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Behavioural probes of Basal ganglia function to further investigate the neurobiology of equine stereotypic behaviour

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Behavioural Probes of Basal Ganglia Function to Further Investigate the Neurobiology of Equine Stereotypic Behaviour

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Coventry University in association with the Royal Agricultural University

DECLARATIONS

This thesis is a product of my own work and is not the work of any collaboration.

I agree that this thesis may be available for reference and photocopying at the discretion of the university.

Kirsty Roberts

This project was granted ethical approval by the Ethics Committee at the Royal Agricultural University prior to data collection.

Director of Studies

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ABSTRACT

The horse (*Equus caballus)* provides a useful model where the study of repetitious behaviour is concerned as they perform three distinct stereotypies including one oral (crib-biting) and two locomotor equivalents (weaving and box-walking). Whilst several preliminary investigations have been performed into the neuro-aetiology of crib-biting, no studies to date have sought to elucidate the brain mechanisms underlying locomotor stereotypy in this species. As such, the primary aim of this investigation was to probe the neural basis of locomotor stereotypy (weaving) and extend current knowledge with regards to the crib-biting response. In this regard, behavioural probes have proved useful in identifying altered striatal functioning in a number of species without the use of invasive methods. Consequently spontaneous blink rate (SBR), behavioural initiation and an extinction-devaluation paradigm were conducted on a sample of crib-biting (n=8), weaving (n=8) and control (n=8) horses to investigate striatal output patterns.

Crib-biting horses demonstrated significantly lower SBR when compared to the control ($p<0.05$) and the weaving ($p<0.01$) animals. Behaviour initiation was significantly increased for the crib-biting ($p<0.01$) and the weaving ($p<0.05$) horses when compared to control equivalents. During the extinction paradigm, the control horses required significantly more trials to reach learning criterion when compared to both crib-biting ($p<0.001$) and weaving ($p<0.001$) animals. The crib-biting horses performed significantly more operant responses during extinction 1 and extinction 2 compared to weaving ($p<0.001$ and $p<0.01$ respectively) and control horses ($p<0.001$ and $p<0.001$ respectively). The crib-biting sample conducted significantly more operant responses during extinction 1 when compared to extinction 2 ($p<0.005$), though no difference was observed for the control or weaving group. Finally, crib-biting horses required significantly more trials to reach total extinction criterion when compared to their control ($p<0.001$) and weaving ($p<0.01$) equivalents.

This data suggests that there is an initial acceleration of ventral-dorsal activity within the striatum of crib-biting horses. However, the significant reduction of operant responses during extinction 2 compared to extinction 1 is indicative of a return to action outcome monitoring in the final stages of the extinction experiment. It is possible that this reduction of operant responses in extinction 2 is resultant of dopamine receptor saturation following devaluation. As the number of operant responses during extinction 2 is significantly higher for the crib-biting horses, the crib-biting horse is therefore responding habitually during extinction 2 in response to the conditioned stimulus as motivation in terms of reward acquisition has ceased. On the other hand the weaving horses did not transit towards stimulus-response learning at any stage of the extinction paradigm. Rather the weaving horse data suggests enhanced motivation as a result of increased phasic dopamine release highlighted by significantly reduced trials to attain learning criterion compared to control animals.

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GLOSSARY

Action-Outcome Behaviours (A-O) – Also known as instrumental behaviours, where the task performed is sensitive to the outcome. Controlled via the associative circuitry.

Appetitive Behaviour – A behaviour which is characterised by associative pathways and therefore the caudate. This behaviour utilises action-outcome monitoring and is indicative of caudate control.

Associative Circuitry – The neural circuitry associated with action-outcome monitoring via connections of the associative cortex and the caudate. Also referred to as the associative network.

Auto-receptor – Located within the pre-synaptic nerve which has a role in negative feedback.

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

Behaviour Sensitisation – The augmentation of the behavioural effect upon re-administration. Can be either pharmacologically induced or environmentally induced. Often results in changes in dopamine receptor physiology, thus the dopamine receptors become sensitised to the dopaminergic response.

Box-walking – Locomotor stereotypy of the horse involving the repetitive circular walking of the stable.

Caudate – The portion of the dorsal striatum associated with action-outcome, instrumental behaviours. Also referred to within this text as the dorsomedial striatum.

Consumatory Behaviour/Goal – The initial goal of the animal, which results in appetitive behaviours being attempted to achieve this goal, grazing is an example of this.

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.

Devaluation – A process utilised in some extinction paradigms which removes the motivation for the food reward. Allows distinguishment between appetitive and habitual behaviours.

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. The dorsal striatum is subdivided into the dorsomedial striatum (see also caudate) and the dorsolateral striatum (see putamen).

Dorsolateral Striatum (DLS) – See putamen.

Dorsomedial Striatum (DMS) – See caudate.

Dynorphin – The neuropeptide that is released upon activation of the direct pathway.

Enkephalin – The neuropeptide that is released upon activation of the indirect pathway.

Extinction Paradigm/Learning – A non-invasive behavioural probe which is utilised to examine the perseverance of a response to a stimulus and is therefore an indication of accelerated habit formation.

Fixed Ratio 1 – Rewarding each individual response, a ratio schedule.

Indirect Pathway – dopaminergic pathway of the striatum which results in behavioural inhibition through the stimulation of D2type receptors, which can be measured via enkephalin levels. Also referred to as the striatopalladial pathway.

Instrumental Behaviour – A behaviour that is controlled by action-outcome monitoring. These can be measured through instrumental tasks for example pulling a lever to achieve a food reward.

Limbic Circuitry – The neural circuitry associated with motivational behaviours via connections between the limbic cortex and the ventral striatum. Also referred to as the limbic network.

Medium Spiny Neurons (MSNs) – GABAergic output neurons of the striatum. There are two types of MSN; those which express D1 type receptors and those which express D2 type receptors.

Mesencephalic/on – The midbrain.

Mesocortical Pathway – Dopaminergic pathway from the ventral tegmental area (VTA) to the frontal cortex.

Mesolimbic Pathway – Dopaminergic pathway from the ventral tegmental area (VTA) to the nucleus accumbens. Also known as the mesoaccumbens pathway.

Nigrostriatal Pathway – Dopaminergic pathway from the substantia nigra to the striatum.

Nucleus Accumbens (NAcc) – A part of the ventral striatum concerned with motivational behaviours. The nucleus accumbens is further divided into core and shell sub-regions. Within this text the terms ventral striatum and nucleus accumbens refer to the same region.

Nucleus Accumbens Core – Anterior aspect of the nucleus accumbens associated with learning, instrumental behaviours and therefore the nigrostriatal system.

Nucleus Accumbens Shell – Ventral-medial aspect of the nucleus accumbens associated with the motivational behaviours and therefore the mesolimbic system.

Operant Response – In relation to the extinction paradigm, each time the horse made contact with the conditioned stimulus card.

Phasic Dopamine – Released in response to environmentally relevant stimuli. Is resultant from burst firing i.e. relatively high levels are released into the synaptic cleft but is quickly removed.

Pre-Pulse Inhibition (PPI) – A non-invasive behavioural probe, often utilising auditory startle stimuli to measure the amplitude of the startle response.

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.

Radial Arm Maze (RAM) – A non-invasive behavioural probe. The win-stay RAM is a measure of hippocampal function, whilst the win-shift RAM is utilised to assess accelerated habit formation.

Rate of Stereotypy – A measure of the amount of stereotypic responses performed within a given time period.

Response Acquisition – If an animal has accelerated response acquisition then they learn the task at an increased rate.

Sensorimotor Circuitry – The neural circuitry associated with habitual behaviours via connections between the sensorimotor cortex and the putamen. Also referred to as the sensorimotor network.

Shaping/Shaped – To train an animal to perform a task.

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

Stimulus-Response Behaviours (S-R) – Habitual type behaviours where the performance of the task is insensitive to the outcome. Controlled via the sensorimotor circuitry.

Striatal Matrix – The portion of the dorsal striatum and the nucleus accumebens core characterised by cholinergic and somatostatin containing neurons.

Striatal Patch – The portion of the dorsal striatum and nucleus accumbens core characterised by low levels of acetylcholine, increased levels of opiates, substance P, D1 type MSNs and D2 type MSNs. Also referred to as the striatal striosomes.

Striatonigral Pathway – See direct pathway.

Striatopalladial Pathway – See indirect pathway.

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

Tolmans Cross Maze – A non-invasive behavioural probe utilised to assess accelerated habit formation.

Tonic Dopamine – Exists within the extracellular space, where the levels change slowly.

Ventral Striatum – The portion of the striatum associated with motivational behaviours and consists of the nucleus accumbens (see also nucleus accumbens). Within this text the terms ventral striatum and nucleus accumbens refer to the same region.

Weaving – Locomotor stereotypy of the horse involving the repetitive weight shift from one forelimb to the other with lateral swaying of the head.

Chapter 1: Introduction

Stereotypic behaviours (STBs) are repetitive, invariant (Pell & McGreevy, 1999; McBride & Hemmings, 2005; Ninomiya *et al.*, 2007) and idiosyncratic (Parker *et al.*, 2009) induced by frustration, repeated attempts to cope or central nervous system (CNS) dysfunction (McBride & Hemmings, 2009). Until recently these behaviours were thought to have no obvious goal or function (Pell & McGreevy, 1999; McBride & Hemmings, 2005; Singer, 2009), though now are reported to have a role in stress control (Hemmings *et al.*, 2007; Wickens & Heleski, 2010) allowing the individual to cope with a sub-optimal environment (Garner *et al.*, 2003a). As such, presence of STBs has been utilised as an indicator of welfare status (Mills *et al.*, 2002; Cooper & Albentosa, 2005; Wickens & Heleski, 2010), although the precise role of stereotypy from an ameliorative stand point remains diffuse. Despite this, the proposed habitual nature of stereotypy performance (Hemmings *et al.,* 2007) could indicate that once established as part of the behavioural repertoire, the behaviour continues irrespective of environmental stimuli.

A number of species exhibit STBs including humans (Harris *et al.*, 2008), typically those which are autistic or suffer from pre-existing brain dysfunctions (Singer, 2009). An estimated 85 million captive animals exhibit STBs (Latham & Mason, 2010) with examples comprising route-tracing in song birds (*Cyanistes caeruleus* and *Poecile palustris*) (Garner *et al.*, 2003a), parrots (*Amazona amazonica)* (Garner *et al.*, 2003b) and carnivores (Wickens & Heleski, 2010) including circus tigers (*Panthera tigris*) (Krawczel *et al.*, 2005). Stereotypy is also observed in farm animals such as bar biting (pigs (*Sus scrofa domesticus*)) and tongue lolling (cattle (*Bos primigenius*)) (Wickens & Heleski, 2010). The most common STBs observed in the horse are crib-biting (CB), weaving and box walking (McBride & Hemmings, 2009).

1.1 Topography and Prevalence of Equine Stereotypic Behaviour

Crib-biting is an oral STB (McBride & Hemmings, 2009) whereby the animal grasps a surface at chest height with the incisors, pulling back creating an arch

with the neck (Moeller *et al.*, 2008; McBride & Hemmings, 2009; Wickens & Heleski, 2010) accompanied by the sucking of air into the proximal oesophageal region, creating an audible grunting sound (Nicol *et al.*, 2002; Moeller *et al.*, 2008; McBride & Hemmings, 2009; Wickens & Heleski, 2010). In contrast, there are two locomotor stereotypies of the horse; weaving which involves repetitive weight shift from one forelimb to the other, often combined with lateral swaying of the head (Cooper *et al.*, 2000; McBride & Hemmings, 2005), and box walking, the repetitive circular walking of the stable (McBride & Hemmings, 2005).

Crib-biting is the most prevalent equine STB, with approximately 4.3% of the horse population exhibiting CB behaviour, in contrast to 3.25% and 2.2% for weaving and box-walking respectively (McBride & Hemmings, 2009). It is worth noting however that when compared to other breeds, thoroughbreds (TBs) are 3.1 times (Bachmann *et al.*, 2003) and warmbloods (WBs) are 1.8 times (Wickens & Heleski, 2010) more likely to exhibit CB behaviour, perhaps suggesting a genetic constitution to causation.

1.2 Clinical Sequalae and Prevention Techniques

Stereotypic behaviours are suggested to have a negative impact on health, all of which are associated with a loss of condition and performance (Cooper *et al.*, 2000; McAfee *et al.*, 2002; McBride & Hemmings, 2009). Crib-biting results in wearing of the incisors (McBride & Hemmings, 2009), with uneven shoe wear due to constant weight shift, leg swelling which can result in lameness (Cooper *et al.*, 2000) and muscle fatigue (Ninomiya *et al.*, 2007) reported in the weaving equine. As the presence of STBs are often viewed as a ‘vice’ (Cooper *et al.*, 2000; Mills & Riezebos, 2005), with a reported 37% reduction in monetary value (Marsden, 2002), there are a number of methods to attempt to reduce the behaviours with 74% of establishments attempting physical prevention (McBride & Long, 2001). For example, owners of weaving horses often utilise anti-weaving bars (Plate 1) (McBride & Long, 2001) so the horse is unable to put the head outside of the stable to conduct the behaviour (McAfee *et al.*, 2002; McBride & Hemmings, 2009). In many cases this is unsuccessful as horses continue to weave within the confine of the stable (McBride & Hemmings, 2009). Attempts to

prevent CB can be severe, including surgical procedures such as a neurectomy or a myectomy, the use of crib-straps and cribbing rings (Plate 1) (McBride & Long, 2001; McBride & Hemmings, 2009) all of which are designed to physically prevent the behaviour. Should the purpose of STBs be to provide a coping mechanism for the animal, the physical prevention of these behaviours could further negatively impact health due to chronic stress (McGreevy & Nicol, 1998; McAfee *et al.*, 2002; Hemmings *et al.*, 2004; Houpt, 2012).

Plate 1: Anti-weave bars (a), crib-biting straps (b) and cribbing rings (c) are often used to prevent weaving (a) crib-biting behaviours (b,c) (McBride & Hemmings, 2009) Source: (a) http://www.horse-training-care.com/horseproblem.html (b) http://www.equineman.com/miracle_collar.htm (c) http://www.holistichorse.com/horse-health/dentistry/265-chapman-on-rings

1.3 Causal Factors

The Hughes and Duncan Model (1988) suggests STBs result from a highly motivated behaviour being prevented, often due to an inadequate environment. Consequently the consumatory goal cannot be achieved, resulting in a highly motivated state, hence the emergence of STBs. Specifically, oral STBs are thought to stem from being unable to fulfil normal oral behaviours such as grazing (Mason & Mendl, 1997). Therefore the CB response acts as a substitute for grazing and as such the behaviour becomes internally rewarded via dopamine release. In contrast, weaving is thought to be associated with frustration due to restriction of social and locomotive behaviours (Pell & McGreevy, 1999; McAfee *et al.*, 2002). Indeed, McAfee *et al.*(2002) demonstrated that when a mirror is placed into a weavers stable to replicate social behaviours such as eye contact, the STB is reduced ($P<0.05$) (Plate 2).

Plate 2: A mirror placed into the stable has been demonstrated to reduce weaving behaviour (McAfee *et al.*, 2002) Source: http://www.equinemanagementandtraining.co.uk/retailarchive.htm

Gastric inflammation is common in CB horses (Nicol *et al.*, 2002), suggesting gastrointestinal discomfort has a role to play in CB development, with Clegg *et al.* (2008) indicating feeding regime is also influential for weaving horses. Horses evolved to consume a forage based diet, with approximately 16-18h of the 24h time budget utilised for mastication in the wild (Cooper *et al.*, 2005). This process produces large volumes of alkaline saliva (Nicol *et al.*, 2002; Nagy *et al.*, 2010). Domesticated horses tend to be fed highly palatable cereal based concentrate feeds to meet high energy requirements (Hemmings *et al.*, 2007; Albright *et al.*, 2009) which reduces mastication, resulting in decreased saliva production and increasing acidity in the foregut (Nicol *et al.*, 2002; Hemmings *et al.*, 2007). This increased acidity results in gastric discomfort, therefore the CB response may attempt to replicate the mastication process to stimulate the parotid salivary gland (Nicol *et al.*, 2002; Hemmings *et al.*, 2007). Indeed, saliva produced during CB is a similar pH to saliva produced during mastication (Moeller *et al.*, 2008). Consequently the function of CB could be to buffer the stomach as a result of gastric pain (Moeller *et al.*, 2008). Studies have suggested the addition of alkaline virginiamycin or antacids resulted in a significant reduction of observed CB response (Mills & MacLeod, 2002; Nagy *et al.*, 2010). However, this reduction of stereotypy could be due to reduced palatability thus increased mastication duration allowing the foregut to be effectively buffered (Johnson *et*

al., 1998). Furthermore, *ad lib* feeding studies have also produced mixed results (Bachmann *et al.*, 2003; Fenn *et al.*, 2008; McCall *et al.*, 2009) highlighting feeding regime in terms of stereotypy development as an area that requires further research.

With regards to the precise effects of stress, chronic stress induces significant alterations to dopamine physiology in a genotype dependent manner (Cabib *et al.*, 1998), with similar alterations recorded in the horse (McBride & Hemmings, 2005). Feral horses are not exposed to chronic stress in the way domesticated horses are, explaining why feral populations rarely exhibit STB compared to domestic managed horses (Cooper & Albentosa, 2005). In rodents with pharmacologically induced STB, an up-regulation of dopamine transmission within the mesoaccumbens pathway was observed (Cabib *et al.*, 1998; Parker *et al.*, 2009). Similar results were also observed in CB horses (McBride & Hemmings, 2005; McBride & Hemmings, 2009). These alterations in basal ganglia dopamine physiology are thought to be essential for STB formation and continuation, as one of the primary roles of the basal ganglia is learning and motor control (Pell & McGreevy, 1999; McBride & Hemmings, 2009). Indeed, this study will investigate further into basal ganglia dysfunction as causal of STB by utilising previously proven behavioural probes conducted in an array of species (Karson, 1983; Garner & Mason, 2002; Garner *et al.*, 2003a; Parker *et al.*, 2009) as a method of determining striatal functioning through non-invasive means.

As of yet little has been done to investigate the neurobiology of weaving horses. Previous research (McBride & Hemmings, 2005) demonstrates neurological adaptations within the dopaminergic pathways and basal ganglia structures of CB horses utilising invasive methods, combined with other non-invasive behavioural probes (Parker *et al.*, 2009). Non-invasive behavioural probes (e.g. Karson, 1983; Hausberger *et al.*, 2007; Parker *et al.*, 2009) could be utilised to test for basal ganglia dysfunction to determine if the same neurological adaptations are observed in weaving horses to increase the understanding of equine STB. In order to contribute further to this growing evidence of neurological based STB development, to observe the same in a sample of weaving horses and an additional sample of CB horses would provide further

empirical evidence of a neurobiological component to STB development in contrast to other theories. Therefore, this study will be divided into two key objectives:

- Objective 1 – to examine and conduct basic behavioural probes including spontaneous blink rate (SBR) and rate of behaviour initiation to determine if they are successful as indicators for altered striatal functioning in stereotypy horses;
- Objective 2 – to undertake an extinction paradigm to investigate rate of habituation, something as of yet not examined within the horse.

Chapter 2: Literature Review

2.1 Review of Basal Ganglia Anatomy

Whilst the brain as a whole exhibits a range of complex and anatomically specific functions, in terms of STB the structures thought to be involved are those collectively known as the basal ganglia (McBride & Hemmings, 2009) (Fig. 1). The basal ganglia, and its corresponding dopaminergic (Da) pathways (Canales, 2005; Zellner & Ranaldi, 2010) are associated with learning, motivation, motor control (Afifi, 2003; Canales, 2005; Nicola, 2007; Moustafa *et al.*, 2008) and action selection (Koch *et al.*, 2000). Furthermore, it is the basal ganglia that allow the continued adaptation of behaviours depending on the most appropriate circumstance via a series of cortical loops (Graybeil, 2004). Overall, the structures that comprise the basal ganglia include the caudate, putamen, globus pallidus, nucleus accumbens (NAcc), olfactory tubercule, substantia nigra (SN) and the subthalamic nucleus (STN) (Afifi, 2003). The globus pallidus comprises of an internal (GPi) and external (GPe) segment (Hikosaka *et al.*, 2000). Furthermore, the substantia nigra is divided into the pars reticula (SNpr) and the pars compacta (SNpc) (Hikosaka *et al.*, 2000).

Figure 1. The placement of the striatal structures within the human brain. Source: http://figshare.com/articles/_Sagittal_view_of_subcortical_structures_with_segmentation_examples_from_FreeSurfer_of_Caudate_light_blue_Putamen_hot_pink_Thalamus_green_Globus_Pallidus_dark_blue_Nucleus_Accumbens_light_brown_Amygdala_turquoise_and_Hippocampus_yellow_/944165.

One of the key structures involved in the constant adaptation of behaviours is the striatum (Nicola, 2007). The striatum is constructed of three conjoined nuclei, each with a unique function (Fig 2). The dorsal striatum consists of the putamen, or the dorsolateral striatum, and is associated with the sensorimotor networks which are characterised by stimulus-response (S-R) learning and motor control (Canales, 2005; Yin & Knowlton, 2006; Nicola, 2007) and the caudate, also known as the dorsomedial striatum, which is involved with associative action-outcome (A-O) learning. The ventral striatum, or the nucleus accumbens (NAcc), is often considered part of the limbic network rather than the basal ganglia and is characterised by stimulus-outcome (S-O) responses (Yin & Knowlton, 2006). Despite this, the NAcc holds a distinct function in the adaptation and motivation of new behaviours in relation to current environmental conditions, specifically those relating to survival methods (Nicola, 2007). As the functions of these structures differ, it is best to consider them individually in terms of their corresponding neural circuitry. Therefore, the ventral and dorsal striatal circuits will be examined in further detail below.

Figure 2. A simplistic overview of the striatum including functions. During habit formation there is a ventral to dorsal shift in control of the striatal circuitry.

2.1.1 The Dorsal Striatum

Medium spiny neurons (MSNs) of the dorsal striatum are output neurons and account for approximately 90% of striatal neurons (Packard & Knowlton, 2002; Humphries & Prescott, 2010; Lovinger, 2010), with as many as 10,000 cortical inputs on each projection neuron (Graybiel, 1998). These neurons are GABAergic; i.e. the neurotransmitter released by these neurons is gamma-amino butyric acid (GABA) (Lovinger, 2010). Aspiny interneurons are also observed within the striatum, however these are cholinergic (Packard & Knowlton, 2002; Humphries & Prescott, 2010; Lovinger, 2010).

Whilst the dorsal striatum is divided into two distinct nuclei with the caudate receiving input from association cortices (consequently is associated with association circuits), and the putamen is innervated by sensorimotor areas (thus is associated with sensorimotor circuits) (Grahn *et al.*, 2008), these are further divided into matrix and striosomal components which are neurochemically and functionally discrete (Packard & Knowlton, 2002; Canales, 2005). Indeed, the striosomes (or the striatal patch) display low levels of acetylcholine, increased levels of opiates, substance P (Packard & Knowlton, 2002) and contains two types of MSNs; those which express D1-type receptors and those which express D2-type receptors (Humphries & Prescott, 2010). Alternatively, the matrix component of the dorsal striatum is characterized by cholinergic and somatostatin-containing neurons (Packard & Knowlton, 2002) with approximately half projecting to the SNpr with the other half projecting to the GP (Humphries & Prescott, 2010). Despite these neurochemical differences, both the striosomes and the matrix are innervated by dopamine (Da) pathways, though the origin of this differs (Packard & Knowlton, 2002). For example, the dopamine pathway innervating the striosomes appears to originate within the ventral tegmental area (VTA), whereas the substantia nigra innervates the matrix (Parkard & Knowlton, 2002). Subsequently, it is thought that the striosomes are associated with motivation and behaviour and therefore the ventral striatum, whereas the surrounding matrix corresponds to locomotive behaviour within the dorsal

striatum via corticostriatal and thalamostriatal projections (Packard & Knowlton, 2002; Canales, 2005).

Indeed, it is the mesencephalic dopamine pathways that are crucial in action selection, a process primarily controlled by the striatum. Dopamine modulates both the striatonigral and the striatopalladial pathways (Fig. 3) (Canales, 2005; Lewis *et al.*, 2006). The striatonigral pathway (direct pathway) and the striatopalladial pathway (indirect pathway) (Canales, 2005; Lewis *et al.*, 2006) have opposing effects whereby the striatonigral pathway has an excitatory effect on the MSNs of the striatum, compared to the striatopalladial pathway which has an inhibitory effect on the MSNs (Canales, 2005; Lewis *et al.*, 2006).

Figure 3: An interpretation of the dorsal striatum striatonigral (direct) and striatopalladial (indirect) pathways. Red lines indicate inhibition, green lines indicate activation. Dyn=dynorphin; Enk= enkaphalin; GPi=Globus pallidus internal segment; SNpr= substantia pars reticulata; Thal=thalamus; GPe=globus pallidus external segment; STN=subthalamic nucleus; SNpc= substantia pars compacta; RN= raphe nuclei (Garner, 2006).

