field. The horses did however, tend to adopt a vigilant posture prior to activation of the audible cue, where their attention was oriented in the direction of arrival of the companion horse, but as discussed in Chapter 3 (Section 3.9) this was not found to be associated with HRV.

At the time of the study half the subjects lived out at grass 24 hours per day and half were stabled during the day and turned out to graze overnight. One of the most notable findings from this study was that during their time in the crew yard significant differences in behaviour were present between horses from these two management regimes; stabled horses walked more, stood less and participated in a greater number of interactive behaviours with their companion than horses kept at pasture. These behavioural observations concur with those of Mal *et al.* (1991a) who reported that confined and isolated horses travelled further, trotted more and stood less during an open field test than horses maintained on pasture with conspecifics. Whilst Grippo *et al.* (2007) found evidence that social isolation reduced HRV and caused depression and anxiety-like behaviours in prairie voles, differences in HRV between stabled and grass-kept horses in Chapter 3 did not reach significance. Although low sample numbers in the present study may be a contributory factor, Mal *et al.* (1991b) were also unable to establish a relationship between physiological indicators of stress (plasma concentrations of thyroid hormones and cortisol, complete blood cell counts and differential leukocyte counts) and behavioural signs of rebound behaviour during increasing levels of confinement and isolation.

Stable-kept horses performed more interactive behaviours in Chapter 3 than their grass-kept counterparts. This implies that a rebound effect occurs in stabled horses in response to their normal daytime regime as has previously been observed in singly housed horses (Christensen *et al*., 2002a) and cattle (Dellmeier, Friend and Gbur, 1985; Jensen, 1999) once free interaction was allowed. As a positive relationship between HRV and 'play' behaviour was identified, this provides additional physiological evidence in support of previous research (e.g. Jensen, 1999) that social interaction, particularly play behaviour, can be associated with positive emotion, especially after a period of social deprivation. Because stable-kept horses performed more interactive behaviours this may indicate that stabled horses could experience a more positive affective state following release from a stressor than is experienced by horses where the contrast between their normal and 'rewarding' environments is not as great.

Further evidence of a positive response to release from confinement was found in Chapter 7 where there was a trend for horses with longer tenure at the Equestrian Centre to have higher HRV in the field than those with shorter lengths of tenure, whereas HRV was similar in all horses when stabled. This implies that horses with longer tenure had a slightly more positive affective state when out at pasture than horses with shorter durations of tenure. It is cautiously proposed that the more an individual is accustomed to the environment and routine of the Equestrian Centre they may find their 'free-time' more rewarding than horses with shorter tenure. It could also be possible that horses with longer tenure were more familiar with their 'field-mates' and that the benefits of group stability, which is a prominent feature of equine social behaviour, were evident in their emotional response. Unstable grouping is a common feature for domestic horses on livery and competition yards and ideally requires careful management to avoid injury, usually via the gradual introduction of new horses from a safe distance. It would be very useful in future studies, with the use of HRV measurements, to investigate whether affective state is influenced by group stability and familiarity in order to improve existing management practices and enhance welfare.

There is at present, no published equine research that has included the measurement of HRV under these conditions therefore these results contribute new and additional findings to this area of study. As recent research has shown that sheep released from restraint (Doyle *et al.,* 2010) and shearing (Sanger *et al.,* 2011) display a positive emotional state compared with control animals it is recommended that future research investigates the use of HRV measurements during cognitive bias assessment in order to further explore affective state in horses. In practical terms, providing variety and contrast via the regular presentation of companionship may serve as a tool to counteract the chronic stress associated with isolation and confinement by compensating negative experiences with positive ones.

Behavioural evidence of negative affective state in response to contrasting circumstances was revealed in Chapter 6, where horses performed significantly more positive social interactions during Week 1 than Weeks 3 and 4 and performed more auto-grooming behaviour in Week 4 than in Week 2. Although physiological data were from a small sample set, HRV during the study was found to be numerically lower in the first week of the study than in subsequent weeks (regardless of housing type). These results imply that there may be a negative effect associated with the initial disruption of the horses' routine as they had spent two weeks turned out to pasture in their respective groups in order to become accustomed to each other prior to the study which took place during what was usually their 'summer holiday'. A similar finding was evident after analysis of baseline measurements in Chapter 7 as HRV was found to be significantly lower when horses were at liberty in the field than in their usual stable (or crew yard). Horses at Brackenhurst Equestrian Centre are familiar with the fitting and removal of HRV monitoring equipment whilst stabled, but due to the risk of equipment being lost or damaged it is not normally fitted whilst at liberty in the field. Therefore, it is plausible that they did not expect HRV equipment to be fitted whilst at pasture so may have associated the procedure with being caught and taken in from the field to be ridden, and the anticipation of having to 'work' resulted in lower HRV measurements.

