Narcoleptic Type Behaviours of the Horse: an exploratory study into temperament differences and neural function.

Josephine Gleeson

Declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, in any previous application for a degree. Except were states otherwise by reference or acknowledgment, the work presented is entirely my own.

DOI: 10.46289/7XU3P45U

Josephine Gleeson

Ethical Approval

Research was carried out in accordance with the Royal Agricultural University Ethics Policy prior to data collection (20224519-Hemmings). Approval for the collection of temperament data via questionnaire was dealt with and granted under a separate request (20215317-Hemmings).

Contents

Acknowledgements

I would firstly like to thank my supervisor Dr Andrew Hemmings, for all his help and support throughout this year (and throughout my whole time at the Royal Agricultural University, in both my undergraduate dissertation and in this masters degree). After many hours of meeting, hundreds of emails, and questions this project is complete. I really could not have completed this project without you Andrew, thank you!

I would also like to thank Dr Mark Ebert, for his guidance as to the set up and use of the Heart Rate Monitor used to collect data for this study into Equine Narcolepsy. Without his kindness to help me set up the Polar Equine V800 belt and watch, I would not have been able to collect the data needed for this particular study, thank you.

Finally, I would like to thank all horse owners who have taken part this this study. All those who filled in questionnaires and allowed their horses to be used to study and collect data, thank you too all.

Abstract

Narcoleptic Type Behaviours (NTB's*) are characterised by intermittent periods of apparent somnolence followed by loss of muscle tone resulting in instability of the front limbs, creating potential risks for handlers and bystanders. This exploratory study examined the potential drivers of NTB's in horses (*Equus caballus*) with a focus upon temperament variables, autonomic nervous system function and inferred measures of dopamine transmission. Thirteen horses of varying age (5 years old – 24 years old) and breed all exhibiting NTB's, were recruited for the study along with thirteen control equivalents all confirmed as being free from the condition. A previously validated temperament questionnaire was initially applied to all animals by the horse owners, followed by recordings at rest of Heart Rate Variability (HRV) on 3 occasions for 30 minutes to assess the integrity of the autonomic nervous system. Finally, Spontaneous Blink Rate (SBR) and Behavioural Initiation Rate (BIR) were applied in triplicate (30 minutes) as inferred indicators of dopamine system tone in all animals. Curiosity scores were significantly lower ($p<0.05$) in NTB versus control horses, as were those for vigilance ($p<0.05$). Conversely, scores for Cooperation were higher in the NTB cohort ($p<0.05$). There were no differences between groups for any of the physiological variables, although there was a significant negative correlation between SBR and Low Frequency / High Frequency (LF / HF) ratio ($r_s= -0.56$, $p<0.01$) for the control, but not the NTB equivalents. Similarly, a strong negative correlation between BIR and LF/HF ratio was uncovered in the control group ($r_s= -0.68$, $p<0.01$) which was absent in NTB animals. The differences in temperament variables pave the way towards possible diagnostic / predictive indicators, whilst the lack of correlation between central and peripheral nervous system indicators in the NTB cohort tentatively implies a decoupling of these systems, although further research is required to ascertain the functional dimensions of these findings.

Ruffian Narcolepsy.mp4

https://1drv.ms/v/s!Ao_-1-8_HjbrjC4BmCGEZzsOYSv0?e=TBQmCi

Pages

List Of Figures

List Of Tables

Abbreviations

Behaviour Initiation Rate (BIR) – A non-invasive behavioural probe which is utilised to determine activation of the direct or indirect pathways.

CNS- Central Nervous System.

Crib-biting – Oral stereotypy of the horse whereby a surface is grasped at chest height with the incisors, the arching of the neck and the sucking of air into the proximal oesophageal region creating a grunting noise.

D1 Type Receptors – Associated with the direct pathway of the striatum, includes the D1 receptor and the D5 receptor.

D2 Type Receptors – Associated with the indirect pathway of the striatum, includes the D2 receptor, D3 receptor and D4 receptor.

Direct Pathway – dopaminergic pathway of the striatum which results in behaviour activation through stimulation of D1type receptors, which can be measured via dynorphin levels. Also referred to as the striatonigral pathway.

Dopamine (Da) – The key neurotransmitter of the striatum. See also tonic dopamine and phasic dopamine.

Dorsal Striatum – The portion of the striatum associated with learning behaviours and movement.

HR – Heart Rate.

NC- Narcolepsy with cataplexy.

NTB- Non Thoroughbred Breeds.

PPID- Pituitary pars intermedia dysfunction.

Putamen – The portion of the dorsal striatum associated with stimulus-response habitual behaviours and movement. Also referred to within this text as the dorsolateral striatum.

REM- Rapid eye movement.

SWS- Slow wave sleep.

Spontaneous Blink Rate (SBR) – A non-invasive measure of dopamine levels within the dorsal striatum.

Striatum – The portion of the basal ganglia that is implicated with motivation, learning and motor behaviours. (plural; striatal).

UPDRS- The Unified Parkinson's Disease Rating Scale.

1.0 Introduction

Narcolepsy is an unusual and incurable sleep disorder of the central nervous system (CNS). It is characterised by spontaneous "sleep attacks" at inappropriate times. This is usually accompanied by an uncontrollable loss of muscle tone known as cataplexy (McBride et al., 2017) also described as spontaneous collapse by other authors (see Greening and McBride for recent review). This disorder is a unique, non-progressive and incurable neurological disease. An indication of this behaviour is shown when the horse's legs buckle suddenly, before the horse either wakes up or sinks totally. The horse will often collapse forwards violently, causing trauma to the fronts of the knees and/or fetlocks. Sleep disorders have been studied in many different species but are generally not common and not well understood (Longstreth et al., 2007). Although several preliminary equine investigations have taken place (Ludvikova et al., 2012), to date the research in this area is minimal, even to the extent that predicted prevalence figures are not in the published domain. One main reason for limited scientific research into equine sleep patterns, is the difficulty in performing non-invasive data collections in order to collect the necessary recording of the electrical activity of the brain for example, without financially inviable and logistically difficult measurement tools. However, the recent discovery and validation

of behavioural monitoring protocols such as SBR (McBride et al, 2022) there now exists the possibility to infer brain activity (with specific reference to dopamine [DA] systems) using non-invasive means. As such this became one of the primary aims of the work reported herein.

In summary, this research aims to investigate the development of endophenotypes in the horse for different dopamine functions by forming the initial relations between behavioural indicators which may include dopamine function, behavioural markers in the horse's temperament and the cognitive ability of each individual horse. The ambition was to see a lower BIR, SBR and HR from the horses showing narcoleptic-like behaviours, than the case control horses, thereby establishing a direct correlation between measurable indicators of DA release and narcolepsy. The following hypothesis was created to drive this work;

1) There will be no significant differences in either BIR or SBR between control and NTB horses.

2) There will be no significant differences in HRV Variables between Control and Narcoleptic Type horses.

3) There will be no significant difference between temperament variables between control and Narcoleptic type horses.

1.1 Narcolepsy and Dopamine Dysfunction

Burgess et al., (2010) discovered increased binding to D2-like DA receptors that was strongly correlated with the loss of muscle tone (cataplexy) element of narcolepsy in human patients. As cataplexy is one of the most reliable dimensions of the equine NTB phenotype, the dopaminergic links are certainly worthy of further research. Moreover, there exists two better studied equine phenomena (Pituitary Pars Intermedia Dysfunction [PPID] and stereotypic behaviour, both of which have a dopamine component (Burgess et al., 2010), from which testable hypotheses can be developed.

Pituitary Pars Intermedia Dysfunction (PPID) also known as "Cushing's disease", occurs because of the collapse of the periventricular hypophyseal dopaminergic neurons, paired with the hypertrophy, hyperplasia, and adenoma formation on the Pars Intermedia (PI) of the pituitary gland (McFarlane et al*.,* 2003); the combination of depleted DA concentration and increased cell synthesis and growth in the PI leads

to the dysregulation of many hormones resulting in the vast array of symptoms displayed. Theoretically a horse with PPID should present a lower SBR than a healthy aged, matched control horse due to the decreased levels of DA. This theory can be supported by findings from human neuroscientific research; Parkinson's disease (PD) is a comparable disease to PPID because of the similarities in the dopaminergic degeneration in the brain. The Unified Parkinson's Disease Rating Scale (UPDRS) is the most widely used clinical rating scale for PD to determine treatment related benefits. SBR is included on this rating scale with specific reference to the measurement of decreased blinking in the early stages of the disease and to be indicative of disease progression (Goetz et al*.,* 2008). This emphasises the importance and potential influence SBR could have in aiding current diagnostic tools in the early stages of PPID. Both SBR and BIR have been used by Roberts et al*.,* (2016), to examine the role of DA in the generation of temperament. This raises the possibility that DA is also involved in narcolepsy, since it is understood that a biochemical malfunction within the network of neurotransmitters in the brain may be related to sleep disorders such as Narcolepsy in the horse.

1.2 Narcolepsy and Stereotypic Behaviours

The DA measures used in the study are not all-inclusive. Dopamine system variation has been linked to the study of stereotypy development in a variety of mammals, including the horse (Olguín et al., 2016). Furthermore, stereotypic behaviour can be used to illustrate how dopamine functions in relation to behavioural output. Thus, in addition to HR, SBR, and BIR measurements, the horses studied were also examined for stereotypic behaviour, because horses with high levels of stereotypy are also very likely to have divergent levels of DA.

1.3 Narcolepsy and Autonomic Nervous System Function

Narcolepsy with cataplexy (NC) occurs because of a loss of hypothalamic neurons that release the neuropeptides – Orexin. Human studies such as Grimaldi et al., (2010), states that there is a normal functioning of cardiovascular reflexes in NC but encourage an impairment of HR modulation at rest in favour of an enhanced sympathetic activity. In this study HR is tested on both Narcoleptic and Control to compare LF/HF ratios and HR Mean, to find a link between Equine Narcolepsy and Autonomic Nervous System Function.

1.4 Importance of Sleep and Links to Narcolepsy

Research has shown that poor management or physical problems (such as arthritis in lower limbs of a horse) can lead to horses becoming injured and at risk due to sleep deprivation (Klerk., 2019). There are three main stages of physiological sleep that being: light sleep, which is similar to the second type which is deep sleep (slow-wave sleep) while standing up. However, the third stage is rapid eye movement (REM) which only happens while the horse is lying down because the animal has complete muscle relaxation (Carson & Wood-Gush, 1983).

Compared to other mammals the horse needs little sleep (averaging three hours per day). Equine sleep occurs in cycles of NREM and REM sleep, as this does in Humas as well, however as stated the horse needs less REM sleep compared to other mammals (Belling, 1990). This may well be because the horse is naturally a prey species. The sleeping cycle of a horse is shown through short periods of time, through slow wave sleep (SWS). On average a horse dozes off every forty five minutes as discussed throughout Billing (1990) study inro equine sleep patterns. The horse will then eventually laydown and reach REM sleep. The understanding into equine sleep patterns is vital to understand further how quality of sleep may influence the behavioural profile of a horse during wakefulness.

Sometimes there may be physical reasons for sleepiness- for example, horses with medical conditions such as arthritis, may find it uncomfortable to try to roll or lie down for the needed REM sleep (Kelemen et al., 2021). During REM sleep, muscles totally relaxed and are not tense, and the eyelids are fully shut. Because of the discomfort, an arthritic horse may choose to not lay down fully and therefore not be able to reach adequate REM sleep (Klerk, 2019). A horse with arthritis may need to live out in a field to avoid muscles seizing up, if kept in the stable the horse is unable to move around a great deal. Another example might be a horse with kissing spine; the aftercare from any of the treatments is vital for the recovery and recuperation of the horse. The horse must be kept comfortable during recovery, for example, a deep bed of straw or bedding of owners choice, for the horse to comfortably lie down. This will help prevent slipping, and will provide a padding between the horse and the floor. A recent ground breaking review by Greening & McBride (2022), suggests that further

research into the truth of equine sleep patterns is essential from a welfare and performance standpoint. Also stated was that, whilst sleep quantity and quality are connected, they are also often detached as to how it is important to take separate measures into both the concept of sleep deprivation and animal welfare. With reference to NTB's, whilst Fuchs et al., (2016) recorded episodes of spontaneous collapse that were linked to refusal to lie down at night due, in part, to husbandry shortcomings and sleep deprivation, the study did not attempt any detailed analysis of central or peripheral nervous system activity. Therefore neurological dysfunction cannot be ruled out. Furthermore, given the clear linkage between cataplexy, dopamine (Burgess et al., 2010) and autonomic function (Grimaldi et al., 2010) in other species, this study sought to determine whether or not such links exist in the horse.

2.0 Review of Literature

2.1 Equine Narcolepsy

Clinical signs of equine narcolepsy can range from lowering of the head to buckling of the knees and sometimes complete collapse (Ludvikova et al., 2012). Affected horses may be seen to frequently rest their heads or hindquarters on fences or other objects (Klerk., 2019). They may display swaying and frequent stumbling. These alarming behavioural symptoms stem from a lack of muscle tone and reflexes (cataplexy) that can be caused by stimuli such as sounds, feeding time, saddling, leading, and other environmental triggers (Chen et al., 2009). The precise causal factors of the disorder remain to be elucidated, although comorbidity with pituitary pars intermedia dysfunction (PPID) has been reported. (Young, 2021) suggesting that age related neurodegeneration may be a factor in some cases.

Narcolepsy is a neurological and rapid eye movement (REM) sleep disorder. The behaviours such as buckling of the knees, lowering of the head, and swaying can be due to the sudden onset of sleep during the daytime (Klerk., 2019).

Due to the lack of targeted research in this area prevalence figures are not available for the UK or indeed other countries. Moreover, from a diagnostic standpoint, Cataplexy remains the best indicator for the condition, but narcolepsy can also occur in the absence of cataplexy. Despite this confounding factor, behavioural monitoring for cataplectic episodes with the aid of video recording, accurate records of the

horse's sleep behaviour, and evidence of wounds on the fetlocks, knees, hocks, and face remains the most reliable diagnostic policy in the absence of other means.

Studies carried out by Ludvikova et al., (2012) and Lunne et al., (1993) show that through genetics, certain breeds and types of horse may carry a higher chance of developing narcolepsy, compared to other breeds. Ludvikova et al., (2012), studies the Lipizzaner horse breed and Lunne et al., (1993) studied the miniature horse. However, without further work no concrete conclusion could be reached through these studies. Both studies collected only a few narcoleptic samples, therefore further data was needed to identify noticeable effect on genetics through the breeds.

It is important to note that narcolepsy is uncommon in horses. Behaviours described by some can often be a result of sleep deprivation rather than equine narcolepsy. Sleep deprivation and narcolepsy are both forms of equine sleep disorders, which can lead to a sudden or partial collapse in horses. Unlike sleep deprivation, narcolepsy is an incurable neurological disease that causes excessive sleepiness and sudden attacks of REM sleep that can be triggered by external stimuli (Belling., 1990). As stated, sleep deprivation can be due to pain which prevents the horse from reaching adequate REM sleep. In this study horses were chosen due to a lack of medical issues that prevented adequate sleep, owners where asked through the temperament questionnaire (Question 8) if their horses lay down to sleep (included asking about the type of bedding used- Question 12), so that a distinct line could be drawn to separate that of sleep deprivation and possible narcolepsy.

Belling, (1990) makes the point that horses developed as prey animals, and for prey animals the familiarity of one's surroundings goes hand in hand with survival. Whereas predators tend to sleep for longer hours, prey animals must be consistently alert. It is therefore important for horses to feel safe enough in their surroundings to reach REM Sleep patterns.

2.2 Narcolepsy in humans and other species

The definition for narcolepsy in dogs has been stated as, a disorder of the nervous system with narcoleptic episodes of sudden collapse and loss of movement (Chen et al., 2009). Scientists researching human narcolepsy have discovered that it is

usually accompanied by a loss of a neurotransmitter in the brain called hypocretin (also known as orexin) (Mayhoney et al., 2019). Hypocretin is important for regulating the sleep/ wake cycle including the REM (Chen et al., 2010) sleep state. Moreover, a mutation in the Hypocretin gene is associated with low levels of hypocretin and predisposition to narcolepsy. It is believed that this hereditary deficiency, along with some autoimmune dysfunctions contributes to the narcoleptic phenotype. Other factors, such as stress may also play a role, not only in humans but other species as well (Mayhoney et al., 2019).

Despite the fact that human narcolepsy was depicted for the first time a century ago, a clinical study was not available until the 1970s. With establishments such as the Stanford Canine Narcolepsy Colony, researchers were able to conduct multiple neurochemical studies to explore the pathophysiology of Narcolepsy. Moreover, in 1999, two independent studies discovered that hypocretin neurotransmission deficiency was critical to the development of narcolepsy with cataplexy (Chen et al., 2009).

Hypocretins are novel hypothalamic neuropeptides involved in a variety of hypothalamic mechanisms, such as energy homeostasis and neuroendocrine function. Hypocretin peptides are produced by a group of neurons in the lateral hypothalamus but are released by projections of these neurons to much of the CNS, from the cortex to the spinal cord (Birt et al., 2015). Hypocretin neurons strongly innervate several regions that promote wakefulness and suppress REM sleep, including the basal forebrain, tuberomammillary nucleus (TMN), periaqueductal grey (PAG), dorsal raphe (DR) and locus coeruleus (LC) (Mahoney et al., 2019).

As mentioned previously, hypocretin ligand and hypocretin receptor genes are important to the pathogenesis of narcolepsy in animals. Mutations in hypocretin-related genes are unusual in humans, but hypocretin-ligand deficiency is found in many cases (Nishino, 2003). Hypocretin-deficient human narcolepsy appears to be a more complex condition than a simple sleep disorder, further investigation into not only human but other mammals is vital.

According to Chen et al., (2009), the regularity of narcolepsy is higher among close human relatives than in the general population, indicating that this disorder does indeed have a genetic predisposition. However, in a subsequent rodent study, a pair of monozygotic twins were found to be narcoleptic, indicating that a strong environmental influence causes the onset of the narcoleptic condition (Chen et al., 2010).

Provided that the hypocretin system is still present in mammals and that narcolepsy has been discovered in a variety of species, it is likely that more animal models will be produced in the future. For example, consider this equine study of narcolepsy and its possible link to stereotypy, seasonality, and the analysis of HR, SBR, and BIR data collections.

2.2.1 Dopamine Systems and Narcolepsy

Clinical studies conducted by Burgess et al., (2010), revealed that human narcoleptics have an altered striatal dopaminergic system. Brain imaging studies, in particular, show that people with narcolepsy have increased D2-like receptor binding, which is strongly linked to cataplexy. D2 receptors are also linked to sleep attacks in Parkinson's disease patients, who, like narcoleptics, have hypocretin cell loss. Furthermore, animal research has demonstrated that the dopamine system can impact sleep and cataplexy. Cataplexy in narcoleptic canines is modulated by chemical manipulation of D2-like, but not D1-like, receptors in dopaminergic brain areas (e.g., substantia nigra and ventral tegmental area). Sleep is regulated by dopaminergic mechanisms; for example, in rats, the decline of wake-active dopamine cells in the ventral periaqueductal grey encourages sleep (Burgess et al., 2010).

The clear dopaminergic links to the cataplectic element of human narcolepsy are the basis for the hypothesis that equine narcolepsy may be connected to DA production and mechanisms.

Although, in relation to the study's hypothesis, while hypocretin neuron loss underpins human narcolepsy, the specific neurochemical mechanisms that cause sleepiness and cataplexy remain unknown (Burges et al., 2010). As a result, there is not a complete human model to be found in horses.

2.2.2 Treatments

The availability of various mammal studies into narcolepsy could provide multiple avenues for testing the mechanisms underlying current treatments while also exploring new therapeutic and non-invasive potentials. The effects of orexin supplement therapy on narcoleptic dogs varied. An early study found that systemic administration of orexin-A (3g/kg) significantly reduced cataplexy in narcoleptic Dobermans. This finding, however, was not replicated when orexins were administered systemically or centrally (Chen et al., 2009).

According to Szabo et al. (2019), only a few drugs have received regulatory approval for narcolepsy. To diversify medications, tests and treatments have been trialled on various neural circuits and targets. Amphetamine, methylphenidate, antidepressants (tricyclic antidepressants), selective serotonin reuptake inhibitors, sodium oxybate, and the H3-receptor inverse agonist/ antagonist pitolisant have all been shown in clinical trials to be effective narcolepsy treatments (Szabo et al., 2019).

Most of these compounds' therapeutic activity is likely dependent on increased catecholamine availability and regulation of locus coeruleus (LC) norepinephrine (NE) neuron activity. The role of LC and NE neurons in sleep-wake regulation and muscle tone may be related to narcolepsy/cataplexy symptoms (Szabo et at., 2019).