The way in which the direct and indirect pathways allow for this is dependent on which dopamine receptors are co-localised with the glutamate receptors on the MSNs (Canales, 2005; Lewis *et al.*, 2006). The cells of the striatonigral pathway express high numbers of D1 type dopamine receptors (D1 and D5) coupled with

the glutamate receptors (Canales, 2005; Lewis *et al.*, 2006). When these D1 type Da receptors are activated, the positive effect on adenyl cyclase results in increased excitability of MSNs (Lewis *et al.*, 2006). As a consequence of this increased excitability of the MSNs, there is increased GABAergic inhibition of the output nuclei of the basal ganglia; the SNpr and the GPi (Lewis *et al.*, 2006). As seen in Fig. 3, the output nuclei inhibit the thalamus when the direct pathway is not active. However, when the output nuclei are under inhibition as a consequence of direct pathway stimulation, the thalamus is no longer subjected to inhibition (Lewis *et al.*, 2006). As a result, the thalamocortical motor relay is stimulated, consequently as is the supplementary motor cortex (Lewis *et al.*, 2006). The net result of this is increased behaviour.

Conversely for the striatopalladial pathway, the SNpc activates the D2 like dopamine receptors (D2, D3, D4) on the MSNs via dopamine projections (Hemmings, 2010). Unlike the D1 type receptors, the D2 type Da receptors are negatively coupled with adenyl cyclase, therefore when stimulated reduce the excitability of the MSNs. Reduction of excitability of the MSNs results in decreased GABAergic inhibition of GPe which in turn inhibits the STN. Inhibition of the STN has the opposite effect on the thalamus than the direct pathway in that the thalamus once again becomes inhibited (Presti & Lewis, 2005). Consequently, the thalamocortical motor relay is inhibited, so the supplementary motor cortex is no longer stimulated. The net result of this is decreased behaviour (Lewis *et al.*, 2006). In summary, stimulation of the striatonigral (direct) pathway results in behaviour activation, whereas stimulation of the striatopalladial (indirect pathway) inhibits behaviour.

However, this model of the direct and indirect pathways can be considered overly simplistic. For example, it should be noted that stimulation of the striatonigral pathway also expresses neuropeptides dynorphin, substance P and A1 adenosine receptors (Lewis *et al.*, 2006) and the striatopalladial pathway expresses enkephalin and A2 adenosine receptors (Lewis *et al.*, 2006). Whilst this means that during stereotypy performance the concentrations of dynorphin

and enkephalin can be measured to determine activation of the direct or indirect pathway respectively (Lewis *et al.*, 2006), these opioids have a modulatory effect on the pathways themselves. Indeed, dynorphin is thought to reduce the excitability of D1 activation within the direct pathway, with a similar process occurring with enkephalin within the indirect pathway (Lewis *et al.*, 2006).

2.1.2 The Ventral Striatum

Where the caudate and putamen are innervated by the associative cortex and sensorimotor cortex respectively, the ventral striatum is linked with the limbic cortex. Like the dorsal striatum, the ventral striatum, i.e. the NAcc, is also divided into two functionally and anatomically discrete areas; the ventral-medial aspect commonly known as the shell and the anterior aspect, or the core (Cardinal *et al.*, 2003). Furthermore, these two distinct anatomical areas are innervated in different ways. For example while the hippocampus innervates both the shell and the core, it is the ventral subiculum that innervates the shell compared to the dorsal subiculum projecting to the core (Kelley, 2004). Additionally, the shell is innervated by the infralimbic and piriform cortices of the prefrontal cortex (PFC) compared to the prelimbic area projecting to the core (Kelley, 2004). Differing aspects of the amygdala also project to both the core and the shell, though the increased projection of the amygdala to the shell results in this part of the NAcc being known as the ‘extended amygdala’ (Kelley, 2004).

The outputs of the shell and the core also differ, with the core projecting to output nuclei such as the ventral palladium (VP) and the STN (Kelley, 2004; Humphries & Prescott, 2010). In comparison the shell projects to limbic regions including the ventral tegmental area (VTA) and the lateral hypothalamus amongst others (Kelley, 2004). Subsequently, it has been suggested that the function of the core and shell subregions of the NAcc differ (Kelley, 2004). Indeed, due to the differing input and output projections of both the core and the shell it has been suggested that the core is more characteristic of the dorsal striatum in terms of function (Kelley, 2004) in that the core is involved with the

learning and adaptation of instrumental behaviours and therefore the nigrostriatal system (Deutch & Cameron, 1992). In contrast, the shell is more concerned with motivational behaviours such as food intake (Kelley, 2004) and consequently the mesolimbic system (Deutch & Cameron, 1992).

As the functions of the NAcc core and the dorsal striatum are highly interlinked this can also be observed in the neuro-anatomy of the core (Humphries & Prescott, 2010). Indeed, the NAcc core also has the same two MSN sub-types as the dorsal striatum; those which express D1-type receptors and those which express D2-type receptor subtypes (Lu *et al.*, 1998; Humphries & Prescott, 2010). In addition, the D1-type MSNs project to the SNpr in an identical manner to the direct pathway of the dorsal striatum (Zhou *et al.*, 2003; Humphries & Prescott, 2010). The core also projects to the VP in a similar fashion as the dorsal striatum, though two origins of the projection have been observed. The first is from the MSNs which express D2-type receptors, and to a smaller degree D1-type receptor MSNs (Lu *et al.*, 1998; Zhou *et al.*, 2003; Humphries & Prescott, 2010). Finally, the VP projects back to the GABAergic neurons within the core 'matrix' (Fig. 4) in the same fashion as the dorsal striatum circuits, giving some evidence for the similarities between the NAcc core and the dorsal striatum (Humphries & Prescott, 2010). A simplistic overview of the ventral striatal circuits can be seen in Fig. 4.

Figure 4: An overview of the nucleus accumbens shell and core ventral striatum circuits. STN, subthalamic nucleus; NAcc, nucleus accumbens; VTA, ventral tegmental area; SNpc, substantia nigra pars compacta; VP, vental palladium; SNpr, substantia nigra pars reticula; PPn, pedunculopontine nucleus; LPO, lateral pre-optic area (Humphries & Prescott, 2010).

As the NAcc core is not entirely dissimilar to the dorsal striatum, attempts have been made to define 'patch' and 'matrix' components of the core (Berendse *et al.*, 1992; Humphries & Prescott, 2010). Within the caudal regions of the core are discrete regions of calbindin poor areas, whereas in the rostral core are discrete calbindin rich regions (Berendse *et al.*, 1992; Humphries & Prescott, 2010). Further confusion arises when accounting for the enkephalin rich regions which are also known as patches (Berendse *et al.*, 1992; Humphries & Prescott, 2010). As the MSNs in the calbindin poor regions project to dopaminergic cells, these have been defined as the 'patch', whereas the MSNs in the calbindin rich rostral core regions project to non-dopaminergic cells and are subsequently known as the matrix (Berendse *et al.*, 1992). However, in contrast to the dorsal striatum where the striosomes (or patches) contain both D1 and D2 receptor subtypes, the dense enkephalic staining of the patches in the caudal core suggest

dominance of D1-type MSNs in this area (Berendse *et al.*, 1992; Humphries & Prescott, 2010).

Attempting to separate the projections from matrix and patch regions of the core is proving difficult in terms of hindbrain connections. Indeed, in their review of ventral striatum function, Humphries & Prescott (2010) summarise that no distinction can be made between the dopaminergic neurons which target the patch or matrix regions of the core as can be done for the dorsal striatum as both regions receive dense innervation from the lateral VTA and to a lesser degree, the SNpc to the medial core (Humphries & Prescott, 2010). However in terms of projections, some evidence suggests that the patches target medial SNpc and the lateral VTA and are thought to have an inhibitory effect on these regions. Further electrophysiological research is required for clarification (Humphries & Prescott, 2010).

In contrast, the NAcc shell has no distinct 'patch' or 'matrix' areas, and is divided into a medial and lateral region with distinct pathways of D1-type and D2-type MSNs, for example there is a dense projection from D1-type MSNs to the VTA (Lu *et al.*, 1998). Despite this, the projections from medial and lateral regions of the shell do differ. The lateral shell has mutual connections with the lateral regions of the VTA and projects to the SNpc like the core, as well as ventrolateral VP, whereas the medial shell has mutual connections with the medial VTA as well as the lateral VTA, with projections also to the medial VP (Zhou *et al.*, 2003; Ikemoto, 2007; Humphries & Prescott, 2010). As with the core, these projections are thought to be inhibitory, but further work is required to analyse this in more detail. As the D1-type MSNs project only to the VTA and the D2-type MSNs project only to the VP these pathways are clearly distinguishable (Humphries & Prescott, 2010).

The pathways of the NAcc shell are unique to those of the core in two main ways. The first is that the NAcc shell is the only structure within the striatum that

has direct connections to structures external to the basal ganglia (such as the lateral hypothalamus and the lateral pre-optic area (LPO) Fig. 4), however, this ability to project to external structures appears to be limited to the medial shell (Humphries & Prescott, 2010). Secondly, the striatopalladial pathway differs when compared to the rest of the basal ganglia. Whilst the core striatopalladial pathway results in further projections to the SNpr or the STN, the NAcc shell does not (Humphries & Prescott, 2010). In comparison, the NAcc shell striatopalladial pathway results in projections external to the basal ganglia (Groenewegen *et al.*, 1993). These structures include the lateral hypothalamus, the mediodorsal hypothalamus and the pedunculopontine nucleus (Groenewegen *et al.*, 1993; Humphries & Prescott, 2010).

2.1.3 A Summary of Limbic, Associative and Sensorimotor Circuit Functions of the Striatum: Connecting the Striatum Nuclei

It is a combination of the limbic, associative and sensorimotor circuits discussed in the above sections that allow the acquisition of new behaviours as well as the ability to choose the correct response to a stimulus (Haber *et al.*, 2000). Consequently, it is the way these three circuits interface with each other within the striatum of the basal ganglia that allow an animal to choose appropriate responses to a specific stimulus, known as action selection (to be discussed in more detail in section 2.1.4) utilising motivation, reward and cognition (Haber *et al.*, 2000). This sub-circuit between these three networks is known as the striatonigrostriatal circuit (Haber *et al.*, 2000). A model of the interaction between the three loops proposed by Yin & Knowlton (2006) suggested a hierarchy, commencing with the limbic circuit within ventral areas, to the associative involving dorsal and rostral areas and finally to the sensorimotor networks with the more caudal areas (Grahn *et al.*, 2008) (Fig. 5).

Figure 5. The model of hierarchy of the limbic, associative and sensorimotor loops proposed by Yin & Knowlton (2006). (From Yin & Knowlton, 2006). Blue triangular arrows indicate excitation, blue circular arrows indicate dopamine modulation, blue square arrows indicate inhibition, purple triangular arrows indicate disinhibition.

However, this model could be considered overly simplistic. Indeed, Haber *et al.* (2000) suggests a more complex model of this hierarchy based on the placement of bi-directional tracers into different regions of the striatum and the ventral midbrain of adult macaques (*Macaca Mulata* and *Macaca Nemistrina)*. These data suggest that each of the limbic, associative and sensorimotor networks has three substantia nigra components; a group of nigrostriatal cells which project dorsally, a central region containing projecting nigrostriatal cells and terminating regions, and finally, a ventral region (Haber *et al.*, 2000). From this, the authors proposed an ascending spiral between the networks rather than the direct links previously suggested. For example, this study suggests that the NAcc shell doesn't just influence the NAcc core via previously suggested striatopalladial pathways but also utilising a series of reciprocal connections (Haber *et al.*, 2000). Primarily, the VTA and the shell form one closed reciprocal loop, while the medial substantia nigra completes the first spiral by projecting towards the core (Haber *et al.*, 2000). The striatonigrostriatal projections allow the continuation of the spiral from the core towards the dorsal striatum, thus indirectly allowing the ventral striatum to influence the dorsal striatum (Haber *et al.*, 2000). Haber *et al.* (2000) further suggest that the projections which terminate directly onto dopamine cells result in inhibition, whereas those which indirectly terminate on a dopamine cell result in disinhibition. These pathways can be viewed diagrammatically in Fig. 6.

Figure 6: An interpretation of the ascending spiral from the NAcc Shell to the Putamen suggested by Haber *et al.* (2000). Red arrows indicate the limbic circuits, green arrows indicate associative circuits and blue arrows indicate sensorimotor circuits. IC, internal capsule; VTA, ventral tegmental area; SNpc, substantia nigra pars compacta; SNpr, substantia nigra pars reticula.

2.1.4 The Role of the Striatum: Action Selection, Instrumental Behaviours and Habit Formation

The ability for an animal to select the correct behaviour (or action) is essential to be able to exploit the ever changing environment it is presented with for survival (Graybiel, 2008). In this way, an animal can utilise action selection as part of a learning process to effectively exploit the environment (Graybiel, 2008). For example, in a novel environment which the animal is unfamiliar with, the environment needs to be explored and correct actions either selected or deselected to exploit that environment to achieve a pre-determined goal (Graybiel, 2008). Consequently, the discussed neural circuitry of the striatum would suggest that this structure has an important role in this action selection process in terms of motivation, reward, locomotive and cognitive behaviours (Graybiel, 2008). Moreover, this can be divided into functions of the NAcc, caudate and the putamen as a part of this learning process.

Hughes and Duncan (1988) proposed a model of motivation (Fig. 7) whereby appetitive behaviours are attempted (i.e. action selection) to fulfil a consumatory goal e.g. ingestion in response to falling blood glucose levels, a model which fits well with the theory of learning suggested above. The initial phases of the appetitive behaviours are thought to be controlled by the NAcc. Nucleus accumbens dopamine has been demonstrated to be involved in the reward functions of behaviour (Graybiel, 2008), with animals readily self-administering dopamine receptor agonists and pharmaceuticals such as amphetamine or cocaine which increase extracellular dopamine levels directly into the NAcc (Ikemoto & Panksepp, 1999). While appetitive behaviours are being conducted, the motivation is self-reinforcing due to a positive feedback loop as the consumatory goal has not been acquired (Fig. 7). Once the consumatory goal has been achieved, a negative feedback loop is activated so the animal is no longer motivated to conduct that particular behaviour; hence the behaviour ceases (Fig. 7). The NAcc shell and the NAcc core are again thought to have differing roles, for example during cocaine administration the NAcc shell was associated with the pleasurable effects whereas the NAcc core was associated with the locomotive effects (Alcaro *et al.*, 2007). When one considers the projections and innervations of these two distinct anatomical areas, this is not a surprising revelation.

In terms of dopamine release within the NAcc, a quick note is required. Dopamine can be released in either a tonic or phasic manner (Alcaro *et al.*, 2007). Tonic dopamine exists within extracellular space in very small concentrations where the levels also change very slowly (Alcaro *et al.*, 2007). In contrast, phasic dopamine is a result of ‘burst firing’ resulting in relatively high levels of dopamine within the synaptic cleft which is then quickly removed (Alcaro *et al.*, 2007). When considering the motivation function of the NAcc, it is the phasic dopamine that is released in novel situations, thus it is this dopamine required for the appetitive behaviours. It should also be noted that high levels of tonic dopamine can have an inhibitory action on phasic dopamine potentially resulting in less excitable neurons (Alcaro *et al.*, 2007). To summarise, the NAcc is required for the initial phases of learning in novel situations via appetitive

behaviours. Lesion studies of the NAcc suggest that the NAcc is not required for instrumental learning or stimulus response learning suggesting these behaviours implement the dorsal rather than the ventral striatum (Graybiel, 2008). This could allow for the NAcc to always be investigating new ways to exploit the current environment.

Figure 7: The Hughes and Duncan Model (1988). The blue arrows demonstrate adaptation of this during stereotypic behaviour, which will be discussed in section 2.2.

Instrumental behaviours are those which are action-outcome (A-O) dependent i.e. the performance of the task is sensitive to the outcome (Yin & Knowlton, 2006). As mentioned before, action-outcome behaviours are those controlled by the associative circuits; that is they are controlled by the caudate (Yin & Knowlton, 2006). In contrast, stimulus-response (S-R) dependent behaviours are those which are considered to be habitual i.e. the performance of the task is not sensitive to the outcome (Graybiel, 2008). The performance of habitual behaviours is essential in allowing the animal to focus attention on other A-O type goals in order to continually adapt to suit the current environmental factors (Schwabe & Wolf, 2011). Furthermore, where A-O learning is under the influence of the caudate, S-R learning is controlled by the putamen via the sensorimotor circuitry (Yin *et al.*, 2006). Unlike A-O learning which is flexible and susceptible to goal devaluation S-R habits are inflexible and are not influenced by goal devaluation (Yin *et al.*, 2006). During the initial phases of learning, for example a laboratory animal learning to press a lever for reward, the associative neural

circuits are recruited (Yin *et al.*, 2006). However, following over-training these instrumental behaviours can become habitual and thus no longer controlled by outcome expectancy (Yin *et al.*, 2006) but relating the situation to a previous goal and the antecedent behaviour that achieved the consumatory goal (Graybiel, 2008). Thus, S-R learning is characterised by a ventral to dorsal shift of neural circuits recruited. Indeed, lesions of the putamen reduce the insensitivity to reward devaluation i.e. the behaviour cannot become habitual and will always remain sensitive to reward devaluation via A-O learning (Graybiel, 2008). However, lesions of the caudate impact A-O learning in such a way that the sensitivity to reward devaluation is reduced i.e. the animal relies on habit S-R learning (Graybiel, 2008).

2.1.5 Interim Summary

To summarise, new behaviours are initiated within the NAcc, though the caudate is necessary for the continuation and adaptation of behaviours relying on A-O learning, so if a previously successful behaviour no longer yields a reward, the behaviour (action) will cease. In contrast, when the putamen or sensorimotor circuits are utilised the behaviours are characterised as inflexible and are not sensitive to reward devaluation. Subsequently, if a behaviour is habit driven, even though that behaviour no longer receives a reward the behaviour continues regardless. Thus habit formation demonstrates a ventral to dorsal shift in control of the behaviour. This hierarchal structure of learning is also referred to as serial adaptation (Yin & Knowlton, 2006). Maze studies, lesion studies and extinction studies are utilised when monitoring basal ganglia dysfunction, however these will be described in section 2.3.

2.2 The Basal Ganglia and Stereotypic Behaviour

Basal ganglia dysfunction can result in a number of negative pathologies. For example in humans basal ganglia dysfunction is linked to Parkinson's disease (Chen *et al.*, 1996), Schizophrenia (Swerdlow *et al.*, 2002), and obsessive compulsive disorder (OCD) (Campbell *et al.*, 1999). Parkinson's disease is

characterised by decreased dopamine levels in the basal ganglia (Chen *et al.*, 1996), whereas increased endogenous dopamine levels are observed in patients with schizophrenia (van den Buuse, 2004). It is now proposed that basal ganglia dysfunction is causal of stereotypic behaviour in a number of species including rodents (Cabib & Bonaventura, 1997; Cabib, 2006; Tanimura *et al.*, 2009) and higher vertebrate species such as the horse (McBride & Hemmings, 2005; 2009; Parker *et al.*, 2009). Here, these will be examined in more detail, summarised by highlighting potential similarities between pharmacologically induced and environmentally induced stereotypy to determine if altered basal ganglia physiology is causal of STBs.

2.2.1 The Basal Ganglia and Pharmacologically Induced Stereotypy

Dopamine plays an important role in stereotypy formation. Indeed, research has demonstrated that administration of dopamine agonists, resulting in dopaminergic stimulation of the striatum, can induce stereotypic behaviour (Canales & Graybiel, 2000). In contrast, administration of dopamine antagonists, thus blocking dopaminergic transmission, reduces and even ceases stereotypic behaviour (Canales & Graybiel, 2000). Given the direct and the indirect pathways, one would expect that the administration of D1 receptor agonists would induce locomotion, and at higher levels stereotypy. In contrast the administration of a D2 receptor agonist would not perhaps immobilise the animal but certainly reduce observed behaviour.

Surprisingly, a number of studies have found this is not the case. For example, Moore and Axton (1988) attempted to induce stereotypic cage climbing in a population of mice by administrating the partial D1 receptor agonist SKF383939, the D2 receptor agonist quinpirole and a mixed D1/D2 receptor agonist apomorphine. Interestingly, the administration of SKF38393 and quinpirole individually failed to induce stereotypic climbing (Moore & Axton, 1988). However, apomorphine resulted in a dose-related increase of stereotypic climbing, an effect lasting up to 60 minutes (Moore & Axton, 1988). Similarly,

when both SKF38393 and quinpirole were administered together, intense stereotypic cage climbing resulted which had a longer lasting effect of up to two hours (Moore & Axton, 1988). Furthermore, stereotypy induced by either apomorphine or a SKF38393/quinpirole combination was abolished by either D1 antagonist SCH23390 or the D2 antagonist clebopride, suggesting that the activation of both D1 and D2 receptor subtypes is necessary for pharmaceutically induced stereotypy (Moore & Axton, 1988).

Other authors have reported similar results. For example Delfs and Kelley (1990) investigated administration of D1 and D2 receptor agonists within the ventrolateral striatum on the development of oral stereotypy in rodents. Previously, microinjection of amphetamine into the ventrolateral striatum had been observed to result in intense oral stereotypies in the rodent, though the dopamine receptors activated remained unknown (Delfs & Kelley, 1990). Microinjection of SKF38393 initially had no effect, however 3-4 hours post injection intense self-biting behaviour was observed. In addition, quinpirole resulted in a dose dependent increase of orofacial behaviours but not intense oral stereotypy (Delfs & Kelley, 1990). However, when a mixed D1/D2 agonist was administered intense oral stereotypy was observed such as that when amphetamine was injected. The authors summarised that for the pharmaceutical induction of oral stereotypy both D1 and D2 receptor activation is required (Delfs & Kelley, 1990).

In agreement, Arnt *et al.* (1988) observed that neither partial nor full D1 agonists induced stereotypy whereas full D2 agonists resulted in hyperactivity. Again the mixed D1/D2 agonists such as apomorphine resulted in both oral stereotypy and hyperactivity (Arnt *et al.*, 1988). Furthermore, by inhibiting dopamine synthesis the authors established that D1 receptor tonus is necessary for the effect of a dopamine agonist. For example, hyperactivity observed as a consequence of D2 agonist administration appears to depend on D1 tonus mediated by endogenous dopamine activity, though increased activation of the D1 receptor subtype is required to result in oral stereotypy (Arnt *et al.*, 1988). Consequently the authors

summarise that ultimately the resultant behaviour from a dopamine agonist depends on D1/D2 receptor affinity (Arnt *et al.*, 1988). This result is mirrored by the findings of Braun and Chase (1986) who also reported that the pharmacologically induced behaviours expressed required both D1 and D2 receptor activation, though the nature of that stereotypy ultimately results from the ratio of D1/D2 activation (Braun & Chase, 1986).

Other evidence implicates the dorsal striatal striosome and matrix components in stereotypy development. Canales and Graybiel (2000) injected the D1 and D2 agonists amphetamine (whose mode of action is to increase phasic dopamine release) and cocaine (which effectively blocks re-uptake of dopamine resulting in high synaptic levels of dopamine) twice-daily for one week, followed by one week of drug withdrawal. On Day 15 the rats were presented with a drug challenge dependent on the pre-treatment. Interestingly, this evidence suggested not only an imbalance in activation of the striosome and matrix components, but that the degree of increased activation of the striosomes over the matrix predicted the degree of motor stereotypy (Canales & Graybiel, 2000), a finding confirmed by Capper-Loup *et al.* (2002). In contrast, Tanimura *et al.* (2011) found no dominance of striosomal components (measured by metabolic activity, volume and cell counts) of the striosomes over the matrix during stereotypy formation but instead implicated reduced activation of the indirect pathway as causal for stereotypy, something that would be expected considering the roles of the direct and indirect pathway. The conflicting evidence presented here suggests this is an area that requires further research.

During pharmaceutically induced stereotypy there appears to be a shift from A-O to S-R habit formation (see section 2.3.1) (Parker *et al.*, 2009). Nelson and Killcross (2006) highlight that 6-OHDA lesions of the nigrostriatal pathway disrupts habit formation, as well as noting that an intercaudate amphetamine injection accelerates S-R type learning. Consequently, the authors investigated the direct impact of amphetamine exposure on habit formation utilising an instrumental devaluation task. One group of rats were sensitised to

amphetamine prior to task training whilst another group were sensitised during and post training with both populations being compared to a control group (Nelson & Killcross, 2006). As hypothesised, sensitisation to amphetamine prior to training in an instrumental devaluation task resulted in accelerated A-O to S-R habit formation process, however post training sensitisation had no effect (Nelson & Killcross, 2006). These results highlight the impact that amphetamine, and therefore elevated dopamine levels, can have on striatal circuit dominance within instrumental tasks.

Dopamine deficient mice, in comparison to the control wild type mice, appear to have a dopaminergic threshold for stereotypic induction (Chartoff *et al.*, 2001). Within the Da depleted mice, when dopamine levels accumulated to >50% of wild type (WT) levels stereotypy was initiated (Chartoff *et al.*, 2001). However, once dopamine levels fell back below this 50% level only hyperactivity was observed until dopamine levels fell below 3% whereby hypoactivity was resultant (Chartoff *et al.*, 2001). The authors suggest this is due to the dopamine deficient mice exhibiting a sensitisation to dopamine, as hyperactivity and stereotypy were observed at much lower levels when compared to WT mice. Indeed, the levels of D1 and D2 agonists which induced high levels of stereotypy in the dopamine deficient mice had no impact on the WT mice (Chartoff *et al.*, 2001). Furthermore, within this population of mice the data suggested that whilst D1 and D2 agonists result in hyperlocomotion, only activation of D1 agonists resulted in stereotypy performance perhaps implicating the direct pathway as causal to stereotypy performance in mice with intact dopaminergic neurons (Chartoff *et al.*, 2001). Berridge and Aldridge (2000) also found results suggesting it is the D1 receptor agonists that results in intense stereotypy performance, highlighting the conflicting results within this field of research.