These findings have important implications for the organisation of management regimes in relation to equine welfare particularly for horses that have their routine disrupted such as performance horses or bloodstock. Kuwahara *et al.* (2004) reported that a change from individual to paired housing activated the sympathetic nervous system in miniature swine which persisted for up to two weeks before returning to normal levels. Recent research also indicates that a diurnal rhythm exists in porcine (Kuwahara *et al.*, 2004) and equine HRV (Gehrke, Baldwin and Schiltz, 2011) due to increased parasympathetic activity at night during sleep. Further study of the diurnal rhythm in horses living under different management routines may therefore be worthwhile; if sleep patterns are disrupted by changes in routine, light, noise, travel, discomfort i.e. inadequate bedding, then this may have welfare implications. As these are novel findings with physiological data in support of behavioural observations, further research is recommended to investigate whether horses habituate to constantly changing routines.

Analysis of baseline HRV data in Chapter 7 suggested that there may be a positive benefit associated with the provision of a haylage diet (rather than hay) for stabled horses. Previous research has indicated that a preference for haylage over hay exists in horses (Goodwin, Davidson and Harris, 2002; Muller and Uden, 2007) and that horses also prefer haylage cut earlier rather than later in the year (Muller, 2011). The exact reason for this is not known but could be attributable to differences in gustatory or olfactory qualities particularly if haylage tastes 'sweeter' as foods containing high levels of sucrose are considered to have a high hedonic value (Chandrashekar, 2006) and are used as a reward in studies of emotion in other animals (Spruijt, van den Bos and Pijlman, 2001; Van der Harst, J. E. and Spruijt, 2007). Human research also suggests that palatable foods can improve mood and mitigate effects of stress via brain opioidergic and dopaminergic neurotransmission (Gibson, 2006). Further study is necessary in order to verify these results but it may be possible to use haylage as a form of reward or positive stimuli for stabled horses if it can be reliably associated with affective state.

8.1.2. Individual differences in affective state

Despite HRV appearing to be a promising indicator of affective state in horses, factors affecting HRV have not been satisfactorily explored. Significant inter-individual differences in HRV were present in Chapters 3-6 which, as purported by other researchers, might be associated with underlying affective state, temperament or breed (Visser *et al*., 2002; Visser *et al*., 2003a; Eager *et al*., 2004) and could account for the absence of significant differences in mean HRV between time-periods. High standard deviations in SDNN and RMSSD have recently been acknowledged in horses by Gehrke, Baldwin and Schiltz (2011) and it has previously been reported in other species that basal levels of physiological parameters as well as behavioural responses may differ between individuals depending on breed, age, sex, management and previous experience (Dechamps *et al.,* 1989; von Borell and Ladewig, 1989). Often the inter-individual variation of such parameters is higher than the intra-individual difference between basal and experimental levels which makes inter-individual comparisons difficult, complicates the interpretation of results and may prove to be a limiting factor.

Numerical differences in HRV according to sex were present in Chapters 4, 5 and 6, with mares recording consistently higher HRV than geldings, a pattern that is in accordance with human research (Rossy and Thayer, 1998). As discussed in Chapter 1 (Section 1.5.1.4) previous studies relating to the different factors that might affect HRV in horses have yielded mixed results. For example, existing equine HRV research either reports no effect of sex, makes no reference to the possible effects of sex when using mixed sex subjects, or lacks detail as to the sex of participating subjects.

The presence of these inter-individual differences in HRV highlighted the need for baseline data for each horse and the identification of factors affecting individual HRV. In order to explore some of these issues, Chapter 7 provided a comprehensive investigation of whether sex, age, breed and other management factors could be attributable to differences in baseline HRV measurements taken at rest both in the stable and at pasture. In addition, as responses of the

autonomic nervous system may also be useful in differentiating between individual horses in terms of temperament, a questionnaire was used in order to collect observer ratings of temperament to establish whether this was a factor affecting HRV in horses.

There is a general preference for and dominance of male horses in equestrian sports (Whitaker, Olusola and Redwin, 2008). While sex-related differences in the behaviour and temperament of horses have previously been reported (mares were associated with aggressive, tense, anxious, suspicious, excitable and panic traits whereas geldings were described as affable and more easily desensitised; Momozawa *et al*., 2007; Duberstein and Gilkeson, 2010), very little physiological evidence exists to quantify such differences in equine behavioural characteristics between sexes. Chapters 4, 5 and 6 identified numerically higher HRV in mares than geldings, mirroring previous findings from human research (Rossy and Thayer, 1998). Using a larger sample size, Chapter 7 confirmed that mares had significantly higher HRV than geldings when at liberty in the field with similar (non-significant) results found in the stable. This suggests that sex-based differences in HRV occur naturally and are a factor requiring control when designing HRV studies using mixed sex subjects. Additionally, there is also a potential benefit to the equine industry in categorising personality differences and isolating strengths and weaknesses of each sex using physiological measurements such as HRV alongside behavioural observations. This could potentially lead to the refinement of training programmes, or selection for a particular niche based on sex-related differences. As the participants used for the present study were a mixture of mares and gelded males, it is not known whether sex differences would also exist in stallions in comparison with mares or geldings. Further research would therefore be necessary to establish whether this is the case.