2.3 Normal Functioning of Dopamine Systems

Dopamine is a monoamine neurotransmitter that is produced in the substantia nigra, ventral tegmental area, and hypothalamus of the brain (Burgess et al., 2010). The level of dopamine transmission increases in response to any positive reward and by a large number of strongly addictive drugs (Olguin et al., 2016). Dopamine plays a role in many important body functions, including movement, memory, pleasurable reward and motivation. High or low levels of dopamine are associated with several mental health and neurological diseases (Bayer & Glimcher., 2005).

According to Bhatia, Lenchner, and Saadabadi., (2022), there are five types of dopamine receptors: D1, D2, D3, D4, and D5. Each receptor serves a distinct purpose. D1 regulates memory, attention, impulse control, renal function, and

locomotion. D2 is responsible for locomotion, attention, sleep, memory, and learning. D3 is responsible for cognition, impulse control, attention, and sleep. D4 is associated with cognition, impulse control, attention, and sleep. D5: decision-making, cognition, attention, renin secretion (Chen et al., 2010).

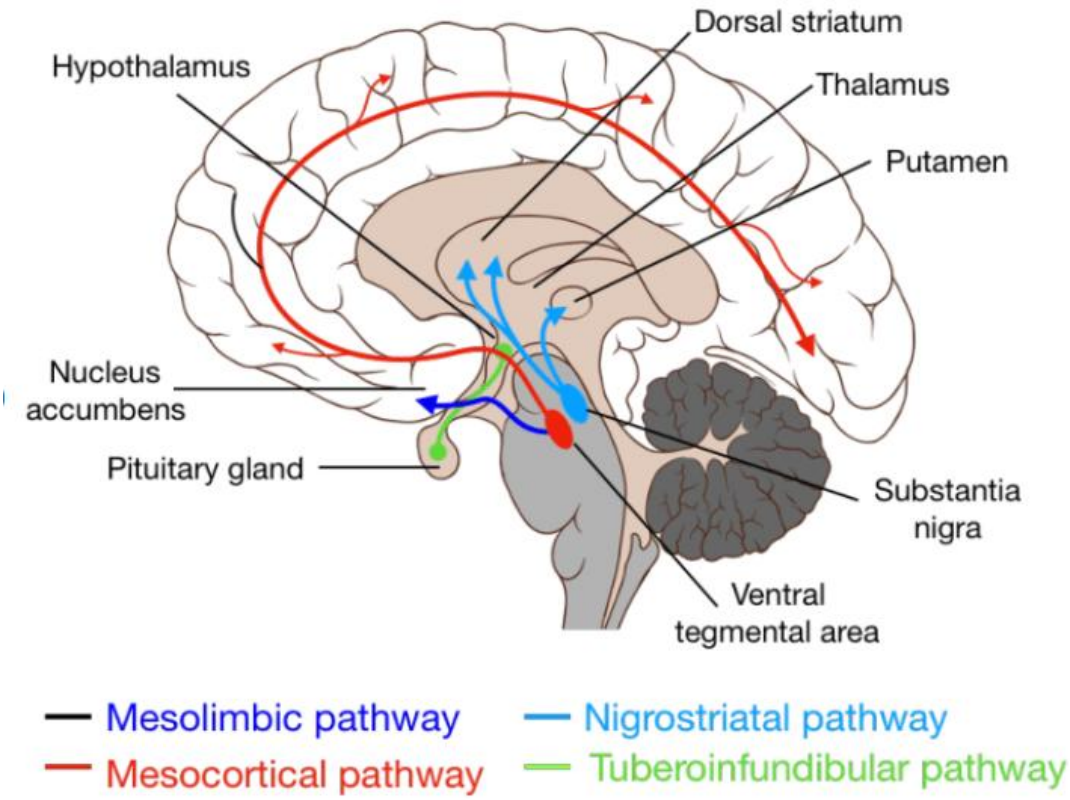
According to Hemmings et al. (2007), striatal infusion of NDMA or dopamine D1 receptor antagonists can start reducing stereotypic behaviour by altering the balance of direct and indirect basal ganglia activity to regulate striato-cortical output. Dopamine can operate on either D1 (D1 and D5) or D2 receptor subtypes (Missale et al., 1998). D1 receptors are affiliated with movement activation in the basal ganglia, whilst the D2 receptors are associated with movement inhibition (Lewis et al., 2006).

2.3.1 Brain Structures in Dopamine Circuitry

Two distinct midbrain structures (Ventral Tegmental Area and Substantia Nigra) are responsible for modulation of a much broader set of brain systems known as the basal ganglia (McBride & Hemmings, 2005). In order to understand the precise role of dopamine in the operation of the basal ganglia circuitry, the individual nuclei and their interrelationships will be explored in the following section.

The ventral and dorsal striatum, globus pallidus, and olfactory tubercle are all part of the basal ganglia (Yin & Knowlton, 2006- see figure 1).

FIGURE 1: The four dopaminergic pathways of the brain (Ayvazyan, 2002). The figure shows the basil ganglia from an equine source.



2.3.2 Direct and Indirect Pathways of the Dorsal Striatum

Even though strategic decision is thought to rely on a large neural network that includes cortical, limbic, and midbrain regions, efferent projections from these structures are known to unite within the basal ganglia's striatum (Alexander & Crutcher,1990).

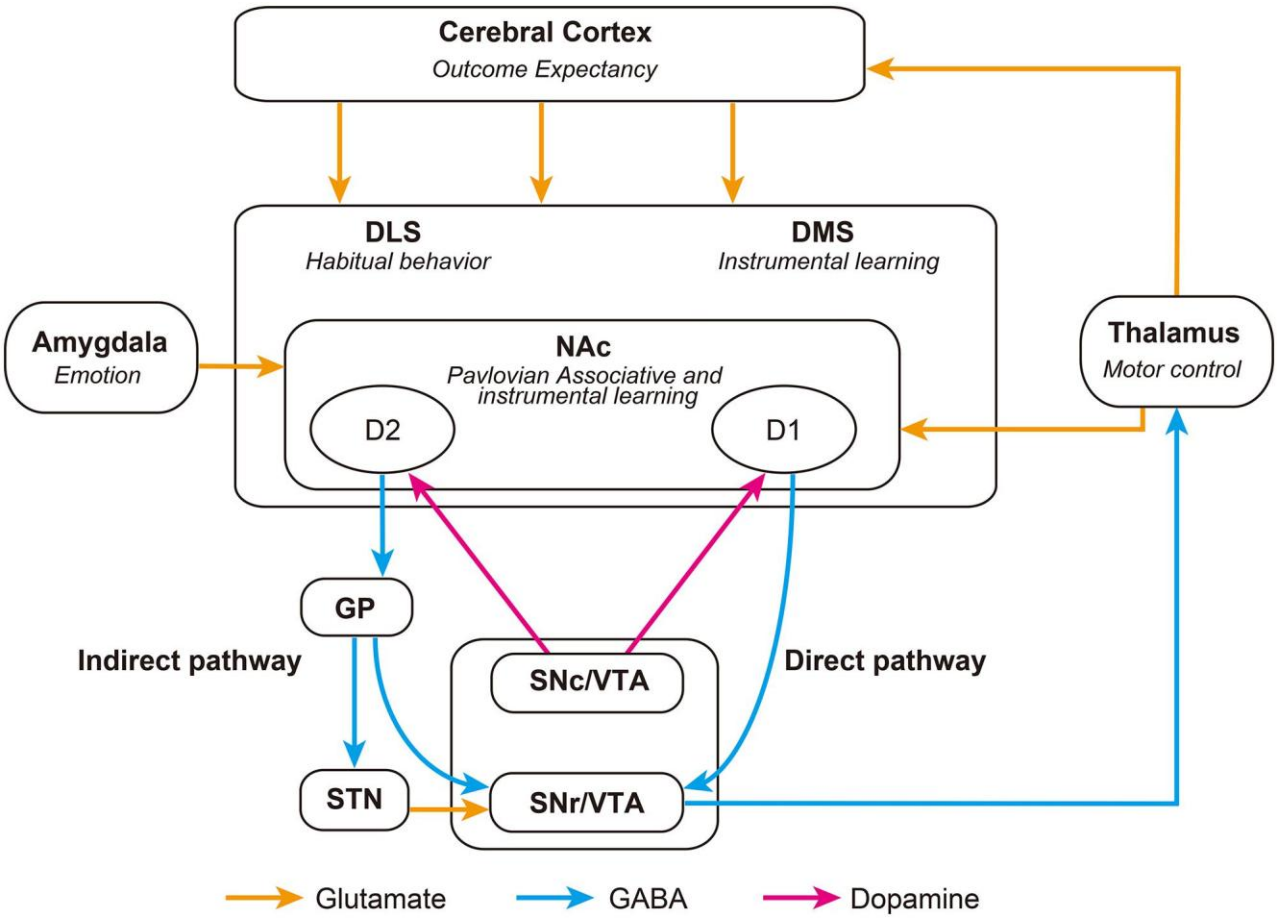
The striatum is the basal ganglia's leading component and primary afferent structure. It is anatomically linked to the cerebral cortex, limbic system, and thalamo-cortical motor system via a number of both structurally and functionally distinguishable cortico-subcortical circuits (Holland and Rescorla, 1975).

The striatum is a structure of the basal ganglia that includes the caudate nucleus and putamen in the dorsal area, as well as the nucleus accumbens and olfactory tubercle in the ventral area (Canales 2005; Yin & Knowlton, 2006; Nicola, 2007). The putamen is a highly interconnected structure including connections with the

substantia nigra, and the globus pallidus (see Figure 1). The putamen has strong links to motor control and motor learning with the additional links to stimulus-response learning (Yin & Knowlton, 2006).

Dopamine regulates the striatonigral (direct) and striatopalladial (indirect) mesencephalic pathways that moderate action selection (Canales 2005, Lewis et al., 2006). Many D1 type receptors (D1 and D5) are colocalised with glutamate receptors in the striatal neural cells associated with the direct pathway and have a beneficial impact on adenylyl-cyclase (Canales 2005; Lewis et al., 2006). This enhances the excitability of striatal MSNs, which have direct inhibitory GABAergic connections with the interior globulus pallidus (GPi) and the substantia nigra pars reticula (SNpr), the basal ganglia's two significant output nuclei (Lewis et al., 2006). When the direct pathway is stimulated by cortical projections, the thalamus becomes disinhibited due to increased inhibition of the output nuclei. As a result, the thalamocortical motor relay and the supplementary motor cortex are stimulated, leading to an increase in the number of behaviours performed (Lewis et al., 2006). Dopamine projections from the substantia nigra pars compacta (SNpc) instead stimulate the indirect pathway, activating the D2 type receptors (D2, D3, and D4) on the MSNs (Hemmings, 2010). The D2 like receptors vary from the D1 receptors in that they are negatively coupled with adenylyl cyclase, causing the excitability of the MSNs to decrease when stimulated.

FIGURE 2. Schematic representation of striatal-associated decision-making neurocircuitry.



D1 neurons inhibit the SNr and release inhibition of thalamic activity, thereby promoting behaviour. D2 indirect pathway neurons, on the other hand, inhibit the GP, disinhibiting the STN and exciting the SNr, which eventually blocks the thalamus and thereby suppresses behaviour. Glutamatergic and dopaminergic afferents, as well as GABAergic signalling within the striatum, are likely to regulate the balance between all these opposing projections (Macpherson, Morita & Hikida, 2014).

The nucleus accumbens of the ventral striatum is implicated in motivation and reward, with a lesser role in fear and impulsivity (Yin & Knowlton 2006). When considered as a whole, the striatum is collectively associated with reward based learning, four motivations, and particularly importantly with action selection and inhibition (Stocco et al., 2010). The importance of these structures is emphasised by the multiplicity of conditions associated with their dysfunction, including Parkinson's

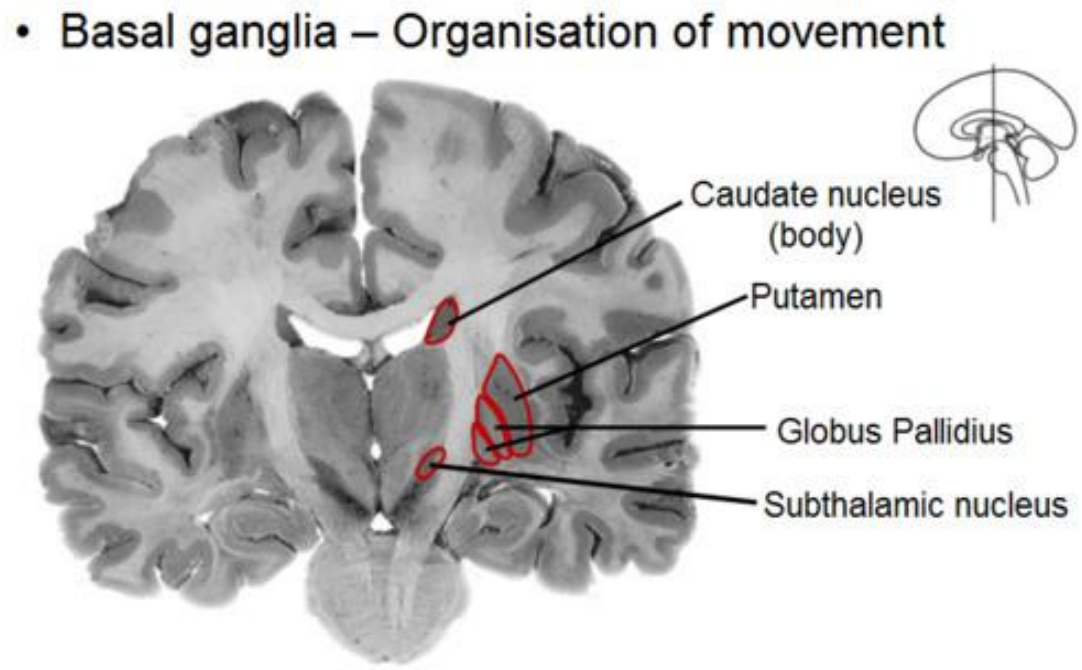
disease, Huntington's disease, addiction, and schizophrenia as some examples (Marsdon, 1982 & Browne et al., 1997).

2.3.3 The Basal Ganglia and its role in behavioural mediation

Multiple brain structures and neurotransmitters are required for cognition and behavioural output. The Basal Ganglia arrange decision making by allowing adaptive frontal motor commands while suppressing others (Olguin et al., 2016). Furthermore, parallel circuits support other basal ganglia functions that involve associative and limbic territories. Several movement disorders are caused by disruptions in the basal ganglia network (Lanciego, Luquin & Obeso., 2012).

The ventral and dorsal striatum, substantial nigra, globus pallidus, and olfactory tubercle are all part of the basal ganglia (Yin & Knowlton, 2006). According to research, cerebral information is moved from the Basal Ganglia to the Thalamus and back to the Cortex via three distinct pathways: the direct and indirect pathways through the striatum, and the hyperdirect pathway through the Subthalamic Nucleus (Gerfen & Bolam, 2010). (see figure 3).

FIGURE 3. Shows the structure of the equine brain in relation to motor control and motor learning.



2.3.4 Role of Dopamine in Appetitive or Aversive Stimuli

With an increase in concern for animal welfare, the equine world has started testing positive reinforcement (R+); as such, horses often experience a combination of negative reinforcement (R-) and R+. An example could be having rein pressure released and being given a treat, or positive reinforcement with loading a horse onto a trailer with pressure and release or with food rewards (Taylor, 2021). An appetitive stimulus is an object or event which is rewarding, acting as a positive reinforcer, whereas an aversive stimulus is a punishing object or event, serving as a negative reinforcer (Schultz, 1998). The more consistent the behaviour is rewarded and repeated, the behaviour becomes reinforced as the object or shown behaviour becomes routine, and the foundations of the learnt behaviour will become stronger. It is likely that this process is connected with the release of Dopamine (DA). Dopamine is the major catecholamine neurotransmitter in the mammalian brain, it has a central role in many cognitive processes, including motor function, working memory, reward-related decision making and behavioural change (Schultz, 2002 & Schultz, 2007).

McBride et al., (2017), states that it is vital to take note of fluctuations in dopamine release. This is defined by the difference between what the animal expects as a reward as to what is delivered. This differential value is referred to as the ‘reward prediction error’ (Schultz et al., 1997). The dopamine response is at its highest at the first time the reward is presented (because it is different and unexpected), but then with multiple presentations the reward becomes less interesting to the horse, and is only re-sparked when the reward value is increased or if sequential unpredictability is re-added (Bayer and Glimcher, 2005). Equally, if the reward is expected but not presented, this then results in a reduction in tonic dopamine release.

The reason that this is relevant to this study is that narcoleptic like behaviour may haves a connection with the horse’s own pleasure release and reward. It is therefore necessary to further understand Dopamine release from a horse who shows narcoleptic behaviours in particular, narcolepsy may cause injury - some horses have been known to injure themselves from falling over onto their knees and fetlocks – so a potentially harmful aversive stimulus is already present. The question is therefore whether a potentially positive pressure and release might overcome this.

Dopamine is also crucial in determining the day-today welfare of the animal. Whether this narcoleptic like behaviour has been brought to attention as a possible learnt behaviour due to stress related factors and could potentially be seen as a stable vices, as down-regulation of dopamine, also because of stress, can lead to behavioural depression.

There are three diseases that will be discussed in this review, as they have relevance and important findings in relation to the brain and the function of Dopamine in a variety of mammals. The first being PD; in PD midbrain dopamine cells are lost, the dorsal striatum can be divided into two major sub-zones, one with inputs from motor related cortical areas and engaged in selecting motor responses the second being, the inputs from associational areas of cortex and engaged in more cognitive parts of behaviour selection.

To conclude, the normal function of dopamine as a neurotransmitter is connection to the reward centre in the brain. DA controls movement by complex actions on striatal neurones operating the direct and indirect motor pathways within the basal ganglia which influence the output of the striato-pallidal complex to premotor cortical areas via the thalamus (McBride et al., 2017). Learning reinforcement is found to have increased with higher DA levels in humans, this could apply to horses, with their learnt behaviours such as stereotypy, studies such as Roberts et al., (2015) have suggested similar results found in horses.

2.4 Diseases and Conditions Relating to Dysregulation of Dopamine Systems

Burgess et al., (2010), found that cataplexy is modulated by a D2-like receptor mechanism, while dopamine modulates sleep attacks by a D1-like receptor mechanism. These results support a role for the dopamine system in controlling sleep attacks and cataplexy in a murine model of narcolepsy. Whereas hypocretin deficiency is the primary reason of narcolepsy, abnormalities in dopaminergic neurotransmission can also contribute to the disorder's sleepiness and cataplexy. Controlled trials, for example, show that people with narcolepsy have a distorted striatal dopaminergic system. According to brain imaging studies, narcoleptics have increased D2-like receptor binding, which is strongly linked to cataplexy. D2

receptors have also been linked to sleep attacks in Parkinson's patients, who, like narcoleptics, have hypocretin cell loss (Burgess et al., 2010).

In this section, diseases which relate to dopamine dysfunction in other species (i.e. Parkinson's) will be discussed, due to co-morbidity of these conditions with narcolepsy. Moreover, given the dopaminergic dimensions of narcolepsy in rodents, reviewing the behavioural impact of altered dopamine will enable the establishment of testable hypotheses in horses.

2.4.1 Parkinson's Disease

Parkinson's disease is a common neurological disorder characterised by a degeneration of dopamine neurons in the substantia nigra and a loss of dopamine in the putamen (Burgess et al., 2009). Parkinson's is classified as a motor disease, but it also creates cognitive and behavioural symptoms. A common treatment is through dopamine replacement therapy, which involves the administration of levodopa (L-Dopa) or dopamine agonists (such as pramipexole or ropinirole) to patients. Dopamine replacement therapy is well known to enhance motor symptoms but its effects in cognitive and behavioural symptoms are much more complex (Lanciego, Luquin & Obeso., 2012).

Studdies carried out by Gurthrie, Myers & Gluck, (2009) on, human behaviour have shown that subjects with Parkinson's disease (PD) who are treated with dopaminergic medication are impaired on learning cognitive tasks.

Parkinson's disease, which is mainly characterised by lack of spontaneous movements (hypokinesia) and slowness of voluntary movement (bradykinesia), as well as increased muscle tone (rigidity) and tremor at rest. Dopamine striatal depletion secondary to cell loss in the SNc is the cause of the major clinical features of Parkinson's disease (Merims & Giladi, 2008). Experimental studies have accumulated from both experimental models and patients with Parkinson's disease, to show that dopamine reduction shifts the balance of the basal ganglia activity toward the indirect circuit, leading to extreme activity of the STN that overstimulates the GPi/SNr. Increased output from the GPi/SNr overinhibits the thalamocortical

projection, reducing cortical neuronal activation associated with movement initiation (Lanciego, Luquin & Obeso., 2012).