2.2.1.1 Pharmacologically Induced Behaviour Sensitisation

The above studies utilise the effects of receptor sensitisation in terms of pharmaceutically induced stereotypy. Behavioural sensitisation is that which, in

terms of drug induced behavioural sensitisation, arises from repeated drug administration and can be defined as the augmentation in the behavioural effect of a psychostimulant upon re-administration (Pierce & Kalivas, 1997). Behavioural sensitisation is thought to initiate from the ventral aspects of the basal ganglia such as the VTA and the NAcc (Pierce & Kalivas, 1997). McBride and Hemmings (2005) identified an increase in dopamine receptor availability within the NAcc of stereotypic horses, demonstrating receptor sensitisation occurring in the absence of pharmaceuticals. Consequently the animal would still demonstrate behavioural sensitisation as a result of this increased response to dopamine release within the NAcc, despite not being exposed to dopaminergic agonists. Subsequently, hyperlocomotion and stereotypic behaviours would be observed in these animals. This will be further discussed in section 2.2.2.

The effects of behaviour sensitisation can be relatively long lasting. For example an early study conducted by Robinson *et al.* (1988) noted that amphetamine pre-treatment resulted in increased hypersensitivity to the motor stimulant effect of an amphetamine challenge 15-20 days after withdrawal. Importantly, the amphetamine pre-treatment had no impact on basal extracellular concentrations of dopamine, but rather the sensitisation is resultant from alterations in the way phasic dopamine is released (Robinson *et al.*, 1988). Similar results were reported by Nishikawa *et al.* (1983) whereby repeated administration of d-methamphetamine for 3-14 days resulted in marked behaviour sensitisation to drug challenges of d-methamphetamine 44-89 days post withdrawal but also to apomorphine and nomifensine. Furthermore, the observed increase in central dopaminergic transmission within the striatum and mesolimbic area suggests that the drug challenges result in increased dopaminergic release within the ventral striatal circuitry (Nishikawa *et al.*, 1983).

Further research has also suggested that dopamine transmission is essential for the initiation of behavioural sensitisation, resulting in altered dopaminergic transmission in axon terminal fields within the nucleus accumbens being critical for the expression of sensitisation (Kalivas & Stewart, 1991). Indeed, the authors

found that when somatodendritic dopamine release is increased the consequential activation of D1 type receptors generates long term adaptations within the ventral striatum allowing behavioural sensitisation, though how these alterations occur is unknown (Kalivas & Stewart, 1991). The authors suggest that the dopamine neurons of sensitised animals become overly sensitive to pharmaceutical or even environmental stimuli, though potentially the direct pathway may have become desensitised (Kalivas & Steward, 1991). Indeed Capper-Loup *et al.* (2002) highlight the importance of both D1 and D2 dopamine receptor subtype activation for the occurrence of behavioural sensitisation within the striatum, in parallel with the above findings suggesting both subtypes of dopamine receptors are required for stereotypy formation.

Whilst the aforementioned studies all tend to implicate dopamine as solely influential on pharmaceutically induced stereotypy or behavioural sensitisation, other evidence also implicates the glutamate system and the GABAergic system (Karler *et al.*, 1995). In fact, Karler *et al.* (1995) suggest psychostimulant induced stereotypy is actually facilitated by NMDA activated GABAergic inhibition of GABA outputs from the striatum resulting in a decrease of the inhibitory influence, which considering that the MSNs within the striatum are GABAergic is entirely feasible (Karler *et al.*, 1995). Therefore hyerlocomotion and stereotypy induced by psychostimulants may result from an inhibition of the indirect pathway ultimately mediating excitation of the cortex (Karler *et al.*, 1995). The authors summarise that the effects of such psychostimulants are facilitated by dopaminergic activation of the glutamatergic and subsequently GABAergic pathways, an area which requires further research (Karler *et al.*, 1995).

2.2.1.2 Interim Summary

To date, data appear to suggest that dopamine agonists reliably induce stereotypy, in contrast dopamine antagonists reliably inhibit the onset of stereotypy and even ceases it altogether in animals expressing stereotypic behaviours. Consequently, these results certainly implicate dopamine in the

initiation of pharmacologically induced stereotypy with other research also ascertaining the effects dopamine has on the glutamergic and GABAergic systems. Importantly, the effect of behavioural sensitisation on repeated administration of dopamine agonists in response to a drug challenge can allow for similarities to be proposed between pharmacologically and environmentally induced stereotypy, ensuring further understanding of the latter. Despite this, the conflicting evidence produced between some studies does suggest this is an area that requires further research.

2.2.2 The Basal Ganglia and Environmentally Induced Stereotypy

Dopamine appears to be an important neurotransmitter in pharmaceutically induced stereotypy. Evidence appears to suggest that dopamine is also highly influential in spontaneous, environmentally induced stereotypy. Indeed, Presti *et al.* (2003) demonstrated just this by attenuating spontaneous stereotypy utilising the D1 antagonist SCH23390 in a population of deer mice (*Peromyscus maniculatus*). Significantly, only the stereotypic jumping behaviour of the mice was attenuated whereas all other behaviour remained unaffected as a result of the dose levels utilised (Presti *et al.*, 2003). Due to the *modus operandi* of the D1 antagonist, the MSNs of the direct pathway were less excitable, decreasing activity required for positive feedback (Presti *et al.*, 2003). In turn this reduced the inhibitory effect at the GPi and SNpr ultimately leading the decline of positive feedback (Presti *et al.*, 2003). Therefore repetitive persistent behaviours could no longer be maintained, ceasing the stereotypy performance (Presti *et al.*, 2003). This study also provided limited support for the similar neural mechanisms between spontaneous and drug induced stereotypies (Presti *et al.*, 2003).

Presti and Lewis (2005) further investigated direct/indirect pathway activity by measuring the dynorphin and enkephalin ratios of the dorsolateral striatum in high-stereotypy compared to low-stereotypy male deer mice. Importantly, the stereotypy was spontaneously induced not pharmaceutically induced. The high-

stereotypy deer mice demonstrated significantly increased dynorphin/enkephalin content ratios compared to the low-stereotypy mice (Presti & Lewis, 2005). In addition, a significant negative correlation between enkephalin content and frequency of stereotypy, and a significant positive correlation between the dynorphin/enkephalin content ratio and frequency of stereotypic performance was observed (Presti & Lewis, 2005). As dynorphin is a measure of direct pathway stimulation, these results suggest stereotypy is consequential of hyperactivity of the direct pathway (Presti & Lewis, 2005). However, this hyperactivity of the direct pathway may result from alterations of the indirect pathway characterised by the reduction of enkephalin (Presti & Lewis, 2005). The authors summarise that spontaneous stereotypy therefore results from decreased activation of the indirect pathway, allowing hyperactivation of the direct pathway (Presti & Lewis, 2005). Furthermore, research by Tanimura *et al.* (2011) also indicates alterations resulting in reduced activation of the indirect pathway as causal for hyperactivity of the direct pathway resulting in repetitive behaviours.

The effects described above suggest that elevated dopamine has a definitive role to play in pharmacologically induced behavioural sensitisation and stereotypy development (McBride & Hemmings, 2004). However, for spontaneous stereotypy to occur in the absence of dopamine agonists there must be an alternative manner of sensitisation of the striatum (the genetic component of behaviour sensitisation will be reported in section 2.2.3). Indeed, there is evidence to suggest such sensitisation does occur in response to environmental stress (Cabib & Bonventura, 1997). A number of stressors are known to cause dopaminergic release into the striatum including high intensity foot shock (Inoue *et al.*, 1994), isolation rearing (Robbins *et al.*, 1996) and social defeat (Tidey & Miczek, 1996) amongst others (McBride & Hemmings, 2004), highlighting potential similarities between pharmaceutical and environmentally induced sensitisation. Furthermore, Cabib and Bonaventura (1997) concluded from their investigation into both amphetamine induced behavioural sensitisation and stress induced behavioural sensitisation via feed restriction that spontaneous stereotypies result from upregulated dopaminergic pathways as a

consequence of chronic stress (Cabib & Bonaventura, 1997). In contrast, Tanimura *et al.* (2009) suggest that sensitisation has no impact on subsequent stereotypy development, as sensitised animals did not show increased levels of spontaneous stereotypy. Similarly animals demonstrating stereotypy did not necessarily demonstrate an increased sensitisation response to a drug challenge (Tanimura *et al.*, 2009). However, as these deer mice were subjected to drug induced behaviour sensitisation rather than stress induced sensitisation this may have had an impact on the results when compared to Cabib & Bonaventura (1997).

The concept of environmentally induced sensitisation can be applied to the horse. Indeed within this species, stereotypy is thought to occur as a coping response (Hemmings *et al.*, 2004), with authors suggesting links between weaning stress and increased performance of stereotypic behaviour (Waters *et al.*, 2002). Furthermore, lack of social contact is thought to result in weaving behaviour (McAfee *et al.*, 2002) as is stable and feed type (Ninomyia *et al.*, 2007). In addition crib-biting was found to increase under a feed stress test investigation (Nagy *et al.*, 2009). All of these findings are strikingly similar to those conditions known to induce sensitisation within rodent models. Stereotypy is intrinsically linked to feeding in the horse with palatable foods contributing to an opioid mediated dopaminergic response (McBride & Hemmings, 2004). Consequently, in a stress sensitised animal the palatable feed results in stereotypic response as a result of increased activation of the direct pathway (McBride & Hemmings, 2004).

It is important to note that stress induced neural sensitisation has not yet been directly linked to stereotypy in the horse. However, within the rodent models discussed, stress is known to contribute to sensitisation of dopamine circuitry leading to eventual propagation of the stereotypic motor response in some individuals (Cabib & Bonaventura, 1997) perhaps as a result of a genetic predisposition (McBride & Hemmings, 2004; Hemmings *et al.*, 2004). Figure 8

demonstrates the potential causal factors of stereotypic behaviour considering altered striatal physiology.

Figure 8: A model of causal factors resulting in stereotypy. (CS = conditioned stimulus). As this demonstrates, behavioural sensitisation is a key precursor to stereotypy development caused by the previously discussed Hughes and Duncan Model (Taken from McBride & Hemmings, 2004).

2.2.2.1 Interim Summary

Evidence from studies utilising deer mice appears to suggest that alterations of the indirect pathway allows hyperactivation of the direct pathway. This adaptation of the direct and indirect pathways result in the disinhibition of repeated behaviours hence the observation of stereotypic behaviours. However, it would appear that behavioural sensitisation is a necessary precursor to stereotypy development as no direct link between stressors and stereotypy performance has been observed without prior behavioural sensitisation. In contrast to pharmaceutically induced behavioural sensitisation, for spontaneous stereotypy the behavioural sensitisation is induced by chronic stressors, potentially in response to genetic predisposition to stress which will be reported below.

2.2.3 Genetic Susceptibility to Stress

Stress has an impact on stereotypy development and although approximately 85 million captive animals exhibit stereotypy, it is important to realise that not all animals exposed to the same environment develop stereotypic behaviours or the same level of stereotypic behaviour (Garner *et al.*, 2006). This proposes that there is a genetic predisposition to stress induced changes to the mesoaccumbens dopaminergic system ultimately resulting in behavioural sensitisation (Cabib & Puglisi-Allegra, 1996; Cabib & Puglisi-Allegra, 2012). The brain is unique in that it maintains functional and morphological neuroplasticity into maturity to ensure homeostasis, so to a degree some adaptation is normal (Cabib, 2006). However, under chronic stress conditions this adaptation can occur at a greater degree resulting in alteration of behaviour. Indeed, such alteration can be applied to the performance of stereotypy (Cabib, 2006). It should be noted that in relation to neurological adaptation, stressors are those which involve appraisal, and therefore require the limbic system and higher cortical areas in contrast to the stressors which activate the HPA axis (Cabib & Puglisi-Allegra, 2012). Stressors can be species specific and include barren or unvarying environments, as discussed in section 2.2.2 (Garner *et al.*, 2006).

Dopamine has an important role in motivational behaviours, particularly those involving the more limbic areas of the basal ganglia such as the ventral striatum, and in some cases the striosomes (Cabib & Puglisi-Allegra, 2012). However these dopaminergic pathways also have a role when an animal is undergoing stress (Cabib & Puglisi-Allegra, 1996; Cabib, 2006; Cabib & Puglisi-Allegra, 2012). For example dopamine, particularly tonic dopamine, has a modulatory role on the 'coping' response (Cabib & Puglisi-Allegra, 1996; Cabib *et al.*, 2002; Cabib & Puglisi-Allegra, 2012). Coping refers to the animals efforts to gain control over the environment; Under these circumstances, there is an increase in dopamine release from the NAcc (Cabib, 2006). However, should the animal fail to cope, it succumbs to 'helplessness' whereby under these circumstances the initial dopamine levels released from the NAcc falls below basal levels in a biphasic manner (Cabib, 2006; Cabib & Puglisi-Allegra, 2012). This highlights

that stress induced adaptations within the brain tend to have a greater impact on the areas which receive dopaminergic projections from the mesencephalon (Cabib, 2006). Initially, an imbalance between cortical and subcortical regions of the brain such as the basal ganglia is observed, consequently the pathways of the basal ganglia are imbalanced between the ventral and dorsal striatum (Cabib, 2006). The resultant behaviour outcomes are inflexible, impulsive and compulsive, all behaviours stereotypic animals are known to exhibit (Garner & Mason, 2002; Cabib, 2006). Furthermore, following chronic stress the initial activation of mesoaccumbens dopamine for 'coping' is progressively eliminated, though the point at which the dopamine release is inhibited during 'helplessness' remains unaffected (Cabib & Puglisi, 2012; Cabib, 2006). Thus the mesocortical dopamine response becomes sensitised, though there is a reduction in mesoaccumbens dopamine response (Cabib *et al.*, 2002; Ventura *et al.*, 2002).

The adaptation of dopaminergic response due to chronic stress is dependent on genetic and environmental interactions. Indeed, the inbred strain of C57BL/6 (C57) mouse is susceptible to this inhibition of dopamine release during failed coping (Cabib, 2006). In comparison, for mice of the inbred strain DBA/2 (DBA) previous experience heavily impacts the dopaminergic response (Cabib & Bonaventura, 1997; Cabib, 2006). For example, group housed DBA mice with *ad lib* food demonstrated the normal biphasic mesoaccumbens dopamine response, though under repeated stress trials a reduction in initial dopamine release was observed (Cabib & Bonaventura, 1997; Cabib, 2006). In stark contrast to this the individually housed free-fed DBA mice demonstrated an increased onset of dopamine inhibition in response to repeated restraint, with the individually housed food restricted DBA mice exhibiting increased levels of mesoaccumbens dopamine release (Cabib, 2006). Further evidence utilising only the individually housed DBA mice which were either free-fed or food restricted suggests that social isolation alone results in the mice responding to restraint as though they had been previously restrained. Furthermore, the addition of food restriction had the opposite effect (Cabib, 2006).

Cabib (2006) suggests that this adaptation of dopaminergic pathways implicates dopamine receptors, particularly D2 type receptors within the shell region of the NAcc (Cabib & Puglisi-Allegra, 2012). The D2 receptor subtypes expressed by post-synaptic non-dopamine neurons mediate the effects of dopamine transmission and once activated results in locomotion and stereotypy. In comparison the auto-receptor dopaminergic neurons have an inhibitory effect on dopamine synthesis and neuronal activity, thus when activated inhibit locomotion and stereotypy (Cabib, 2006). Interestingly, for non-stressed animals the location of increased D2 receptors differs for C57 and DBA mice (Cabib *et al.*, 1998; Cabib, 2006). For example, when D2 receptors are activated the C57 mice exhibit increased locomotion and stereotypic climbing suggesting an increased sensitivity of post synaptic D2 receptor subtypes, whereas the DBA mice demonstrated inhibition of locomotion and stereotypies suggesting increased sensitivity of the auto-receptor dopaminergic neurons (Cabib, 2006). Furthermore, the analysis of D2 receptor densities in the NAcc within both strains highlighted increased auto-receptor D2 dopamine neurons for DBA mice compared to increased D2 receptors for post-synaptic non-dopamine neurons for the C57 strain (Cabib, 2006).

However, adaptations as a result of chronic stress reverse these differences; C57 mice develop increased auto-receptor D2 dopamine neurons whereas the DBA mice develop increased D2 receptors on non-dopamine neurons as a result of gene-environment interactions (Cabib & Bonaventura, 1997; Cabib, 2006). The resultant effect is a predisposition to behavioural sensitisation (see Chpt 2.1.1). Indeed, environmentally induced sensitisation effects of amphetamine are demonstrated in DBA mice but not C57 mice which are known to be more susceptible to the effects of amphetamine under standard husbandry conditions, with this susceptibility being reduced following stressful situations such as restricted feeding and repeated restraint (Cabib & Bonaventura, 1997; Cabib, 2006). In contrast, C57 mice under a feed restriction regime and individually housed DBA mice are more predisposed to helplessness, though food restricted DBA mice are less predisposed to helplessness and are more predisposed to stereotyped behaviour in drug-free conditions (Cabib & Bonaventura, 1997).

Therefore, the adaptations which result in increased mesoaccumbens dopamine transmission result in behavioural sensitisation, active coping resulting in stereotypy development, though adaptations which inhibit dopamine transmission results in helplessness (Cabib, 2006; Cabib & Puglisi-Allegra, 2012).

In addition, repeated stress also has significantly different effects on dopamine receptor density between the C57 and DBA mice as measured by quantitative autoradiography (Cabib *et al.*, 1998). For example, DBA mice having undergone repeated stress demonstrate increased D1 type dopamine receptors in the NAcc, though C57 mice exhibit reduced D1 receptors within the dorsal striatum (Cabib *et al.*, 1998). Furthermore, D2 receptor densities were increased within the NAcc within DBA mice but reduced D2 densities were observed within the substantia nigra, though no differences in these loci were observed in C57 mice (Cabib *et al.*, 1998). However, C57 mice expressed increased D2 receptor density on the VTA compared to a significant decrease in DBA mice (Cabib *et al.*, 1998). These results indicate a relationship is required for stereotypy development between genetic predisposition and environmental interactions (Cabib *et al.*, 1998; Cabib *et al.*, 2002; Cabib, 2006).

Recent unpublished data also implicates a genetic predisposition to altered dopamine receptor subtypes in the stereotypic horse (Hendry & Hemmings, 2012). In fact, within a relatively small population of crib-biting horses significant genetic mutations were observed, perhaps suggesting a genetic predisposition to behavioural sensitisation, and consequently, the performance of stereotypic behaviour (Hendry & Hemmings, 2012). Interestingly, the loci of these genetic mutations corresponds to D1 dopamine receptor subtypes not D2 receptor subtypes with one mutation at base pair 66, and another at base pair 135 (Hendry & Hemmings, 2012). Whilst this tends to disagree with the findings presented by Cabib (2006), McBride and Hemmings (2005) identified that within a population of 9 crib-biting animals compared to a sample of 9 control horses, the crib-biting horses had significantly higher D1 and D2 receptor densities within

the NAcc (McBride & Hemmings, 2005). Additionally, significantly lower D1 receptor densities and D2 receptor affinity were observed within the caudate (McBride & Hemmings, 2005). However, this is still in agreement with previous evidence in that it is the mesoaccumbens dopaminergic pathway that is altered during stereotypy/behavioural sensitisation. In regards to the horse, TB's and WB's are 3.1 times (Bachmann *et al.*, 2003) and 1.8 times (Wickens & Heleski, 2010) respectively more likely to exhibit crib-biting behaviour, again hinting towards a genetic predisposition to stereotypy formation, though the differing management regimes of these horses compared to other breeds should not be discounted.

Evidence from bank voles also suggests a potential genetic predisposition to stress resulting in stereotypy performance (Schoenecker & Heller, 2000). Previously, evidence from a number of species appeared to suggest that offspring were more likely to exhibit stereotypic behaviour if at least one of the parents were also stereotypic (Schoenecker & Heller, 2000). Consequently, Schoenecker and Heller (2000) selectively bred bank voles to investigate the impact of having stereotypic parents. Interestingly, not only did having stereotypic parents increase the likelihood of the offspring exhibiting stereotypic behaviour, but the mothers type of stereotypy also had an effect on the stereotypy performed (Schoenecker & Heller, 2000). The authors argue that the genetic predisposition in combination with the mothers stereotypic behaviour being a stressful or frustrating environment may result in the acquisition of stereotypy in offspring (Schoenecker & Heller, 2000).

Additionally, research into a genetic component to schizophrenia also appears to suggest dopamine genetics as influential on psychotic behaviour development (Eells *et al.*, 2006). Indeed, four patients with schizophrenia and one patient suffering from bipolar disorder all demonstrated a mutation at nuclear receptor Nurr1 which is expressed in dopamine neurons (Eells *et al.*, 2006). In rodents, these mutations have been found to reduce mesolimbic and mesocortical dopamine levels and importantly elevate locomotor activity, a behaviour strongly

characterised by dopamine (Eells *et al.*, 2006). The authors highlight that while this mutation was only evident in 1% of the schizophrenic population tested, the mutation was never observed in control individuals (Eells *et al.*, 2006). Again, this highlights genetics as influential to the occurrence of psychotic behaviour.

2.2.3.1 Interim Summary

Overall, this is an area that requires more research. Whilst there are evident strain differences observed between the inbred C57 and DBA mice, contrasting evidence from the horse in regards to dopamine receptor subtypes underlying stress induced sensitisation resulting in stereotypy requires further investigation. Indeed, crib-biting is but one of a number of equine stereotypies and current genetic evidence from the horse investigating stereotypy is limited in terms of sample size. Therefore, this is a potential area for future research. Utilising a larger population of both crib-biting and weaving horses would be beneficial in further determining the impact of the genetic-environment relationship for stereotypic development.

2.3 Behavioural Probes of Altered Brain Function

As discussed, basal ganglia dysfunction may be causal of stereotypic behaviours. Indeed, invasive studies have determined altered basal ganglia physiology in the rodent as influential on stereotypic behaviour development. Non-invasive behavioural probes are an effective measure of dopaminergic physiology within the striatum due to the control of instrumental behaviours utilising both A-O learning and S-R habit formation (Yin & Knowlton, 2006; Balleine *et al.*, 2009). Furthermore, other authors have determined that midbrain dopamine sensitivity can also be measured utilising non-invasive methods. These are discussed in more detail below.

2.3.1 Tolmans Cross Maze

As the name suggests, the Tolmans cross maze was first implemented by Tolman in 1932 on a population of rats with results concluding that the striatum is important for utilising a 'response' strategy (Yin & Knowlton, 2006; Shiflett & Balleine, 2011). Indeed, lesions of the dorsal striatum in rodents resulted in predominance of 'place' strategy compared to hippocampal lesions resulting in predominance of 'response' strategy during a cross maze example, despite overtraining which is known to induce response strategies (Yin & Knowlton, 2004; 2006). In particular, it is the putamen/sensorimotor circuits that are required to allow fixed S-R response strategies to be formed successfully, as the caudate is implicated in the adaptation of A-O strategies (Johnson *et al.*, 2007). Subsequently, the cross maze has since been utilised by a number of researchers on an array of species, including higher vertebrate species such as horses, and has been found to be an effective non-invasive method of determining basal ganglia dysfunction (Parker *et al.*, 2009). The Tolmans cross maze investigates basal ganglia dysfunction as a means of testing the acceleration of A-O, or place strategy to S-R, or response strategies (Yin & Knowlton, 2004).

Figure 9: The Tolmans Cross Maze. The baited arm is indicated with a blue and yellow square; the green arrow in the probe phase indicates the correct response seen in a control animal, the red arrow represents the response from an animal with basal ganglia dysfunction. Adapted from Yin & Knowlton (2006).

The cross maze is constructed of a north, east, south and west facing arm (Fig. 9) with a number of external visual cues present (Yin & Knowlton, 2006). The procedure can be divided into two main phases; the training phase and the probe phase (Yin & Knowlton, 2004; Yin & Knowlton, 2006; Parker *et al.*, 2009). For example, during the training phase the east arm may be baited and the west arm would not, though the visual cues would be identifiable in both arms (e.g. a food bowl). The animal would be placed first through the south arm of the maze and utilise trial and error to determine the location of the food reward (Johnson *et al.*, 2007). This training stage is repeated to ensure the animal has successfully identified the correct location of the food reward (Parker *et al.*, 2009). In contrast, the probe phase is undertaken only once. During the probe test, the animal is entered into the opposite arm of the maze, in this example that's the north arm, though neither bucket is baited (Parker *et al.*, 2009). Animals with normally functioning basal ganglia will enter the maze and turn left as they have learnt that the food reward is contained within the east arm. However, animals with a sensitised dopaminergic response will not turn towards the east arm, rather they would turn right towards the west arm, as these animals are utilising a response, not a place, strategy (Yin & Knowlton, 2004).