Whilst collecting HRV data for the present study it was intended that a comparison would be made between mares during oestrus and dioestrus. The horses were all aged above the onset of puberty (~18 months of age) and the reproductive cycle occurs during spring and summer throughout the mare's lifetime (Witherspoon, 1971). It is usually possible to visually identify if

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a mare is in oestrus by their behaviour i.e. posturing, lifting the tail and winking (Rossdale and Carson, 2002). Two sets of measurements were collected from the group of mares during the breeding season, one whilst stabled and the other from the field two weeks later, but during these times none of the mares showed any behavioural or visible indication of being in oestrus. As each cycle is usually 21 days long, with five days of oestrus and 16 of dioestrus (Rossdale and Carson, 2002) further data collection was intended later in the summer. Despite daily checks by the Equestrian Centre staff throughout the month of August mares showed no determinable signs of returning into oestrus. One possible reason for this may be the management regime imposed on these horses. Because males can 'over-bond' with females and guard their movements aggressively (Hartmann, Søndergaard and Keeling, 2012), pasture grazing at the Equestrian Centre is permitted in groups segregated according to sex and the field in which the mares were kept over summer was not within visible distance of the nearest group of geldings. The absence of any form of contact with the opposite sex may suppress visible signs of oestrus in mares, similar to the 'bachelor stallion effect' described by McDonnell and Murray (1995); that is, a socially mediated suppression in testosterone production and reproductive function in subordinate stallions within a group, hence it being undetectable in these mares on this occasion. If it is possible to monitor oestrus and dioestrus using HRV there are potential applications for the use of this measurement in the breeding industry, especially as human studies such as Rossy and Thayer (1998) suggest that the effects of oestrogen promote vagal cardiac control in women. Further investigation is therefore necessary to establish whether this is the case.

As discussed in Chapter 7 (Section 7.5), the remaining factors tested (breed, coat colour, age, height, background and medical history, bedding, concentrate feed and temperament score) were not found to significantly affect HRV and can be disregarded when selecting subjects for participation in future HRV studies.

8.1.3. Social and spatial factors

Chapter 3 identified behavioural and HRV responses that appeared to be related to management regime and the presence or absence of social contact. Further investigation into the effect of housing types offering different levels of social interaction on equine HRV and behaviour was explored in Chapter 6. A comparison was made between HRV and behavioural data from horses kept in four different housing systems, each offering increasing levels of social contact and physical space, to examine the effects of social contact and visual horizons on affective state and establish whether a relationship existed between behaviour and HRV. The study identified that housing system significantly affected behaviour and that overall HRV was not affected by housing system. The most isolating housing type with limited visual horizons (farmhouse stables) was associated with significantly less browsing behaviour, more standing still and a greater incidence of stereotypic behaviour (all of which correlated with low HRV) than the other housing types. Although not significant, the results of Chapter 7 followed a similar pattern to those obtained in Chapter 6 where HRV was numerically highest in horses living in paired housing in a crew yard and lowest in individually housed horses from the farmhouse stables that faced outward over the yard.

Chapter 6 revealed that certain behaviours (standing, auto-grooming and stereotypic behaviour) were indicative of negative affective state and could be associated with particular housing types. Standing behaviour occurred significantly less often in the paddock and crew yard than in the farmhouse or lodden stabling. Auto-grooming was performed significantly less often in the paddock than the other housing types and stereotypic behaviour occurred significantly more in the farmhouse stables than in the other housing types. Little information exists regarding the function of auto-grooming but it appears to indicate negative affective state and, as it did not appear to replace mutual grooming may be a behavioural indicator of sparse environmental stimuli.

Browsing and play fighting behaviour (as was previously observed in Chapter 3) were indicative of positive affective state and could also be associated with certain housing types. Browsing behaviour was performed for significantly longer in the paddock than the crew yard (horses attempted to graze the short grass and browse the blackthorn hedgerow) and was observed least often in the farmhouse stables despite identical bedding substrates being used in the crew yard and lodden stables. While there was no significant difference in HRV between the paddock and crew yard (both of which permitted access to a companion and provided space to move around freely), significantly more standing and play fighting behaviour was observed in the crew yard than the paddock.

Of the two types of individual housing, internal dimensions and bedding substrate were identical but lodden stables had superior visual horizons towards neighbouring horses and the external environment than farmhouse stables. Horses stood still for similar durations in both housing types but farmhouse stables were associated with numerically lower HRV and significantly more stereotypic behaviour whereas horses in the lodden stables browsed the bedding for significantly longer. The presence of an inverse relationship between stereotypic behaviour and HRV provides unique physiological evidence linking the lack of visual contact with conspecifics and restricted visual horizons with negative affective state. These results reinforce previous research by McGreevy *et al.* (1995) and Redbo *et al.* (1998) highlighting the positive benefits of social housing and also that of Cooper, McDonald and Mills (2000) and Ninomiya *et al.* (2008) who related increased visual horizons with a reduction in stereotypic behaviour and frustration in stabled horses.

Evidence in further support of this theory was found in Chapter 4 where an association between HRV (and therefore affective state) and the position of the horse's hay net was revealed. Rear placed nets were linked with low HRV, high levels of vocalisation, frequent orientational changes and weaving. This suggested that the position of fixtures and fittings inside the stable, such as the hay net, could influence the horse's capacity for environmental monitoring and

seemed to affect their emotional experience. Recent research in goats has shown that feeding stations allowing greater all round vision of the surrounding environment significantly reduces HRV and faecal cortisol levels compared with designs that impair visual fields (Nordmann *et al.*, 2011). Repositioning the location of the hay net closer to the doorway may therefore provide a simple enrichment to their environment that induces a positive affective state with the prospect of improving welfare.