The DA precursor levodopa (L-Dopa) is the gold standard treatment used to manage the motor symptoms of PD. Considering the dopaminergic neurodegenerative nature of the disease and the subsequent effect this has on motor control, L-Dopa is a logical treatment to use (Khor and Hsu, 2007; Salat and Tolosa, 2013). DA is unable to cross the blood brain barrier; therefore, L-Dopa is used as a prodrug for DA (Khor and Hsu, 2007). Pergolide, the gold standard treatment for PPID, was once used on humans for treatment of PD, however, the drug was removed from the human pharmaceutical market because of severe side effects, including restrictive valvular heart disease (van Camp *et al.,* 2004) however, this side effect has not been reported in horses. The use of pergolide in the human market emphasises the similarities between PD and PPID, although the neurodegeneration occurs in anatomically distinct structures, the type of neuron and the progressive aspects of the diseases, enables extrapolated research from PD to be plausibly applied to equine neurodegenerative disease, particularly the effect of depleted DA on learning and motivation.

One hypothesis is that the cognitive impairment in medicated PD is due to an overdose effect of the medication on the ventral part of the striatum where there has been less damage to the dopaminergic input (Frank, Seeberger & O'Reilly, 2004). The outcomes of the loss of the dopaminergic cells in PD on the phasic changes (directly on learning), has proven challenging to investigate mammal experiments. The use of computational modelling to simulate human learning data in PD therefore provides an unusual method for examining the mechanisms that lead to learning impairment in PD. Collecting cognitive data from a horse is difficult, but a variety of tests, including the GO-NO-GO Task, the Tolman's cross maze (Parker et al., 2009), and extinction paradigms, have been performed on horses in an attempt to discover the cognitive effects of dopamine dysfunction (Hemmings et al., 2007).

In relation to equine narcolepsy, the decision to collect SBR, HR and BIR from horses that show narcoleptic like behaviours, was taken on the basis that this would

be of less disturbance for the horse and owners taking part, whilst also being the least invasive to the horse and its wellbeing.

2.4.2 PPID

Pituitary Pars Intermedia Dysfunction (PPID), also known as Equine Cushing's disease (ECD), is a frequently diagnosed disease of aged horses with a pathophysiology similar to Parkinson's disease (PD), with rising levels of -synuclein (-syn), possibly leading to dopaminergic neuron loss (Fortin et al., 2021). ECD is a long-term and progressive disease of the pituitary gland. Other medical issues in horses with Cushing's disease have been discovered, including laminitis, chronic infections, pseudolactation, and other issues (McCue, 2002). According to research, different areas of the horse's brain (particularly the Hippocampus, Amygdala, and Cerebellum) associated in Cortisol regulation can undergo structural changes (Cushing's Disease News, 2017). Horses and ponies with Cushing's don't produce enough dopamine which means that the pituitary gland becomes uncontrolled and produces excessive hormones.

Pergolide, sold under the brand name Permax and Prascend among others, is an ergoline-based dopamine receptor agonist used in some countries for the treatment of Parkinson's disease (Papich., 2021). Furthermore, Clinical Veterinary Advisor, 2012 states that horses with PPID have been seen to have improvements from up to just six weeks of treatment using Pergolide, a dopamine D2 receptor agonist, with doses of 0.75 to 1.5 mg/d.

2.4.3 Stereotypy

According to Hemmings, McBridge, and Hale (2007), environmental mediated (spontaneous) stereotypies result from basal ganglia dysregulation. Anomalies in learning task performance can also indicate basal ganglia dysfunction. Consequently, studies have shown a strong link between inappropriate repeat responding within an extinction learning paradigm and stereotypy performance. Throughout this study Stereotypy will be mentioned as not only do the horses used in this study show stereotypic behaviour, but there is possibly a connection between how the equine brain works and narcolepsy.

The connection to dopamine and its relation towards reward and reinforcement in the brain is the ‘control centre’ of the nervous system it coordinates all voluntary and involuntary actions.

Motor stereotypies are abnormally repetitive behaviours that can improve with excessive dopaminergic stimulation and are characteristics of some neurologic disorders. Many of the horses studied (both narcoleptic and case control) showed stereotypical behaviours, such as weaving, box-walking and crib-biting. This data sits alongside the SBR and BIR data as a measure of how important dopamine is within the NTB horse.

2.5 Measuring Dopamine

In order to understand if whether dopamine dysfunction has a relationship to that of horses who show narcoleptic behaviour, robust and non-invasive measurements of neural activity is required. Behavioural indicators of central dopamine function have been applied with success to horses in the past, including spontaneous eye blink rate, behaviour initiation rates, and cognitive testing (Roberts et al., 2015). Due to the potential application of such measurements to the experimental work proposed, they are reviewed in detail in sections 2.8, 2.9 and 2.10 of this literature review.

2.5.1 Spontaneous Blink Rate

Spontaneous blink rate (SBR) has previously been used and successfully as a means of measurement for basal ganglia dopamine function by Karson (1983). Replicated by Roberts et al., (2015) in the horse. This is due to the influence of dorsal striatal dopamine circuity in the generation of spontaneous blinks, where increased Da activity results in a higher SBR (Karson, 1983). The suitability of spontaneous blink rates for assessing basal ganglia function must be scrutinised due to the influence other factors may have over the animal’s blink rate (Karson 1983). Certainly, it could be expected that external factors may have an influence on the observed blink rates. Consequently, during the data collection horses were observed in the home stable where external factors can be minimised and to an extent controlled. Moreover, anomalous results may occur due to human error in miscounting. To make the data collection as valuable and accurate as possible, the

data will be collected from the same person each time, and from only the left eye of the horse being used for data collection. Moreover, making it clear that both full blinks and half blinks will be collected over three thirty minute intervals, so over the final time of an hour and a half. Therefore, blinks counted can be as accurate as possible and the results will not be biased by multiple different data collectors.

Regarding stereotypic behaviour, SBR has also been used to measure comparing crib-biting horses and control study horses. Within the group of horses selected for this study in narcolepsy, some horses showed stereotypic behaviours. For these horses, SBR data collections where of most significance. This can be traced back to a widespread increase in the sensitivity of the receptors in the striatum (Roebel & MacLean, 2007). Roebel and MacLean (2017) discovered that horses exhibiting a stereotypic behaviour (crib-biting) had a lower SBR when compared to their control counterparts. This finding was previously observed in stereotypic rodents (Roebel & MacLean, 2007).

In addition, people who use cocaine compulsively have been discovered to have a lower SBR, in addition to a lower number of D2 receptors located inside the striatum (Colzato et al., 2008). The reduction of D1 receptor density and D2 receptor affinity in the caudate of crib-biting horses (McBride & Hemmings, 2005) could advance into a hypodopaminergic state in the caudate, which could lead to a decrease of SBR in these mammals (McBride & Hemmings, 2005).

The ventral striatum may also play a role in SBR control, as the centre of the nucleus accumbens travels towards the SNpr in the same way that the dorsal striatum does. It is possible that the sensitisation of both D1 and D2 receptors discovered in the ventral striatum could result in the inhibition of SNpr control over the superior colliculus, thereby lowering SBR. More research is needed to understand the pathways and neural mechanisms that contribute to the reduction of SBR in animals.

Moreover, linking SBR back to PD in humans, several conditions are particularly well studied including how 28 dopaminergic nigrostriatal cells in the caudate degenerate, increase the activity in the SNpr (Chen et al., 1996). Blink amplitude increased in this study and consequently the SBR was reduced. Some Parkinson’s patients have

been observed to have a SBR of just 3 blinks per minute (Karson, 1983). This provides another clear link between dopamine function and SBR.

2.5.2 Temperament Questionnaire

Investigations have previously indicated a link between dopamine and temperament in human participants (Gerra et al., 2000). However, Roberts et al. (2016), investigated the relationship that exists between dopamine systems and temperament of horses specifically. Using a temperament questionnaire Roberts et al. (2016) ascertained behavioural characteristics of individual horses and how this relates to dopaminergic activity of the basal ganglia, which was itself determined using spontaneous blink rates to indicate high medium and low dopamine activity. It was determined that anxiety was correlated positively with SBR, whereas docility was negatively correlated with SBR, indicating that basal ganglia dopamine may be linked with temperament.

This is similar to this study on equine narcolepsy. A temperament questionnaire was provided to the owners of the horses that showed signs of narcolepsy or narcoleptic like behaviours, following the collection of SBR and HR data collections. This gives an indication to basal ganglia activity and this can be compared to the case control study horses.

Moreover, having completed data collection, one further question was asked of all participants of this investigative study into Narcolepsy, that being; had the horse owners ever noticed their horse sleeping led down, or evidence of REM sleep (bedding being flattered). All owners came back as responded “YES”. This can therefore allow to cancel out sleep deprivation in this study.

Finally, the horse’s diet was also considered in the questionnaire (Question 16) as there is a link between SBR and the gut-brain axis link to starch content of horse diet as this is an interesting point. However, it was not analysed in the context in this investigation and will be considered for further investigation in future work.

2.6 Dysfunction of the Peripheral Nervous System in Narcolepsy

Narcolepsy, although an unusual disease, is one of the most common causes of chronic sleepiness. Patients with narcolepsy typically show cataplexy and a dramatic

reduction in the cerebrospinal fluid concentration of the neuropeptide orexin (hypocretin), which results from extensive loss of the orexin-producing neurons in the hypothalamus (Burgess et al., 2010). Sachs & Kaiser., (1982) stated, smaller increases in human heart rate (HR) and blood pressure (BP) at the handgrip test and smaller changes in HR during deep breathing and the Valsalva maneuver, but no significant difference in patients with narcolepsy compared with healthy controls.

However, according to Fronczek and Thijs. (2013), a reduced heart rate response to arousals and leg movements in human narcoleptic patients may be due to a lack of hypocretin-1. Other factors including age, gender, disease duration, and BMI may also contribute to cataplexy and reduced HR. The researchers come to the conclusion that the hypocretin system is important in autonomic regulation during sleep. Furthermore, the study Fronczek & Thijs., (2013) found that secreting neurons enhanced sympathetic output parameters such as HR, blood pressure, and body temperature when hypocretin was administered or stimulated. In contrast, hypocretin neuron-ablated rats and hypocretin deficient mice had lower HR and blood pressure throughout wakefulness. This fit with the theory that hypocretin deficiency would lead to a lower sympathetic tone. This, in turn, could explain the lowered metabolic rate seen in narcolepsy.

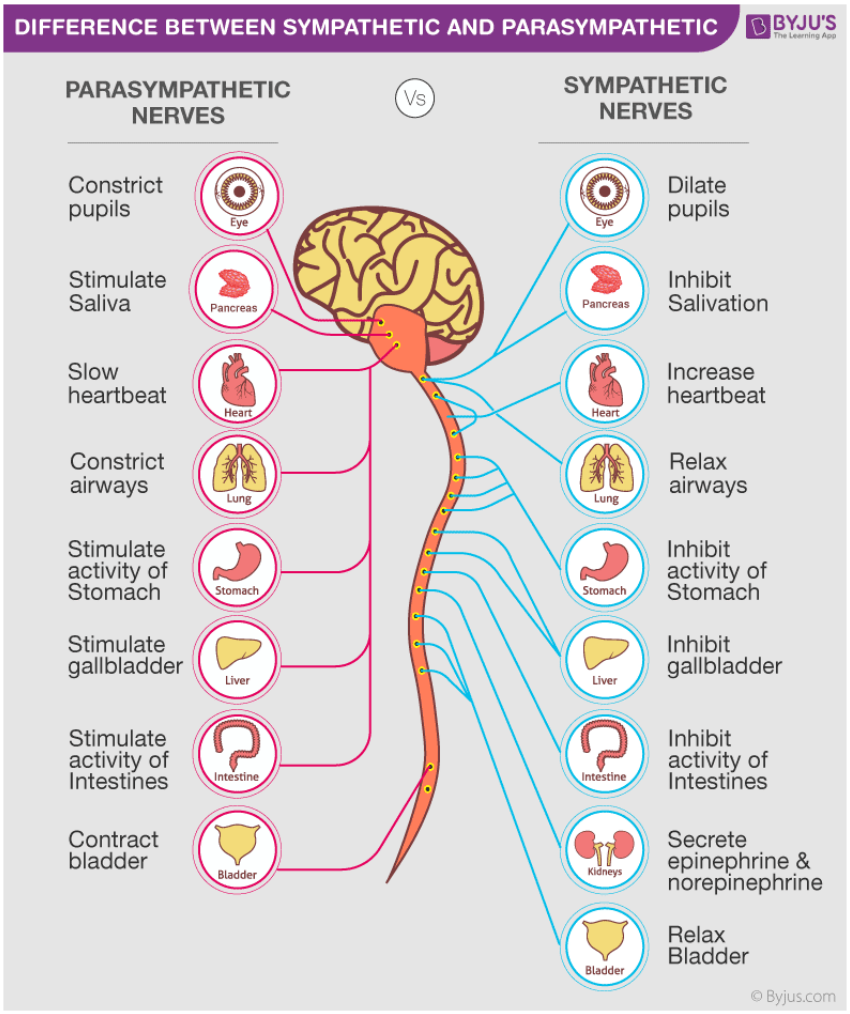
2.7 Sympathetic Parasympathetic Nervous System

Despite the differences among horses and humans, there are several shared similarities in the very basic development and structure of the nervous systems. Both systems have an autonomic nervous system (ANS), this regulates organs, muscles, glands, etc. The parasympathetic and sympathetic nervous systems are the chief element of ANS.

The ANS comprises of two parts: the sympathetic and parasympathetic nervous systems. The sympathetic nervous system triggers the fight or flight response during a threat or for any perceived danger, and the parasympathetic nervous system puts the body back into a state of calm. The sympathetic nervous system releases epinephrine and norepinephrine, that increases the heart rate. The parasympathetic

nervous system releases acetylcholine, the hormone that reduces the heart rate (Byjus, 2020).

FIGURE 4. Difference Between Sympathetic and Parasympathetic**.** (Byjus, 2020).



Throughout this study the connection between HR, SBR and BIR will be connected to this narcoleptic behaviour shown by case studies. By collecting HR analysis, this could be used to provide insight into responses of the autonomic nervous system (ANS) in a range of physiological and pathological conditions.

Stereotypy is included in this study from a neural viewpoint, specifically focusing on crib-biting horses as from the temperament questionnaire completed by owners with horses who show signs of narcolepsy; some are crib-biters. A further understanding

into the striatum region not only in the equine, but also in other species might give some insight into this behaviour.

The temperamental questionnaire offered to owners and trainers has allowed for data collection in if the horse suffers from any other illness or issue the owner would like to state, and what medicine if taken was used. To get a well-rounded picture of each horse and its background, it was found important to know if the horse suffered any other medical history that may have a connection to either sleep deprivation or narcolepsy.

At last, owners and trainers should ensure that their horses are in peak physical condition for their respective disciplines. As previously stated, sleep deprivation may be the result of poor management. Management advice for example, regular respiratory system checks and heart rate checks, should be introduced to a management routine for horses after exercise (such as interval training) if the horse is having to go under a lot of physical stress, such as eventing or racing.

2.8 Behavioural Initial Rate

The behaviour initiation rate (BIR) is where the number of times an animal switches between one behaviour to another, in a set amount of time. It is also a non-invasive behavioural probe into dopamine function in the striatum of the basal ganglia (Garner 2003; Garner & Mason 2006, Roberts et al., 2015). In this study BIR analysis has been carried on thirteen horses who show narcoleptic behaviour and thirteen case control horses that are similar in type and age. Alongside counting behavioural switching, a heart rate monitor was used to collect data so that a contrasting connection can be analysed. Garner & Mason., (2006), concluded that significantly higher HR increase was observed in stereotypic horses compared to horses which did not display any stress-related behaviours, reflecting a correlation between behavioural and physiological responses.

A variety of different behavioural expressions can be represented by different coping strategies of individuals in stressful situations (Roberts et al., 2015). A fundamental link between behavioural responses and the activation of the physiological stress

response and DA has proven to be of great value in understanding the neurology of the equine brain and its response to stress.

However, it must be noted that that as an indirect measurement, it cannot offer a complete insight into the brain of the animal under investigation, and as such cannot provide an exact numerical representation of dopamine or receptor concentration in the brain (Garner & Mason, 2002). An example of some uncertainty for collecting BIR, might be the existence of busy environments - such as other horses being brought in or turned out – causing the study horse to become alert or unsettled, therefore affecting BIR harder and possibly making data less accurate. In this study, data collection for narcoleptic like behaviours was as far as possible conducted when horses were in a familiar stable with access to hay/hayladge and water. When it was possible, the observations for each horse was carried out during a peaceful and quiet time of day in the stable. This was done to reduce the likelihood of any disturbances affecting the environmental stimuli, which in turn could affect the rate at which the horse's BIR was measured.

Stereotypic behaviours were observed, however, and when the behaviour started (such as crib-biting) the start of that visual behaviour was counted as one BIR, and when that behaviour stopped and changed to another; again, it was counted as one BIR. If each individual stereotypic behaviour was counted (each individual crib-bite), then the BIR would not be consisted if other horses did not show stereotypic behaviour and results would look unfamiliar from one another.

Furthermore, evidence from more studies will be useful in understanding neurological disorders of a lesser studied species (i.e., equids). The inferred measures of DA release in this investigation have all been used and seen in other species and studies, and such studies may be cross-references to this study to confidently identify DA functioning.

2.9Heart Rate Variability

Anxiety is associated with activity of monoaminergic systems of the brain, including dopaminergic and serotonergic. When under physical or psychological stress, which could include anxiety, it is not uncommon to observe a cardiac response. Heart Rate

changes are the first indicator that a subject is observed when under stress, when the number of beats per minute increases. However other cardiac responses are known to occur, including changes in heart rate variability. Within this study of Narcolepsy, it is most likely that the HR will be seen to decrease with horses that show narcoleptic like behaviours, in comparison to case control horses.

Heart rate variability (HRV) is a physiological occurrence referring to the variability in length of the beat to beat interval, usually measured between the R peaks of the QRS complex. Therefore, animals that show stress or stereotypic behaviours may have altered dopamine function within the mesocorticolimbic dopaminergic circuitry, which could demonstrate reduced HRV compared with those which are do not show signs of stress or stereotypy. Dishman et al., (2000) also found that vagal control of the heart has a connection to emotional stress in an animal. Regardless of different factors including heart rate, blood pressure and physical fitness therefore ruling out several potential confounding variables in the measurement of HRV. This indicates that HRV is a potentially reliable measurement of narcolepsy as this behaviour has a connection to stereotypy. However, factors such as coat length may have an influence over transference of electrical signal to the electrodes of recording equipment, which likely affects longitudinal studies. Therefore, water was be used as a lubricant underneath the HR monitor thereby allowing for the best connection and readings from each horse.

HRV was collected utilising the Polar® Equine V800 Science system during all observations. Data was be downloaded via Polar Flow® and Kubios version 2.2 software. For each collection of data, the owners completed a consent form as well as the temperamental questionnaire before each site visit.

2.10Conclusion

Dopamine has been referred to as a highly significant behavioural output modulator across a number of species, including humans, dogs, and horses, amongst others. Given the significance of the horse's disposition and the behavioural characteristics it possesses in relation to the protection of humans and the fulfilment of equine functions, such as the horse's involvement in the (eventing, dressage or hacking). It is necessary to conduct additional research into the effect that dopamine has on the

behavioural output of horses. However, practises such as the experimental modification of the horse's dopamine levels might raise ethical difficulties. However, horses who exhibit spontaneous dopamine adaptations may also demonstrate hyperdopaminergic stereotypy in addition to hypodopaminergic PPID. In previous studies, the researchers focused their attention on mammals that exhibited signs of dopaminergic changes as well. Their goal was to determine the extent to which these adaptations influence the cognitive and behavioural output of an organism. In this review, previous research has provided useful non-invasive behavioural markers such as SBR and BIR of underlying dopamine changes. Dopamine in the horse may be the subject of additional in-depth research as a result of SBR and BIR. The link between dopamine and temperament is one of the topics covered in this study. If additional research into dopamine changes can be conducted prior to the onset of disease symptoms, as has been done in humans, it may be possible to develop better treatments (the same non-invasive data collections could be used on the horse).

Regarding this study into equine narcolepsy, as well as SBR and BIR; HR and a detailed temperament questionnaire form was also part of the data collection into narcolepsy. As mentioned previously all these methods are the safest and least invasive to horse and owner. Both narcoleptic and case control horses were as similar a possible in age and type allowing for the data to be as directly comparable as possible. In summary, this research aims to investigate the development of endophenotypes in the horse for different dopamine functions by forming the initial relations between behavioural indicators which may include dopamine function, behavioural markers in the horse’s temperament and the cognitive ability of each individual horse. The ambition was to see a lower BIR, SBR and HR from the horses showing narcoleptic-like behaviours, than the case control horses, thereby establishing a direct correlation between measurable indicators of DA release and narcolepsy. This result was not confirmed to a sufficiently high level of proof by the study. However, a correlation was established between the temperamental review and narcolepsy. A deep investigation into temperamental factors is therefore required.