This differential response during the maze test is dependent on the dominant striatal circuit. As discussed, during habit formation there is a shift from flexible learning controlled by the limbic and associative circuitry, though as the behaviour becomes under the control of sensorimotor circuitry the behaviour becomes fixed (Yin & Knowlton, 2004). It is suggested that stereotypic animals exhibit an accelerated shift from the flexible A-O learning to inflexible S-R learning characterised by the increasing control of the sensorimotor circuits as a result of the altered dopamine sensitivity (Parker *et al.*, 2009). Therefore, during the probe test control animals utilise flexible A-O place strategy as the number of repeat trials is not enough for a habit to have formed. Inversely, the stereotypic animals will not be able to utilise flexible A-O learning during the probe phase due to accelerated sensorimotor circuitry control; thus will utilise a habitual, motor response strategy (Yin & Knowlton, 2004).

Indeed, Parker *et al.* (2009) demonstrated such a phenomenon in a population of horses displaying the equine oral stereotypy crib-biting in comparison to non-stereotypic horses, building on previous work suggesting accelerated A-O to S-R processes in sensitised animals (Parker *et al.*, 2008). Despite the relatively small population size of just 5 crib-biting and 5 control horses, the evidence highlighted that crib-biting animals display preferential S-R type learning, suggesting acceleration from flexible limbic and associative striatal circuits culminating with the sensorimotor circuits, resulting in accelerated habit formation within these animals (Parker *et al.*, 2009). The authors also highlight the alteration of balance between matrix and striosome activation with the striosomal cells becoming dominant during the shift from A-O to S-R learning, corresponding with the changes observed in the striatum when an animal is exposed to pharmaceutical induced stereotypy (Parker *et al.*, 2009). This suggests that alteration of the basal ganglia circuits does indeed correspond to cross maze performance in stereotypic individuals. Therefore, the cross maze is a good, well established non-invasive probe utilised to investigate basal ganglia dysfunction (Yin & Knowlton, 2006) which has previously been successfully applied to the stereotypic horse to highlight basal ganglia dysfunction as causal to STB (Parker *et al.,* 2009), though due to the small sample size utilised, further investigation is required.

2.3.2 Radial Arm Maze

The radial arm maze (RAM) consists of eight arms and is an adaptation of the Tolmans cross maze originally designed by Olton & Samuelson (1976) to measure spatial learning and memory tasks (Janitzky *et al.*, 2011). During initial investigations into spatial learning and memory utilising the RAM each of the eight arms were fully baited and the rat (*Rattus norvegicus*) was required to visit each arm once to receive the food reward (Dubreuil *et al.*, 2003). Since then, adaptations of this original design have been utilised (He *et al.*, 2002; Dubreuil *et al.*, 2003) whereby only certain arms are baited. Trials where the same arms are baited during each run are known as fixed position of reward task (FPRT) compared to the variable position of reward task (VPRT) whereby only four of

the arms are baited but the placement of these are varied between each trial (He *et al.*, 2002). Interestingly, in trials utilising VPRT reinforcement the rats chose to enter each arm only once during each trial and did not immediately re-enter the arms that were rewarded in the previous task (He *et al.*, 2002).

The original version of the RAM proposed by Olton & Samuelson (1976) is known as a win-shift task (Packard & Knowlton, 2002; Dubreuil *et al.*, 2003). During win-shift tasks animals undergo daily training sessions whereby there are no visual or auditory cues indicating the location of the reward, rather the animal is required to traverse the maze and learn the location of maze arms already visited to achieve the food reward (Packard & Knowlton, 2002; Yin & Knowlton, 2006). Furthermore, the animal would need to learn to not return to previously visited maze arms during the same trial, with re-entries recorded as errors (Packard & Knowlton, 2002; Yin & Knowlton, 2002). As the win-shift task requires the animal to remember which arms have been visited during each daily trial, this type of task is successfully utilised to examine spatial working memory (Packard & Knowlton, 2002). However, the suggestion that Tolmanian cognitive mapping strategy may also influence performance in win-shift tasks should also be taken into account (Packard & Knowlton, 2002). Indeed, win-shift tasks are sensitive to reward devaluation and due to the impairment effect of hippocampal lesions on win-shift performance the hippocampus has been selected as a possible structure controlling win-shift type tasks (He *et al.*, 2002; Yin & Knowlton, 2002). Interestingly, these hippocampal lesions have no impact on win-stay type tasks (Packard & Knowlton, 2002; Debreuil *et al.*, 2003), however as the hippocampus is known to have 'place' cells, allowing the animal to succeed during spatial memory tasks by utilising extra-maze place cues (Poucet *et al.*, 1991) unlike the striatum, this is perhaps unsurprising (Retailleau *et al.*, 2012).

In contrast, a relatively new adaptation of the RAM known as the win-stay paradigm utilises a stimulus such as a light (Fig. 10) to indicate the position of the food reward (Yin *et al.*, 2006). Gradually, the rodent would learn that the

presentation of the light bulb indicates the location of baited maze arms, consequently would select these arms to retrieve the reward (Yin & Knowlton, 2006). Errors are recorded when the animal incorrectly enters an unlit maze arm (Packard & Knowlton, 2002). After extensive training the animals' response becomes insensitive to reward devaluation, suggesting attenuation of S-R habit formation rather than stimulus-stimulus association (Packard & Knowlton, 2002; Yin & Knowlton, 2006). Subsequently, it is thought that win-stay tasks are under the influence of the dorsolateral striatum (Yin & Knowlton, 2006). Indeed, dorsal striatal lesions were demonstrated to impair performance of the win-stay task, though no impact was observed during win-shift type tasks (Packard & Knowlton, 2002; Delgado *et al.*, 2005). In addition, post-training injection of dopamine D2-receptor agonists into the dorsal striatum impaired the performance of rodents during the win-stay task but not the win-shift type task (Lovinger, 2010). When combined with the knowledge that dopamine agonist injection has been demonstrated to increase the acquisition of S-R habit formation (Yin *et al.*, 2006) and even induce stereotypy (Parker *et al.*, 2009), the dorsal striatum, particularly the putamen, is highlighted as a key structure for habit formation.

Figure 10: An example of a rodent in a radial arm maze. In this example, the rodent is undergoing a win-stay type task to examine striatal functioning (Yin & Knowlton, 2006).

In summary the dorsolateral striatum has a distinct role in habit formation. Indeed, the use of lesion studies has demonstrated the importance of the putamen for the acquisition of S-R habit formation. The Tolmans cross maze reviewed in section 2.3.1 highlighted that stereotypic animals exhibit enhanced S-R habit formation as a result of altered dorsal striatum function. Consequently, the win-stay RAM could also be utilised to test for altered basal ganglia function in stereotypic animals in a similar manner. If the proposed causality of stereotypic behaviour is indeed altered basal ganglia function, one would expect impairment of performance in the win-stay type RAM task in a population of stereotypic animals in comparison to control animals. However, the author is currently unaware of any studies that have so far tested this hypothesis, highlighting this as an area that requires further research.

2.3.3 Extinction learning

Extinction paradigms with a devaluation phase are the gold standard method undertaken to analyse habit behaviour (Schwabe & Wolf, 2011). Indeed, Faure *et al.* (2005) successfully implicated dopamine as influential in habit formation with an extinction paradigm. Subsequently extinction, or devaluation tasks, give an indication of A-O learning or S-R habitual responding (Yin & Knowlton, 2006; Grahn *et al.*, 2008). As discussed, this process is a natural, beneficial one allowing the animal to focus attention elsewhere in the face of an ever changing environment (Schwabe & Wolf, 2011). However, the acceleration of this process in stereotypic animals is not necessarily beneficial, and is a potential indication of stress induced changes within the striatum (Hemmings *et al.*, 2007; Dias-Ferreira *et al.*, 2009; Schwabe & Wolf, 2011).

The procedure of an extinction paradigm is a relatively simple but effective one (Schwabe & Wolf, 2011). For example, the animal (be it rodent or otherwise) is shaped to perform an action in return for a food reward, often in response to a stimulus (Hemmings *et al.*, 2007; Schwabe & Wolf, 2011). Initially, this behaviour is controlled by flexible A-O circuitry, though when overtraining occurs the

behaviour is controlled via inflexible S-R circuitry resulting in habit formation. In some extinction paradigms, once the association is acquired the reward undergoes devaluation, however this is not apparent in all extinction paradigms (Hemmings *et al.*, 2007; Schwabe & Wolf, 2011). At this stage the animal then undergoes extinction i.e. the food reward is no longer delivered when the animal performs the previously rewarded action (Hemmings *et al.*, 2007; Schwabe & Wolf, 2011). An animal that utilises flexible A-O learning would be sensitive to reward devaluation and extinction; in contrast an animal with accelerated ventral to dorsal circuitry control would exhibit S-R habitual responding, in that the previously rewarded behaviour will continue despite lack of reward attainment (Hemmings *et al.*, 2007; Schwabe & Wolf, 2011). Furthermore, rodents exposed to amphetamine, which increases dopamine levels within the striatum and perhaps mimics the increased sensitivity to dopamine during stereotypy, demonstrated a rapid progression from A-O to S-R (Nelson & Killcross, 2006) similar to that observed in stressed and stereotypic animals (Hemmings *et al.,* 2007; Dias-Ferreira *et al.,* 2009).

In terms of stereotypy, the proposed accelerated shift of control from the ventral to dorsal striatal circuitry (Parker *et al.,* 2009) suggests one would expect to observe a lack of reward devaluation or reward extinction (i.e. increased perseverance) under an extinction paradigm. Evidence suggests increased dopaminergic sensitivity is causal of this increased perseveration (Garner, 2006; Hemmings *et al.*, 2007). As there is a potential link between stereotypy and stress induced changes within the striatum, the work of Schwabe and Wolf (2011) is worthy of mention. Indeed in a population of seventy-six students, those who were exposed to stressful situations prior to an extinction paradigm indicated a reduced sensitivity of instrumental responding to extinction, i.e. the individuals exposed to a brief stressor demonstrated enhanced use of S-R type responding rather than flexible A-O learning (Schwabe & Wolf, 2011).

Furthermore, Dias-Ferreira *et al.* (2009) examined chronic stress of the striatal circuitry, namely the associative and sensorimotor circuits. A sample of 8 rodents

were exposed to an established chronic stress paradigm, with a further 8 rodents utilised as a control group to determine the effect of stress on striatal circuit dominance (Dias-Ferreira *et al.*, 2009). The rats were shaped to press a lever for a food reward, counterbalanced between either sucrose or pellets under a ratio schedule as opposed to interval schedule, as ratio schedules are known to create a stronger bias towards A-O type behaviour (Yin & Knowlton, 2006; Dias-Ferreira *et al.*, 2009). Indeed, the stressed animals were insensitive to reward devaluation, which the authors proposed is resultant of the rapid shift to a habitual response rather than A-O learning (Dias-Ferreira *et al.*, 2009) highlighting acceleration of ventral-dorsal circuitry control.

In a second study by the same authors, a separate group of control and chronically stressed rats were shaped to press one lever for a sucrose reward and another lever for a pellet reward (Dias-Ferreira *et al.*, 2009). During this extinction paradigm one of the levers was subjected to reward devaluation. The control rodents significantly reduced lever pressing for the devaluated reward in comparison to the non-degraded lever. In contrast the stressed rodents continued to press both levers similarly suggesting their behaviour was habitual (Dias-Ferreira *et al.*, 2009). Subsequent neuro-analysis demonstrated atrophy of the associative striatal circuit and hypertrophy of the sensorimotor striatal circuit suggesting that the altered behaviour in stressed rats is consequential of decreased associative monitoring and an increased reliance on S-R learning (Dias-Ferreira *et al.*, 2009). Therefore, as stress is thought to contribute to the onset of stereotypic behaviour, the same insensitivity to reward devaluation and extinction should be observed in a group of stereotypy prone animals.

Indeed, Hemmings *et al.* (2007) investigated just this in a sample of control (n=10) and crib-biting (n=10) horses though in the absence of reward devaluation. Horses were shaped to press a square button in response to a conditioned stimulus (CS), which in this case was both auditory and visual, presented simultaneously (Hemmings *et al.*, 2007). As expected the crib-biting horses required significantly more operant responses ($P<0.01$) and unreinforced

trials to extinction (P<0.01) demonstrating that stereotypy appears to have the same rapid shift from ventral to dorsal circuitry control within the striatum as observed in the chronically stressed rodents described above. In contrast to this concept, the alterations discussed in section 2.2.2 whereby crib-biting horses exhibited significantly increased D1 and D2 receptor activation in the NAcc and significantly decreased D1 receptor activation in the caudate (McBride & Hemmings, 2005) may result in increased responding to predictive cues even when they are no longer as predictive as they once were, implicating the ventral striatum in enhanced perseveration (Garner, 2006; Hemmings *et al.*, 2007). Similarly, Garner and Mason (2002) demonstrated that stereotypy frequency is positively correlated with impaired extinction learning in stereotypic bank voles (*Myodes glareolus*) (Garner, 2006). Indeed, further research in other species including blue tits, marsh tits and two species of bears (*Ursus thibetan* and *Helarctos malayanu*) has also demonstrated stereotypy is highly correlated with impaired performance in an extinction paradigm (Vickery & Mason, 2003; Garner, 2006).

The evidence presented here appears to suggest a) a link between stress and stereotypy and b) stressed and stereotypic animals exhibit insensitivity to reward devaluation and extinction. Furthermore, this insensitivity appears to be highly correlated with the level of stereotypy exhibited by individuals of a number of species. Consequently, the extinction paradigm is the gold standard in determining basal ganglia dysfunction in a variety of animals and thus is a good measure of determining altered striatal functioning in stereotypic animals.

2.3.4 Behavioural Initiation

A relationship has been observed between stereotypy performance and extinction learning. Evidence from bank vole and mice (*Mus musculus*) studies suggests behavioural initiation (the rate at which animals switch between different behaviours) is also correlated with stereotypy and performance in extinction tasks (Garner, 2006; Würbel, 2006). In addition, strain differences in

mice populations have also been observed, with the more hyper-vigilant strains exhibiting higher levels of stereotypic behaviours (Würbel, 2006). While accelerated habit formation explains impaired performance in extinction tasks for stereotypic or stressed animals, the same S-R type habit formation would not explain increased levels of overall activity. However, the concept of an up-regulated direct pathway would (Tanimura *et al.,* 2009).

Drug and lesion studies of the direct pathway by acting on the D1 type receptors is known to result in activation or disinhibition of behaviour, whilst the same on the indirect pathway results in either the suppression or activation of stereotypy (Garner & Mason, 2002). The administration of dopaminergic agonists such as amphetamine to induce stereotypy is known to involve suppression of the indirect pathway by acting on the D2 type receptors (Garner & Mason, 2002; Garner *et al.*, 2003b; Garner, 2006), subsequently behavioural disinhibition results due to the resulting dominance of the direct pathway (Garner, 2006). As stereotypic behaviour develops in amphetamine administered animals, there is also an increase in the rate of initiation between different behaviours (Garner, 2006). As administration of dopamine agonists also results in hyperactivity (Arnt *et al.,* 1988) perhaps a chain of events occurs during stereotypy development commencing with increased locomotor behaviour, leading to increased rate of behaviour initiation, culminating in the occurrence of stereotypic behaviour. Indeed Chartoff *et al.* (2001) demonstrated this chain of events in their sample of dopamine deficient mice. Garner (2006) suggests that stereotypic animals undergo changes in other behaviours also, including enhanced rates of behavioural initiation characterised by increased impulsivity and impairments in extinction learning, a phenomenon known to be true (Garner & Mason, 2002). Indeed, during their study on a population of bank voles (n=8) by utilising observation and calculating mean number of behaviours initiated per minute a significant correlation was observed between behavioural initiation and stereotypy performance (Garner & Mason, 2002).

Garner *et al.* (2003a) also implicates dorsal striatal circuitry as being causal of stereotypy and increased rates of behavioural initiation. Having already demonstrated that captive bank voles exhibit increased behavioural initiation and that this was correlated with stereotypic performance there is scope to examine this in other captive animals. To examine the potential for a correlation between behavioural initiation and stereotypic performance in a different species, in this case blue tits and marsh tits (Garner *et al.*, 2003a), would be beneficial. The birds were video recorded and the mean rate of behavioural initiation per second was calculated over a four minute period (Garner *et al.*, 2003a). However, in contrast to the evidence in the bank vole population, the results from the marsh tit and blue tit populations were not so clear cut. Initially, as behavioural initiation rates increased as did the stereotypy level, though as the behavioural initiation rates continued to increase the level of stereotypy dropped (Garner *et al.*, 2003a). However, the authors suggest this may have simply been an artefact of the experimental methods as the birds tended to switch behaviours at such a speed that correct and proper observations were not possible (Garner *et al.*, 2003a). Furthermore, the relative complexity of tit stereotypic behaviour (route tracing) could also have had an impact on the results (Garner *et al.*, 2003a). Other research in some bear species has also suggested that this link may not be as simple as that observed in bank voles (Garner, 2006). Despite this, it could still be concluded that there is a correlation between stereotypic performance and rate of behavioural initiation.

Behavioural initiation is an area that requires further research. Whilst some evidence suggests a positive correlation between behavioural initiation and levels of stereotypic behaviour performance, other studies provide conflicting arguments. Consequently, investigating behavioural initiation in other species would be influential in determining whether alteration of the direct and indirect pathways is causal to stereotypic behaviour, and the other behavioural side effects observed as a result of stereotypy. The horse could provide a good indicator of this, as these stereotypic behaviours are relatively simple. Furthermore, as strain differences have been suggested in rodents this could also be tested in horses. For example, TBs exhibit increased occurrence of

stereotypic behaviour compared to other horse breeds, however there is a lack of scientific information regarding behavioural initiation in the horse. Subsequently, further research utilising stereotypic horses as a higher vertebrate model for examining rate of behaviour initiation in comparison to control horses would provide further weight to the concept of basal ganglia dysfunction as causal to stereotypic behaviours.

2.3.5 Pre-pulse Inhibition

Pre-pulse inhibition (PPI) is the suppression of a startle reflex when a weak pre-pulse (or stimulus) precedes the startling stimulus by approximately 30 to 500 milliseconds (Caine *et al.*, 1995; Swerdlow *et al.*, 2002; Howland *et al.*, 2004). Research has demonstrated that PPI is an effective operational measure of sensorimotor gating (Cadenhead *et al.*, 1993). In other words, PPI is an effective method of determining whether animals are effective at filtering/buffering the large array of sensory information presented to the brain (Cadenhead *et al.*, 1993). Where animals are ineffective at this, there is potential for sensory overload and secondary cognitive fragmentation and disorganisation (Cadenhead *et al.*, 1993; van den Buuse, 2004). Often, these studies utilise auditory startle methods by first exposing the animal to low levels of 'white' background noise (typically 70dB (Eells *et al.*, 2006)). Once complete, louder sound levels are utilised (perhaps 120dB (Eells *et al.*, 2006)) to generate a startle response. Altered PPI is thought to be indicative of basal ganglia dysfunction. A number of disorders are thought to result from basal ganglia dysfunction including Tourette's Syndrome and schizophrenia (Swerdlow *et al.*, 2002). Indeed, humans diagnosed with schizophrenia indicate a decreased PPI (Cadenhead *et al.*, 1993; van den Buuse, 2004). Subsequently, an underlying commonality could be relevant to further investigating PPI and stereotypic behaviour (Swerdlow *et al.*, 2002).

Altered cortico-limbic-striatal circuitry, such as increased dopamine levels, is apparent in patients with schizophrenia, particularly in the forebrain structures

such as hippocampus and NAcc (Howland *et al.*, 2004; Ojima *et al.*, 2004). In combination with rodent models investigating PPI, the mesolimbic dopaminergic system is implicated as causal to reduced PPI (van den Buuse, 2004). Furthermore, the administration of dopaminergic agonists such as apomorphine and amphetamine also results in a disruption of PPI in a rodent model (Swerdlow *et al.*, 2002; van den Buuse, 2004). In particular, these dopamine studies suggest that the D2-type receptors rather than D1-type receptors are responsible for the alteration of PPI (Cadenhead *et al.*, 1993; Caine *et al.*, 1995; van den Buuse, 2004; Eells *et al.*, 2006; Onogi *et al.*, 2010; Weber *et al.*, 2010). Indeed, research has identified that the cortico-striato-pallido-pontine circuitry is crucial in effectiveness of sensory gating (Cadenhead *et al.*, 1993; Koch *et al.*, 2000; Howland *et al.*, 2006). As discussed it is the NAcc shell and striosomes of the caudate (Koch *et al.*, 2000), that have prominent projections to the ventral pallidum. The ventral striatum is also the primary innervation and projection site from more limbic areas of the basal ganglia, suggesting alterations in this striatal area reduces PPI, with Koch *et al.* (2000) identifying SNpr lesions as a commonality in stereotypy performance and decreased PPI. Indeed, the dopaminergic projections to the NAcc may be implicated in modulation of PPI (Powell *et al.*, 2003; van den Buuse, 2004; Howland *et al.*, 2004). Yamada *et al.* (1997) suggest that it is the sensitivity of the dopamine autoreceptors within the NAcc that may impact PPI, with low PPI being associated with low autoreceptor sensitivity. Furthermore, the authors also suggest that when a startling stimulus is presented, there is negative feedback inhibition of dopamine neurons, resulting in decreased endogenous dopamine levels. However, when a pre-pulse stimulus is presented the release of dopamine prevents this negative feedback (Yamada *et al.*, 1997). Again, this suggests that dopamine activity is key for the PPI response.

As this evidence of dopaminergic influence was primarily based on pharmacological manipulations (Yamada *et al.*, 1997; Swerdlow *et al.*, 2001; Powell *et al.*, 2003), Swerdlow *et al.* (2002) investigated endogenous dopamine levels in relation to PPI. During this study, endogenous dopamine levels were measured utilising eye blink rate (see section 2.3.6), and utilised auditory startle

response as a measure of PPI (Swerdlow *et al.*, 2002). The authors hypothesised that should PPI be regulated by dopamine, the individuals whom exhibit high levels of endogenous dopamine (i.e. the individuals with increased eye blink rate) would exhibit low levels of PPI (Swerdlow *et al.*, 2002). Indeed, this was found to be case in a population of 83 right handed males, providing some evidence for a link between dopaminergic activity and PPI (Swerdlow *et al.*, 2002). However, PPI is also thought to be regulated by other neurotransmitters such as glutamate and serotonin (Cadenhead *et al.*, 1993; Swerdlow *et al.*, 2002; Howland *et al.*, 2006), with Furuya *et al.* (1999) and Wan *et al.* (1996) suggesting PPI disruption is as a consequence of NMDA receptor-mediation and is in fact independent of dopamine receptors. Subsequently, this is a potential area for further research.

There is a general consensus that the dopaminergic systems in the striatum are involved with the alteration of PPI highlighted by a number of studies administering dopamine agonists, with this alteration being reversed by the administration of dopamine D2 receptor antagonists (Cadenhead *et al.*, 1993). As stereotypy could well be the result of altered dopaminergic function within the basal ganglia striatum, PPI could be an effective behavioural probe of altered basal ganglia function as causal for stereotypy development. For example, in stereotypic animals there is believed to be an alteration of the sensitivity of dopamine receptors with increased D1 and D2 type receptors in the ventral striatum and decreased D1 receptors in the caudate (McBride & Hemmings, 2005), perhaps replicating the effects of increased exogenous dopamine levels in basal ganglia disorders such as schizophrenia (van den Buuse, 2004).

Consequently, one would expect to observe altered PPI in a stereotypic animal. Whilst Koch *et al.* (2000) attempted to integrate stereotypy performance into their study of PPI on SNpr lesions, and observed that animals with SNpr lesions both had a reduction in PPI and increase in stereotypy performance, further research is required. Furthermore, work conducted by Li *et al.* (2011) administering dopamine agonists observed that it was the dosing schedule that impacted PPI

rather than the type of D2 agonist administered. As a similar incremental process of dopamine efflux is observed in stereotypic animals this provides evidence that PPI would be decreased in stereotypic animals. In contrast, Piljman *et al.* (2003) demonstrated that emotionally stressed animals did not differ from control animals when testing PPI. However there is potential that at this stage the animals had not been exposed to a prolonged period of stress neither were they genetically predisposed to altered basal ganglia function. Powell *et al.* (2003) observed disrupted PPI in socially isolated rodents and further implicated the NAcc as causal for PPI deficits. Further evidence (Swerdlow *et al.*, 2001; Eells *et al.*, 2006) indicates a genetic difference in PPI response. Therefore, the proposed function of dopaminergic systems in stereotypy performance as well as the reduction in PPI suggests that PPI could be a useful behavioural probe in determining basal ganglia dysfunction as the causal pathology in stereotypic animals (Koch *et al.*, 2000).

2.3.6 Spontaneous Blink Rate

On the surface, the observation of spontaneous blink rate (SBR) may seem irrelevant to determining basal ganglia dysfunction. However SBR has been successfully utilised as a measure of dopamine release within the striatum (Karson, 1983). A blink is described as a temporary closure of both eyes using movement from both the upper and the lower eyelids, where the pupil is temporarily hidden from view though the position of the eyeball does not necessarily change (Blount, 1927). SBR in primates is described as bilateral paroxysmal brief repetitive eye closures that occur continuously and in the absence of obvious external stimuli, and is not affected by external stimuli such as light, heat or humidity (Karson, 1983).