These findings confirm that where management practices involve individual stabling, a stable design with visual access to conspecifics and a view of the external environment is a preferable alternative to stabling where such contact is severely restricted. There also seems to be no greater benefit associated with providing access to multiple companions in a larger area than was gained in the crew yard with a single companion. These results also reinforce the findings of Chapter 3 that facilitating play behaviour via social housing seems to positively influence affective state. In order to incorporate more positive emotional experiences and enhance welfare, it is therefore recommended that management regimes involving individual stabling are adapted to incorporate regular access to a companion in an environment where there is sufficient space to move around freely.

8.1.4. Interpretation of behaviour and underlying affective state

Chapter 4 explored the relationship between HRV and pre-feeding anticipatory behaviour in order to investigate the high levels of behavioural activity observed during the initial pilot study (Chapter 2: Section 2.3). Observations carried out prior to the study identified that a period of anticipatory behaviour existed before feeding in the form of vocalisation, orientation and movement of the head and ears, aggression directed towards neighbouring horses and locomotion around the stable. Assessing HRV and behaviour during a pre-conditioned response to a positive reward also had the advantage of supplementing the findings of Chapter 3, where horses were experimentally conditioned to expect a positive reward in the form of a companion.

The study compared HRV data obtained during anticipation and consumption of their usual feed with baseline HRV measurements and explored the relationship between HRV and behaviour during the anticipatory phase.

Similar to the findings in Chapter 3, HRV in Chapter 4 was not significantly affected by time-period for the group as a whole. This suggests that although the behaviour displayed in anticipation of feeding shared some similarities with stereotypic behaviour, in that it was repetitive and invariant, there was no evidence of negative affective state. It was therefore possible to dissociate anticipatory behaviour from true stereotypic behaviour that is related to opioid and dopaminergic systems and is indicative of poor welfare. Research in this area is scarce but these results are not uncommon as Reefmann *et al.* (2009a) also found non-significant patterns of HRV change in sheep during anticipation and receipt of feed. Given that there was no evidence of negative affective state during oral or locomotor anticipatory behaviour there appears to be no reason why such pre-feeding activities should be discouraged.

Analysis of specific behaviours in Chapter 4 did reveal that high levels of behavioural transitions, ear movements and incidences of nodding behaviour could be associated with low HRV. The frequency of ear posture changes has previously been identified as a method of assessing emotional reactions in sheep. Reefmann *et al.* (2009b) found that negatively valenced situations (social separation) induced a high number of ear posture changes whereas positive situations (feeding) were characterised by few ear posture changes. Reefmann *et al.* (2009b) and Reefmann, Wechsler and Gygax (2009) hypothesise that because ears are essential for gathering, integrating and appraising information from the environment, negative situations may require increased attention to environmental surroundings to allow for problem-solving, whilst ear activity decreases once attention to external stimuli is no longer necessary. Results from the present study provide additional physiological evidence to suggest that this may be the case in horses, i.e. that a high number of ear posture changes may signify negative affective state. Further research would be necessary using HRV to elucidate whether ear frequency

measurements could distinguish between stimuli that is negative (e.g. frightening) versus that which is positive (e.g. exciting) where it is often difficult to differentiate objectively using behavioural observations and where physiological measurements such as heart rate are of limited use.

In order to achieve optimal welfare in all aspects of the horse's lifestyle, the psychological well-being of a ridden horse is also an important consideration. As HRV appears to be a useful aid to interpreting behaviours in the stable, it may also be of use when evaluating the emotional effect of ridden and training exercises and could identify whether any aspects of riding and training are rewarding. For example, Schmidt *et al.* (2010c) have recently discovered that HRV decreased to its lowest point whilst young horses in training were being mounted by a rider. This suggests that an emotional response occurs to a situation resembling an imminent and potentially lethal threat under natural conditions.

In addition, public denunciation regarding the use of certain training methods used to warm up elite dressage horses led van Breda (2006) to use HRV to assess stress in elite dressage horses 30 minutes after being schooled in a coercively obtained Rollkur (hyperflexion) posture compared with the HRV of recreational horses 30 minutes after their basic dressage training. Results of the study implied that recreational horses experienced more stress (lower HRV) than elite horses trained according to the Rollkur method. Conversely, a later behavioural study by von Borstel *et al.* (2009) reported that horses ridden in Rollkur demonstrated higher levels of discomfort compared to regular poll flexion and would avoid being ridden in Rollkur if given the chance. As previous research has established that HRV is probably a useful technique for the evaluation of autonomic activity up to ~130bpm (Physick-Sheard *et al.,* 2000), which is around the average HR during trot (Rossdale and Young, 2002) further assessment of HRV *whilst* horses are ridden in Rollkur would elucidate this matter.