3.0 Methodology

3.1 Sample Population

Although veterinary advice had previously been sought by all horse owners, due to the lack of formal diagnostic indicators in equine medicine (Young 2021), none of the animals in this study had been diagnosed as narcoleptic. Therefore, in selecting horses for this study we identified a 'narcoleptic type behaviour' (NTB) phenotype characterised by:

- Somnolence / drowsiness at a standstill
- Progressive lowering of the head
- Buckling of the forelimbs followed by partial loss of muscle tone leading to temporary postural instability.

The definition owners had to confirm they saw, with their own horse was "the buckling of the forelimbs with possible drastic collapse and instability".

In the temperament questionnaire questions were asked about the horse's sleep patterns, if it was common to see them sleeping on the ground or any evidence in their stables of flattered bedding. Moreover Question 8 in the questionnaire asks about the type of bedding used as studies have shown this to affect the quality of the horses REM quality of sleep. Therefore, precautions were taken place before further contact to owners with horses showing signs of Narcoleptic type behaviours compared to sleep deprivation, furthermore, in collection of data some horses actually preformed the behaviour described in real time, therefore confirming even further the difference between Narcolepsy and sleep deprivation.

All NTB horses (n=13) were identified using author contacts and posts on social media platforms i.e. Facebook containing a detailed description of the NTB phenotype. The animals were in ridden exercise, being used for either competition or leisure purposes with the exception of 2 that had been retired from ridden work (see table 1a). A population of control animals (n=13) were selected on the basis that they did not show the NT phenotype (see table 1b). Following consultation with the owners, none of the animals recruited were reported to have limb based pathologies which would have otherwise confounded the findings.

TABLE 1a. Summary of the thirteen NTB Horses

Horse	Age	Gender	Breed	Discipline	Other Notable Behavioural Anomalies
1	10	Gelding	Warmblood	Eventing & Hunting	Crib-biting
2	14	Mare	Irish Sports Horse	Retired	NONE
3	23	Mare	Thoroughbred	Fun Rides & Dressage	Box Walking
4	21	Mare	Warmblood	Hunting & Show jumping	Crib-biting and Weaving
5	17	Gelding	Irish Sports Horse	Leisure rides & Fun Rides	NONE
6	25	Gelding	Irish Draught X Thoroughbred	Leisure rides	NONE
7	8	Gelding	Warmblood	Leisure riding, Show jumping & Eventing	Crib-biting
8	9	Gelding	Warmblood	Hunting, Eventing, Show jumping & Dressage	Crib-biting & Weaving
9	24	Mare	Thoroughbred	Event	Crib-biting
10	14	Gelding	Connemara	Leisure riding, Fun Rides & Dressage	NONE
11	20	Gelding	ISH	Retired	Box walking, Door kicking & Barging
12	13	Gelding	Thoroughbred	Hunting & Eventing	Box Walking
13	20	Mare	ISH	Leisure riding & Fun Rides	Crib-biting

TABLE 1b. Control Horses

Horse	Age	Gender	Breed	Discipline	Other Notable Behavioural Anomalies
1	20	Gelding	Connemara	Retired	NONE
2	19	Gelding	Warmblood	Hunting	NONE
3	7	Gelding	Irish Sports Horse	Hunting & Team Chasing	NONE
4	22	Mare	Thoroughbred	Retired	Box walk & Weaving
5	12	Gelding	Irish Sports Horse	Eventing & Fun Rides	Crib-biting
6	9	Gelding	Thoroughbred	Fun Rides & Dressage	NONE
7	14	Gelding	Warmblood	Hunting, Eventing & Run Rides	Crib-biting
8	20	Gelding	Irish Sports Horse	Eventing & Hunting	Weaving & Kicking door
9	5	Gelding	Warmblood	Dressage & Fun Rides	Crib-biting
10	13	Mare	Warmblood	Show Jumping	Box walking
11	22	Mare	Thoroughbred	Retired	Crib-biting
12	17	Mare	Irish Sports Horse	Dressage, Fun Rides & Hunting	Weaving & Box Walking
13	7	Mare	Connemara	Fun rides & Hacking	NONE

3.2 Study Location

The horses used in the investigation were situated at yards in the South East and West of the UK (figure 5). Due to the unusual and unique characteristics shown by NTS's, recruiting thirteen NTB's was a huge achievement, however this did take

several months between first communications and yard visits. Despite disparate location certain management factors were put in place to minimise confounding variables, these are discussed below.

FIGURE 5. Shows a map of the UK and the areas visited for both control and NTB horses' data collections.

3.3 Management Conditions

Stress has been shown to bring about fluctuations in both SBR (Roberts et al., 2016) and heart rate (Roberts et al., 2016). As a result, the horse's home stable was chosen as the location for the observations in order to reduce the amount of stress the animal experienced. If a horse did become stressed or unsettled at any point during the procedures, then the data collection was paused, and an assessment was made on whether it was suitable to continue.

Water was presented and given to horses after thirty minute intervals between each data collection.

3.4 Temperament Questionnaire

After Roberts et al., (2016) a temperament questionnaire was chosen to be the most suitable method to gain information which relates to temperament data from the sample cohort. The questionnaire consisted of two sections, the first section of the questionnaire asked for details about the horses' basic information (age, sex, breed and height) and management (diet and any health parameters). The second assessed the temperament utilising a series of 9 point Likert scaled questions. Which were more specific to narcolepsy and how it effects the rideability and management of the horses. The survey can be viewed in full at the link provided below:
https://rau.onlinesurveys.ac.uk/equine-temperament-questionnaire-v2
The survey was completed by the owners of all 26 horses featured in the study.

3.5 Heart Rate Variability

Heart Rate (HR) was collected by using the Polar Equine V800 belt and watch (a system capable of recording heart rate variability) fitted as per manufactures instructions (seen in Figures 6 & 7). To ensure sufficient signal conduction between the horse's skin and the electrodes, the hair was moistened using a damp sponge. Before collecting HR results, the belt was put on the horse to acclimatise the animal to the pressure of the Belt and to avoid any anomalous results derived from novel stimulus arrival. Heart rate recording was conducted for 30 minutes at rest on 3 separate occasions.

FIGURE 6. Shows the Belly Strap of the Polar V800 HR Monitor on a Case Study.

The data was uploaded to the web-based Polar Flow system, before transferral to KUBIOS software version 2.2, HRV Standard, utilising the smoothness priors function, with the smoothness parameter set at 500ms.

3.6 Spontaneous Blink Rate

SBR observations were carried out on all of the animals who participated in this research, in a manner that was analogous to that of Chen et al., (1996), in the same way that prior unpublished data on the horse (Issaoui, 2011) were also collected and documented by Roberts et al., (2015). In order to minimise the amount of stress the horses experienced during the process, the evaluation of SBR was carried out in the same stable in which the horses where kept. While being loosely tied up for data collection, horses were allowed access to forage and water and were kept in their yards at periods when there was less noise. This was done to reduce the impact of environmental factors on the blink rate.

A timer was used to ensure accuracy of duration which stated previously was three lots of thirty minute intervals. This ensures as far as possible that the horse was relaxed, whilst simultaneously allowing the observer to have an unobstructed view of the left eye. The left eye was the only side observed through out all data collections (control and NT). Due to the anatomy of the horse, it is difficult to obtain an accurate

bilateral blink count with a single observer, therefore only blinks from the left eye were recorded for consistency throughout the trail.

As soon as the timer was started, each full and half blink was counted for thirty minutes (carried out for a total of three times). This was carried out by using a mechanic counter (see Figure 7); this was also used to record BIR.

FIGURE 7. Shows a Blue and Yellow Mechanical Counter used to collect SBR and BIR Data.

3.7 Behavioural Initial Rate

BIR data collections were carried out at the same time as SBR with its own separate mechanical counter (See Figure 7). The mechanical counters used where two separate colours used on separate hands so that the data collector can avoid human error as much as possible. During the duration of data collection the horse had access to forage and water. Horses were free to roam so that the BIR did not have any restrictions for the horse.

During the BIR observation, the sort of behaviour that the horse was performing was neither taken into consideration nor recorded individually; rather, the only thing that was documented was the number of fresh initiations of behaviour. For example, a

crib-biting horse may start the behaviour and then stop and change to looking alert, this was counted as two BIR collections, rather than counting each individual crib-bite the horse preformed.

3.8 Statistical Analysis

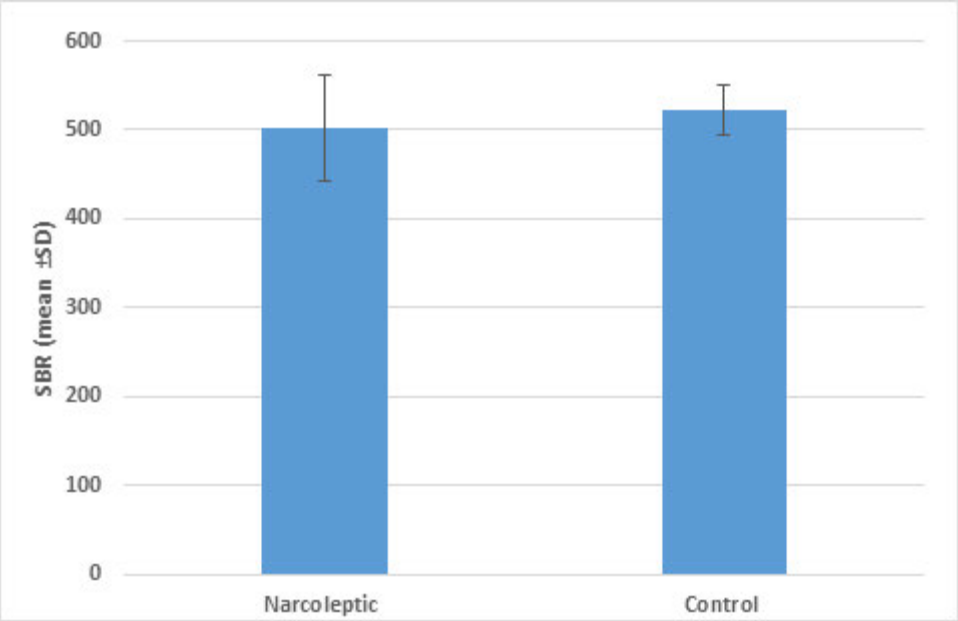
Data were organised on Microsoft® Excel and analysed using GenStat 20th Edition. Following the application of the Shapiro-Wilk test for Normality, normal distribution was established for the physiological data and thus a parametric approach (2 sample t-test) was applied. On the other hand, for comparisons of the scoring data obtained from the questionnaire, the Mann-Whitney U test was applied. Finally, Linear Regression and Spearman's Rank Correlation were utilised to investigate relationships between the physiological data and temperament scoring data respectively.

3.9 Ethical Approval

Ethical approval for the behavioural and physiological comparisons was granted by the RAU Ethics Committee (20224519-Hemmings). The NTB phenotype was measured and observed, with no attempts made to induce the behaviours or alter the day to day management conditions of the animals in any way. Approval for the collection of temperament data via questionnaire was dealt with and granted under a separate request (20215317-Hemmings).

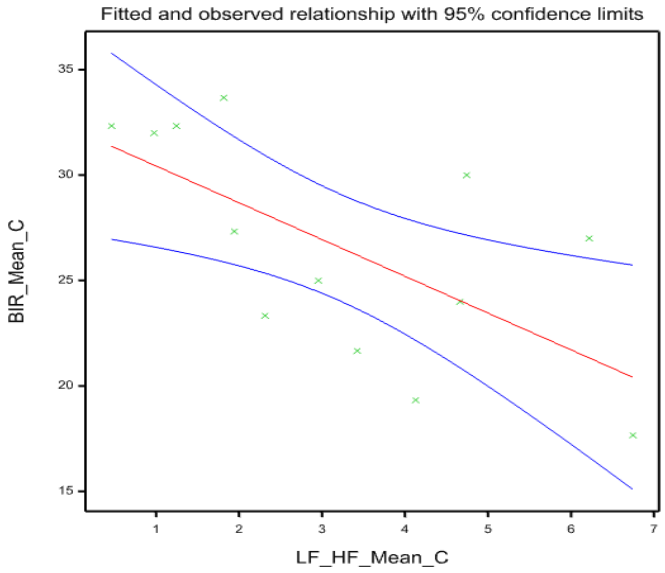
4.0 Results

FIGURE 1. Mean SBR values of control versus NTB's horses.



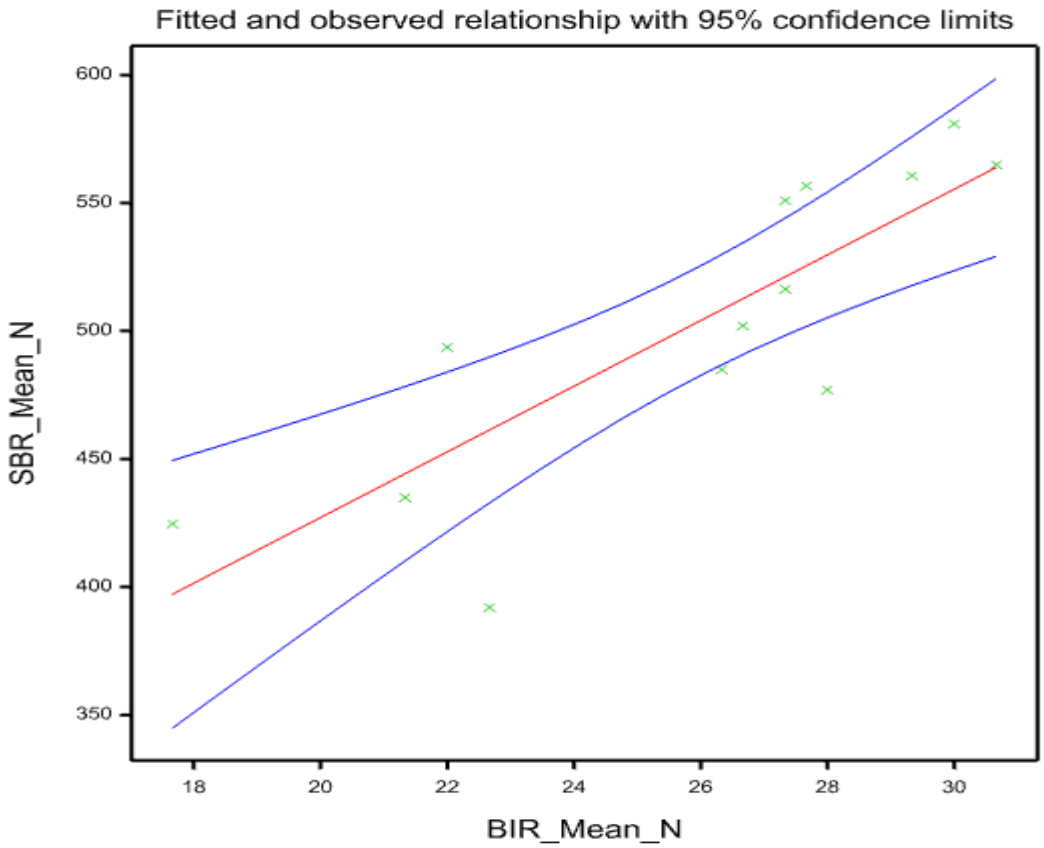
Analysis via t-test, revealed no significant differences for any of the physiological variables (SBR, BIR, LF/HF Ratio, Mean HR, RMSSD) between NTB's versus control horses. Figure one shows an example comparison of SBR values ($p>0.05$, $t=-1.08$, $df = 17.08$). See appendix 1 for all other comparisons.

FIGURE 2. Regression plot displaying LF/HF ratio versus BIR mean for control horses.



A significant negative correlation was revealed between BIR and LF/HF ratio for control animals (R^2 = 0.38, p<0.05, figure 2). However, a corresponding correlation was not evident for narcoleptic counterparts.

FIGURE 3. Regression plot displaying SBR and BIR values for narcoleptic horses



On the other hand, a strong significant positive correlation was revealed between SBR, and BIR mean values for the narcoleptic horses (R^2 = 0.65, p<0.01, figure 3). A corresponding correlation was not evident for the control animals.

TABLE 1. Physiological Correlations, ** p<0.01, NS = Non Significant, N = NTB's, C = Control

Physiological Correlations	**Correlation Coefficient (R^2)**	**Significance**	**Control / NTB's**
RMSSD Mean Vs. LF HF Mean	-0.594	**	N
RMSSD Mean Vs. LH HF Mean	-0.621	**	C

SBR Mean Vs. LH HF Mean	-0.56	**	C
SBR Mean Vs. LH HF Mean	0.168	NS	N
LF/HF Mean Vs. BIR Mean	-0.688	**	C
LF/HF Mean V.s BIR Mean	0.162	NS	N

Further regression analysis revealed significant moderate to strong negative correlations between RMSSD and LF/HF ratio for both control and narcoleptic groups. Finally, for control horses only, a significant negative correlation between SBR and LH / HF ratio was evident (see table 1). All other physiological correlations can be viewed in appendix 1.

TABLE 2. Correlations between temperament and physiological variables, ***p<0.001, **p<0.01, *p<0.05, N = NTB's, C = Control

Temperament Correlations	**Correlation Coefficient (r_s)**	**Significance**	**Control/ NTB's**
Memory Vs. BIR Mean	0.682	**	C
Patience Vs. BIR Mean	0.621	**	C
Slow Vs. BIR Mean	0.749	***	C
SBR Mean Vs. Confident	-0.569	**	C
SBR Mean Vs. Docility	0.681	**	C
SBR Mean Vs. Reliable	-0.521	**	C
Stubbornness Vs. RMSSD Mean	-0.758	***	C
Subordinate Vs. SBR Mean	0.48	**	C
Timid Vs. RMSSD Mean	0.471	**	C
LF HF Mean Vs. Competitiveness	-0.572	**	N
LF HF Mean Vs. Cooperation	0.652	**	N
Narcoleptic Vs. Competitiveness	-0.785	***	N
Narcoleptic Vs. Concentration	-0.527	*	N
LF HF Mean Vs. Friendliness horse	0.542	*	N
LF HF Mean Vs. Friendliness People	0.55	*	N
RMSSD Mean Vs. Hardworking	0.674	**	N
NTB Frequency Vs. Friendliness horse	0.544	*	N
Narcoleptic Vs. Hardworking	-0.524	*	N

Narcoleptic Vs. Hardworking	-0.529	*	N
Narcoleptic Vs. Inconsistent	0.798	***	N
Subordinate Vs. LF HR Mean	0.619	**	N
Narcoleptic Vs. Irritable	0.532	**	N
Narcoleptic Vs. Irritable	0.532	**	N
RMSSD Mean Vs. Playful	-0.575	**	N
Narcoleptic Vs. Panic	0.743	**	N
SBR Mean Vs. Reliable	-0.575	**	N
Sociable Vs. RMSSD Mean	-0.475	*	N
Stubbornness Vs. RMSSD Mean	-0.594	**	N
Tense Vs. SBR Mean	0.555	*	N
NTB Frequency Vs. RMSSD Mean	-0.468	*	N
NTB Frequency Vs. SBR Mean	0.436	*	N
NTB Frequency Vs. RMSSD Mean	-0.498	*	N
NTB Frequency Vs. Solitary	-0.442	*	N
NTB Frequency Vs. Subordinate	0.627	**	N
NTB Frequency Vs. Tense	0.599	**	N

4.1 The Relationship between Temperament and Physiological Variables

A strong significant positive correlation was revealed between Slow vs. BIR Mean for control horses. On the other hand, a strong negative correlation between Stubbornness vs. RMSSD was evident for the control group. With reference to the NTB cohort, strong negative and positive correlations were uncovered for NTB frequency versus competitiveness and inconsistent respectively (see table 3).