Consideration of the neuroanatomical and physiological control of blinking would be worthwhile if dopamine is influential on SBR, as a number of factors can influence blink rate. For example, reflex blinking occurs in response to sudden sensory stimuli and is often thought of as a trigeminofacial reflex consisting of

two main parts; R1 and R2, where R1 is a simple pontine reflex and R2 includes the spinal pathway of the trigeminal nerve (Karson, 1983). Similarly, dry spots in the corneal tear film also elucidates a blink response, however as dry spots appear around 15 to 30 seconds after a blink, meaning on average 2 to 4 blinks per minute would be required to maintain corneal lubrication, a number far below the average adult blink rate, this may not be an influential factor on SBR in any case (Karson, 1983).

However, upon investigation there also appears to be a link between SBR and dopamine within the basal ganglia. Basal ganglia dopamine levels are thought to alter trigeminal reflex blink amplitude via SNpr inhibition of the superior colliculus, which excites the nucleus of the raphe magnus which results in inhibition of the spinal trigeminal complex (Kaminer *et al.*, 2011). Swerdlow *et al.* (2002) also suggest a link between SBR and PPI, which is also under the control of SNpr indirectly where low levels of PPI are associated with increased SBR, further implicating dopamine levels for altered SBR (Swerdlow *et al.*, 2002). Indeed, Taylor *et al.* (1999) found robust evidence to suggest strong positive correlations between SBR and dopamine levels within the rostral ventromedial caudate nucleus in a population of 9 adult male monkeys (*Cercopithecus aethops sabaeus*). Furthermore, this is just one study of a number conducted on an array of species including other breeds of monkey, rodents and humans highlighting a link between dopamine levels and SBR (Karson, 1983). Consequently, administration of dopamine agonists and antagonists has been utilised to determine further effects of dopamine on SBR (Jutkiewicz & Bergmen, 2004; Colzato *et al.*, 2008).

Indeed, prior to the evidence provided by Taylor *et al.* (1999), a large body of work was conducted by Karson *et al.* (1981a; 1981b; 1983). One of the primary studies implicating dopamine as influential on SBR utilised a population of four rhesus monkeys and the administration of 0.36mg/kg dopamine agonist apomorphine (Karson, 1983). Astonishingly, 30 minutes post subcutaneous injection the SBR was quadrupled, though this effect was inhibited when

sulphuride (a D2 receptor blocker) was administered three hours prior to apomorphine (Karson, 1983). Again, as suggested with PPI, it would appear that D2 receptors are implicated in basal ganglia dysfunction.

Thus, when considering basal ganglia dysfunction, SBR has been utilised to test for altered dopamine levels within the striatum. For example, patients with Parkinson's disease exhibit a reduced SBR (Chen *et al.*, 1996), though interestingly in patients with Tourette Syndrome SBR was found to be correlated with the intensity of the tics (Chen *et al.*, 1996). Furthermore, schizophrenia was found to have increased SBR (Chen *et al.*, 1996; Aarts *et al.*, 2012). This would again suggest the role of dopamine sensitivity in basal ganglia dysfunction and the ability to assess this utilising SBR, as Parkinson's disease is characterised by a loss of nigrostriatal dopaminergic cells within the caudate (Delgado *et al.*, 2005) in stark contrast to schizophrenia, which is characterised by elevated endogenous dopamine levels. The dopamine depletion observed in Parkinson's disease would increase the activity of the SNpr thus increasing the blink amplitude, consequently observed SBR would be reduced, with the opposite occurring in schizophrenic patients (Kaminer *et al.*, 2011). Therefore, this suggests that reduced dopamine levels, or increased receptor sensitivity decrease the SBR in comparison to elevated dopamine levels increasing SBR (Kaminer *et al.*, 2011).

Not only has SBR been utilised to investigate known basal ganglia pathologies, SBR has also been utilised as an indicator to determine basal ganglia dysfunction as causal of stereotypic behaviour (Roebel & MacLean, 2007). Indeed, research has now indicated that the SBR of stereotypic individuals is in fact lower than SBR observed in their control counterparts (Roebel & MacLean, 2007). This evidence is in line with what is already known; i.e. stereotypic animals have decreased dopamine availability but have increased postsynaptic receptor sensitivity (McBride & Hemmings, 2005; Roebel & MacLean, 2007), therefore stereotypic animals would exhibit decreased dopamine levels within the midbrain. In contrast, Lewis *et al.* (1990) observed increased stereotypic

behaviour and SBR in monkeys (*Macaca mulatta*) which had previously been socially isolated when administered with apomorphine highlighting the use of SBR as a measure of dopamine in stereotypic animals. This study also implicated early life experience as being causal to these dopaminergic changes as discussed in section 2.2.2 (Lewis *et al.*, 1990). More recently, Colzato *et al.* (2009) successfully utilised SBR as a measure of dopaminergic functioning, further implicating dopamine as a modulator for action control.

Therefore, SBR is a useful non-invasive measure of determining altered dopamine sensitivity within the striatum, particularly the rostral part of the ventromedial caudate. When considering a stereotypic animal one would expect to observe reduced SBR in line with previous studies. As the methodology is reasonably straight forward in that eye blinks could be measured for a period of time, SBR is a robust, practical, proven and critically non-invasive (Barbato *et al.*, 2012) method that can be utilised as a behavioural probe in determining whether altered dopaminergic levels in the striatum are causal of STB by observing SBR in animals with environmentally induced stereotypy.

2.4 Final Conclusions and Recommendations

Despite a number of potential aetiologies being suggested as causal to stereotypic behaviour (Chapter 1), the literature provides strong evidence for basal ganglia dysfunction, in particular the striatum, as a key stereotypic output. For example, having understood the different pathways of the striatum as controlled by either the ventral striatum (limbic circuitry), caudate (associative circuitry) or the putamen (sensorimotor circuitry) as well as investigating previous studies on environmentally and pharmacologically induced stereotypy there appears to be a relationship between stereotypy and the dopaminergic pathways, particularly the direct and the indirect pathways. The ability to determine which striatal circuit is activated by utilising non-invasive behaviour probes such as the cross maze and an extinction paradigm has proved invaluable in contributing to the knowledge of stereotypic behaviour.

Furthermore, the ability to measure dopamine release within the striatum with SBR, PPI and effect of this alteration on the direct and indirect pathway activation with behavioural initiation has also enhanced the knowledge of dopaminergic stimulation of the striatal pathways as key to stereotypy development. In combination with emerging genetic evidence towards a predisposition to stress induced sensitisation of the striatum, thus inducing stereotypy, further convinces this author that the alterations of the striatum are key to stereotypy development.

The horse as a model of stereotypy has often been utilised successfully in studies of basal ganglia dysfunction (McBride & Hemmings, 2005, 2009; Hemmings *et al.*, 2007; Parker *et al.*, 2009; Issaoui & Hemmings, 2011; Hendry & Hemmings, 2012). Therefore this study will also be utilising the horse to further implicate altered basal ganglia dysfunction as causal to stereotypic behaviour development. To enable this to be successful, the following experiments have been selected as a result of the literature reviewed which are all well within the scope of this present study:

- Experiment 1: Stereotypic response to food reward
- Experiment 2: Spontaneous blink rate
- Experiment 3: Behavioural initiation
- Experiment 4: Extinction paradigm to investigate rate of habituation

Previously, increased SBR has been reported in the CB horse (Issaoui & Hemmings, 2011) suggesting an increase in elevated dopaminergic levels within the striatum. However, Roebel and MacLean (2007) reported conflicting results with decreased SBR in a sample of stereotypic humans. With McBride and Hemmings (2005) reporting increased dopamine receptor sensitivity within the NAcc of crib-biting horses, it is surprising a decreased SBR was not observed by Issaoui and Hemmings (2011). As such, it would be beneficial to repeat this work in a further sample of crib-biting horses and expand it by also investigating SBR in weaving horses due to the distinct lack of literature regarding the neuro-

aetiology of weaving animals. This will provide further insight into the dopamine levels within the stereotypic animals' striatum.

Behavioural initiation is also an area that requires further research. Whilst some evidence suggests a strong correlation between behavioural initiation and stereotypic behaviour performance, other studies provide conflicting arguments. Consequently, investigating behavioural initiation in other species would be influential in determining whether alteration of the direct and indirect pathways is implicated in stereotypic behaviour development. The horse could provide a good indicator of this, as these stereotypic behaviours are relatively simple. Furthermore, as strain differences have been suggested in rodents this could also be tested in horses. For example, TBs exhibit increased occurrence of stereotypic behaviour compared to other equine breeds, however there is a lack of scientific information regarding behavioural initiation. Subsequently, further research utilising stereotypic horses as a higher vertebrate model for examining rate of behaviour initiation in comparison to control horses would provide further weight to the concept of basal ganglia dysfunction as causal to stereotypic behaviours.

The extinction task utilised in this study will be parallel to that conducted by Dias-Ferreira *et al.* (2009) on a sample population of stressed rodents and used as an improvement on Hemmings *et al.* (2007). Indeed, Dias-Ferreira *et al.* (2009) highlighted that stressed rodents demonstrate an accelerated shift of control from the ventral to dorsal striatal circuits, though surprisingly a similar study has yet to be undertaken in a population of stereotypic animals of any species despite the distinct relationships noted by a number of authors between stress and stereotypy. Hemmings *et al.* (2007) utilised an extinction task within the horse to further highlight altered dorsal striatal circuitry as causal to stereotypic behaviour, though failed to implement sensory specific satiety with the use of a devaluation phase. Therefore, this model of the extinction task could not distinguish between appetitive and habitual behaviours. Consequently the extinction paradigm here will utilise a two-phase extinction paradigm

incorporating sensory specific satiety, effectively removing feeding motivation. The motivation to continue with the task will cease, thereby only habitual behaviours will be conducted, not appetitive tasks.

This will determine whether there is in fact accelerated shift from the ventral to dorsal striatal circuitry control within stereotypic animals as proposed by Parker *et al.* (2009) with their cross maze. However, the sample size utilised by Parker *et al.* (2009) was not large enough to draw any generalizable conclusions. Importantly the extinction task is less open to external stimuli than the cross maze (i.e. the horse utilising distal cues during the probe phase) thus providing clear evidence for either ventral or dorsal circuitry control.

To date, little research has been conducted on the neuro-aetiology of weaving horses. Previous authors (McBride & Hemmings, 2005; Hemmings *et al.,* 2007; Parker *et al.,* 2009) have only utilised crib-biting animals. Therefore the results of these studies can only be applied to the oral stereotypy crib-biting. To contribute further to the knowledge of stereotypy in horses as a whole it is important to consider locomotor stereotypy also, as there is no evidence to suggest the neuro-aetiology of these two stereotypies are comparable. Subsequently, this study will incorporate the use of crib-biting and weaving horses to further investigate the neuro-aetiology of equine stereotypy.

Building on previous research by Hemmings *et al.* (2007), Parker *et al.* (2009) and Dias-Ferreira *et al.* (2009) could have potential implications for the way in which stereotypic horses should be trained, as well as the maintaining welfare standards for horses displaying stereotypy. The methodology of these experiments is continued in Chapter 3.

Chapter 3: Methodology

3.1 Sample Population

A sample group consisting of crib-biting (n=8), weaving (n=8) and control horses (n=8) was selected following behavioural observation and personal communications with owners (Table 1). Stereotypic horses were required to express stereotypic behaviour as described by the owner and confirmed by the researcher. Weaving and crib-biting behaviour was observed in every case with evidence of CB such as chewed surfaces being observed in the horses stable (McBride & Hemmings, 2009).

Table 1 The Physical Characteristics of the Sample Population (CB=crib biting, W=weaving, C=control).

Horse	Age (Years)	Gender	Breed
CB1	5	Gelding	Argentinian Polo Pony
CB2	4	Mare	Argentinian Polo Pony
CB3	14	Gelding	Argentinian Polo Pony
CB4	8	Mare	Argentinian Polo Pony
CB5	25	Gelding	Argentinian Polo Pony
CB6	20	Gelding	Thoroughbred (TB)
CB7	19	Gelding	Welsh Section A
CB8	18	Gelding	Irish Sports Horse x TB
W1	16	Gelding	TBx
W2	18	Gelding	WB
W3	14	Mare	TB x Hanoverian
W4	12	Mare	Gypsy Cob
W5	9	Gelding	Belgian WBx
W6	6	Mare	Connemara x TB
W7	11	Gelding	Irish Draught
W8	14	Gelding	TB
C1	19	Gelding	TB x Hanoverian
C2	12	Gelding	TB x Argentinian Polo Pony
C3	32	Gelding	Argentinian Polo Pony
C4	22	Gelding	Argentinian Polo Pony
C5	12	Mare	Argentinian Polo Pony
C6	15	Mare	Argentinian Polo Pony
C7	20	Gelding	Welsh Section A
C8	7	Gelding	Irish Sports Horse

3.2 General Management Factors

Management factors were considered throughout data collection and are discussed further below. Please note that due to the horses being privately owned, observations were undertaken at a total of 8 different yards.

3.2.1 Feeding

As horses are trickle feeders, it was not necessary to deprive horses of food prior to any of the experiments as there would still be adequate motivation to obtain the food reward (Parker *et al.*, 2008). Similarly, the diet was not manipulated during the course of any study as not to induce stress. Furthermore when considering the individual workloads of the horses, it was important not to alter the diet to ensure individual dietary requirements were met, as well as to standardise food motivation in terms of nutrition (Hemmings *et al.*, 2007). In addition, for one hour pre- and one hour post feeding, data collection was avoided to minimise the distracting effects of feeding (Issaoui & Hemmings, 2011).

3.2.2 Stress Reduction

Stress is thought to result in increased occurrence of stereotypy due to the coping function (Wickens & Heleski, 2010). Reducing this stress by ensuring observations were conducted within the horses usual stable was essential as not to artificially increase stereotypic response and therefore alter blink rate (Cadenhead *et al.*, 1995) or behavioural initiation (Garner & Mason, 2002). To attempt to reduce stress the horse had eye-contact with conspecifics during the experiments. If the horse was thought to be subjected to undue stress as a result of the procedures, particularly upon presentation of the operant device during the extinction paradigm, the horse was removed from the study and a replacement found for ethical reasons.

3.3 Pilot Studies

To ensure methods selected were both practical and effective to achieve the aims and objectives, the methods described below were subjected to a prior pilot study to highlight any potential issues at an early stage. Consequently, any potential problems were highlighted prior to data collection, allowing data collection to be as efficient and valid as possible. A full write up of each pilot study and subsequent protocol amendments can be found in Appendices B, C and D.

3.4 Rate of Stereotypy

Stereotypic horses within this study were observed for rate of stereotypy both pre- and post-delivery of a small quantity (~5g) of a commercially available pelleted feed (SPILLERS® High Fibre Cubes). This feed was selected due to the low energy value (Digestible Energy: 8.4MJ/kg) therefore was unlikely to result in behaviour un-characteristic of the horse as a result of high energy content. This category of observations was undertaken within the horses' usual stable as not to induce stress, for the reasons previously described. The horse was allowed free movement of the stable, with *ad lib* forage based feed (i.e. hay or similar) and water available for the duration of the observation. As a consequence of the usual stable being utilised, a crib-biting surface was also available.

The animal was placed into the stable and allowed to habituate to the observers' presence for ten minutes. Should a crib-biting horse be wearing a crib-biting strap, this was removed immediately prior to habituation as were weaving bars. Following the habituation period, the observer recorded every stereotypic response within a fifteen minute period utilising a mechanical counter. Every crib-biting response (every individual crib-bite, not crib-biting bout) was recorded for the cribbing horses, with every weaving response being recorded (whereby one 'left-right-left' sequence or 'right-left-right' sequence equated to one weave) for the weaving horses. Once this fifteen minute period was complete, the horse was given 5g portion of commercial high fibre feed and immediately observed for

a further fifteen minutes. As with the pre-feed interval, every stereotypic instance was recorded. Weaving is thought to be cued as an anticipatory response performed in the context of food predictive conditioned stimulus (McAfee *et al.,* 2002), therefore, the weaving horses were alerted to the imminent attainment of feed by shaking a feed bucket 15 minutes before attaining the food. In contrast crib-biting behaviour is described as post-prandial, arising as a consequence of the act of feeding itself, therefore the prior shaking of the feed bucket was not required for these horses.

Once the final fifteen minutes was complete any further stereotypy responses were no longer recorded. The horse was then returned to the field or remained in the stable dependent on owner preference. This method was repeated in the same manner for three consecutive days permitting a mean to be calculated for pre- and post-feed for individual horses, as well as per stereotypy category.

Statistical analyses were performed utilising the Statistical Package for Social Sciences (SPSS) v.20.0. Mean stereotypic rate per 15 minute period of observation before and after feeding was calculated for each individual subject. In order to determine whether a significant difference was present before and after feeding for both crib-biting and weaving horses, the Kolmogorov-Smirnov test was conducted to test for normality (Hosker, 2008). Data were found to be normally distributed, subsequently a Paired Samples T Test was applied to compare the individuals stereotypy rate prior to and post feeding. In addition linear regression was undertaken to determine if age had a significant impact on rate of stereotypy pre- and post-feed. Finally, Independent Samples T Tests were conducted to determine if a significant difference in rate of stereotypy pre-feed and post-feed occurred between mares and geldings within the crib-biting and the weaving sample groups.

3.5 Spontaneous Blink Rate

The observation to measure SBR was conducted in a similar fashion to that of Chen *et al.* (1996), in line with previous unpublished data also conducted on the horse (Issaoui & Hemmings, 2011). Where possible the observation was undertaken at a quiet time of day at the yard to minimise the effect of external stimuli on blink rate. Where Chen *et al.* (1996) measured their human subjects in both a relaxed and attentive state, it was assumed that in this study the horses were in an attentive state due to the difficulty in determining and ensuring the horse remained in a relaxed state (Issaoui & Hemmings, 2011).

Initially, the horse was brought in from the field and placed into their usual stable and was allowed to habituate to the observers' presence for ten minutes. Should crib-biting horses be wearing a crib-biting strap, this was removed immediately before habituation as were any weave bars. Prior to this habituation process, the horse was loosely tethered to ensure that the observer had sufficient view of the horses left eye. Water was available *ad lib* during this observation. Following habitation, a timer was programmed to alert the observer when the half hour data collection duration was complete. As soon as the timer was initiated, each full blink (defined by Karson (1983) as bilateral paroxysmal brief repetitive eye closures occurring continuously) was recorded utilising a mechanical counter. Considering the anatomy of the horse, it was difficult for a solo observer to record true bilateral eye closures, so only the left eye was observed for all horses.

Following the half hour data collection period, the horse was either released back into the stable or the field depending on owner preference. This procedure was repeated for three consecutive days, allowing the blinks per 30 minute period were to be recorded a) each day, b) the overall mean blinks per 30 minute period for each horse and c) overall mean SBR per 30 minute period for control, crib-biting and weaving horses.

Mean SBR was calculated for cribbing, weaving and control groups utilising the mean SBR from each individual horse (n=8). To determine whether there was a significant difference in SBR between these three groups' data were first tested for normality. As data were normally distributed (as determined by a Kolmogorov-Smirnov test) an Analysis of Variance (ANOVA) was conducted with a post-hoc Least Significant Difference (LSD) test applied to highlight where these differences were observed. Linear regression analysis was employed to determine whether there was a significant relationship discovered in this sample of stereotypic animals between rate of stereotypy and blinks 30/minute (Roebel & MacLean, 2007). Furthermore linear regression was undertaken to determine if age had a significant impact on SBR. Finally, Independent Samples T Tests were conducted to determine if a significant difference in SBR occurred between mares and geldings of the control, crib-biting and the weaving sample groups.

3.6 Rate of Behaviour Initiation

The procedure for measuring rate of behaviour initiation within this sample of horses was adapted from the method utilised by Garner and Mason (2002) on a sample of bank voles within their home cage. Consequently, rate of behaviour initiation for the horse was also conducted within the horses own stable in line with this, and the aforementioned stress reduction. For the duration of this observation *ad lib* hay and water was available, and as with the previous observations the horse was brought in from the field and habituated to the observers presence for ten minutes, or should the horse already be within their stable the horse was simply habituated to the observer. Where possible, these observations were conducted at a quiet time of day at the yard to minimise the distracting effect external stimuli could have on the rate of behaviour initiation of the horse. Where crib-biting horses were wearing a crib-biting strap, this was removed immediately prior to habituation, as were weave bars.

Following habitation, a timer was programmed to alert the observer when the half an hour observation period was complete. Once the timer had been initiated,

every behaviour initiation was recorded utilising a mechanical counter. Following the half hour recording period the horse was returned to the field or left within the stable depending on owner preference. This procedure was completed in the same manner for three days allowing a) behaviour initiations per 30 minutes to be calculated for each individual per day, b) mean behaviour initiations per 30 minutes over the three day period to be calculated and c) overall mean behaviour initiation per 30 minutes for control, weaving and crib-biting sample groups.

All behaviours performed were defined by a pre-determined ethogram as described by McDonnell (2003) and included the different types of standing behaviour. During the course of this observation the type of behaviour the horse performed was not taken into consideration, only the number of new initiations of behaviour was recorded. To ensure rate of behavioural initiation was not over or under represented, stereotypic behaviour was included under the ethogram with each bout being recorded as a new initiation of behaviour as per Garner and Mason (2002). Furthermore, each bout of behaviour was recorded as one initiation irrespective of the previous behaviour, consequently the sequence 'Feeding – Grooming – Feeding – Drinking – Standing Rest' was recorded as four initiations (Garner & Mason, 2002). Consequently, sub-movements of behaviour, for example raising the head whilst still undergoing mastication as part of feeding was not recorded as an initiation of a new behaviour.

The Kolmogorov-Smirnov test found data to be normally distributed. Subsequently, to analyse significant differences between the means calculated for rate of behaviour initiation for crib-biting, weaving and control groups (calculated utilising the mean data from each individual horse) an ANOVA was employed with a post-hoc LSD test. Furthermore for stereotypic horses mean number of behaviours initiated per 30 minutes was calculated and correlated with linear regression analysis with stereotypy as per Garner and Mason (2002) to determine whether there was a significant relationship between rate of behaviour initiation and stereotypy rate. Linear regression was also utilised to

determine if age had a significant impact on rate of behaviour initiation. Finally, Independent Samples T Tests were conducted to determine if a significant difference in rate of behaviour initiation occurred between mares and geldings of the control, crib-biting and the weaving sample groups.

3.7 Extinction Paradigm

To determine whether there is an accelerated shift from ventral to dorsal striatal circuitry control in stereotypic horses an extinction paradigm utilising sensory specific satiety was employed to build on the results of previous authors (Hemmings *et al.*, 2007; Dias-Ferreira *et al.*, 2009; Parker *et al.*, 2009). To date, the methods utilised to determine accelerated S-R learning in horses (Hemmings *et al.,* 2007; Parker *et al.*, 2009) have been flawed. The sample size utilised by Parker *et al.* (2009) was too small, and only included crib-biting animals, to allow any extrapolations to be made to the general stereotypic horse population. In addition Hemmings *et al.* (2007) did not employ any devaluation stage, so was not a true measure of habit formation as appetitive behaviours were also be recorded as operant responses, and again only crib-biting horses were utilised. The use of sensory specific satiety in combination with extinction employed here was essential in determining control of either ventral or dorsal striatal circuitry upon data collection. As the more ventral striatal circuits are characterised by A-O learning, the devaluation of the food reward thus reducing motivation to feed would result in faster extinction rates in control horses. In contrast the more dorsal striatal circuits are characterised by S-R learning, consequently if stereotypic horses were insensitive to reward devaluation and still attempted to attain the food reward following reward devaluation and extinction then the behaviour would be habitual. Indeed Dias-Ferreira *et al.* (2009) demonstrated just this in a population of stressed rodents. Consequently a suitable adaptation based upon Dias-Ferreira *et al.* (2009) and Hemmings *et al.* (2007) was utilised on this population of horses.

For the successful completion of the extinction paradigm the horse was required to learn to press a triangle conditioned stimulus (CS) card adhered to an operant device (Plate 3) to achieve a food reward.

Plate 3: The operant device utilised during the extinction task. Hooks on the reverse of the device allows the board to be attached to the stable door.

As for the previous tasks which required a food reward, 5g of SPILLERS® High Fibre Cubes was administered on successful muzzle contact at fixed ratio 1 (Hemmings *et al.*, 2007). For ease of data collection the extinction task was divided into eight distinct phases as seen in Figure 12, each explained in more detail below.

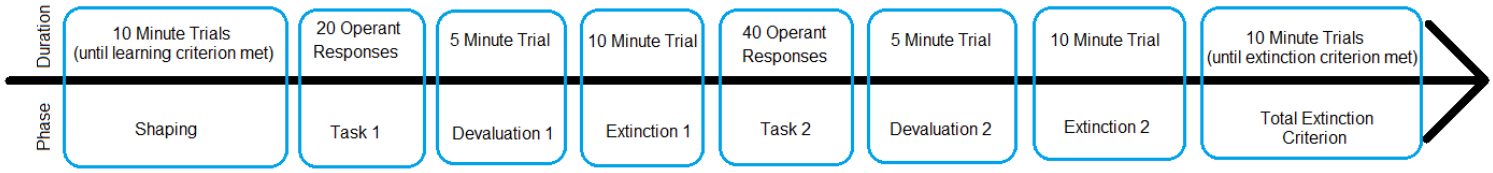

Figure 11. The 8 distinct phases of the Extinction Paradigm in the order conducted.

3.7.1 Shaping Phase

In an attempt to minimise the horse associating any food reward with the observer, the horse was initially habituated to the operational device under supervision. Crib-biting straps were removed from the crib-biting horses where present immediately prior to habituation. As the operant device utilised was mobile, the extinction paradigm could be conducted within the horses usual

stable. During habituation 5g of high fibre cubes were placed within the feeding bucket of the device to encourage the horse to investigate. The habituation process was complete when the horse consumed the food within the bucket, demonstrating they were comfortable with the device.