In contrast to the behavioural and HRV responses of horses anticipating positive rewards that were explored in Chapters 3 and 4, Chapter 5 investigated the effect of sham-clipping, a common but potentially negatively valenced management practice that often necessitates physical or chemical restraint. Horses were selected according to their behaviour during clipping sessions prior to the study where they were classified as either compliant or non-compliant as described in Chapter 5 (Section 5.2). The study investigated whether HRV differed prior to, during and after sham-clipping or in comparison with baseline measurements and explored the relationship between HRV and behaviour.

Non-compliant horses were found to have numerically lower HRV throughout the study and exhibited significantly more behavioural activity during and after sham-clipping than compliant horses. While this may be indicative of their affective state throughout the process, sex based differences could be implicated as a causal factor because a) the compliant group comprised a greater number of mares than geldings and b) non-significant sex-based differences in HRV were present. However, there was no difference in behavioural score between the two groups prior to sham-clipping and the magnitude of difference in HRV between groups was greatest during sham-clipping (although this did not reach significance), which implies that affective state (or emotional response) was the contributory factor rather than sex. These findings concur with those of Eager *et al*. (2004) who related HRV components to equine temperament and both Visser *et al*. (2002) and Visser *et al*. (2003a) who suggest that HRV could be a useful measure for quantifying behavioural traits in horses. As discussed in Chapter 5, it is also possible that females have a naturally higher tolerance to aversive stimuli than males which might explain why there was a greater proportion of mares in the compliant group upon selection.

Behaviour, but not HRV was significantly affected by time-period which suggests that the sham-clipping period was neutrally valenced despite the fractious behaviour observed in some individuals, although significant individual differences in HRV were noted which may have neutralised any differences in mean HRV. The results of this study suggest that, perhaps with a

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larger sample group, HRV could be a useful tool when determining the relationship between externally manifested behaviour and internal affective state. The compliant horses did not appear to be concealing a negative affective state beneath a calm exterior and sham-clipping did not appear to induce a negative affective state even in horses displaying non-compliant behaviour. HRV measurements could therefore prove useful in supplementing behavioural observations when detecting underlying affective states, emotional responses to different management practices and ultimately assessing the welfare of horses, particularly how they are coping with their environment and the challenges they are presented with.

As evidence from Chapter 5 indicated that practical exposure to an aversive stimulus (sham-clipping) might be useful for identifying the relationship between behaviour and HRV, Chapter 7 investigated these findings using resting measurements from a larger sample size where horses were assigned to one of four groups according to their level of compliance during clipping. It was not possible using HRV measurements taken at rest to distinguish between horses according to their level of compliance during clipping. This implies that the numerical differences in HRV that were present during exposure to the stimulus in Chapter 5 occurred in response to the stimulus and did not persist at rest. There is no evidence from Chapter 7 to suggest that non-compliant horses experience a persistent underlying negative affective state. Affective state also appeared to be more accurately identified during exposure to a routine management practice (clipping) than by means of the temperament survey and HRV measurements taken at rest.

Although baseline HRV in Chapter 7 was not found to be affected by the presence or absence of stereotypic tendencies, it is recommended that further study is undertaken using similarly sized groups as previous research has yielded mixed results (Bachmann *et al*., 2003; Nagy *et al*., 2009). However, the presence of sex-based differences in HRV found in this series of studies casts some doubt over the validity of Bachmann and colleagues study (2003) as their protocol

was based on a comparison between matched pairs of crib-biting and control horses that were not matched for sex.

8.2. CONCLUSION

In its natural environment, the horse is a herd-forming, free-ranging herbivore that usually avoids predation by flight and is a social animal, spending most of its time in close contact with conspecifics. Confinement and isolation, riding, training and exposure to management practices therefore offers the domestic horse a vastly different experience than a natural, pasture-like environment. Although many management regimes restrict the horse's ability to move around freely and limit their contact with conspecifics, there may still be a psychological need to respond to environmental factors even when the biological need to perform adaptive behavioural responses has been removed. The disparity between these unfulfilled underlying mechanisms and owner-dictated management practices can lead to the development of physiological and behavioural abnormalities indicative of a negative affective state and poor welfare.

As discussed previously, the concept of animal welfare is not easily defined in scientific terms but it is reasonable to suggest that a description of 'welfare' should include the animals' harmony with its environment and its physical and psychological health. There is increasing acceptance among animal welfare scientists that subjective feelings should be taken into account and that the assessment of emotion is an important factor when determining welfare. If animal welfare is associated with the mental state of an animal then good welfare should be achieved when an animal does not feel any long lasting negative emotions and when they can experience positive ones.

In order to study emotional states scientifically, the development of accurate measures is an essential first step. As animals lack the ability to provide verbal information about their

emotional state, the task of reliably establishing their affective state or emotional reaction is challenging as the subjective component, or emotional experience, can only be inferred from behavioural and/or physiological components. The behaviour or physiology of an individual in response to pleasant or unpleasant stimuli can therefore be used as reasonable evidence for the existence of affective state, thereby aiding welfare assessment. To date, research into equine emotion has attempted to identify 'reactivity', temperament or emotionality (e.g. fear and frustration) that may be relevant for horse owners and trainers on a practical level. However, these studies make no attempt to identify the underlying affective state of the animal when assessing the emotional response to different stimuli nor do they investigate the existence of positive affective states or factors that might influence positive emotions. As such, there is no strong scientific knowledge of what their emotional experiences are.