TABLE 3. Comparison of Temperament variables between control and NTB groups, *p<0.05, NS= Non Significant

Temperament Variable	NTB	Control	Significance	P Value
Calm Vs. Nervous	2.69 ± 1.43	2.92 ± 1.89	NS	1
Concentration	7.23 ± 1.78	6.23 ± 2.04	NS	0.063

Self Reliance	6.31 ± 2.28	5.92 ± 1.89	NS	0.526
Excitability	5.46 ± 1.56	5.84 ± 1.99	NS	0.541
Trainability	7.61 ± 1.93	6.84 ± 1.41	NS	0.064
Friendliness- People	8.15 ± 1.14	8.53 ± 0.77	NS	0.413
Friendliness - Horse	7.46 ± 2.25	8.53 ± 0.77	NS	0.265
Curiosity	5.53 ± 1.85	6.92 ±1.71	*	0.013
Memory	7.15 ± 1.46	6.69 ± 1.93	NS	0.622
Panic	3.84 ± 1.95	3.61 ± 1.98	NS	0.704
Cooperation	7.93 ± 1.32	6.61 ± 1.55	*	0.028
Inconsistant	2.38 ± 1.61	3.61 ± 1.71	*	0.023
Stubborness	4 ± 2.41	4.76 ± 1.73	NS	0.323
Docility	6.46 ± 2.33	4.92 ± 1.8	NS	0.09
Vigilance	4.38 ± 2.18	6.15 ± 1.14	*	0.017
Patiance (Missing)	5.31 ± 2.39	5.38 ± 2.39	NS	0.491
Competitivness	4.92 ± 2.53	5.53 ± 1.81	NS	0.429
Skittishness	3.07 ± 1.55	3.31 ±1.60	NS	0.514
Timid	5.15 ± 2.11	5.76 ± 1.36	NS	0.374
Active	5.61 ± 2.53	6.23 ±1.53	NS	1
Impulsive	3.31 ± 1.70	3.53 ± 2.02	NS	0.926
Aprehensive	3.38 ± 2.32	4.07 ± 2.10	NS	0.279
Confident	7 ± 0.81	6.07 ± 2.10	NS	0.47
Eccentric	5.07 ± 2.69	4.38 ± 2.53	NS	0.404
Equable	5.23 ± 1.83	5.23 ± 1.78	NS	0.988
Fearful	3.46 ± 1.26	2.84 ± 1.14	NS	0.175
Irritable	3.46 ± 1.66	3.38 ± 1.50	NS	0.956
Sociable	7.23 ± 1.09	7.53 ± 0.96	NS	0.384
Opportunistic	4.69 ± 1.88	5.38 ± 1.98	NS	0.328
Permissive	5.30 ± 2.25	3.84 ± 1.95	NS	0.1
Playful	6.23 ± 1.96	7.15 ± 1.62	NS	0.066
Popular	6.92 ± 1.55	7 ± 1.63	NS	0.644
Protective	4.92 ± 2.49	4.15 ± 2.67	NS	0.397
Slow	4.76 ± 2.24	4.07 ± 1.80	NS	0.415
Solitary	2.76 ± 1.64	2.23 ± 1.01	NS	0.468

Subordinate	5.23 ± 2.20	4.76 ± 1.64	NS	0.323
Tense	3 ± 1.87	4.23 ± 2.01	*	0.042
Suspicious	4.61 ± 1.66	5.76 ± 1.87	NS	0.071
Reliable	7.53 ± 0.96	6.92 ± 1.71	NS	0.393
Hardworking	7.84 ± 0.80	7.76 ± 1.36	NS	0.772
Intelligent	7.46 ± 1.94	8.38 ± 0.76	NS	0.14

4.2 Temperament Differences between NTB and Control Groups

Narcoleptic horses displayed significantly lower curiosity, vigilance, tenseness and consistency scores compared to control equivalents. On the other hand, scores for co-operation were significantly higher for narcoleptic versus control (see table 8 in Appendix).

4.3 NTB reported results regarding Narcolepsy compared to Control Groups

In table 4, results show clearly how NTB owners answered the specific questions regarding Narcolepsy and the individual connection to their experiences. The control groups did not report any results in this section of the questionnaire as this did not concern the owners of the control groups, as control horses did not show any signs of this Narcoleptic-like reported behaviour. A 1-9 Scale was used in the questionnaire in this section to help owners choose their correct response (1 being less often and 9 being frequently).

5.0 Discussion

The primary aim of this investigation was to compare physiological and temperament variables between horses that display Narcoleptic Type Behaviours (NTB's) and control equivalents. The following null hypotheses were used to drive this work:

1) There will be no significant differences in either BIR or SBR between control and NTB horses.
2) There will be no significant differences in HRV Variables between Control and Narcoleptic Type horses.

3) There will be no significant difference between temperament variables between control and Narcoleptic type horses.

Overall, there were no differences in any of the physiological variables between NTB and control groups and so hypotheses 1 and 2 are accepted. Conversely, significant differences between groups were uncovered in 3 temperament variables (curiosity, vigilance and cooperation) therefore hypothesis 3 is rejected. The ramifications of these findings are discussed below.

5.1 Inferred Measures of Dopamine

Investigation into BIR and SBR revealed no significant difference between NTB horses and the controls, suggesting that dopamine system dysfunction is not a factor in the NTB phenotype. Both BIR and SBR are used to infer the extent of dopamine transmission through a non-invasive method for the animal. Karson (1983) successfully collected SBR previously as a means of measurement for basal ganglia dopamine function in Humans and Roberts et al., (2015) in the horse. The latter study compared SBR values between crib-biting, weaving and stereotypy free controls. Blink rate values for the crib-biting cohort were significantly lower compared to the weaving and control equivalents. Furthermore, studies carried out by McBride and Hemmings, (2005) state that horses that show crib-biting behaviour have been found to have a significantly higher concentration of D1 receptor densities in the nucleus accumbens compared to controls, similar outcomes were identified with D2 receptor densities. Therefore, the DA receptor based findings of McBride and Hemmings (2005) appear to be reflected in the SBR data reported in the later study by Roberts et al., (2015).

Moreover, studies such as Roberts et al., (2015) who also collected data through a Questionnaire given to horse owners, had opposite results to this study in regard to Docility and SBR Mean. In this study the conclusion was that NTB Horses had a higher SBR Mean (See table 2 in results) compared to Control horses. However, in comparison to Roberts et al., (2015) study, horses who had stereotypic behaviours showed lower SBR Mean compared to Control horses. This is surprising as one would expect horses with Narcoleptic-like behaviours who in some cases also showed stereotypical behaviours, to show a slower SBR collection result in

comparison to a control study horse. This difference in results may exist due to several reasons; one being a slower reaction to the flight or fight response in horses who show Narcoleptic-like behaviours due to dopamine and adrenal gland response. Secondly, being a possible alteration in sleep patterns. Is the quality of sleep from the horse adequate, which earlier in this study states that precaution was taken when choosing horses with Narcoleptic-like behaviours compared to sleep deprived horses, however this reason cannot be ignored.

This lends further credence to the conclusions drawn from the data reported herein, namely that the dopamine systems of the NTB and control do not differ significantly in terms of their functional properties. However, it is important to note that nine out of the thirteen NTB animals were reported by their owners to perform stereotypic behaviour. The link between the NTB and stereotypy phenotype should therefore be investigated in future studies. In particular, it has been reported that horses exhibiting stereotypy have different nocturnal habits (Clegg et al., 2008) which could point towards sleep anomalies as an underlying factor in NTB animals.

Moreover, Garner (2003) and Garner & Mason (2006) suggest that BIR is also another non-invasive investigation method into collecting Dopamine in the mammal. Aside from the dopaminergic correlates, the very nature of the BIR metric means that it can be used as an indicator of activity. Therefore it is surprising that given the links between sleep deprivation and NTB's recorded by other authors (i.e. Fuchs et al., 2016) that differences were not observed. Nevertheless, the lack of BIR differences occurring between NTB and control animals lends further support for the absence of DA dysfunction in the NTB cohort. The change of routine could have also affected the BIR results, as some horses were used to being turned out all hours; being brought in for data collection was out of routine. However, to make data collection equal and non-biased, all horses were given the same instruction as to being always stabled with access to forage and water (to allow minimum human error as possible).

Schultz (2002) and Schultz (2007) states that Dopamine is the major catecholamine neurotransmitter in the mammalian brain, with its main role being in cognitive processes, including motor function, working memory, reward-related decision making and behavioural change. Crib biting horses have been studied in similar

ways to this study, with data collection of SBR and BIR. Hemmings, McBridge & Hale., (2007) suggests that horses who crib bite have a higher SBR and BIR mean (elevated dopamine), than that of the control horses compared. As in this study, the first null hypothesis should in theory be rejected, however there was a slight difference between the narcoleptic behaviour horses compared to the control horses, just not enough to have a significant difference in this study. Further investigation should take place in regard to SBR and BIR with narcoleptic horses; minimal human error and environmental factors should be taken into consideration.

5.2 Findings Related to Heart Rate Variability

Furthermore, HR was also used as a non-invasive method to collect vital data from the horses studied to determine if HR would show a significant difference from a control horse to that which showed narcoleptic behaviours. Again, looking at relaxed horse showing narcoleptic behaviours, one would think in comparison to a control horse, there would be a significant difference in the overall HR mean, however the null hypothesis was accepted. Dishman et al., (2000) also found that vagal control of the heart has a connection to emotional stress in an animal. Therefore, when changing some horse's routine but stabling them for the data collection, this would have naturally increased the animals HR, altering the results to some extent. Moreover, studies such as Grimaldi et al., (2010) and Randle et al., (2017), suggest that mammals with NC showed an increased sympathetic drive on HR with an increased LF/HF ration, compared to the control used in that study. However, in this study, analysis revealed significant moderate to strong negative correlations between RMSSD and LF/HF ratio for both control and narcoleptic groups. This finding states that both Narcoleptic and Control horses showed similar HR results.

5.3 Temperament Differences Between Studied Groups

The third and final null hypothesis is that there will be no significant difference between temperament variables between control and Narcoleptic type horses, which is rejected as this was not the case. This can be seen in the results section in Table 2 and 3 in the results section of this study. There was a connection between Temperament variables and Physiological correlations in both control horses and

Narcoleptic studies, however more so in Narcoleptic. It may be concluded from these results that; Narcolepsy affects the management of the horse as regards to both competitiveness and the rideability of the horse. Furthermore, in table 3, the comparison of temperament variables between control and narcoleptic groups shows that the Narcoleptic horses displayed significantly lower curiosity, vigilance, tenseness, and consistency scores compared to control equivalents. On the other hand, scores for co-operation were significantly higher for narcoleptic versus control. This is significant as findings from Roberts et al., (2015) stated that temperament questionnaires are beneficial for the findings of animals, as owners can describe animals' personalities to the best of their abilities (which can help determine temperament variables), which can help in the development of studies that require further investigation.

Moreover, results shown in Table 2 and 3 says that NTB horses show lower vigilance and tenseness in this study compared to control. This result can be compared to a study carried out on humans by Sachs & Levander (1981), which discusses the personality traits in patients with Narcolepsy. Which states that patients who have Narcolepsy show higher scores in anxiety, compared to control patients and patients with Narcolepsy had unexpectedly low scores in some socialisation scales. In comparison to this study in horses, which shows significantly lower levels in tenseness and vigilance. This difference may exist due to sleep patterns, if the horse has had irregular sleep patterns and showing Narcoleptic-like behaviours regularly, then the horse could result in a more relaxed demeaner as the horse could be like that of a human who is slow to react to a stressful situation due to lack of sleep and therefore a slow response in the brain is a result.

5.4 Correlations Between Studied variables

In relation to this study into narcolepsy, there is a particularly interesting negative correlations between SBR / BIR and LF/HF ratio in control but NOT in NTB horses. This means that the dopamine systems of the control horses are linked to the autonomic nervous system (see Appendix 3, Figure 4). However, the findings implies that this is not the case for NTB counterparts. Through this there might be a connection between the brain and a peripheral link in Control not NTB's. Further research into this must be carried out to understand further.

5.5 Implications for the Training & Management of Horses that show Narcoleptic Behaviours

Owners and trainers will want to understand if this behaviour shown (through data collection) will have any influence on the horse's performance, temperament and any sale potentials. This study should help owners and trainers to understand and manage horses with Narcoleptic behaviour, and avoid its being labelled as a 'stable vice?' Possibly more importantly, the fact of narcolepsy may be an indicator of raised dopamine levels, and this may provide important insights as to the horse's management. Roberts et al., (2016) states that horses with higher levels of dopamine, demonstrate distinct neuro-cognitive differences, and this should have an impact on the type of management and training these horses need. For instance, horses with high dopamine levels in a jumping competition may pre-empt the next move/jump and perform too soon (hitting a pole or turning into the wrong fence), resulting in a lower competition score. Similarly, if a horse with high dopamine levels has learned to perform a specific behaviour for a reward (pressure and release), it may be difficult to stop this behaviour once it has been learned, even in inappropriate situations such as a competition. Therefore, trainers and owners of these horses should be made aware and or educated on these cognitive attributes of horses who may have higher dopamine levels and train them appropriately. This not only means to not over train them, but also means avoiding instilling patterns which reinforce compulsive and impulsive behaviours.

A study carried out by Sivakumar (2016), suggests that children diagnosed with Narcolepsy may show a change in personality. Sivakumar (2016) states that 'children and adolescents with narcolepsy often show changes in personality and behaviour, irritability or aggression, poor school performance and have significantly higher rates of depression'. This finding corresponds with those reported herein as owners and trainers will want to find out if the temperament of the horse is at all affected by this narcoleptic-like behaviour. As suggested, patterns to sleep and quality of REM sleep is very important for any living being as this has proven to affect the behaviour (Sivakumar (2016), therefore, a further study into equine narcolepsy could be the collection and observation of the quality of sleep so that owners and trainers can be aware of its effects.

Although different to Roberts et al., (2016) study, this study into Equine Narcolepsy did not find concrete evidence that horses with performed narcoleptic behaviours have significantly higher dopamine levels compared to the control horses. However as many of the horses collected for study did show signs of stereotypic behaviour, there may be a link between the way the stereotypic horses' brains and narcoleptic horses' brains function.

Moreover, the application of SBR data collection within the horse training and ownership should be considered of a method of examining dopamine physiology to train suitably, especially if any behavioural changes become more obvious as management of the training continues, then there may be indications of adaptations in dopamine physiology. furthermore, due to the SBR/ temperament connections recounted in the results section of this study; affordable and easily applied data collections such as this, could be introduced when buying a horse, at the stage of the pre-purchase veterinary inspections to access the horses suitability for the buyers chosen discipline.

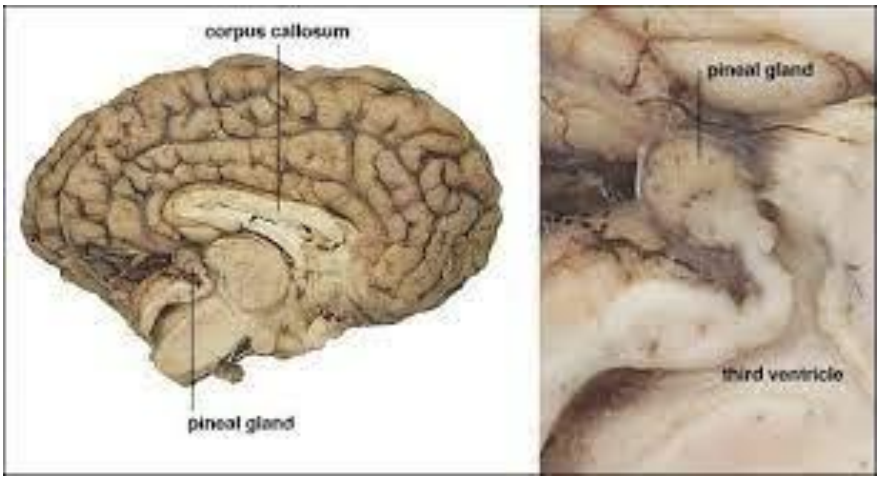
5.6 Pseudo-Narcolepsy

In addition, environmental factors suggested in the introduction of this study (such as quality of sleep), that could encourage apparently narcoleptic behaviour for example type of bedding (as well as depth), feed, length of time the horse is turned out in a field (the overall management of the horse and its routine) and the general health and wellbeing of the horse. In such cases, changes to the management or routine of the horse may address the apparent narcoleptic behaviour. The management factors can all be influenced by the owner of the horse. In regards to management, owners and with assistance from trainers if they so wish, should inform themselves and learn to the best of their abilities to research into how horses can gain and improve from certain changes, such as bedding and or hours spent out in the field. It is common that some horse owners may confuse equine sleep depression and narcolepsy, it is important to learn and understand the differences. A horse with narcolepsy can be seen to be awake one moment and collapse to the flaw the next, in comparison to sleep deprivation, where the horse gradually falls asleep. Studies carried out by Pitman, (2013) have been carried out with miniature horses. Narcolepsy can be a

rather intense behaviour to observe; unlike an animal with sleep deprivation, which will usually show tiredness before trying to sway and potentially collapsing, narcolepsy is quite sudden. Sleep deprivation can sometimes be a result from an underlying health condition such as Arthritis in the horses' joints (making it harder for the horse to lie down and get REM sleep), furthermore, it could be a result of not a deep enough established bed to lay down on (Kwiatkowska-Stenzel, Sowińska, and Witkowska., 2016). Another environmental factor that was used in the temperamental questionnaire was in regard to seasonality. Environmental insecurities could lead to sleep deprivation. An example could be the horse being too hot from being over rugged, and therefore too uncomfortable to lie down. Horses' bodies can adapt brilliantly to its surroundings, therefore when top level competition horses compete abroad in a climate they are not used to, often owners and trainers will travel the horse weeks before competition day to acclimatise the horses body to the heat and humidity. It is common that horses do not commonly lie down in cold snowy conditions, in comparison to a warm sunny day, where it is more often someone may see a horse led down in the sun for a snooze. Every horse is individual and have its own sleep behaviours and patters, it is important for owners to take notice of this (Murphy et al., 2011).

The transmission mechanism between daylight levels and a horse's behaviour is the production of Melatonin. Melatonin is produced in the pineal gland (see Figure 1) and is primarily made during the dark hours and is produced less when a sufficient amount of sunlight and wavelength enters the eye of the mammal. As the seasons change, as does the length of day and therefore this can affect the production of melatonin by the pineal gland. Melatonin has been linked to the sleep–wake cycle in mammals, which may have another connection to seasonal change due to the length of day and night during a specific time of year. This provides a neuroendocrine signal (from clock – seasons, to the body) that communicates seasonal timing (Murphy et al., 2011).

FIGURE 1. Shows a cross section angle of an equine brain, pointing out the Pineal Gland and Corpus Callosum.



In addition, melatonin levels are found to be low during daylight hours and high throughout the night, as a result of study that has been conducted. This enables the horse to transform changes in the seasons into a hormonal signal. Therefore, allowing the horse prepare and adapt its body to the climate, for example, shedding its winter coat for the spring time of year. Older horses may struggle during this process and owners may have to manage their coats by clipping the horse when the weather turns warm (Equilume, 2017).

However, this topic on seasonality is very interesting and subjective feedback from the case studies owners would be an avenue for future research within this area of seasonality and linking it to narcoleptic-like behaviour. Given the significance of melatonin in regulating sleep/ wakefulness cycles, it would be important for further investigation into the operation of these systems in narcoleptic mammals.

Through genetic screening, it could also be possible to identify horses at risk of dopamine physiology adaptations. Genetic testing is well recognised in horse racing and is of huge importance in the yearling sales (for example at Ascott), but genetic testing should also be of crucial importance across all disciplines such as polo ponies, event horses and endurance horses. Equine genomics has led to a growth in the development of devices to help identify traits and diseases, to learn about evaluating gene expression (Finno & Bannasch, 2014). The environment has a huge influence on species such as the horse, and complex diseases may have a strong connection to polygenic traits, this can also vary due to physiologic factors such as age, breed, and sex, even in “normal” individuals. There are diseases currently (such as Wobblers Syndrome) in the horse with presumed heritable basis. However, there is not a genetic test currently available for Wobblers and therefore making it difficult

to understand if there is an inheritance connection (Finno & Bannasch, 2014). Through genetic screening the possibility of identifying horses at risk of dopamine physiology adaptations may be worth investigating. Studies into rodents carried out by Anstrom et al., (2009) suggests that exposure to environmental stressors, prior to dopamine elevation or loss, may be a possible test to carry out with horses. Again, this would mean that the owner/ carer of the horse would have to sign consent forms and an ethical evaluation would have to take place before any data collection. However, this may enable for a suitable management regime to take place for individual horses, in response to any findings. Overall, this may decrease any likelihood of stereotypy onsets.

Ultimately, further improved understanding of these dopaminergic conditions is needed. It is possible that this will allow for improvements to be made to the interaction between humans and horses, as well as allow for more favourable implications to be made towards the performance of the horse. Additionally, this will result in an enhanced equine welfare status.

5.7 Study Limitations

1. Larger sample sizes throughout would have made the results more representative to the horse population who show signs of narcolepsy. However, determining the difference between equine narcolepsy and sleep deprivation without a veterinarian support was a limitation to the population size for this study. In addition, it is essential to emphasise the wide range of horse breeds that were recruited in this investigation. This is because it is probable that breed-related variation played a role in lowering the probability of discovering meaningful results through genetics.
2. All horses were privately owned (not all on privately owned yards, some at livery) and as such were subject to the owner's own management regimes. Whilst steps were taken to ensure similarity when collecting data from each individual horse. For example, ensuring similar stabling and access to forage and water, however some horses were used to being turned out at all times so a change of routine and environment was essential for this particular study; this was outside of the researcher's control. Furthermore, future studies may be improved if owners consented to following a management regime given by

researcher prior to data collection, to investigate further investigation into differing management regimes.