Successive approximation was then utilised to shape the horse to associate the triangle CS card with a food reward, eventually resulting in the horse selecting the CS card on the operant device to ensure that human influence on the task was minimised. The triangle CS card was constructed of laminated A4 paper which was adhered to the operant device with Velcro (Fig. 12). Initially, the triangle CS card was presented to the horse next to the operant device with 5g of SPILLERS® High Fibre Cubes being rewarded via the feeding tube when the horses' muzzle came into contact with the CS card. This process was repeated in 10 minute trials, with a two minute break in between each trial, until the horse successfully made ten successive muzzle contacts with the triangle CS card whilst the observer was present. The process was then repeated in the same manner with the observer behind the operant device in such a way that the observer was no longer visible to the horse, ensuring visual cues from the observer did not impact the horses' behaviour. Learning criterion for the shaping phase was set at ten successive operant responses of the triangle CS card when presented on the device with no human contact. The total number of trials taken to achieve this was recorded as response acquisition. Following response acquisition, the device was removed from the stable door for a two minute break before the start of task 1.

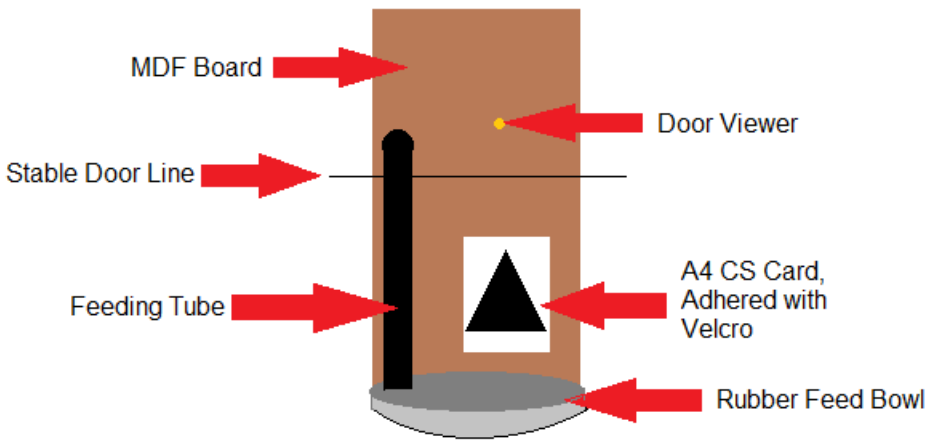

Figure 12. The operant device. The door viewer was utilised so the horse did not associate the researcher with food reward rather than the CS card.

3.7.2 Task 1

The triangle CS card was adhered to the operant device and placed over the stable door, signifying the start of the trial. Each time the horses muzzle came into contact with the triangle CS card, 5g of SPILLERS® High Fibre Cubes was rewarded (fixed ratio 1). The learning criterion for task 1 was set at 20 consecutive operant responses of the CS card. Following the 20th operant response, task 1 was considered complete and the operant device was removed for a 2 minute break prior to devaluation 1.

3.7.3 Devaluation 1

Following the 2 minute break after the completion of task 1, sensory specific satiety devaluation 1 occurred. Exactly 1kg of SPILLERS® High Fibre Cubes was weighed and administered to the horse in a black bucket. The horse was allowed *ad lib* access to this bucket for 5 minutes, importantly in the absence of the operant device and with minimum human contact. Once the 5 minute devaluation phase was complete the bucket was removed from the stable with the horse again allowed a 2 minute break prior to extinction 1. During this time any remaining high fibre cubes were weighed on electric scales, with the resultant weight being recorded.

3.7.4 Extinction 1

The operant device was presented to the horse for a 10 minute trial. During this trial, operant responses of the CS card were not rewarded with any administration of high fibre cubes. During extinction 1 two key observations were recorded; the first was latency of approach to first operant response and the second was the total number of operant responses the horse conducted during the 10 minute trial. Once the trial was complete the operant device was removed from the stable door and the horse allowed a 2 minute break.

3.7.5 Task 2

The operant device was attached to the stable door and again all operant responses were rewarded with 5g of high fibre cubes at fixed ratio 1, as occurred in task 1. In contrast to task 1 the learning criterion for task 2 was set at 40 consecutive operant responses, allowing time for development of habitual responding. Immediately after the 40th operant response of the CS card the operant device was removed as in task 1 and there was a 2 minute break before devaluation 2 was initiated.

3.7.6 Devaluation 2

Devaluation 2 was undertaken in the same manner as devaluation 1.

3.7.7 Extinction 2

Extinction 2 was undertaken in the same manner as extinction 1.

3.7.8 Total Extinction of the Operant Response

The criterion to reach total extinction of the operant response was set at two successive distinct 10 minute trials, with a 2 minute break between each trial, with zero operant responses to avoid the effects of spontaneous recovery. This was undertaken after extinction 2 only, not extinction 1. Following extinction 2 the device was removed for a 2 minute break and then presented for a 10 minute trial with operant responses of the CS card yielding no food reward. As with the previous extinction trials, each new trial required latency of approach to first operant response and total number of operant responses within each trial to be recorded. Once extinction criterion had been met the total number of trials for the horse to achieve total extinction criterion was documented. Upon completion of the extinction task horses were either left untied in the stable or returned to the field dependent on owner preference.

Once data were tested for normalcy of distribution using the Kolmogorov-Smirnov test, a series of parametric ANOVAs were utilised to compare the crib-biting, weaving and control horses' performance within the extinction task. These ANOVAs were used to determine significant differences regarding the following:

- Total number of trials taken to achieve learning criterion between CB, weaving and control horses;
- Total number of trials taken to reach total extinction criterion between CB, weaving and control horses;
- Latency of approach in extinction 1 between CB, weaving and control horses;
- Total number of operant responses in extinction 1 between CB, weaving and control horses;
- Latency of approach in extinction 2 between CB, weaving and control horses;
- Total number of operant responses in extinction 2 between CB, weaving and control horses.

A Paired Samples T Test was utilised to determine if there were any significant differences observed in number of operant responses between extinction 1 and extinction 2 for the control, crib-biting and weaving horses.

Linear regression was undertaken to determine if age had a significant impact the parameters described above. Further linear regression analysis was undertaken utilising the number of operant responses during extinction 1, extinction 2 and trials taken to reach total extinction criterion to evaluate the effect of dopamine use SBR and behavioural initiation data on extinction criterion for stereotypic animals. Finally, Independent Samples T Tests were conducted to determine if a significant difference within the parameters described above occurred between mares and geldings within the control, crib-biting and the weaving sample groups.

3.7.9 Feeding Motivation

As it could be argued that this particular extinction paradigm could simply be a measure of feeding motivation, the amount of food remaining following both devaluation 1 and 2 was recorded. This was subsequently subjected to an ANOVA, whereby no significant differences in remaining feed would suggest that all of the sample groups demonstrated equal motivation for feed. Linear regression analyses were also undertaken to determine if age had a significant impact on the amount of feed remaining following devaluation 1 and 2. Finally, Independent Samples T Tests were utilised to determine if there was a significant difference in feed remaining following devaluation 1 and 2 between mares and geldings of all sample groups.

4.1 Rate of Stereotypy

A Paired-Samples T-Test was utilised to determine if there was a significant difference in the rate of stereotypy performed pre-feed compared to rate of stereotypy performed post-feed for both the crib-biting and weaving sample groups.

Indeed within the crib-biting population there was a significant increase between rate of stereotypy post feed compared to rate of stereotypy pre-feed t_7=-6.442, p<0.001 (Fig.13). This demonstrates that for crib-biting horses feed has a significant impact on rate of stereotypy in crib-biting horses.

In contrast, no significant difference was observed between rate of stereotypy pre-feed and rate of stereotypy post-feed (t_7=0.956, p=0.371) within the weaving sample group (Fig. 13). Consequently, feed had no significant impact on rate of stereotypy for weaving horses.

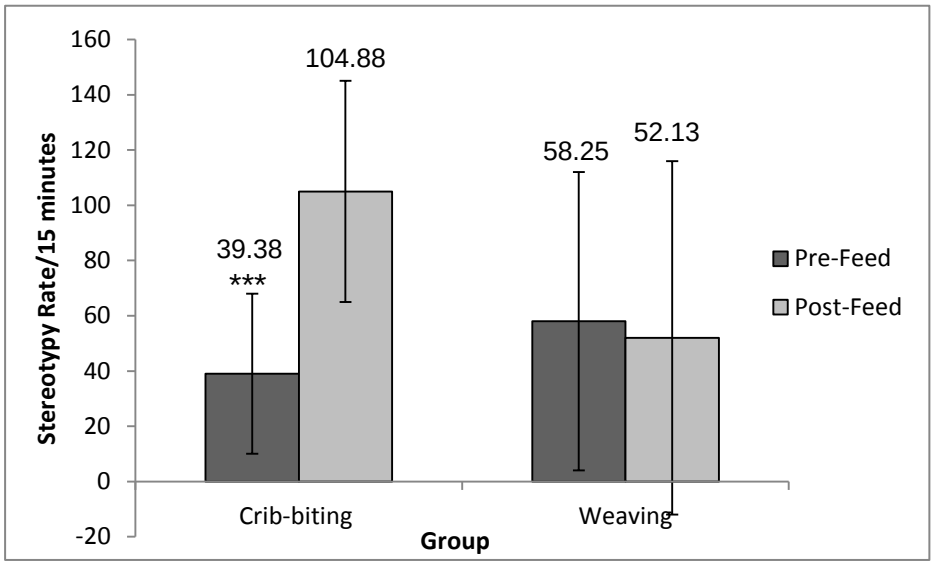

Figure 13. Mean rate of stereotypy (±std dev) observed during the 15 minute duration prior to feeding compared to rate of stereotypy observed during the 15 minute duration post feeding (*=p<0.001).**

To determine if the age of the horses utilised had an impact upon the mean rate of stereotypy performed pre-feed and post-feed for both the crib-biting and the weaving populations linear regression analysis was undertaken. No significant linear relationships were observed pre-feed for either the crib-biting ($F_{1,6}$=0.010, r=0.041, r^2=0.002, p=0.923) or the weaving ($F_{1,6}$=2.370, r=0.532, r^2=0.283, p=0.175) animals. Similarly, no significant linear relationship was discovered post-feed for the crib-biting ($F_{1,6}$=0.001, r=0.013, r^2=0.000, p=0.975) nor the weaving ($F_{1,6}$=1.939, r=0.434, r^2=0.188, p=0.282) horses.

4.2 Spontaneous Blink Rate

A one-way analysis of variance (ANOVA) was conducted to determine whether a significant difference was observed between the spontaneous blink rates of control, crib-biting and weaving horses (Fig. 14). The one-sample ANOVA found there to be a significant difference between the three groups ($F_{2,21}$=7.555, p=0.003). The post-hoc Least Significant Difference (LSD) test highlighted a significantly lower SBR for the crib-biting horses compared to the control horses (p=0.047), as well as a significantly lower SBR for the crib-biting horses compared to weaving animals (p=0.001). However no significant difference was observed between the SBR of the control and weaving horses (p=0.091).

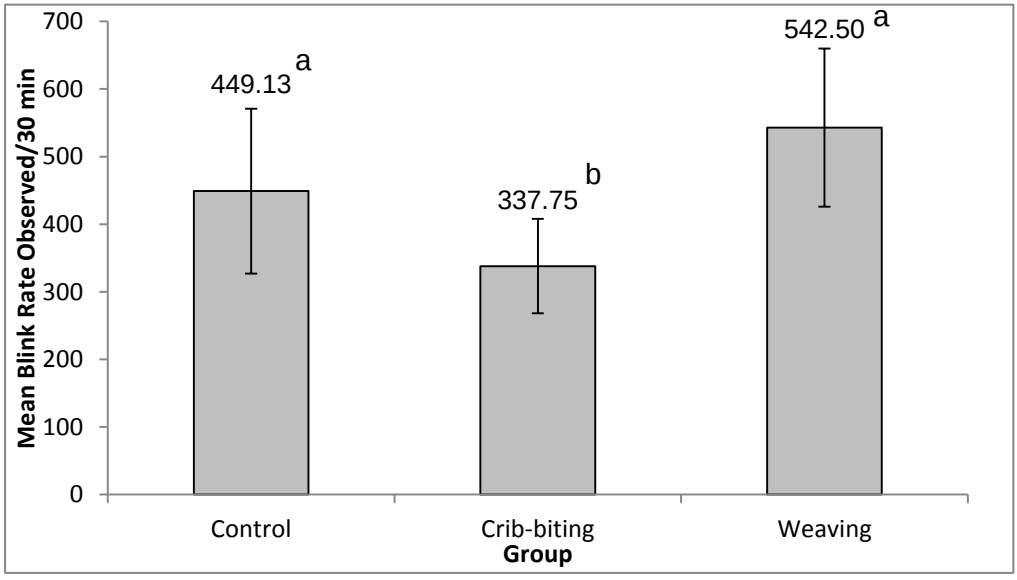

Figure 14. A demonstration of mean blink rate (±std dev) observed between Control, Cribbing and Weaving horses (values sharing superscripts are not significantly different).

Linear regression analysis determined that there was no significant linear relationship between age and mean SBR within all horses ($F_{1,22}$=1.251, r=0.232, r^2=0.054, p=0.275), control horses ($F_{1,6}$=1.428, r=0.439, r^2=0.192, p=0.277), crib-biting horses ($F_{1,6}$=0.777, r=0.339, r^2=0.115, p=0.412) or weaving horses ($F_{1,6}$=0.201, r=0.180, r^2=0.032, p=0.669).

4.3 Rate of Behaviour Initiation

To analyse whether significant differences in number of behaviours initiated/30 minute period were present between control, crib-biting and weaving groups a one-way ANOVA was utilised (Fig. 15). The one-way ANOVA found the differences between groups to be significant ($F_{2,21}$=8.330, p=0.002). Post-hoc LSD analyses found there to be a highly significant increase of behaviour initiations for the crib-biting compared to control group (p=0.001), as well as a significant increase of behaviour initiations for the weaving compared to control animals (p=0.033). No significant difference was observed between the crib-biting and weaving horses (p=0.087).

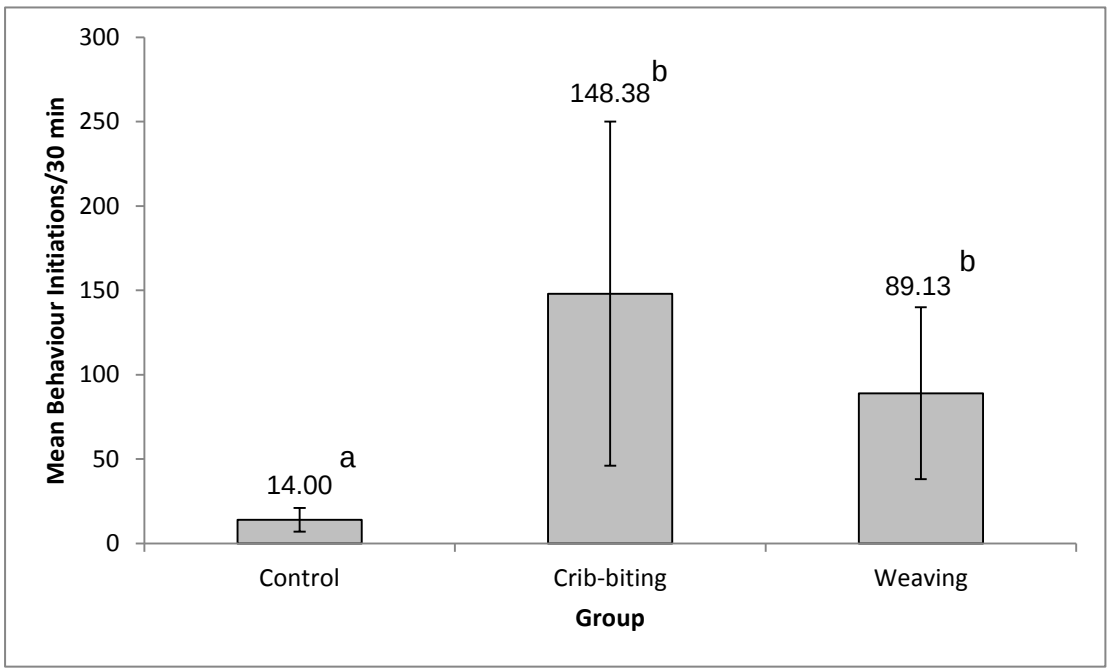

Figure 15. The mean behaviours initiated (±std dev) during the 30 minute observation period for Control, Cribbing and Weaving horses. Values sharing superscripts are not significantly different.

Linear regression analyses demonstrated that there was no significant linear relationship between age of the horse and rate of behaviour initiation when looking at all horses ($F_{1,22}$=0.214, r=0.098, r^2=0.010, p=0.648), control horses ($F_{1,6}$=0.565, r=0.293, r^2=0.086, p=0.481), crib-biting horses ($F_{1,6}$=0.000, r=0.006, r^2=0.000, p=0.989) or weaving horses ($F_{1,6}$=1.975, r=0.498, r^2=0.248, p=0.210).

4.4 Extinction Paradigm

As a number of parameters were recorded during the extinction paradigm, each of these will be analysed in more detail in individual sections below.

4.4.1 Trials Taken to Reach Learning Criterion

The mean trials taken to reach learning criterion was analysed with a one-way ANOVA which found a significant difference between the three groups (Fig. 16) ($F_{2,21}$=28.282, p<0.001). Subsequent post-hoc Least Significant Difference (LSD) tests demonstrated that control horses required significantly more trials to reach learning criterion when compared to both crib-biting (p<0.001) and weaving horses (p<0.001). However no significant difference was found between the crib-biting and the weaving horses (p=0.789).

There was no significant linear relationship between age and trials taken to reach learning criterion when considering all horses ($F_{1,22}$=0.770, r=0.184, r^2=0.034, p=0.390), control horses ($F_{1,6}$=0.231, r=0.193, r^2=0.037, p=0.648), crib-biting horses ($F_{1,6}$=1.016, r=0.380, r^2=0.145, p=0.352) or weaving horses ($F_{1,6}$=1.238, r=0.414, r^2=0.171, p=0.308).

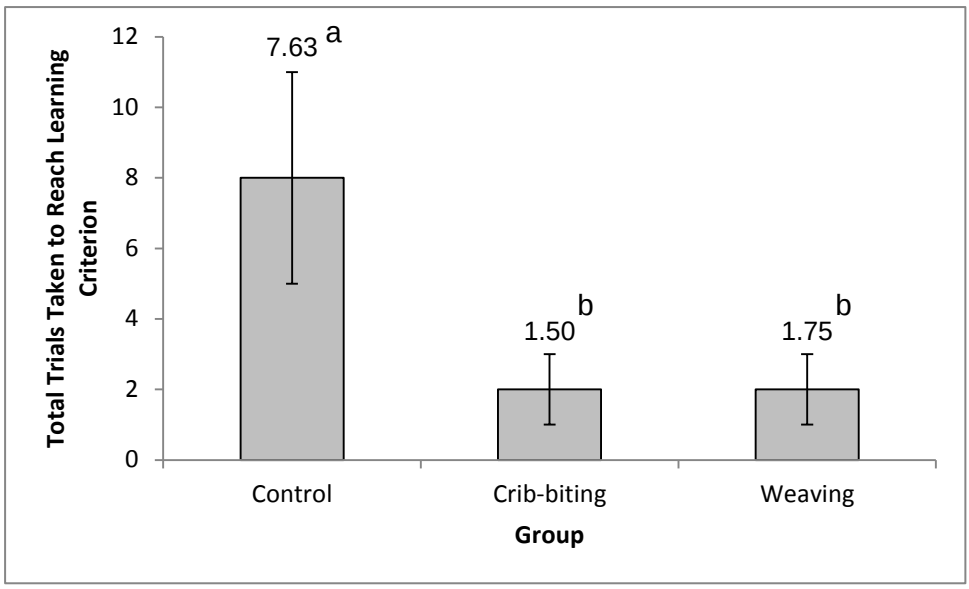

Figure 16. The mean number of trials taken for each group to reach learning criterion (±std dev). Values sharing superscripts are not significantly different.

4.4.2 Feed Leftover following Devaluation 1

The mean mass of feed leftover following devaluation 1 for the control horses was 645.50±205.98g, 526.88±306.70g for the crib-biting and 455.13±238.93g for the weaving horses. A one-way ANOVA indicated that there was no significant difference between the three groups ($F_{2,21}$=1.146, p=0.337). As no difference was found a post-hoc LSD test was not required.

In addition no significant linear relationship was observed when considering age of horse and feed leftover following devaluation 1 when taking into account all horses ($F_{1,22}$=0.294, r=0.115, r^2=0.013, p=0.593), control horses ($F_{1,6}$=2.053, r=0.505, r^2=0.255, p=0.202), crib-biting horses ($F_{1,6}$=0.249, r=0.200, r^2=0.040, p=0.635) or weaving horses ($F_{1,6}$=0.048, r=0.089, r^2=0.008, p=0.834).

4.4.3 The Number of Operant Responses Conducted During Extinction 1

The one-way ANOVA (Fig. 17) found a highly significant difference between sample groups ($F_{2,21}$=26.221, p<0.001). Subsequent post-hoc LSD tests demonstrated that the crib-biting animals conducted significantly more operant responses when compared to the control (p<0.001) and the weaving groups

(p<0.001). However no significant difference was observed between the control and weaving horses (p=0.276).

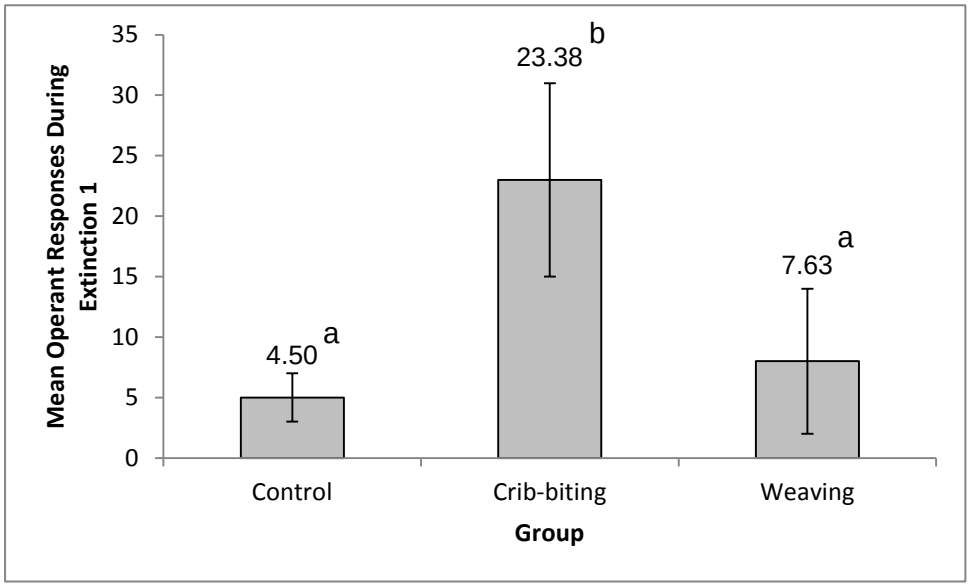

Figure 17. The mean number of operant responses (±std dev) performed during extinction 1 by the control, crib-biting and weaving horses. Values sharing superscripts are not significantly different.

No significant linear relationship was observed between age and all horses ($F_{1,22}$=1.487, r=0.252, r^2=0.063, p=0.236), control horses ($F_{1,6}$=0.005, r=0.027, r^2=0.001, p=0.949), crib-biting horses ($F_{1,6}$=1.980, r=0.498, r^2=0.248, p=0.209) or weaving horses ($F_{1,6}$=0.738, r=0.331, r^2=0.110, p=0.423).

4.4.4 Latency of Approach to the First Operant Response during Extinction 1

Data collected from latency of approach to the first operant response were subjected to a one-sample ANOVA (Fig. 18) which found a significant difference between the three sample groups ($F_{2,21}$=3.626, p=0.044). When data were further subjected to a post-hoc LSD test, there was found to be a significant increase in the mean latency of approach to the first operant response for extinction 1 between the control and crib-biting horses (p=0.035), as well as the control and weaving horses (p=0.026). No significant difference was observed between the crib-biting and the weaving (p=0.887) animals.