In animals, behavioural activity and facial expressions along with alterations in physiology can be measured to indicate an individuals' emotional state. However, many physiological measures involve invasive procedures that can contribute to the stress load of the individual, thus distorting results and, even where non-invasive methods are employed via salivary, faecal or urinary samples, the information gained can at best, only determine the presence or absence of 'stress' and therefore offer limited assessment of positive affect. As a result, accurate interpretation of physiological measures in terms of affective state is not always straight forward. Situations likely to induce different affective states may evoke similar physiological responses that could simply be indicative of arousal. HR and skin temperature, for example, may rise in both fear and excitement inducing situations. So while some physiological indicators offer useful information they are not devoid of interpretive problems in that they may relate to the intensity or arousal of emotional states rather than emotional valence.

One major growth area in the study of emotion is the measurement of HRV alongside behavioural observations. HRV is an established parameter to quantify the state of the autonomic nervous system and there is theoretical and empirical evidence from human and

animal research for the emergence of HRV as an important marker of emotional regulatory ability. Human studies have associated high levels of HRV with pleasant emotions such as cheerfulness, calmness, satisfaction with life and positive self-esteem whereas low HRV has been associated with anxiety, depression, panic disorders, an imbalance of effort and reward, loss of optimism and control, helplessness and hopelessness. In addition, animal research has associated low HRV with stressful situations such as restraint, social isolation, pain, illness, anhedonia and anxiety and high HRV with relaxation, control of their environment, social housing and feed rewards. By presenting horses with stimuli that have specific positive or negative characteristics it may therefore be possible to use both behavioural and physiological responses as an indicator of emotional state and as such, formed the basis of investigation for this research.

The aim of this research was to evaluate the use of heart rate variability alongside behavioural measurements as a non-invasive method of assessing the affective state in horses. This was achieved firstly by investigating whether an emotional response could be ascertained when horses were presented with or exposed to housing and management conditions differing in their emotional valence and secondly by investigating factors that may affect underlying HRV. Social interactions, in addition to food and sexual behaviour are considered to be rewarding to all animals. As horses are a highly social species, routinely subjected to long periods of physical isolation, social companionship was used as a positive reward in Chapter 3. The anticipation of a positive reward in the form of food was investigated in Chapter 4. Chapter 5 studied the response of horses undergoing a potentially negatively valenced procedure in the form of sham clipping. The response of horses kept in four different housing systems offering increasing levels of social contact and physical space was examined in Chapter 6 and a thorough assessment of factors affecting HRV was conducted in Chapter 7.

By presenting or exposing horses to housing and management conditions differing in their emotional valence it was possible to identify factors that significantly affected HRV and

highlight incidences where a significant relationship existed between behaviour and HRV. Rewarding and contrasting circumstances were found to be significantly associated with HRV; social interaction, 'play' behaviour and the provision of a haylage diet were all associated with high HRV and disruption to the horses' leisure time with low HRV. HRV also provided an objective physiological measurement to improve the interpretation of behaviour and assessment of underlying affective state; specific pre-feeding behaviours (behavioural transitions, ear movement and head nodding) could prove a useful indicator of negative affective state in future studies. Specific social and spatial factors significantly affecting HRV were also identified; tactile contact with neighbouring horses was related to high HRV as was the positioning of internal stable fixtures that optimised environmental monitoring. A natural variation in HRV between equine sexes and between individuals was confirmed and changes in HRV were found to be situation-specific.

In addition to highlighting the circumstances during which a relationship existed between HRV and behaviour and identifying factors that affected HRV it was also possible to use these initial data to make suggestions for the establishment of a preferable habitat and management regime for horses. There appeared to be a benefit associated with allowing domestic horses to engage in periods of social interaction, in particular providing the opportunity to engage in 'play' behaviour. Horses that are kept in management regimes typified by confinement and isolation might derive a greater benefit from the 'reward' or contrast of social interaction than horses kept in more social conditions. Where negative experiences are a necessary part of management regimes, these could be mitigated by providing appropriate housing with extensive visual horizons towards neighbouring horses, where the external environment can be monitored and by providing a reward via a preferred forage type. It is also likely that negative responses are situation specific so ensuring these are not prolonged and that the balance of positive experiences outweighs negative ones, quality of life may be enhanced.

As the use of HRV measurements to assess positive and negative emotion in horses is a relatively new area of investigation this study makes a unique contribution to equine welfare research. HRV was found to add vital physiological support to existing findings and although research of this nature is in its infancy, HRV appears to be a useful tool for assessing affective state and interpreting behavioural responses to stimuli in horses. There is extensive scope for further investigation into the use of HRV as a measure of emotional responses to management factors, environmental and ridden/training conditions in order to identify enjoyable or rewarding practices, so that the experience of positive emotion can be incorporated into management and training.