3. The same researcher completed all observations; however, some level of human error would be a possibility thought this study during SBR and BIR observations. For improvement in this area for future research, technology such as a camera systems and accelerometers could be used for SBR and BIR observations to eliminate human error to the best of the researcher's ability.

5.8 Experiment Design for Future Research

Genetic testing such as the study through Orexin through blood tests with veterinarian support would allow for further investigation into specific breeds performing the narcoleptic behaviours (Burgess et al., 2010). Through genetic analysis on blood tests, one may compare results which will allow for more specific findings into the type of horse which may demonstrate equine narcolepsy. Through this, this can then help owners, breeders and trainers determine the purchase, reproduction, and training of individual horses.

Moreover, the importance of equine sleep quality is another important subject for future research into equine narcolepsy. Studies such as twenty four hour surveillance cameras on horses that are stabled could be a test, which would allow for further knowledge into equine sleep patterns. Hartman & Greenings., (2019) investigates the importance of the influence of auditory stimulation on the occurrence of nocturnal equine sleep-related behaviour in stabled horses. With this being a modern study into sleep behaviour patterns into equine REM sleep, a similar study into the different sleep patterns of that of a horse who shows narcoleptic behaviour to a control horse would be valuable data collection into this study of equine narcolepsy.

Likewise, dissection and further study into the equine brain would be hugely beneficial in this study into equine narcolepsy. If a horse's brain, who showed narcoleptic behaviour was compared to that of a case control horses brain, then physical size difference into the basal ganglia could be compared. However, this would not be possible, and any findings would not be significant, as a lack of samples being able to be used would be extremely limited. Moreover, the horse

owners of the study horses used, would have to agree to allow their horse to be used for scientific research and ethics form would have to be agreed.

Finally, in order to advance future research on relaxation, McBride et al., (2004) states that studies have shown that music and physiotherapy are related to opioid system activation. If the horses were observed while listening to music or receiving physiotherapy, data on their behavioural responses could be collected, and a controlled environment could act as another link to relaxation and narcolepsy in the horse. HR could also be collected during a controlled environment data collection as classical music could be used as a relaxant for the horse. This would be an extension onto what was discovered in this specific study, as stated in the temperament questionnaire, some narcoleptic horses performed this behaviour listening to music, for the farrier and the physio. Further research could be collecting BIR, HR and SBR data whilst this narcoleptic behaviour is being performed, however, allowing for an ethics form to be accepted when inducing this behaviour is sensitive.

6.0 Final Conclusions

SBR and BIR are both proven as a reliable indicator of dopamine transmission in other species (Roberts et al., 2016). However, in this study into equine narcolepsy no significant findings into SBR and BIR were concluded from the data collections. Moreover, HR was used to determine if a cardiovascular difference could be found among the case study horses however, again no significance was found. Finally, there was a clear significant finding with narcoleptic horses displaying significant lower curiosity, vigilance, tenseness, and consistency scores compared to control equivalents. On the other hand, scores for co-operation were significantly higher for narcoleptic versus control horses (see table 3 in the results section). Further work is required to investigate the importance of dopamine within equine narcolepsy and as mentioned, further research into BIR, SBR and genetic testing through blood tests should be used as a continuation to this study. The findings of this initial investigation will now pave the way to further understanding and development into prevalence and neural function in equine narcolepsy.

7.0 References

Alexander, G.E. and Crutcher, M.D. (1990) Functional Architecture of Basal Ganglia Circuits: Neural Substrates of Parallel Processing. *Trends in Neurosciences, Volume 13* (1), pages 266-271. https://doi.org/10.1016/0166-2236(90)90107-L

Ayvazyan, A. (2002*). Chemical Transmission in CNS and Antipsychotic Drugs Pt II.* Retrieved from, http://www.pitt.edu/~super1/lecture/lec6001/004.htm.

Bayer, H, M., Glimcher, P. W. (2005). Midbrain dopamine neurons encode a quantitative reward prediction error signal. *Neuron, Volume 47* (1), pages 129-141.

Belling, J. H. (1990). Sleep patterns in Horses. *Equine Practice, volume 12* (8), Pages 22-27.

Bhatia, A., Lenchner, J. R., Saadabadi, A. (2022). Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK538242/#:~:text=There%20are%20five%20types%20of,receptor%20has%20a%20different%20function.

Birt, M. A., Guay, K., Treiber, K., Ramirez, H. R., Snyder, D. (2015). The Influence of a Soft Touch Therapy Flowtrition on Heart Rate, Surface Temperature, and Behaviour in Horses. *Science Direct, volume 35* (8), pages 636-644.

Browne, S.E., Bowling, A.C., Macgarvey, U., Baik, M.J., Berger, S.C., Muquit, M.M., Bird, E.D. and Beal, M.F. (1997). Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. *Annals of neurology, volume 41* (5), pages 646-653.

Burgess, C. R., Tse, G., Gillis, L., Peever, J. H. (2010). Dopaminergic Regulation of Sleep and Cataplexy in a Murine Model of Narcolepsy. *National Library of Medicine, Volume 33* (10), pages 1295-1304.

Byjus. (2020). *Difference Between Sympathetic and Parasympathetic*. Retrieved from https://byjus.com/biology/difference-between-sympathetic-and-parasympathetic/#:~:text=The%20autonomic%20nervous%20system%20comprises,to%20a%20state%20of%20calm.

Canales, J.J., (2005) Stimulant-induced adaptions in neostriatal matrix and striosome systems: Transiting from instrumental responding to habitual behaviour in

drug addiction. *Neurobiology of Learning and Memory, Volume 83* (2), Pages 93-103.

Carson, K., Wood-Gush, D. G. M. (1983). Equine behaviour: II. A review of the literature on feeding, eliminative and resting behaviour. *Science Direct, Volume 10* (3), Pages 179-190.

Chen, E. Y., Lam, L. C., Chen, R. Y. & Nguyen, D. G. (1996) Blink rate, neurocognitive impairments, and symptoms in schizophrenia. *Biological Psychiatry, Volume 40* (7), Pages 597-603.

Chen, L., Brown, R. E., McKenna, J. T., McCarley, R. W. (2009). Animal Models of Narcolepsy. *National Library of Medicine, Volume 8* (4), Pages 296-308.

Chen, S.A., Chiang, C.E., Tai, C.T., Lee, S.H., Chiou, C.W., Ueng, K.C., Wen, Z.C., Cheng, C.C. and Chang, M.S., (1996). Longitudinal clinical and electrophysiological assessment of patients with symptomatic Wolff-Parkinson-White syndrome and atrioventricular node reentrant tachycardia. *Circulation, volume 93* (11), Pages 2023-2032.

Clegg, H. A., Buckley, P., Friend, M. A., McGreevy, P. D. (2008). The ethological and physiological characteristics of cribbing and weaving horses. *Science Direct, Volume 109* (1), Pages 68-76.

Clinical Veterinary Advisor. (2012). Retrieved from https://www.sciencedirect.com/science/article/pii/B9781416099796000866

Colzato, L.S., van den Wildenberg, W.P.M., Hommel, B. (2008) Reduced Spontaneous Eye Blink Rates in Recreational Cocaine Users: Evidence for Dopaminergic Hypoactivity. *Plos ONE, volume 3* (10), Page 3461.

Cushing's Disease News. (2017). *Brain Metabolism Altered in Patients with Cushing's Disease, Study Finds*. Retrieved from https://cushingsdiseasenews.com/2017/12/05/brain-metabolism-altered-in-patients-with-cushings-disease-study-finds/

Dishman, R.K., Nakamura, Y., Garcia, M.E., Thompson, R.W., Dunn, A.L. and Blair, S.N., (2000). Heart rate variability, trait anxiety, and perceived stress among

physically fit men and women. *International Journal of Psychophysiology, volume 37* (2), Pages 121- 133.

Equilume. (2017). *How Light Signals the Breeding Season to the Mare*. Retrieved form https://equilume.com/equine-reproduction/light/

Fortin, J.S., Hetak, A. A., Duggan, K. E., Burglass, C. M., Penticoff, H. B., Schott, H. C. (2021). *Equine pituitary pars intermedia dysfunction: a spontaneous model of synuclein Pathy. Natural Library of Medicine.* Received from, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8346493/

Frank, M. J., Seeberger, L. C., O'Reilly, R. C. (2004). By Carrot or by Stick: Cognitive Reinforcement Learning in Parkinsonism. *Science Direct, volume* 306 (1), Pages 1940–1943.

Fronczek, R & Thijs, R. D. (2013). Autonomic Alterations in Narcolepsy—Contrasting Results in Mice and Men. *Science Direct, Volume 36* (1), Pages 9-10.

Fuchs, C., Kiefner, C., Reese, S., Erhard, M., Wöhr, A. C. (2016). Narcolepsy: Do Adult Horses Really Suffer from a Neurological Disorder or Rather from a Recumbent Sleep Deprivation/Rapid Eye Movement (REM)-Sleep Deficiency? *Equine Veterinary Journal, Volume 48* (50), Pages 9-9.

Garner, J.P., (2006) Perseveration and stereotypy- systems level insights from clinical psychology In: *Mason, G. & Rushan, J., eds. Stereotypic Animal Behaviour Fundamentals and Applications to welfare, Volume 2* (1), Pages 121-153.

Garner, J.P., Mason, G.J. (2002). Evidence for a relationship between cage stereotypies and behavioural disinhibition in laboratory rodents. *Behavioural Brain Research, Volume 136* (1), Pages 83-92.

Garner, J.P., Mason, G.J., Smith, R., (2003) Stereotypic route tracing in experimentally caged songbirds correlates with general behavioural disinhibition. *Animal Behaviour, volume 66* (4), Pages 711-727.

Gerfen, C. R., Bolam, J. P. (2010). Chapter 1 - The Neuroanatomical Organization of the Basal Ganglia, *Science Direct, Volume 20* (1), Pages 3-28. Retrieved from https://www.sciencedirect.com/science/article/pii/B9780123747679000019

Gerra, G., Zaimovic, A., Timpano, M., Zambelli, U., Delsignore, R. and Brambilla, F. (2000) Neuroendocrine correlates of temperamental traits in humans. *Psych neuroendocrinology, Volume 25* (5), Pages 479-496.

Goetz, C.G., Poewe, W., Dubois, B., Schrag, A., Sterm, M.B., Lang, A.E., LeWitt, P.A., Fahn, S., Jankovic, J., Warren-Olanow, C., Martinez-Martin, P., Lees, A., Rascol, O., van Hilten, B., Stebbins, G.T., Holloway, R., Nyenhuis, D., Sampaio, C., Dodel, R., Kulisevsky, J., Tilley, B., Leurgans, S., Teresi, J., Shaftman, S., LaPelle, N. (2008). *MDS-UPDRS. Movement Disorders*. Retrieved from http://www.movementdisorders.org/MDS-Files1/PDFs/MDS-UPDRS-RatingScales/NewUPDRS7308final.pdf

Greening, L., McBride, S. (2017). *A Review of Equine Sleep: Implications for Equine Welfare.* Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9428463/

Grimaldi, D., Pierangeli, G., Barletta, G., Terlizzi, R., Plazzi, G., Cevoli, S., Franceschini, C., Montagna, P., Cortelli, P. (2010). Spectral analysis of heart rate variability reveals an enhanced sympathetic activity in narcolepsy with cataplexy. *National Library of Medicine, Volume 121* (7), Pages 1142- 1147.

Guthrie, M., Myers ,C. E ., Gluck, M. A. (2009). A Neurocomputational model of tonic and phasic dopamine in action selection: A comparison with cognitive deficits in Parkinson's disease. *Natural Library of Medicine, volume 200* (1), Pages 48-59.

Hartman, N., Greening, L. M. (2019). A Preliminary Study Investigating the Influence of Auditory Stimulation on the Occurrence of Nocturnal Equine Sleep-Related Behaviour in Stabled Horses. *Science Direct, Volume 82* (1), Pages 1-4.

Hemmings, A., McBride, S. D., Hale, C. E. (2007). Perseverative responding and the aetiology of equine oral stereotypy. *Science Driect, Volume 104* (1-2), Pages 143-150.

Holland, P. C., & Rescorla, R. A. (1975). The effect of two ways of devaluing the unconditioned stimulus after first- and second-order appetitive conditioning. *Journal of Experimental Psychology: Animal Behaviour Processes, 1*(4), Pages 355–363. https://doi.org/10.1037/0097-7403.1.4.355

Horse & Hound. (2012). *Understanding narcolepsy in horses.* Retrieved from https://www.horseandhound.co.uk/horse-care/vet-advice/understanding-narcolepsy-in-horses-314135

Issaoui, L. (2011) *Further investigation into cognitive alterations and the equine oral stereotype phenotype. BSc Dissertation, Royal Agricultural University.*

Karson, C.N., (1983). Spontaneous eye-blink rates and dopaminergic systems. *Brain, Volume 106* (3), Pages, 643-653.

Kelemen, Z., Grimm, H., Long, M., Auer, U., Jenner, F. (2021). Recumbency as an Equine Welfare Indicator in Geriatric Horses and Horses with Chronic Orthopaedic Disease. *National Library of Medicine, Volume 11* (11), Pages 3189-3189.

Khor, S.P., Hsu, A. (2007). The pharmacokinetics and pharmacodynamics of Levodopa in the treatment of Parkinson's disease. *Current Clinical Pharmacology, Volume 2* (1), Pages 234-243.

Klerk, J. D. (2019). Sleep deprivation in horses. *Veterinary Practice Today, volume 7* (3), Pages 38-40.

Lanciego, J. L., Luquin, N., Obeso, J. A. (2012). Functional Neuroanatomy of the Basal Ganglia. *National Library of Medicine, Volume 2* (12), Pages 9621- 9622.

Lewis, M.H., Presti, M.F., Lewis, J.B. & Turner, C.A. (2006) The Neurobiology of Stereotypy I: Environmental Complexity. *CABI International, Volume 2* (1), Pages 190-227.

Lindstrom, T., Redbo, I. (2000). Effect of feeding duration and rumen fill on behaviour in dairy cows. *Applied Animal Behaviour Science, Volume 70* (1), Pages 83-97.

Longstreth, W. T., Koepsell, T. D., Ton, T. G., Hendrickson, A. F., Van Belle, G. (2007). The epidemiology of narcolepsy. *Sleep, volume 30* (1), Pages 13–26.

Ludvikova, E., Nishino, S., Sakai, N., Jahn, P. (2012). Familial narcolepsy in the Lipizzaner horse: a report of three fillies born to the same sire. *Veterinary Quarterly, Volume 32* (2), Pages 99-102.

Lunn, D. P., Cuddon, P. A., Shaftoe, S., Archer, R. M. (1993). Familial occurrence of narcolepsy in Miniature Horses. *Equine Veterinary Journal BEVA, Volume 25* (6), Pages 483-487.

Macpherson, T., Morita, m., & Hikida, T. (2014). Striatal direct and indirect pathways control decision-making behaviour. *Frontiers in Psychology*. Retrieved from https://www.frontiersin.org/articles/10.3389/fpsyg.2014.01301/full

Mahoney, C. E., Cogswell, A., Koralnik, I. J., Scammell, T. E. (2019). The neurobiological basis of narcolepsy. *Nat Rev Neurosci, volume 20* (2), Pages 83-93.

Marsden, C.D.(1982). Basal ganglia disease. *The Lancet, volume 320* (8308), Pages 1141-1147.

Matthews, N. S., & Carroll, G. L. (2007). *Review of Equine Analgesics and Pain Management.* Retrieved from https://aaep.org/sites/default/files/issues/proceedings-07proceedings-z9100107000240.pdf

McBride, S. D., Parker, M. O., Roberts, K., Hemmings, A. (2017). Applied neurophysiology of the horse; implications for training, husbandry and welfare. *Science Direct, Volume 190* (1), Pages 90-101.

McBride, S. D., Roberts, K., Hemmings, A. J., Ninomiya, S., Parker, M. O. (2022). The impulsive horse: Comparing genetic, physiological and behavioural indicators to those of human addiction. Science Direct, Volume 254 (1), Pages 113896.

McCue, P. M. (2002). *Equine Cushing's disease.* Retrieved from https://europepmc.org/article/med/12516933

McFarlane, D., Donaldson, M.T., Saleh, T.M., Cribb, A.E. (2003). The Role of Dopaminergic Neurodegeneration in Equine Pituitary Pars Intermedia Dysfunction (Equine Cushing's Disease). 4*9th Annual Convention of the American Association of Equine Practitioners.* American Association of Equine Practitioners.

Merims D., Giladi, N. (2008). Dopamine dysregulation syndrome, addiction, and behavioural changes in Parkinson's disease. *Science Direct, Volume 14* (4), Pages, 273–280.

Missale, C., Nash, S. R., Robinson, S. W., Jaber, M. & Caron, M. G. (1998) Dopamine receptors: from structure to function. Physiological Reviews, 78 (1), Pages 189- 225

Murphy, B. A., Martin, A. M., Furney, P., Elliott, J. A. (2011). *Absence of a serum melatonin rhythm under acutely extended darkness in the horse.* Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3103467/

NHS (2019). *Narcolepsy.* Retrieved from https://www.nhs.uk/conditions/narcolepsy/causes/#:~:text=Many%20cases%20of%20narcolepsy%20are,the%20brain%20that%20produce%20hypocretin.

Nishino, S. (2003). The hypocretin/orexin system in health and disease. *Science Direct, volume 54* (2), Pages 87-95.

Olguín, H. J., Guzmán, D. C., García, E. H., Mejía, G. M. (2016). The Role of Dopamine and Its Dysfunction as a Consequence of Oxidative Stress. *National Library of Medicine, Volume 20* (16), Pages 1-20.

Papich, M. G. (2021). Pergolide, Pergolide Mesylate. *Science Direct, Volume 1* (1), Pages 718-720.

Pitman, K. (2013). *Horse Sport*. Retrieved from https://horsesport.com/magazine/health/equine-sleep-disorders/

Presti, M.F., Lewis, M.H. (2005). Striatal opioid peptide content in an animal model of spontaneous stereotypic behaviour. *Behavioural Brain Research, Volume 157* (1), Pages 363-368.

Randle, H., Steenbergen, M., Roberts, K., Hemmings, A. (2017). The use of the technology in equitation science: A panacea or abductive science? Science Direct, Volume 190 (1), Pages 57-73.

Roberts, K., Hemmings, A. J, Moore-Colyer, M., & Hale, C., (2015). Cognitive differences in horses performing locomotor versus oral stereotypic behaviour. *Applied Animal Behaviour Science, volume 168* (1), Pages 37-44.

Roebel, A.M. & MacLean, W.E. (2007) Spontaneous eye-blinking and stereotyped behaviour in older persons with mental retardation. *Research in Developmental Disabilities, volume 28* (1), Pages 37-42.

Sachs, C., Kaijser L. (1982). Autonomic regulation of cardiopulmonary functions in sleep apnea syndrome and narcolepsy. *Sleep, Volume 5* (3), Pages 227–238.

Sachs, C., Levander, S. (1981). Personality traits in patients with narcolepsy. *Science Direct*, *Volume* 2 (4), Pages 319- 324.

Salat, D., Tolosa, E. (2013). Levodopa in the treatment of Parkinson's disease: current status and new developments. *Journal of Parkinson's Disease, Volume 3* (1), Pages 255-269.

Schubert, T. (2019). *Nervous System Disorders and Effects of Injuries in Horses.* Retrieved from https://www.msdvetmanual.com/horse-owners/brain,-spinal-cord,-and-nerve-disorders-of-horses/nervous-system-disorders-and-effects-of-injuries-in-horses

Schultz, W. (1998). Predictive reward signal of dopamine neurons. *Journal of Neurophysiology, Volume 80* (1), Pages 1-27.

Schultz, W. (2002). Getting Formal with Dopamine and Reward. *Neuron, Volume 36* (1), Pages 241-263.

Schultz, W., (2007). Behavioural dopamine signals. *Trends in Neuroscience, Volume 30* (1), Pages 203-210.

Schultz, w., Dayan, P., Montague, P.R. (1997). A neural substrate of predication and reward. *Science Direct, Volume 275* (1), Pages 1593-1599.

Sivakumar, M. (2016). Change in personality and daytime sleepiness in a seven-year-old boy. *Science Direct, Volume 21* (2), Pages 71- 72.

Steiner, H., Gerfen, C.R. (1998). Role of dynorphin and enkephalin in the regulation of striatal output pathways and behaviour. *Experimental Brain Research, Voulem 123* (1), Pages 60-76.

Stocco, A., Lebiere, C., Anderson, J.R. (2010). Conditional Routing of Information to the Cortex: A Model of the Basal Ganglia's Role in Cognitive Coordination. *Psychological Review, volume 117* (2), Pages 541–74.

Stucke, D., Ruse, M. B., Lebelt, D. (2015). Measuring heart rate variability in horses to investigate the autonomic nervous system activity – Pros and cons of different methods. *Applied Animal Behaviour Science, volume 166* (1), Pages 1-10.