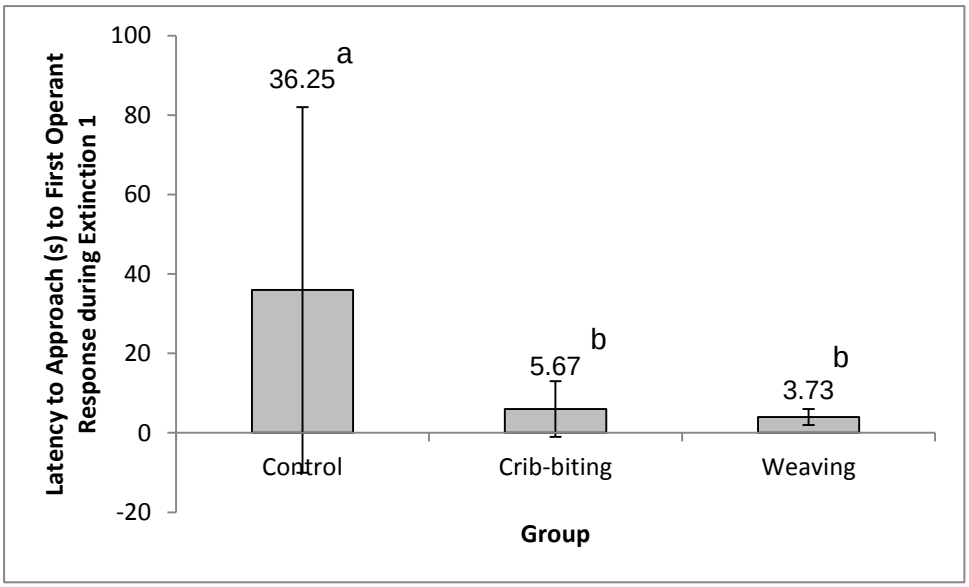

Figure 18. The latency of approach to the first operant response (±std dev) during extinction 1 for control, crib-biting and weaving horses. Values sharing the same superscript are not significantly different.

No significant linear relationships were observed between age for all horses ($F_{1,22}$=0.026, r=0.035, r^2=0.001, p=0.873), control horses ($F_{1,6}$=0.467, r=0.296, r^2=0.072, p=0.520), crib-biting horses ($F_{1,6}$=0.800, r=0.343, r^2=0.118, p=0.405) or weaving horses ($F_{1,6}$=0.004, r=0.027, r^2=0.001, p=0.950).

4.4.5 Feed leftover following Devaluation 2

The one-way ANOVA was undertaken to determine if any significant differences were present between mean amount of feed remaining following devaluation 2 for control horses (695.88±223.45g), crib-biting horses (575.25±263.95g) and weaving horses (570.00±279.28g). The ANOVA stated that there was no significant differences present between any of the three groups ($F_{2,21}$=0.616, p=0.550). Consequently the post-hoc LSD test was not utilised.

There was also no significant linear relationship between mass of feed leftover following devaluation 2 and age when considering all horses ($F_{1,22}$=0.008, r=0.019, r^2=0.000, p=0.929), control horses ($F_{1,6}$=1.061, r=0.388, r^2=0.150, p=0.343), crib-biting horses ($F_{1,6}$=0.013, r=0.047, r^2=0.002, p=0.913) or weaving horses ($F_{1,6}$=0.398, r=0.249, r^2=0.062, p=0.551).

4.4.6 Number of Operant Responses Conducted during Extinction 2

A one-way ANOVA was utilised to determine whether there were any significant differences between the mean number of operant responses performed during extinction 2 for control horses, crib-biting horses and weaving horses (Fig. 19) which was found to be significant ($F_{2,21}$=12.659, p<0.001). The subsequent post-hoc LSD test demonstrated that the mean number of operant responses performed by the crib-biting animals was significantly higher compared to the control horses (p<0.001), and the weaving animals (p=0.001). In comparison no significant difference of mean number of operant responses was observed between the control group and the weaving group (p=0.547).

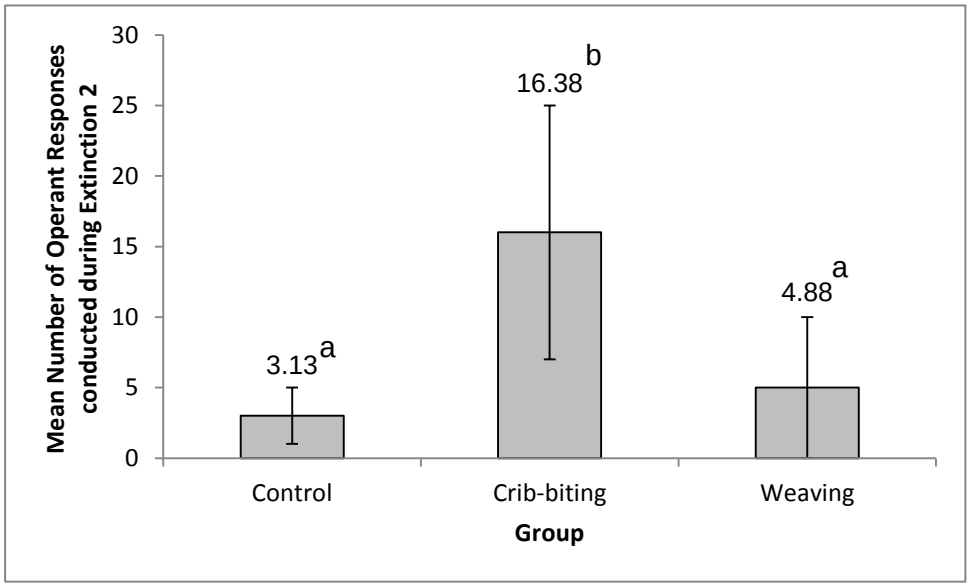

Figure 19. The mean number of operant responses (± std dev) conducted by control, crib-biting and weaving horses during extinction 2. Values sharing the same superscript were not significantly different.

Furthermore, no significant linear relationship was observed between age of the horses and mean number of operant responses conducted during extinction 2 when considering all horses ($F_{1,22}$=0.000, r=0.004, r^2=0.000, p=0.985), control horses ($F_{1,6}$=24.853, r=0.898, r^2=0.806, p=0.002), crib-biting horses ($F_{1,6}$=0.036, r=0.078, r^2=0.006, p=0.855) or weaving horses ($F_{1,6}$=0.574, r=0.295, r^2=0.087, p=0.478).

4.4.7 Latency of Approach to First Operant Response during Extinction 2

The mean latency of approach to first operant response during extinction 2 data were subjected to a one-way ANOVA to determine whether there was significant differences between control, crib-biting and weaving groups. The mean latency of approach to first operant response during extinction 2 was 29.90±28.70s for the control horses, 20.08±39.07s for the crib-biting horses and 14.70±21.93s for the weaving horses. The ANOVA found no significant difference between any of the three groups ($F_{2,22}$=0.504, p=0.611), consequently a post-hoc LSD test was not required.

Linear regression analysis demonstrated there was no significant linear relationship between age and latency of approach to first operant response during extinction 2 when comparing all horses ($F_{1,22}$=0.000, r=0.001, r^2=0.000, p=0.997), control horses ($F_{1,6}$=0.541, r=0.287, r^2=0.083, p=0.490), crib-biting horses ($F_{1,6}$=0.319, r=0.225, r^2=0.050, p=0.593) or weaving horses ($F_{1,6}$=2.900, r=0.571, r^2=0.326, p=0.139).

4.4.8 Trials Taken to Reach Total Extinction Criterion

A one-way ANOVA found that there was significant difference between the mean trials taken to reach total extinction criterion between control, crib-biting and weaving horses (Fig. 20) ($F_{2,21}$=9.8000, p=0.001). Successive post-hoc LSD analysis found that crib-biting animals required significantly more trials to reach total extinction criterion when compared to both the control (p<0.001) and the weaving horses (p=0.009). No significant difference was observed between the control and weaving groups (p=0.162).

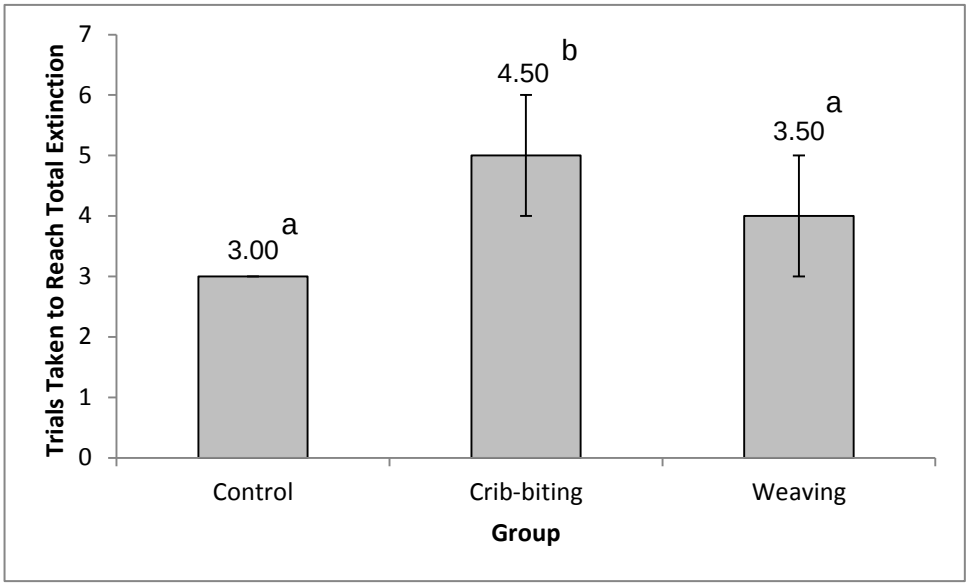

Figure 20. The mean number of trials (±std dev) taken for the control, crib-biting and weaving horses to reach total extinction criterion. Values sharing the same superscript are not significantly different.

No significant linear relationship was observed for trials taken to reach extinction criterion when considering all horses ($F_{1,22}$=0.035, r=0.040, r^2=0.002, p=0.852), crib-biting horses ($F_{1,6}$=2.317, r=0.528, r^2=0.279, p=0.179) or weaving horses ($F_{1,6}$=1.800, r=0.480, r^2=0.231, p=0.228). Linear regression analysis could not be calculated for control horses due to the trials taken to reach extinction was the same for all control animals.

4.4.9 Effect of Stereotypy on Number of Operant Responses Conducted During Extinction 1 and Number of Operant Responses Conducted During Extinction 2

The Paired Samples T-Test determined that there was no significant difference in the number of operant responses conducted between extinction 1 and extinction 2 (see Figures 17 and 19 for means) for the control (t_7=1.629, p=0.147) or the weaving sample groups (t_7=1.528, p=0.170). However the crib-biting horses performed significantly less operant responses during extinction 2 when compared to extinction 1 (t_7=4.365, p=0.003).

4.5 Relationships between Spontaneous Blink Rate and other Parameters within the Stereotypic Sample Groups

To determine whether there were any significant relationships between SBR and other parameters a series of linear regression analyses were undertaken. These are reported in greater detail below.

4.5.1 Spontaneous Blink Rate and Rate of Stereotypy Pre-Feed

For the crib-biting horses there was no significant linear relationship between mean SBR compared to rate of stereotypy pre-feed ($F_{1,6}$=0.314, r=0.223, r^2=0.050, p=0.595). Similarly there was no significant linear relationship between the same parameters for the weaving horses ($F_{1,6}$=0.665, r=0.316, r^2=0.100, p=0.446).

4.5.2 Spontaneous Blink Rate and Rate of Stereotypy Post Feed

There was no significant linear relationship between SBR and rate of stereotypy post-feed for the crib-biting horses ($F_{1,6}$=0.060, r=0.099, r^2=0.010, p=0.815). In addition, no significant linear relationship between SBR and rate of stereotypy post-feed for weaving horses was observed ($F_{1,6}$=0.575, r=0.296, r^2=0.087, p=0.477).

4.5.3 Spontaneous Blink Rate and Number of Operant Responses during Extinction 1

Linear regression analysis demonstrated that there was no significant linear relationship between SBR and mean number of operant responses during extinction 1 within the crib-biting sample ($F_{1,6}$=1.534, r=0.451, r^2=0.204, p=0.262). Additionally no significant linear relationship was observed between SBR and number of operant responses during extinction 1 for the weaving animals ($F_{1,6}$=0.040, r=0.082, r^2=0.007, p=0.848).

4.5.4 Spontaneous Blink Rate and Number of Operant Responses during Extinction 2

There was no significant linear relationship observed between SBR and number of operant responses during extinction 2 for either the crib-biting ($F_{1,6}$=0.758, r=0.335, r^2=0.112, p=0.417) or the weaving horses ($F_{1,6}$=0.795, r=0.342, r^2=0.117, p=0.407).

4.5.5 Spontaneous Blink Rate and Trials Taken to Reach Total Extinction Criterion

The linear regression analysis determined that there was no significant linear relationship between SBR and mean number of trials taken to reach total extinction criterion for the crib-biting horses ($F_{1,6}$=0.442, r=0.262, r^2=0.069, p=0.531). Furthermore there was no significant linear relationship between SBR and mean number of trials taken to reach total extinction criterion for the weaving horses ($F_{1,6}$=0.005, r=0.029, r^2=0.001, p=0.948).

4.6 Relationships between Behaviour Initiation and other Parameters within the Stereotypic Sample Groups

To determine if there were any significant linear relationships between mean behavioural initiation and other parameters linear regression analysis was undertaken. These are described below.

4.6.1 Behaviour Initiation and Rate of Stereotypy Pre Feed

There was a strong positive significant linear relationship (Fig. 21) between mean behaviour initiation and rate of stereotypy pre-feed for the crib-biting horses ($F_{1,6}$=35.717, r=0.925, r^2=0.856, p=0.001).

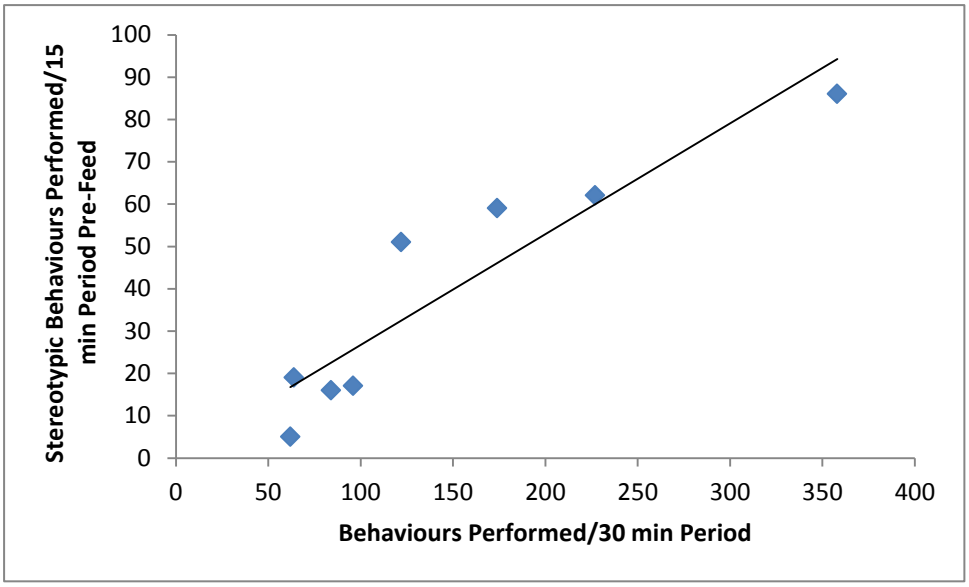

Figure 21. Positive linear relationship observed between number of behaviours performed within a 30 minute period compared to stereotypic behaviours performed within a 15 minute period prior to feeding in the crib-biting sample.

In addition, a medium to strong positive linear relationship (Fig. 22) was observed between mean behaviour initiation and mean rate of stereotypy pre-feed for the weaving horses also ($F_{1,6}$=6.526, r=0.722, r^2=0.521, p=0.043).

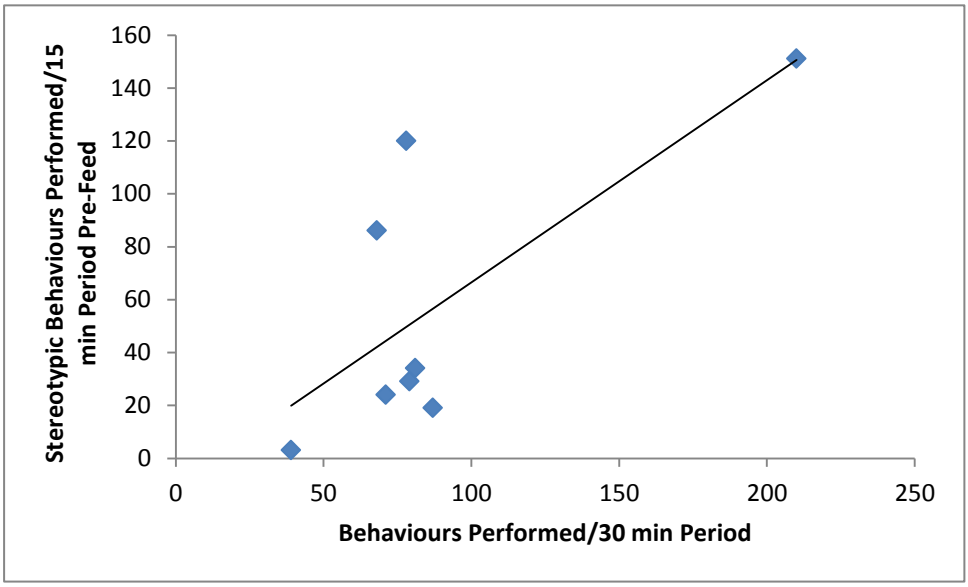

Figure 22. The positive linear relationship observed between behaviours initiated per 30 minute period compared to stereotypic behaviours performed within the 15 minute period prior to feeding in the weaving sample.

4.6.2 Behaviour Initiation and Rate of Stereotypy Post Feed

There was no significant linear relationship observed between mean behavioural initiation and mean rate of stereotypy post-feed for the crib-biting horses

($F_{1,6}$=1.810, r=0.481, r^2=0.232, p=0.227) nor the weaving horses ($F_{1,6}$=2.702, r=0.557, r^2=0.311, p=0.151).

4.6.3 Behaviour Initiation and Number of Operant Responses during Extinction 1

The linear regression analysis determined that there was no significant linear relationship between mean behavioural initiation and mean number of operant responses during extinction 1 for the crib-biting horses ($F_{1,6}$=0.015, r=0.050, r^2=0.003, p=0.906). Indeed, no significant linear relationship was observed within the weaving horses for the same parameters ($F_{1,6}$=0.372, r=0.241, r^2=0.058, p=0.564).

4.6.4 Behaviour Initiation and Number of Operant Responses during Extinction 2

No significant linear relationship was observed between mean behavioural initiation and number of operant responses conducted during extinction 2 within either the crib-biting ($F_{1,6}$=0.016, r=0.052, r^2=0.003, p=0.902) or the weaving population ($F_{1,6}$=0.054, r=0.094, r^2=0.009, p=0.824).

4.6.5 Behaviour Initiation and Trials Taken to Reach Total Extinction Criterion

Within the crib-biting sample group there was no significant linear relationship between mean behavioural initiation and mean number of trials taken to reach total extinction criterion ($F_{1,6}$=0.904, r=0.362, r^2=0.131, p=0.378). There was no significant linear relationship between the same parameters for the weaving population ($F_{1,6}$=0.005, r=0.029, r^2=0.001, p=0.946).

4.7 Effect of Sex

To determine whether the sex of the horse had an impact on the parameters a series of Independent Samples T-Tests were undertaken. The statistical outputs of these can be viewed in Appendix F.

Chapter 5: Discussion

The aim of this study was to extend current knowledge regarding the neuro-aetiology of stereotypy by further examining the role of altered basal ganglia physiology in crib-biting and also weaving horses. The following data gathering strategies were employed in this regard:

1. Rate of stereotypy;
2. Spontaneous blink rate;
3. Behavioural initiation;
4. An extinction paradigm, utilising a sensory specific satiety devaluation phase.

The outcome of each measurement (Table 2) will be discussed separately below.

Table 2: Summary of Results. Values sharing the same superscript for each parameter are not statistically different (SBR=Spontaneous Blink Rate; OR=Operant Response; LA=Latency to Approach).

	Control	Crib-biting	Weaving
Rate of Stereotypy/15 min (pre-feed)	N/A	39.38±28.91	58.25±53.93
Rate of Stereotypy/15 min (post-feed)	N/A	104.88±39.73	52.13±64.41
SBR/30 min	449.13±121.83[a]	337.75±69.97[b]	542.50±116.79[a]
Behaviour Initiation/30 min	14.00±6.89[a]	148.38±102.08[b]	89.13±50.98[b]
Trials Taken to Reach Learning Criterion	7.63±2.88[a]	1.50±0.76[b]	1.75±1.16[b]
Feed Remaining Devaluation 1 (g)	645.50±205.98	526.88±306.70	455.13±238.93
OR Extinction 1	4.50±1.69[a]	23.38±7.50[b]	7.63±5.88[a]
LA to first OR Extinction 1 (s)	36.25±46.32[a]	5.67±7.24[b]	3.73±1.91[b]
Feed Remaining Devaluation 2 (g)	695.88±223.45	575.25±263.95	570.00±279.28
OR Extinction 2	3.13±1.73[a]	16.38±8.68[b]	4.88±4.45[a]
LA to first OR Extinction 2 (s)	29.90±28.70	20.08±39.07	14.70±21.93
Trials To Total Extinction Criterion	3.00±0.00[a]	4.50±0.76[b]	3.50±0.93[a]

5.1 Rate of Stereotypy

Previous reports have suggested that crib-biting behaviour is predominantly a post-prandial response compared to weaving behaviour which is suggested to occur as a pre-prandial response to feed attainment (McBride & Hemmings, 2004). Indeed, these data confirm that within this sample population crib-biting is predominantly a post-prandial behaviour as following consumption of feed the crib-biting response more than doubled when compared to basal levels. Whilst this result may not be entirely surprising having being cited previously, it is most interesting when considering the basal ganglia as causal of stereotypic behaviour. Previously authors have proposed an increased sensitisation to dopamine within the nucleus accumbens (McBride & Hemmings, 2005). Upon consumption of palatable feed there is an increase in dopamine released from the VTA to the NAcc, which in the crib-biting sample here resulted in a stereotypic response due to the increased sensitivity to dopamine, and consequently an increased activation of the direct pathway (McBride & Hemmings, 2004). Therefore the data presented here supports previous theories that crib-biting horses are sensitised to dopamine, and following increased dopamine release as a result of consuming palatable feed, the crib-biting response is observed. Interestingly, weaving behaviour was not found to be solely pre-prandial within this sample of weaving horses, as no significant difference in weaving frequency performed pre-feed and post-feed was observed. When considering that weaving is also proposed to be a response to social isolation (McAfee *et al.*, 2002) perhaps this should be used to stimulate the weaving response in future studies. Whilst this is interesting in terms of increasing knowledge as to the potential social frustration nature to weaving behaviour as opposed to feed related frustration for crib-biting horses, only the weaving animals were aware of feed attainment through the shaking of the bucket. Thus, there is potential that the crib-biting response may also increase as a result of being alerted to feed attainment; as such this is a potential area for further research.

5.2 Spontaneous Blink Rate

A number of authors suggest that the post-prandial crib-biting response is as a result of gastro-intestinal pain (Nicol *et al.*, 2002; Hemmings *et al.*, 2007; Moeller *et al.*, 2008). In contrast, other authors suggest that altered basal ganglia physiology, as revealed by direct measurement of dopamine receptor populations, may result in crib-biting behaviour (McBride & Hemmings, 2005; McBride & Hemmings, 2009). However these methods are expensive and time consuming which limits the scope of such work. In this respect, behavioural probes of dopamine transmissions such as SBR may provide a solution. Previously, behavioural probes of dopamine levels within the mesencephalon had not been collected in combination with stereotypy rate for the horse, allowing room for multiple causes to be speculated.

The data here found that crib-biting SBR was significantly reduced when compared to both control and weaving equivalences. Consequently, sensitisation of the striatum increases stimulation of the SNpr ultimately resulting in a reduction in blink amplitude (Kaminer *et al.,* 2011) for CB horses. This mirrors that observed by Roebel and MacLean (2007) whereby stereotypic adult humans demonstrated a decreased SBR. The authors suggested that this is due to increased post synaptic sensitivity resulting in a sensitisation to dopamine (Roebel & MacLean, 2007; McBride & Hemmings, 2009; Kaminer *et al.*, 2011), a phenomena previously observed within crib-biting horses (McBride & Hemmings, 2005). Whilst Roebel & MacLean (2007) suggest this sensitisation occurs within the caudate, McBride and Hemmings (2005) observed increased D1 and D2 receptor density within the nucleus accumbens but decreased D1 receptor density and D2 receptor affinity within the caudate. Therefore sensitisation of the NAcc results in altered pathways of the motivational limbic system in the striatum in crib-biting horses. The reduced SBR observed in this group of crib-biting horses suggests that sensitisation of the NAcc could also reduce blink amplitude. Whilst this does not agree with previous reports with regards to the location of dopaminergic influenced SBR (Taylor *et al.,* 1999), the NAcc core also projects to the SNpr in a similar manner as the caudate. There is potential that this could

allow for reduced blink amplitude observed by Taylor *et al.* (1999), however due to the conflicting evidence this is an area that requires further research. However, these data presented here would also suggest that altered basal ganglia dopamine physiology is causal of the crib-biting response within the horse, not the gastro-intestinal pain theory previously presented.