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Appendix 1

Ethogram of interactive behaviour observed between horses

(Chapter 3)

Behaviour	Definition
Play fight	- Initiated by a head threat tossing motion in which the head is rapidly flipped up and down, either whilst standing still or moving - The cohorts appear to alternate offensive and defensive roles and spar on, stopping short of injury. - Nip – slight opening of the jaw to take and release small pieces of skin or flesh between the teeth. - Bite – wider opening of the jaws and teeth to take and quickly release a larger piece of flesh and skin between the teeth. - Resting rear - The animal raises its chest and forelegs so that one or both limbs rest across the body of a conspecific, typically with lateral orientation - Neck wrestle – sparring with the head and neck. - Rear – raising of forequarters while hind legs remain on the ground, resulting in a near vertical position - Hind quarter threat – usually with ears back and the rump turned toward conspecific. The animal raises one leg as if aiming to kick, often simultaneously backing towards the target - Evasive jump/spin – contact is avoided by propelling the body off the ground away from the offensive gesture, or by pivoting around one hind leg. Often alternated between attacking and evading roles (McDonnell and Poulin, 2002)
Mutual groom	Two animals standing side by side, facing in opposite directions, grooming each other with their incisors – usually the neck, withers, back or rump (Crowell-Davis, Houpt and Carini, 1986)
Chase	At walk, trot or canter an animal is pursued with the apparent effort to catch up to or over take it (McDonnell and Poulin, 2002)
Close proximity	Two animals standing no more than approximately 50cm distance away from the other, not partaking in any other interactive behaviour
Sniff nose	With the noses of two animals touching or close to touching, and the mouth closed, air is drawn in through the nostrils
Sniff body	With the nose of one animal touching or close to touching an area of the other's body, and the mouth closed, air is drawn in through the nostrils.

Appendix 2

Ethogram of anticipatory behaviour

(Chapter 3)

Behaviour			Definition
Position in Crew Yard	**Front left (facing)**	Any	Horse positioned in front left quadrant of crew yard facing access gateway
		Over left	Horse positioned in front left quadrant of crew yard with head held over the gate & orientated to the right (as viewed)
		Over right	Horse positioned in front left quadrant of crew yard with head held over the gate & orientated to the left (as viewed)
		Over centre	Horse positioned in front left quadrant of crew yard with head held over the gate & orientated centrally
		Behind left	Horse positioned in front left quadrant of crew yard with head held behind the gate & orientated to the right (as viewed)
		Behind right	Horse positioned in front left quadrant of crew yard with head held behind the gate & orientated to the left(as viewed)
		Behind centre	Horse positioned in front left quadrant of crew yard with head held behind the gate & orientated centrally
	Front left (away)		Horse positioned in front left quadrant of crew yard with body facing away from access gateway
	Front right		Horse positioned in front right quadrant of crew yard
	Rear right		Horse positioned in rear right quadrant of crew yard
	Rear left		Horse positioned in rear left quadrant of crew yard
Ear position	**Left ear front**		Left ear canal visibly orientated forwards
	Left ear back		Left ear canal visibly orientated to the rear, either held vertically or flattened to the head
	Left ear side		Left ear canal visibly orientated to the side, either held vertically or loosely drooping
	Right ear front		Right ear canal visibly orientated forwards
	Right ear back		Right ear canal visibly orientated to the rear, either held vertically or flattened to the head
	Right ear side		Right ear canal visibly orientated to the side, either held vertically or loosely drooping
Locomotion	**Standing**		All four feet remain in contact with ground for >2 seconds.
	Walking		Four beat gait of forward movement for >2 seconds.
	Trotting		Two beat, diagonal gait of forward movement for >2 seconds.
	Rearing		Both front feet lifted off the ground while supporting body weight on hind legs
	Bucking		Both hind feet lifted off the ground while supporting body weight on front feet.

Appendix 3

Ethogram of behavioural states

(Chapter 4)

Behaviour		Definition
Position in Stable	**Front**	Head and body orientated towards open front of stable.
	Right	Head and body orientated towards right hand wall of stable.
	Left	Head and body orientated towards left hand wall of stable.
	Rear	Head and body orientated towards rear wall of stable.
Head position when orientated at front of stable	**Left high**	Muzzle above top of stable door, orientated left (as viewed) so only left eye visible.
	Left mid	Muzzle level with top ¼ of stable door, orientated left (as viewed) so only left eye visible.
	Left low	Muzzle in bottom ¾ of stable door, orientated left (as viewed) so only left eye visible.
	Right high	Muzzle above top of stable door, orientated right (as viewed) so only right eye visible.
	Right mid	Muzzle level with top ¼ of stable door, orientated right (as viewed) so only right eye visible.
	Right low	Muzzle in bottom ¾ of stable door, orientated right (as viewed) so only right eye visible.
	Centre high	Muzzle above top of stable door, orientated centrally so both eyes visible.
	Centre mid	Muzzle level with top ¼ of stable door, orientated centrally so both eyes visible.
	Centre low	Muzzle in bottom ¾ of stable door, orientated centrally so both eyes visible.
Ear position	**Left ear front**	Left ear canal visibly orientated forwards.
	Left ear back	Left ear canal visibly orientated to the rear, either held vertically or flattened to the head.
	Left ear side	Left ear canal visibly orientated to the side, either held vertically or loosely drooping.
	Right ear front	Right ear canal visibly orientated forwards.
	Right ear back	Right ear canal visibly orientated to the rear, either held vertically or flattened to the head.
	Right ear side	Right ear canal visibly orientated to the side, either held vertically or loosely drooping.
Oral behaviour	**Chewing**	Circular motion of mouth and lips as though chewing food.