Szabo, S. T., Thorpy, M. J., Mayer, G., Peever, J. H., Kilduff, T. S. (2019). Neurobiological and immunogenetic aspects of narcolepsy: Implications for pharmacotherapy. *Science Direct, Volume 43* (1), Pages 23-36.

Taylor, I. L. (2021). *Impact of Training Method on Behavioural, Physiological, and Relationship Measures in Horses.* Retrieved from, https://vtechworks.lib.vt.edu/handle/10919/101781.

Van Camp, G., Flamez, A., Cosyns, B., Weytjens, C., Muyldermans, L., van Zandijcke, M., De Sutter, J., Santens, P., Decoodt, P., Moerman, C., Schoors, D. (2004). Treatments of Parkinson's disease with pergolide and relation to restrictive valvular heart disease*. The Lancet*, *Volume 36* (3), Pages 1179-1183.

Whittaker, X., Spoolder, H.A.M., Edwards, S.A., Lawrence, A.B., Corning, S. (1998). The influence of dietary fibre and the provision of straw on the development of stereotypic behaviour in food restricted pregnant sows. *Applied Animal Behaviour Science, Volume 61* (1), Pages 89-102.

Wickens, C. L. & Heleski, C. R. (2010) Crib-biting behaviour in horses: a review. *Applied Animal Behaviour Science, volume 128* (1), Pages 1-9.

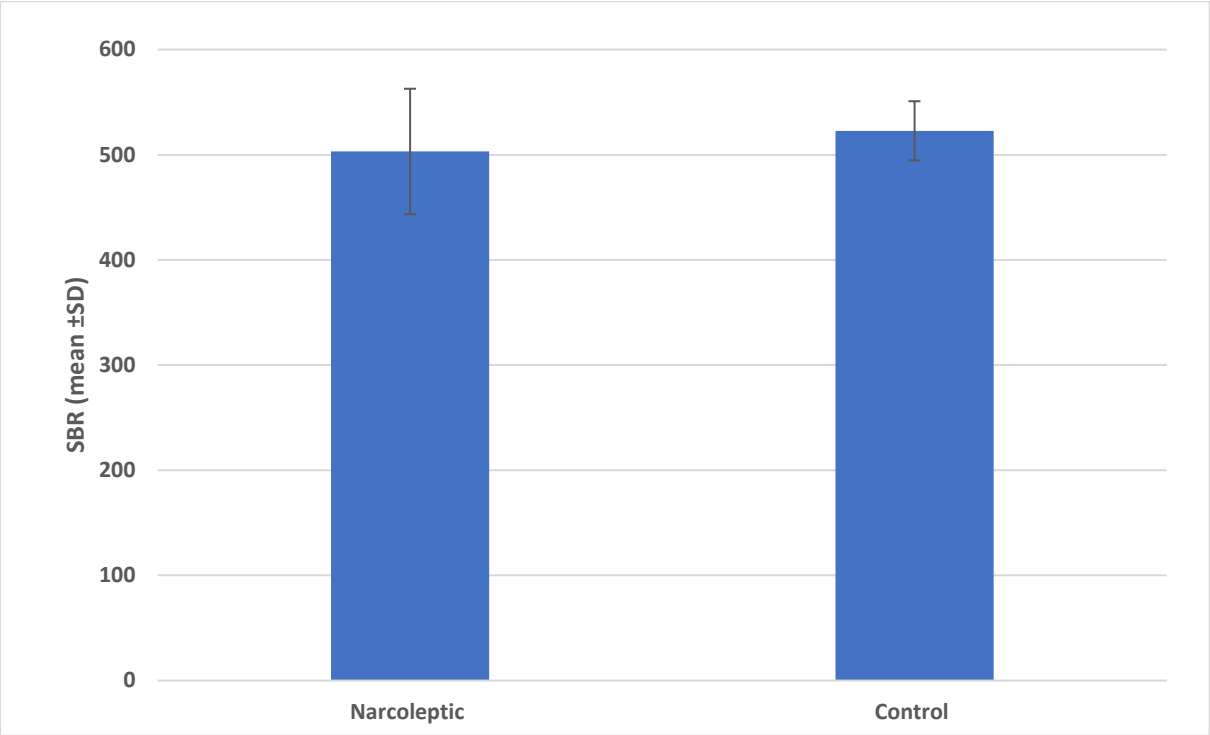
Yin, H.H. & Knowlton, B.J. (2006) The role of the basal ganglia in habit formation. *Nature Reviews Neuroscience, Volume 7* (6), Pages 464-476.

Young, A. (2021). *Narcolepsy.* Retrieved from https://ceh.vetmed.ucdavis.edu/health-topics/narcolepsy#:~:text=Clinical%20signs%20of%20narcolepsy%20can,exhibit%20swaying%20and%20frequent%20stumbling.

Appendices

Appendix 1: Comparison of Physiological Indicators**.**

Figure 1.



p>0.05, t=-1.08, df = 17.08

Figure 2.

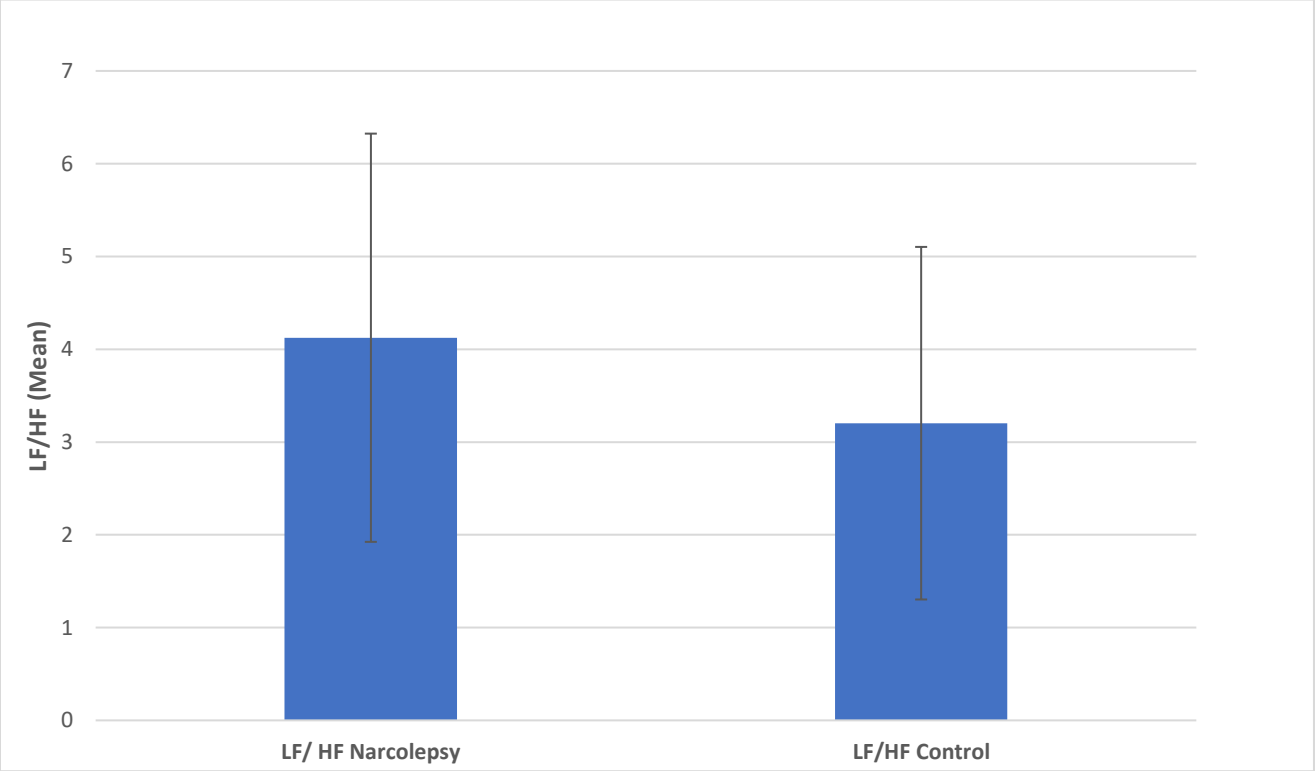
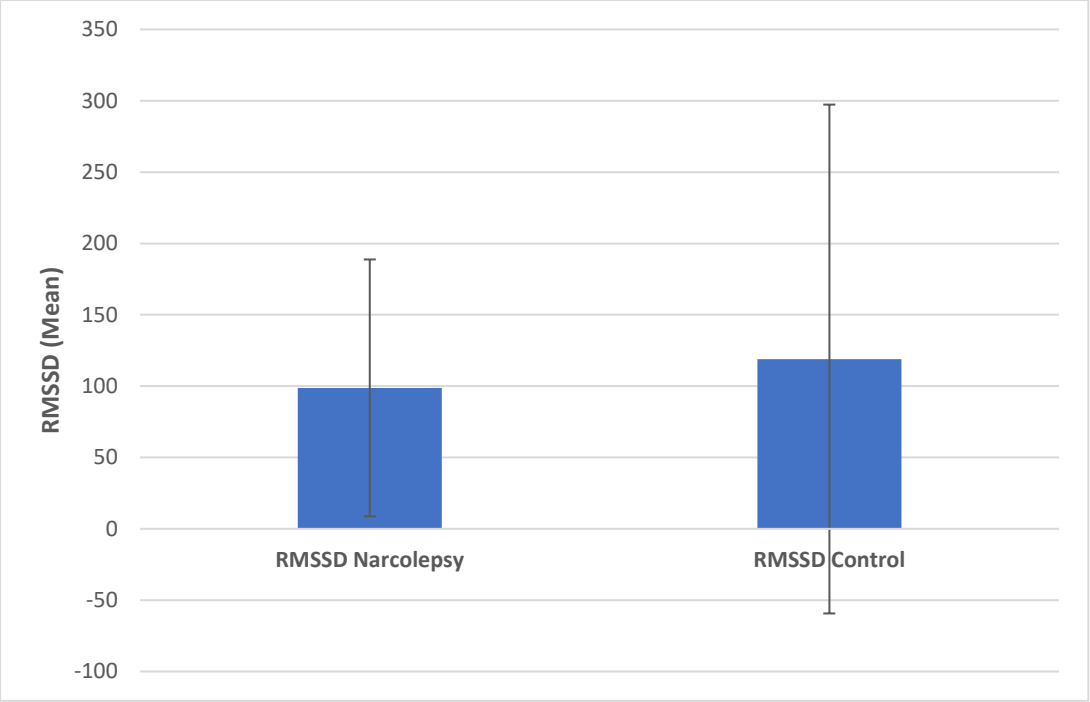


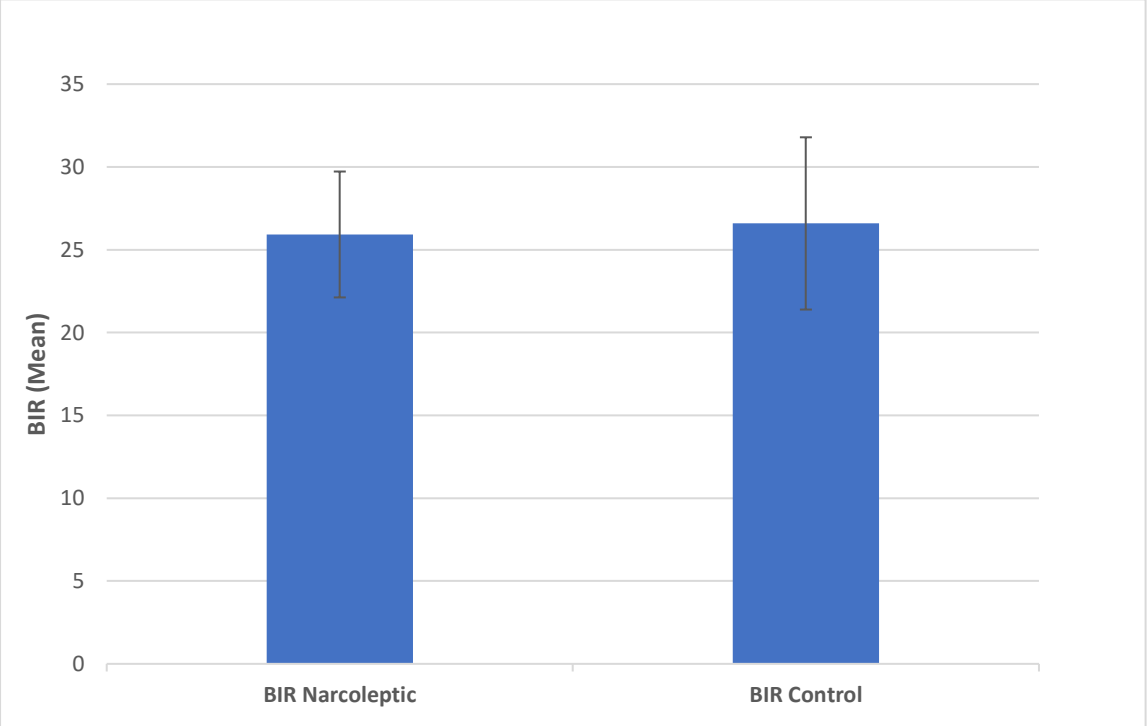
Figure 3.

P<0.05, t= 1.09, df= 24



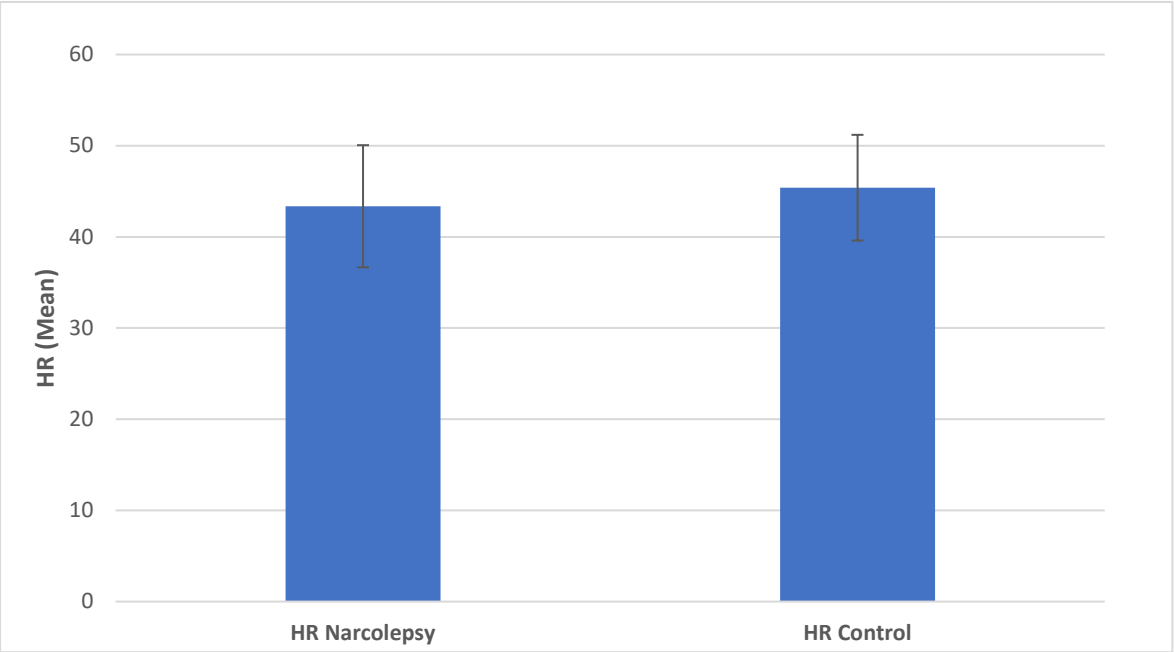
P<0.01, t= 4.50, df= 12

Figure 4.



p>0.05, t= 0.37, df =24

Figure 5.



p>0.05, t= 1.32,df= 12

Appendix 2: Owners Consent Form (Blank, to keep owners anonymous).

Animal Owner Consent Form

Consent:

- I have been given a full explanation by the investigators of the nature, purpose, location and likely duration of the study, and of what I will be expected to do. ☐

- I have been advised about any risks/possible ill-effects on *my / my animal's/s'* health and well-being which may result. I have been given the opportunity to ask questions on all aspects of the study and have understood the advice and information given as a result. ☐

- I agree for *my and my animal's/s'* anonymised data and their samples to be used for this study ☐

- I agree for *my and my animal's/s'* anonymised data and their samples to be used for future research that will have received all relevant legal, professional and ethical approvals. ☐

- Should I chose to withdraw *myself/ my animal(s)* from the study or any of my animals require to be withdrawn by the researchers I give consent to the use of data collected up to that point. ☐

- I agree for the researchers to contact me to provide me with a study results summary. ☐

- I understand that I am free to withdraw from the study at any time without needing to justify my decision, without prejudice and without my legal rights and or relationship with the RAU being affected. ☐

- I confirm that I have read and understood the above and freely consent to participating in this study. I have been given adequate time to consider my participation. ☐

Name of participant (BLOCK CAPITALS)

.....

Signed

.....

Date

.....

Name of researcher/person* taking consent

….....

(BLOCK CAPITALS)

Signed …….....

Date

……..

Appendix 3: Full Original Data Collection Results from Temperament Questionnaire, HR, SBR and BIR.

Figure1. Significant Findings from Control Horses Results from Temperament Questionnaire (With Key in Yellow).

Control Variables	Correlation Coefficient	P Value	Significance			
LF/HF mean Vs. BIR Mean	-0.688	0.003	**		*	p<0.05
Concentration Vs. Aperhensive	-0.76	0.001	***		**	p<0.01
Confident Vs. Aperhensive	-0.733	0.001	***		***	p<0.001
Curiosity Vs. Competitivness	-0.618	0.007	**			
Eccentric Vs. Age	0.59	0.009	**			
Friendliness Horse Vs. Calm & Nervous	-0.519	0.018	**			
Hardworking Vs. Aperhensive	-0.561	0.012	**			
Inconsistant Vs. Active	0.637	0.005	**			
Inconsistant Vs. Aperhensive	0.752	0.001	***			
Irritable Vs. Aperhensive	0.664	0.004	**			
Self Reliance Vs. Aperhensive	-0.591	0.009	**			
Self Reliance Vs. Calm & Nervous	-0.67	0.003	**			
Memory Vs. BIR Mean	0.682	0.003	**			
Panic Vs. Age	-0.593	0.008	**			
Panic Vs. Aperhensive	0.603	0.008	**			
Panic Vs. Calm & Nervous	0.618	0.007	**			
Patiance Vs. BIR Mean	0.621	0.006	**			
Reliable Vs. Aperhensive	-0.909	0	***			
Self Reliance Vs. Aperhensive	-0.591	0.009	**			
Self Reliance Vs. Calm & Nervous	-0.67	0.003	**			
Skittishness Vs. Calm & Nervous	0.632	0.006	**			
Slow Vs. Active	-0.711	0.002	**			
Slow Vs. BIR Mean	0.749	0.001	***			
Solitary Vs. Aperhensive	0.668	0.004	**			
Solitary Vs. Calm & Nervous	0.607	0.008	**			
Tense Vs. Aperhensive	0.675	0.003	**			
Trainability Vs. Age	0.673	0.003	**			
Trainability Vs. Aperhensive	-0.751	0.001	***			
Vigilance Vs. Aprehensive	0.628	0.006	**			
Confident Vs. Concentraition	0.558	0.012	**			
Cooperation Vs. Concentration	0.633	0.006	**			
Cooperation Vs. Confident	0.758	0.001	***			
Friendliness horse Vs. Concentration	0.607	0.008	**			
Friendliness people Vs. Concentration	0.607	0.008	**			
Hardworking Vs. Concentraition	0.615	0.007	**			
Inconsistant Vs. Concentration	-0.61	0.007	**			
Inconsistant Vs. Confident	-0.69	0.003	**			
Inconsistant Vs. Cooperation	-0.818	0	***			
Irritable Vs. Concentration	-0.616	0.007	**			
Memory Vs. Cooperation	0.799	0	***			
Opportunistic Vs. Cooperation	-0.559	0.012	**			
Panic Vs. Confident	-0.836	0	***			
Panic Vs. Cooperation	-0.704	0.002	**			
Patiancce Vs. Cooperation	0.734	0.001	***			
Reliable Vs. Concentration	0.618	0.007	**			
Reliable Vs. Confident	0.639	0.005	**			
SBR Mean Vs. Confident	-0.569	0.011	**			
SBR Mean Vs. Docility	0.681	0.003	**			
Self Reliance Vs. Concentration	0.704	0.002	**			
Self Reliance Vs. Confident	0.593	0.008	**			
Self Reliance Vs. Cooperation	0.559	0.012	**			
Skittishness Vs. Concentration	-0.557	0.012	**			
Sociable Vs. Curiosity	0.719	0.002	**			
Solitary Vs. Concentration	-0.738	0.001	***			
Subordinate Vs. Eccentric	-0.63	0.006	**			
Tense Vs. Concentration	-0.59	0.009	**			
Trainability Vs. Concentration	0.622	0.028	*			
Trainability Vs. Confident	0.655	0.014	**			
Trainability Vs. Cooperation	0.608	0.093	*			
Hardworking Vs. Fearful	-0.615	0.007	**			
Impulsive Vs. Fearful	0.626	0.006	**			
Irritable Vs. Hardworking	-0.584	0.01	**			
Panic Vs. Hardworking	-0.632	0.006	**			
Skittishness Vs. Hardworking	-0.664	0.004	**			
Irritable Vs. Intelligent	-0.611	0.007	**			
Panic Vs. Inconsistant	0.595	0.008	**			
Patiance Vs. Memory	0.929	0	***			
Popular Vs. Intelligent	0.592	0.009	**			
Protective Vs. Impulsive	-0.601	0.008	**			
Reliable Vs. Inconsistant	-0.645	0.005	**			
RMSSD Mean Vs. LH HF Mean	-0.621	0.006	**			
SBR Mean Vs. LH HF Mean	-0.56	0.012	**			
Self Reliance Vs. Impulsive	-0.732	0.001	***			
Skittishness Vs. Impulsive	0.883	0	***			
Skittishness Vs. Memory	-0.614	0.007	**			
Sociable Vs. Impulsive	-0.681	0.003	**			
Stubborness Vs. LF HF Mean	0.606	0.008	**			
Tense Vs Inconsistant	0.726	0.002	**			
Tense Vs. Irritable	0.601	0.008	**			
Vigilance Vs. Irritable	0.565	0.011	**			
Self Reliance Vs. Patiance	0.664	0.004	**			
Skittishness Vs. Patiance	-0.581	0.01	**			
Slow Vs. Patiance	0.616	0.007	**			
Subordinate Vs. Permissive	-0.628	0.006	**			
Timid Vs. Playful	-0.551	0.013	**			
Trainability Vs. Panic	-0.598	0.008	**			
SBR Mean Vs. Reliable	-0.521	0.017	**			
Skittishness Vs. Protectivness	-0.572	0.011	**			
Sociable Vs. Skittishness	-0.587	0.009	**			
Solitary Vs. Self Reliance	-0.728	0.002	**			
Stubborness Vs. RMSSD Mean	-0.758	0.001	***			
Subordinate Vs. SBR Mean	0.48	0.161	*			
Suspicious Vs. Reliable	-0.748	0.001	***			
Tense Vs. Reliable	-0.75	0.001	***			
Timid Vs. RMSSD Mean	0.471	0.026	**			
Trainability Vs. Self Reliance	0.67	0.003	**			
Tense Vs. Suspicious	0.633	0.006	**			
Vigilance Vs. Trainability	-0.594	0.008	**			

Figure 2. Significant findings from NTB Horses, from Temperament Questionnaire (With Key in Yellow, see Figure 1).