Appendix 4

Ethogram of behavioural events

(Chapter 4)

Behaviour	Definition
Weave	Repetitive lateral (swinging) movement of the head, typically but not always whilst held above the stable half-door.
Nod	Repetitive vertical movement of the head, typically but not always whilst held above stable door.
Aggression	Observation of any of the following: head and neck stretched forward in a lunging motion towards neighbouring horse, both ears laid back onto the head, opening and closing of jaws as if to bite.
Oral stereotypy	Visible protrusion of tongue beyond lips, tongue rolling, repeated licking of stable fittings, sham-chewing.
Vocalisation	Emissions of any vocal sound e.g. whinny, nicker.
Box-walk	Repeatedly moving between two locations in stable or repetitively walking a route around the stable.

Appendix 5

Ethogram of behavioural states

(Chapter 6)

Behavioural state	Definition
Browsing	Ingestion of grass, plants or bedding. Oral investigation of bedding or licking mineral block
Standing	Standing still, either alone or with another horse but with no visible interaction between individuals
Lying	Horse positioned in either lateral or sternal recumbency, includes rolling.
Active	Forward movement either in walk (four beat gait) trot (two beat, diagonal gait) or canter (three beat gait)
Social (Positive)	Physical or olfactory contact with another horse e.g. nuzzling/sniffing. Mutual grooming demonstrated by two horses standing head to tail using teeth to firmly manipulate an area of the other horse
Social (Negative)	Aggressive posture, ears laid back, lowered head and neck, threat or actual kick/bite
Stereotypy	Includes box-walking - repetitive pacing around the enclosure usually starting from and returning to same point, weaving - repetitive swinging of head from side to side, nodding – repetitive swinging of head up and down, crib-biting (biting or chewing a fixed surface/object with teeth), or repetitive licking of an inanimate object
Autogroom	Self-grooming e.g. scratching an area of the body using teeth, foot or another object

Adapted from Cooper, McDonald and Mills (2000), Heleski (2002) and Visser, Ellis and Van Reenen (2008).

Appendix 6

Nutritional content of concentrate feed

(Chapter 7)

		Pasture cubes	Competition mix	High fibre nuts	High energy mix	Chaff	
Crude Protein		10	12	9	12	4	%
Crude Oils and Fats		3.5	3	3	3.5	0.5	%
Crude Fibre		15	11	20	11	24	%
Crude Ash		7.5	8	9	8	13	%
Copper		35	35	50	35	-	mg/kg
Sodium		0.5	0.4	0.5	0.4	0.1	%
Lysine		3.5	5	3.5	5.5	-	g/kg
Potassium		-	-	-	0.9	-	%
Vitamin	**A**	10000	12000	15000	12000	-	iu/kg
Vitamin	**D**	1500	1400	2000	1400	-	iu/kg
Vitamin	**E**	200	325	250	340	-	mg/kg
Vitamin	**B1**	-	12	-	12	-	mg/kg
Vitamin	**B2**	-	4.5	-	4.5	-	mg/kg
Vitamin	**B6**	-	6.5	-	-	-	mg/kg
Vitamin	**B12**	-	0.02	-	0.02	-	mg/kg
Biotin		-	0.9	-	0.9	-	mg/kg
Digestible Energy		10	11.5	9.5	11.5	8	MJ/Kg
Feeding requirements		600	600	300	600	-	mg/100kg bwt

Dodson and Horrell (2011)

Pasture cubes
Suited to horses and ponies in light-medium work but can also be fed to horses in medium-hard work if the feeding levels are adjusted accordingly.
Contain vitamins & chelated minerals, added mint & garlic. Oat and barley free.

Competition mix
Source of quality protein with added B vitamins and electrolytes.
Ideal for horses and ponies that are competing and require instant bursts of energy.
Contains uniquely cooked cereals for muscle glycogen replenishment & a bespoke B vitamin package.
High electrolyte levels to aid hydration status work.

High fibre nuts
Ideal for all adult horses and ponies that are either at rest or in light work and hold their weight well.
Low calorie, low intake formula. High in fibre & low in starch, providing slow release energy.
Contains vitamins & chelated minerals. 98% cereal free, suitable for laminitics.
Suitable as a partial forage replacer for older horses with chewing difficulties as can be fed soaked.

High energy mix
Source of quality protein, benefits from added B vitamins and electrolytes.
Ideal for horses and ponies that are competing and require instant bursts of energy.
Contains uniquely cooked cereals for muscle glycogen replenishment & a bespoke B vitamin package.
High electrolyte levels to aid hydration status work

Chaff
Complementary feeding stuff specifically for horses and ponies requiring a low-calorie chaff with the exception of laminitics.
Ideal way to add fibrous bulk to a horse's or pony's diet.
Comprised of Chopped Oat Straw, Cane Molasses and Limestone Flour (7%).