Narcoleptic			
Variables	Correlation Coefficient	P Value	Significane
Age Vs. Active	-0.72	0.002	**
Calm & Nervous	0.721	0.002	**
Concentration Vs. Aperhensive	-0.754	0.001	***
Confident Vs. Aperhensive	-0.605	0.01	**
Docility Vs. Aperhensive	-0.652	0.006	**
Excitability Vs. Active	0.574	0.013	**
Fearful Vs. Calm & Nervous	0.716	0.003	**
Impulsive Vs. Aperhensive	0.861	0	***
Impulsive Vs. Calm & Nervous	0.598	0.011	**
Inconsistant Vs. Aperhensive	0.797	0.001	***
Irritable Vs. Aperhensive	0.831	0	***
Irritable Vs. Calm & Nervous	0.711	0.003	**
Panic Vs. Aperhensive	0.729	0.002	**
Patiance Vs. Aperhensive	-0.66	0.005	**
Protective Vs. Active	-0.697	0.004	**
Protective Vs. Age	0.708	0.003	**
Self Reliance Vs. Aperhencive	-0.903	0	***
Self Reliance Vs. Calm & Nervous	-0.665	0.005	**
Skittishness Vs. Aperhensive	0.702	0.003	**
Skittishness Vs. Calm & Nervous	0.621	0.008	**
Solitary Vs. Active	-0.59	0.011	**
Solitary Vs. Age	0.706	0.003	**
Stubborness Vs. Active	0.724	0.002	**
Tense Vs. Age	-0.613	0.009	**
Vigilance Vs. Aperhensive	0.62	0.009	**
Cooperation Vs. Competitivness	-0.581	0.012	**
Docility Vs. Concentratio	0.775	0.001	***
Docility Vs. Confident	0.618	0.009	**
Friendliness horse Vs. Competitivness	-0.806	0.001	***
Friendliness people Vs. Competitivness	-0.662	0.005	**
Hardworking Vs. Concentration	0.846	0	***
Impulsive Vs. Concentration	-0.776	0.001	***
Inconsistant Vs. Concentration	-0.672	0.005	**
Intelligent Vs. Concentration	0.666	0.005	**
Intelligent Vs.Confiden	0.614	0.009	**
Irritable Vs. Concentration	-0.761	0.001	***
Irritable Vs. Confident	-0.614	0.009	**
LF HF Mean Vs. Competitivness	-0.572	0.014	**
LF HF Mean Vs. Cooperation	0.652	0.006	**
Memory Vs. Curiosity	0.652	0.006	**
Panic Vs. Concentration	-0.551	0.017	*
Patiance Vs. Concentraition	0.746	0.002	**
Patiance Vs. Cooperation	0.66	0.005	**
Self reliance Vs. Concentration	0.802	0.001	***
Self reliance Vs. Confident	0.69	0.004	**
Skittishness Vs. Concentration	-0.588	0.011	**
Skittishness Vs. Confident	-0.727	0.002	**
Subordinate Vs. Competitivness	-0.93	0	***
Subordinate Vs. Cooperation	0.701	0.003	**
Tense Vs. Concentration	-0.693	0.004	**
Tense Vs. Confident	-0.687	0.004	**
To what extent does narcoleptic Vs. Competitivness	-0.785	0.001	***
To what extent does narcoleptic Vs. Concentration	-0.527	0.02	*
Trainability Vs. Concentration	0.567	0.014	**
Ecitability Vs. Docility	-0.576	0.013	**
Equable Vs. Eccentric	-0.737	0.002	**
Friendliness people Vs. Fearful	-0.625	0.008	**
Hardworking Vs. Docility	0.724	0.002	**
Hardworking Vs. Ecitability	-0.764	0.001	***
Impulsive Vs. Docility	-0.736	0.002	**
Intelligent Vs. Excitability	-0.588	0.011	**
Irritable Vs. Docility	-0.759	0.001	***
Panic Vs. Eccentric	0.676	0.005	**
Panic Vs. Equable	-0.687	0.004	**
Patiance Vs. Docility	0.761	0.001	***
Self reliance Vs. Docility	0.704	0.001	***
Skittishness Vs. Docility	-0.792	0.019	*
Solitary Vs. Ecitability	-0.573	0.013	**
Stubborness Vs. Eccentric	0.64	0.007	**
Stubborness Vs. Ecitability	0.623	0.008	**
Tense Vs. Docility	-0.631	0.007	**
Friendliness people Vs. Friendliness horse	0.841	0	***
Impulsive Vs. Friendliness People	-0.574	0.013	**
Inconsistant Vs. Impulsive	0.593	0.011	**
Irritable Vs. Hardworking	-0.718	0.003	**
Irritable Vs. Impulsive	0.728	0.002	**
Irritable Vs. Inconsistant	0.585	0.012	**
LF HF Mean Vs. Friendliness horse	0.542	0.018	*
LF HF Mean Vs. Friendliness People	0.55	0.017	*
Panic Vs. Inconsistant	0.901	0	***
Patiance Vs. Friendliness Horse	0.608	0.009	**
Patiance Vs. Friendliness People	0.819	0	***
Patiance Vs. Impulsive	-0.875	0	***
Playful Vs. Hardworking	-0.595	0.011	**
RMSSD Mean Vs. Hardworking	0.674	0.005	**
Reliable Vs. Impulsive	-0.568	0.014	**
Self reliance Vs. Impulsive	-0.918	0	***
Self reliance Vs. Inconsistant	-0.695	0.004	**
Skittishness Vs. Friendliness people	-0.684	0.004	**
Skittishness Vs. Hardworking	-0.607	0.009	**
Skittishness Vs. Impulsive	0.648	0.006	**
Subordinate Vs. Friendliness horse	0.845	0	***
Subordinate Vs. Friendliness People	0.778	0.001	***
To what extent does narcoleptic Vs. Friendliness horse	0.544	0.017	*
To what extent does narcoleptic Vs. Hardworking	-0.524	0.02	*
To what extent does narcoleptic Vs. Hardworking	-0.529	0.02	*
To what extent does narcoleptic Vs. Inconsistant	0.798	0.001	***
Trainability Vs. Friendliness people	0.556	0.016	**
Vigilance Vs. Friendliness People	-0.677	0.004	**
Vigilance Vs. Impulsive	0.838	0	***
Panic Vs. Irritable	0.646	0.006	**
Patiance Vs. Irritable	-0.662	0.005	**
Popular Vs. Irritable	-0.656	0.005	**
RMSSD Mean Vs. LF HF Mean	-0.594	0.011	**
Self reliance Vs. Irritable	-0.815	0	***
Skittishness Vs. Irritable	0.858	0	***
Subordinate Vs. LF HR Mean	0.619	0.009	**
Tense Vs Intelligent	-0.616	0.009	**
To what extent does narcoleptic Vs. Irritable	0.532	0.019	**
Trainability Vs. Intelligent	0.641	0.007	**
Protective Vs. Playful	-0.727	0.002	**
RMSSD Mean Vs. Playful	-0.575	0.013	**
Self reliance Vs. Panic	-0.654	0.006	**
Self reliance Vs. Patiance	0.821	0	***
Skittishness Vs. Patiance	-0.741	0.002	**
Stubborness Vs. Playful	0.744	0.002	**
Subordinate Vs. Patiance	0.697	0.004	**
To what extent does narcoleptic Vs. Panic	0.743	0.002	**
Trainability Vs. Patiance	0.649	0.006	**
Vigilance Vs. Patiance	-0.806	0.001	***
SBR Mean Vs. Reliable	-0.575	0.013	**
Skittishness Vs. Self reliance	-0.724	0.002	**
Sociable Vs. RMSSD Mean	-0.475	0.029	*
Stubborness Vs. Protevtive	-0.665	0.005	**
Stubborness Vs. RMSSD Mean	-0.594	0.011	**
Tense Vs. SBR Mean	0.555	0.016	*
Tense Vs. Self Reliance	-0.604	0.01	**
To what extent does narcoleptic Vs. RMSSD Mean	-0.468	0.03	*
To What Extent does narcoleptic Vs. SBR Mean	0.436	0.039	*
To what extent does narcoleptic Vs. RMSSD Mean	-0.498	0.025	*
Trainability Vs. Self Reliance	0.554	0.016	**
Suspicious Vs. Sociable	-0.701	0.003	**
Tense Vs. Stubboness	0.633	0.007	**
To what extent does narcoleptic Vs. Solitary	-0.442	0.037	*
Vigilance Vs. Skittishness	0.572	0.013	**
To what extent does narcoleptic Vs. Subordinate	0.627	0.008	**
To what extent does narcoleptic Vs. Tense	0.599	0.011	**
Trainability Vs. Suspicious	-0.608	0.009	**

Figure 3. The Key used to determine the Significance in Results.

Correlation Coefficient	Strength
0-2	No correlation between variables
2 to 4	weak positive correlation
4 to 6	weak to moderate correlation
6 to 8	moderate to strong
8+	strong

Figure 4. SBR, BIR, Heart Rate and NTB score.

Physiological Correlations	Correlation Coefficient	Significance	Control / NTB
RMSSD Mean Vs. LF HF Mean	-0.594	**	N
RMSSD Mean Vs. LH HF Mean	-0.621	**	C
SBR Mean Vs. LH HF Mean	-0.56	**	C
SBR Mean Vs. LH HF Mean	0.168	NS	N

Figure 5. Intercorrelations between temperament variables.

Temperament Correlations	Correlation Coefficient	Significance	Control / NTB
LF/HF mean Vs. BIR Mean	-0.688	**	C
Memory Vs. BIR Mean	0.682	**	C
Patiance Vs. BIR Mean	0.621	**	C
Slow Vs. BIR Mean	0.749	***	C
SBR Mean Vs. Confident	-0.569	**	C
SBR Mean Vs. Docility	0.681	**	C
SBR Mean Vs. Reliable	-0.521	**	C
Stubborness Vs. RMSSD Mean	-0.758	***	C
Subordinate Vs. SBR Mean	0.48	**	C
Timid Vs. RMSSD Mean	0.471	**	C
LF HF Mean Vs. Competitivness	-0.572	**	N
LF HF Mean Vs. Cooperation	0.652	**	N
To what extent does narcoleptic Vs. Competitivness	-0.785	***	N
To what extent does narcoleptic Vs. Concentration	-0.527	*	N
LF HF Mean Vs. Friendliness horse	0.542	*	N
LF HF Mean Vs. Friendliness People	0.55	*	N
RMSSD Mean Vs. Hardworking	0.674	**	N

To what extent does narcoleptic Vs. Friendliness horse	0.544	*	N
To what extent does narcoleptic Vs. Hardworking	-0.524	*	N
To what extent does narcoleptic Vs. Hardworking	-0.529	*	N
To what extent does narcoleptic Vs. Inconsistant	0.798	***	N
Subordinate Vs. LF HR Mean	0.619	**	N
To what extent does narcoleptic Vs. Irritable	0.532	**	N
To what extent does narcoleptic Vs. Irritable	0.532	**	N
RMSSD Mean Vs. Playful	-0.575	**	N
To what extent does narcoleptic Vs. Panic	0.743	**	N
SBR Mean Vs. Reliable	-0.575	**	N
Sociable Vs. RMSSD Mean	-0.475	*	N
Stubborness Vs. RMSSD Mean	-0.594	**	N
Tense Vs. SBR Mean	0.555	*	N
To what extent does narcoleptic Vs. RMSSD Mean	-0.468	*	N
To What Extent does narcoleptic Vs. SBR Mean	0.436	*	N
To what extent does narcoleptic Vs. RMSSD Mean	-0.498	*	N
To what extent does narcoleptic Vs. Solitary	-0.442	*	N
To what extent does narcoleptic Vs. Subordinate	0.627	**	N
To what extent does narcoleptic Vs. Tense	0.599	**	N

Figure 6. Control and NTB Results from HR, BIR, LF/HF & RMSSD Means.

	Control			
HR Mean	SBR Mean	BIR Mean	LF/HF Mean	RMSSD Mean
54.34133333	551.6666667	32.33333333	0.465073333	703.5366667
56.08766667	485.3333333	33.66666667	1.81618	103.633
48.57966667	572.6666667	32	0.976846667	126.35
39.10933333	541	27.33333333	1.943503333	36.36033333
47.72533333	541.3333333	27	6.218333333	37.223
42.18233333	526.3333333	21.66666667	3.424633333	54.94333333
51.028	513	19.33333333	4.1288	58.43066667
43.498	496.6666667	25	2.955666667	120.3633333
38.04	514.6666667	24	4.6637	43.435
41.27033333	477.3333333	17.66666667	6.745766667	37.81566667
41.28966667	516	23.33333333	2.313243333	80.272
40.01166667	553	32.33333333	1.246476667	59.77366667
46.95	507.6666667	30	4.7425	84.84266667
	Narcoleptic			
HR Mean	SBR Mean	BIR Mean	LF/HF Mean	RMSSD Mean
39.988	435	21.33333333	4.643866667	36.47966667
42.021	424.6666667	17.66666667	3.158716667	59.62733333
61.536	516.3333333	27.33333333	1.121353333	126.9266667
51.637	551	27.33333333	5.7228	77.74933333
43.94933333	477	28	7.038333333	48.796
38.09466667	392	22.66666667	5.675666667	95.012
40.94733333	581	30	3.049856667	386.7433333
37.73566667	502	26.66666667	1.593123333	78.06566667
38.81766667	556.6666667	27.66666667	7.019366667	63.96266667
38.53566667	565	30.66666667	7.683233333	47.51966667
45.13533333	560.6666667	29.33333333	2.753566667	72.89166667
39.57366667	493.6666667	22	2.387123333	82.783
45.68966667	485	26.33333333	1.7675	107.7776667

Figure 7. Data Collected from NTB and Control Horses to find out the significance of the P Value, gathered from the Temperament Questionnaire.

Temperament Variable	NTB	Control	Significance	P Value (less than 0.05)
Calm Vs. Nervous	2.69 ± 1.43	2.92 ± 1.89	NS	1
Concentration	7.23 ± 1.78	6.23 ± 2.04	NS	0.063
Self Reliance	6.31 ± 2.28	5.92 ± 1.89	NS	0.526
Excitability	5.46 ± 1.56	5.84 ± 1.99	NS	0.541
Trainability	7.61 ± 1.93	6.84 ± 1.41	NS	0.064
Friendliness-People	8.15 ± 1.14	8.53 ± 0.77	NS	0.413
Friedliness - Horse	7.46 ± 2.25	8.53 ± 0.77	NS	0.265
Curiosity	5.53 ± 1.85	6.92 ±1.71	S	0.013
Memory	7.15 ± 1.46	6.69 ± 1.93	NS	0.622
Panic	3.84 ± 1.95	3.61 ± 1.98	NS	0.704
Cooperation	7.93 ± 1.32	6.61 ± 1.55	S	0.028
Inconsistant	2.38 ± 1.61	3.61 ± 1.71	S	0.023
Stubborness	4 ± 2.41	4.76 ± 1.73	NS	0.323
Docility	6.46 ± 2.33	4.92 ± 1.8	NS	0.09

Vigilance	4.38 ± 2.18	6.15 ± 1.14	S	0.017
Patiance (Missing)	5.31 ± 2.39	5.38 ± 2.39	NS	0.491
Competitivness	4.92 ± 2.53	5.53 ± 1.81	NS	0.429
Skittishness	3.07 ± 1.55	3.31 ±1.60	NS	0.514
Timid	5.15 ± 2.11	5.76 ± 1.36	NS	0.374
Active	5.61 ± 2.53	6.23 ±1.53	NS	1
Impulsive	3.31 ± 1.70	3.53 ± 2.02	NS	0.926
Aprehensive	3.38 ± 2.32	4.07 ± 2.10	NS	0.279
Confident	7 ± 0.81	6.07 ± 2.10	NS	0.47
Eccentric	5.07 ± 2.69	4.38 ± 2.53	NS	0.404
Equable	5.23 ± 1.83	5.23 ± 1.78	NS	0.988
Fearful	3.46 ± 1.26	2.84 ± 1.14	NS	0.175
Irritable	3.46 ± 1.66	3.38 ± 1.50	NS	0.956
Sociable	7.23 ± 1.09	7.53 ± 0.96	NS	0.384
Opportunistic	4.69 ± 1.88	5.38 ± 1.98	NS	0.328
Permissive	5.30 ± 2.25	3.84 ± 1.95	NS	0.1
Playful	6.23 ± 1.96	7.15 ± 1.62	NS	0.066
Popular	6.92 ± 1.55	7 ± 1.63	NS	0.644
Protective	4.92 ± 2.49	4.15 ± 2.67	NS	0.397
Slow	4.76 ± 2.24	4.07 ± 1.80	NS	0.415
Solitary	2.76 ± 1.64	2.23 ± 1.01	NS	0.468
Subordinate	5.23 ± 2.20	4.76 ± 1.64	NS	0.323
Tense	3 ± 1.87	4.23 ± 2.01	S	0.042
Suspicious	4.61 ± 1.66	5.76 ± 1.87	NS	0.071
Reliable	7.53 ± 0.96	6.92 ± 1.71	NS	0.393
Hardworking	7.84 ± 0.80	7.76 ± 1.36	NS	0.772
Intelligent	7.46 ± 1.94	8.38 ± 0.76	NS	0.14

Figure 8. Comparison of NTB and Control horses in relation to Narcoleptic-specific Questions. C= Control, N= NTB's

Frequency of Narcolepsy - Occurs very occasionally vs Occurs very regularly	**To what extent does narcoleptic-like behaviours influence the management of the Horse?**	**To what extent does narcoleptic-like behaviours influence the rideability of the Horse?**	**Control/ NTB's**
			C
			C
			C
			C
			C
			C

			C
			C
			C
			C
			C
			C
			C
8	7	2	N
3	4	2	N
2	1	1	N
7	5	3	N
6	4	5	N
9	2	1	N
5	3	2	N
7	6	5	N
8	7	7	N
8	8	3	N
5	7	2	N
9	9	1	N
7	7	2	N

Raw Data from the Questionnaires is Available on Request.