Surprisingly, the weaving horses displayed a significantly increased SBR compared to the crib-biting horses but not the control horses. It should be noted that whilst there was a trend for increased SBR for the weaving horses compared to the control animals this was not found to be significant. This suggests there is no receptor based sensitisation of the striatum in weaving horses, but rather there may be elevated dopamine levels within the striatum accounting for the increased SBR in these animals (Kaminer *et al.,* 2011) although direct measurement of receptor populations and Da metabolites would be required to confirm this. Receptor up-regulation has been shown previously to be genotype dependant (Cabib *et al.,* 1998) suggesting that the locomotor STB phenotype lacks this genetic tendency. Previously no distinction has been made between the differences in neural physiology between crib-biting and weaving horses, the data reported herein is unique in this respect. The crib-biting cohort classically matched the SBR findings of Roebel and MacLean (2007) which again suggests increased dopamine receptor sensitivity in these animals, rather than elevated dopamine levels. As the weaving horses' SBR was not significantly different to the control horses then perhaps there is no difference in striatal physiology for weaving horses compared to the control equivalences. However, when considering the weaving horses' behavioural initiation results (to be discussed in detail below) then this is unlikely to be the case, as the weaving horses demonstrated approximately 6 times the number of behaviour initiations when compared to the control horses, suggesting that there are indeed differences within the neural physiology.

It should be noted that previously unpublished data observed an increased in SBR in a sample of crib-biting horses (Issaoui & Hemmings, 2011), which

opposes the findings presented here. During the half hour observation period utilised in this study it was noticed that blinks appeared to occur in bouts, whereby the horse may not blink for a minute or so but following this a number of blinks would occur in quick succession with this pattern being repeated. The crib-biting sample for Issaoui and Hemmings (2011) were only observed for a period of three minutes. Subsequently, it is entirely probable that this study observed a series of blinks in quick succession and not properly accounting for the duration between these sets of blinks within the three minute time frame. Indeed considering that both this study and Roebel and MacLean (2007) observed a decreased SBR in the crib-biting horse and stereotypic humans respectively, and considering the link between decreased SBR and increased post synaptic receptor density within the NAcc (McBride & Hemmings, 2005) it is far more likely that a decreased SBR would be observed within the crib-biting horse.

5.3 Behavioural Initiation

Crib-biting horses performed almost 11 times the number of initiations when compared to the control horses ($p=0.001$). Garner and Mason (2002), Garner *et al.* (2003a) and Garner (2006) have previously utilised behavioural initiation as a probe of altered dorsal basal ganglia dopamine physiology. In this respect they discovered an increased rate of behaviour initiation in high stereotypy bank voles (Garner & Mason, 2002), marsh tits and blue tits (Garner *et al.,* 2003a). Indeed, drawing from the bank vole study in particular (Garner & Mason, 2002) and the large body of amphetamine studies reviewed in chapter 2, this increase in behavioural initiation is resultant from altered dorsal striatal circuitry. In particular, the suppression of the indirect pathway, allowing the direct pathway to dominate (Garner, 2006).

As a result of altered dopamine physiology in crib-biting animals (McBride & Hemmings, 2009) perhaps the direct pathway is allowed to dominate, as the indirect pathway is being suppressed due to dopamine acting on inhibitory D2 type receptors. However, McBride and Hemmings (2005) observed decreased

D2 receptor affinity in combination with decreased D1 receptor density in the caudate. This would suggest that both the direct and indirect pathways of the caudate have reduced output. However, the putamen demonstrated no difference in dopamine receptor affinity or density (McBride & Hemmings, 2005). As the putamen is implicated with motor control then perhaps it is here that the up-regulation of the direct pathway occurs rather than in the caudate for crib-biting horses. Previous research into dynorphin/enkephalin ratios has determined stereotypy occurs as a result of direct pathway activation (Presti & Lewis, 2005) though rate of behaviour initiation was not measured. Therefore, it would be most interesting to investigate dynorphin and enkephalin levels within the putamen to determine direct/indirect pathway dominance as causal to the increased rate of behaviour initiation observed in the crib-biting horses. Ultimately this would allow an increase in the number of behaviours performed. Consequently, action selection is not being modulated as normal hence the increase in behavioural initiation observed within this crib-biting sample group. Interestingly both this study and Garner and Mason (2002) found a significant linear relationship between behaviour initiation and basal stereotypy rate in partial agreement with the crib-biting data presented herein.

In addition, a significant linear relationship was also observed with the weaving horses and rate of stereotypy pre-feed, perhaps due to the increase in dopamine release in anticipation of feed. Perhaps then, the increase in behaviour initiation for the weaving horses in the absence of decreased SBR could suggest that weaving horses do not express dopaminergic receptor sensitisation, and simply express an up-regulation of the direct pathway as a result of elevated phasic dopamine levels. Cabib and Puglisii-Allegra (2012) demonstrate that within chronically stressed rodents a reduction in tonic dopamine levels are apparent. In addition, evidence suggests that a reduction of tonic dopamine levels results in an increase in the phasic dopamine response in reaction to environmentally relevant stimuli (Grace, 1991; Floresco *et al.,* 2003), which in this case would be social isolation frustration.

As administration of dopamine agonists (elevating the levels of dopamine within the striatum) also results in an increased rate of behaviour initiation (Garner, 2006) perhaps this is the case here, particularly as it is the phasic dopamine that is required for motivation and appetitive behaviours (Alcaro *et al.,* 2007). In addition a trend for increased SBR was also apparent in the weaving horses, further suggesting elevated dopamine levels within the weaving horses. This would suggest again that altered striatal physiology is causal of stereotypic behaviours not gastrointestinal discomfort. Genetic analysis would be beneficial to determine if there are genetic mutations within crib-biting and weaving horses, particularly if the loci of these mutations differ. Such mutations, and the loci of these mutations, would potentially predispose these horses to become either sensitised to dopamine (crib-biting horses) or to enhance motivation as a result of an increase in phasic dopamine release (weaving horses). Consequently this would allow the up-regulation of the direct pathway, the altered SBR observed and ultimately the emergence of the stereotypic response within the weaving horses. However, the measurement of dopamine metabolites such as DOPAC (as an indicator of dopamine levels) and receptor density/affinity would be required to confirm this.

5.4 Extinction Learning

In order to fully gauge the impact of altered dopamine transmission on cognitive processing the extinction paradigm was utilised, which has been described as the gold standard (Schwabe & Wolf, 2011) to determine rate of habituation. It has previously been suggested that stereotypic animals learn differently compared to control animals, in that they show an enhanced S-R type learning over A-O learning. Consequently this suggests crib-biting horses are more predisposed to habit formation (Parker *et al.*, 2009) as a result of stress induced changes within the striatum (Hemmings *et al.*, 2007; Dias-Ferreira *et al.*, 2009; Schwabe & Wolf, 2011) despite being fully capable of utilising place A-O type learning (Parker *et al.*, 2009) at initial stages of task acquisition. Whilst Parker *et al.* (2009) and Hemmings *et al.* (2007) were instrumental in suggesting altered dorsal striatal circuitry in a population of crib-biting horses, the methods utilised

had noticeable weaknesses. In particular, the small sample group of only crib-biting horses utilised does not consider the striatal functioning of weaving animals. Therefore, a generalizable overview of striatal functioning in stereotypic horses is not provided. Furthermore, Hemmings *et al. (*2007) did not dissect appetitive drive from habitual responding as no devaluation phase was conducted. Subsequently no differentiation between appetitive and habitual behaviours could be made as the horses would still be motivated in terms of reward to perform the task. Therefore, the application of sensory specific satiety during the devaluation phase, as utilised by Dias-Ferreira *et al.* (2009), during this study accounted for this, allowing an accurate measure of habit formation.

Again differences were observed between all three groups though not in the way previous research would have suggested. Indeed, in line with previous research the weaving and the crib-biting horses would learn the task much faster than the control horses and would perform increased operant responses during extinction 1 and 2, as well as require increased number of trials to reach total extinction criterion. However this was not found to be the case.

5.4.1 Trials Taken to Reach Learning Criterion

Initially the results were in line with previous research (McBride & Hemmings, 2009; Parker *et al.*, 2009) as both the crib-biting horses and the weaving horses required significantly less trials to reach learning criterion than the control horses, with no significant difference observed between the crib-biting and the weaving animals. As significantly less trails were required to attain learning criterion for the stereotypic sample groups then it could be suggested that stereotypic horses form response acquisition at a much faster rate when compared to control horses, as a result of increased motivation due to increased phasic dopamine release (weaving) or increased dopamine receptor sensitivity (crib-biting). This suggests that at this stage the horses are still utilising the ventral striatal circuitry to effectively learn to respond to a cue for a food reward. In the latter stages of the task previous research would suggest an increase in

control of the more dorsal striatal circuitry ultimately observing a shift of control from the NAcc to the caudate with neural activity spreading to the putamen following repetition. The data presented herein would tentatively suggest that crib-biters transit to putamen control more rapidly, whilst weavers remain embedded in the realms of appetitive drive (at least for the trial duration) (Nelson & Killcross, 2006; Parker *et al.*, 2009).

5.4.2 Extinction 1

Following devaluation 1, distinct differences emerged between the crib-biting and the weaving horses. Indeed the crib-biting horses demonstrated a significantly increased number of operant responses during extinction 1 when compared to both the control and the weaving horses. Drawing from previous devaluation and extinction paradigms (Yin & Knowlton, 2006; Hemmings *et al.*, 2007; Dias-Ferreira *et al.*, 2009) this suggests that crib-biting horses are demonstrating the same increased shift from ventral to dorsal circuitry observed in chronically stressed rodents, highlighting altered striatal physiology as causal for the habitual crib-biting response at this early stage within the extinction paradigm. Further conclusions could also be drawn from Dias-Ferreira *et al.* (2009) regarding the effect of chronic stress on extinction learning. Indeed Dias-Ferreira *et al.* (2009) suggested that previous exposure to chronic stress was implicated in the altered performance during extinction tasks, and further determined that within a chronically stressed sample of rodents there was a distinct reorganisation of corticostriatal circuitry, with atrophy of the associative circuitry in conjunction with hypertrophy of the sensorimotor circuitry being observed. Consequently these animals are biased towards habitual responding rather than utilising flexible A-O type learning. McBride and Hemmings (2005) also reported decreased output from the caudate, characterised by the decrease in D1 receptor density and D2 receptor affinity in their sample of crib-biting animals, further supporting the concept of increased reliance on S-R type learning. This appears to be the case in this sample of crib-biting horses. As of yet, no assays have been performed on the weaving horse striatum, so receptor dynamics following chronic stress/stereotypy formation is still unknown.

However, there was no significant difference between the number of operant responses conducted between the control horses and the weaving horses during extinction 1. This suggests that at this stage within the extinction paradigm the weaving horses had not yet shifted to habitual responding as the crib-biting horses had, but were rather still utilising the more ventral associative circuits. However, as little research has been conducted regarding the learning mechanisms of weaving animals this does require further investigation. As there was no significant difference in feed remaining following devaluation 1 between any of the three sample groups this again highlights that the crib-biting horses were behaving habitually to the presentation of the CS card and not as a result of differential feeding motivation.

Further similarities arose when considering the latency of approach to the first operant response during extinction 1. The number of operant responses performed by the weaving horses suggests that at this stage of the extinction paradigm they were utilising a more flexible approach to the task governed by A-O monitoring at the level of ventral and dorsomedial striatum. However, the latency of approach to the first operant response of the weaving horses was more in line with the crib-biting horses as no significant difference in LA was observed between these two sample groups, despite there being a significant difference between LA of approach to first operant response between both the weaving and control horses and the crib-biting and the control horses. As discussed it is apparent that the crib-biting horses were acting in a habitual manner, however the weaving horses were not. This increase in LA to the CS card does suggest that the weaving horses are in a greater motivational state compared to the control horses, perhaps suggesting the ventral striatum is more associated with weaving behaviour due to elevated phasic dopamine levels, compared to the dorsal striatum being associated with crib-biting behaviour.

5.4.3 Extinction 2

Furthermore, during extinction 2 similar results were observed in terms of the number of operant responses conducted. As with extinction 1 the crib-biting horses conducted significantly more operant responses compared to the control and weaving horses. Again, there was no significant difference in the number of operant responses conducted between the control horses and the weaving animals. This highlights that altered dorsal striatal circuitry as a result of chronic stress plays a highly influential role on the behaviour of crib-biting horses, but also demonstrates the difference between crib-biting and weaving horses' striatal functioning. In contrast to extinction 1 there was no significant difference between the latency of approach between the three sample groups during extinction 2. This could be a potential manifestation of reducing feeding motivation within all three groups as once more there was no significant difference between the feed remaining following devaluation 2.

5.4.4 Trials Taken to Reach Total Extinction Criterion

The crib-biting horses required significantly more trials to reach total extinction criterion requiring a mean of 4.50±0.76 trials compared to 3.00±0.00 and 3.50±0.93 trials for the control and weaving horses respectively. In contrast there was no significant difference between control and weaving horses in the number of trials to reach total extinction criterion. To a degree, these data support previous results from extinction paradigms (Nelson & Killcross, 2006; Hemmings *et al.*, 2007; Dias-Ferreira *et al.*, 2009) with the crib-biting horses demonstrating accelerated habit formation when compared to the control horses, suggesting the same atrophy of the associative circuitry and hypertrophy of the sensorimotor circuitry observed in chronically stressed rodents (Dias-Ferreira *et al.,* 2009). Indeed, this fits well with the altered dopamine receptor densities/affinity reported by McBride and Hemmings (2005). However as the weaving horses did not demonstrate accelerated habit formation, it is clear from the difference in performance during the extinction paradigm as a whole that the cause of this altered circuitry appears to differ when comparing the oral and locomotor stereotypies.

5.5 Altered Dorsal vs. Altered Ventral Striatal Circuitry

If the difference in the number of operant responses conducted during extinction 1 and extinction 2 are also considered, further evidence for the difference in neural circuitry between crib-biting and weaving horses arises. Crib-biting horses performed significantly more operant responses during extinction 1 than extinction 2. It is known that the crib-biting horse striatal receptors are sensitive to dopamine and as a result are highly motivated (McBride & Hemmings, 2005). Perhaps then during extinction 1 the crib-biting horse is highly motivated to respond to the CS due to previously attaining food reward for performing the task, despite no longer achieving the food reward. However the number of operant responses during extinction 2 was significantly lower when compared to extinction 1. As it has been determined that crib-biting horses demonstrate increased Da receptor densities within the NAcc (McBride & Hemmings, 2005; McBride & Hemmings, 2009) then perhaps in extinction 2 there is saturation of dopamine receptors due to *ad lib* feeding during devaluation 1 and 2, as well as successfully attaining a food reward during the trials. Consequently the CB horse is not as motivated to perform the task during extinction 2. However as this study, in line with others (Nelson & Killcross, 2006; Hemmings *et al.*, 2007; Dias-Ferreira *et al.*, 2009) determined that stressed/stereotypic animals form habits at a faster rate when compared to control animals, the crib-biting horse is responding as a result of an increased dominance of sensorimotor circuitry rather than ventral striatal circuitry. Consequently, the CB horses are performing the task habitually during extinction 2 despite being less motivated to do so in terms of ventral striatal circuitry. In contrast the weaving horses did not demonstrate an increase in rate of habit formation nor did they demonstrate a significant difference in number of operant responses performed between extinction 1 and extinction 2, further suggesting a difference in striatal circuitry between two different stereotypies within a single species, a concept not previously suggested in any species.

When considering all the behavioural probes there is a clear difference between control and crib-biting horses, crib-biting and weaving horses and in some cases

control and weaving horses. What is most interesting is that both the way in which both the control horses and the crib-biting horses are distinctly different with starkly differing SBRs, behavioural initiation and the way in which both groups behaved within the extinction paradigm, almost as if they are at opposite ends of a spectrum. In contrast the way in which weaving horses behaved was not so predictable, in terms of SBR and the extinction paradigm the weaving horses behaved more like control horses, though in terms of behavioural initiation behaved more like the crib-biting horses. Consequently, it is clear that the neural circuitry behind crib-biting and weaving behaviour differs.

The crib-biting data here does support previously suggested theories regarding altered dorsal striatal circuitry (Hemmings *et al.,* 2007; Parker *et al.*, 2009). Crib-biting horses demonstrate decreased SBR, increased behavioural initiation and accelerated S-R habit formation as demonstrated from the extinction paradigm, suggesting alterations such as sensitisation of the ventral circuitry and reduced output of the caudate, allowing the sensorimotor circuit to dominate, perhaps as a result of chronic stress as other authors speculate (McBride & Hemmings, 2005; McBride & Hemmings, 2009). In contrast, the weaving horse data is not so entirely clear-cut. From the extinction paradigm it can be concluded that the dorsal striatum appears to function normally in weaving animals. Perhaps then it is the ventral striatum that is not functioning as it should be for weaving horses, as it has already been established that the ventral striatum has no role in appetitive behaviours (controlled by the associative circuits) or habitual (controlled by sensorimotor circuits), such as those utilised during extinction tasks (Graybiel, 2008).

The ventral striatum, as discussed in section 2.1.4, is a key structure for motivation. When considering the Hughes and Duncan Model (1988) it is clear that there is a distinct flow of events during action selection commencing at motivation and finishing at consumatory behaviour. As the proposed stressor for weaving behaviour is social isolation (McAfee *et al.,* 2002) as opposed to weaning stress perhaps the neural circuits react differently. Crib-biting horses

become highly motivated to feed, so upon consuming palatable feed there is an internal reward with dopamine, which as the horse is already sensitised potentially due to a genetic predisposition in combination with chronic stress (McBride & Hemmings, 2005) stimulates the habitual crib-biting response. In contrast, weaving horses would be highly motivated to remove themselves from the situation which is keeping them in social isolation and is unrelated to feeding, as the rate of stereotypy pre- and post-feed data presented here demonstrate.

Further support for altered ventral striatal circuitry within weaving horses is that weaving is a locomotive stereotypy. Following administration of cocaine, which actively blocks the uptake of dopamine resulting in high levels of extracellular dopamine, the NAcc shell (Ikemoto & Panksepp, 1999) is responsible for the pleasurable effects and the NAcc core is responsible for locomotive aspect of cocaine administration (Alcaro *et al.*, 2007) allowing some similarities to be made with weaving behaviour. Initially both the weaving horses and the crib-biting horses are 'trapped' in the appetitive stage of the Hughes and Duncan (1988) model. For crib-biting horses as a result of chronic stress altering the striatal circuitry allowing the sensorimotor circuits to dominate, the crib-biting response becomes habitual (Dias-Ferreira *et al.*, 2009). Indeed, when the data from the rate of stereotypy observed pre-feed and post-feed in conjunction with the number of operant responses conducted during extinction 1 and extinction 2 are considered there appears to be high motivation and habitual control in the crib-biting horses, in that the habitual nature of crib-biting resulted in the pre-feed crib-biting response as well as resulted in the operant responses during extinction 2 despite decreased motivation.

In contrast, perhaps for the weaving horses upon presentation of social isolation stress the ventral striatum is internally rewarded with high levels of extracellular phasic dopamine, stimulating pleasure to act as a coping mechanism, resulting in the locomotive movement observed during weaving behaviour similar to that observed within cocaine administration. Therefore the weaving horse is 'stuck' in a highly motivated state, such as the appetitive phase of the Hughes and

Duncan model (1988), however this behaviour is not habitual. Indeed, the extinction paradigm further suggested that for the weaving horses, weaving behaviour was not under the control of the putamen as no evidence of habit formation was observed within this study. As the NAcc core is not dissimilar to the dorsal striatum, it is entirely possible that the increase in phasic dopamine release proposed here would act on the D1 and D2 receptors in a similar manner as occurs in the dorsal striatum. Consequently, the weaving horses would still demonstrate increased behavioural initiation as a result of D2 activation on the indirect pathway allowing dominance of the direct pathway, but not decreased SBR as a result of increased dopamine receptor sensitivity (Roebel & MacLean, 2007). However, this is an area that requires further research to fully investigate the interaction of these pathways in the weaving horse as a result of altered ventral striatal functioning. The trend for increased SBR combined with the enhanced motivation during the extinction paradigm within this sample of weaving horses, suggests that an increase in phasic dopamine release resulting in increased endogenous levels of dopamine is associated with locomotor stereotypy, contrasting to the results extrapolated from crib-biting horse data.

Subsequently, conclusions can be drawn from these behavioural probes when considering striatal functioning. Crib-biting horses demonstrate both altered ventral and dorsal striatal functioning. Previous research (McBride & Hemmings, 2005) demonstrated sensitised ventral striatal circuitry utilising assays, with the reduced SBR observed here also highlighting this sensitivity to the dopaminergic response in crib-biting horses. In addition, the accelerated ventral to dorsal circuitry activity within the crib-biting horses was also demonstrated by significantly increased rate of behaviour initiation due to reduced output of the caudate allowing up-regulation of the direct pathway within the putamen. Alterations in the dorsal striatal circuitry are also highlighted via accelerated habit formation characterised by enhanced perseveration during the extinction paradigm. Thus, crib-biting horses demonstrate a much increased motivation within the ventral striatum, atrophy and hypertrophy of the associative and sensorimotor circuitry respectively, characterised by the increased reliance on S-R learning during the extinction paradigm and increase in behaviour initiation.

Thus during the initial stages of crib-biting development appetitive behaviours are attempted, though due to altered dorsal striatal circuitry, quickly become habitual.

In contrast, weaving horses only demonstrate altered ventral striatal circuitry demonstrated by the trend for increased SBR and significantly increased behavioural initiation. These results suggest weaving horses are in a highly motivated state as a result of increased phasic dopamine release within the NAcc and are therefore trapped in the appetitive loop of the Hughes and Duncan model (1988). Further research is required to fully investigate the alterations within the striatal functioning within stereotypic animals, particularly as this is first known case in any species suggesting differing neural alterations between locomotor and oral stereotypy.

5.6 Effect of Age and Sex

Murphy *et al.* (2004) reported significant differences in visuospatial processing between genders. As such the effect of age and sex were analysed to minimise the impact these could have potentially had on the data collected. Sex generally had no impact on the results, though when considering the latency of approach to first operant response during extinction 1 there was a significant difference between the number of operant responses conducted between control mares and control geldings. However, this is potentially a manifestation of the small sample size used, as well as the unequal number of mares and geldings within the group, particularly as this was the only significant difference observed between geldings and mares. In addition age had no effect on the data when tested with linear regression analysis.

5.7 Limitations of the Study

There were a number of significant limitations that in future could be built upon to effectively increase the reliability and the validity of the study. The first and

foremost of these limitations is the small sample group used. To be able to generalise the results across the equine population a much larger sample group would need to be utilised. In addition, several different breeds of horses were utilised and this may have had an effect of the results collected as it is known that TBs and WBs do exhibit increased levels of STB, and there may be significant differences between these breeds and other breeds that were used such as Argentinian Polo Ponies etc. In future this would try to be accounted for by utilising one breed where possible to minimise the impact of individual breed characteristics on the results.

In addition, as all horses were privately owned there were noticeable differences between the routines of the horses. For example feeding regime, types of feed, turnout, stabling etc. all differed between each yard and each owner. This is quite noticeable when comparing the extinction results found here to those of Dias-Ferreira *et al.* (2009) who also devalued home cage food. This was simply not possible for these horses due to owner preference and the large variety of different feeds the horses were given. Furthermore, some of the owners of the crib-biting horses chose to utilise cribbing collars, and whilst these were removed for the duration of the observations, it has previously been recorded that the removal of the cribbing collar results in increased crib-biting behaviour (McGreevy & Nicol, 1998). However, the ten minute habituation period would have reduced the impact this may have had on the results. Similarly some of the weaving horses had weave bars on the stable door, though again these were removed during observations. If this study was to be repeated in the future then perhaps keeping all the horses at one yard and on the same management regime could be an option to completely minimise all external environmental effects where possible. Whilst every possible step was taken to reduce observer error, for example only one observer undertaking all observations in the same manner it is inevitable that small anomalies may have occurred, particularly when measuring blink rate and behaviour initiation. In future to prevent this, all observations could also be recorded with a discrete camera to allow repeated observation away from the yard to completely minimise any indiscretions.

Finally, to improve on the extinction task, an automated computer operant device would be an advantage, such as that utilised by Hemmings *et al.* (2007). This would completely remove any observer error as the observer would not be present and therefore would not be able to affect the horse in anyway, either consciously or sub-consciously. In addition, the feed could be dispensed in a much faster and much more accurate way which could vastly alter the results observed here, particularly the trials taken to reach learning criterion and the number of trials taken to reach extinction criterion.

5.8 Further Research

Further research is essential to determine the cause of the observed differences within these data. These data provide a firm starting point demonstrating that there are distinct differences between the neuro-aetiology of crib-biting and weaving horses which previously have been grouped together as stereotypic horses.

Future Study 1 – Measure of Dopamine

In particular, it would be interesting to perform assays to determine the dopamine receptor densities/affinity within the weaving horses as has been done so in crib-biting horses (McBride & Hemmings, 2005). It would be most interesting to further determine if this a) differs from control horses and b) differs from crib-biting horses. This would again provide evidence as to whether altered ventral striatal circuitry is more influential on weaving behaviour in the same way altered dorsal striatal circuitry appears to be influential for crib-biting animals. In addition, measuring DOPAC would determine whether endogenous dopamine levels are elevated in the weaving horses when compared to the crib-biting horses. Furthermore, measuring the dynorphin and enkephalin levels within the crib-biting putamen and weaving striatum would shed more light on the behaviour initiation results presented here.

Future Study 2 – Behavioural Probes

Utilising a greater number of behavioural probes could also provide more answers combining those chosen here. For example, although the weaving horses' performance was not altered during the extinction task there is potential that they may perform differentially for a Tolman's cross maze, a radial arm maze or even PPI, particularly in combination with SBR due to the proposed links between these two behavioural probes. Of particular note is the Tolmans cross maze, as Parker *et al.* (2009) previously observed altered performance within this task in a small sample of crib-biting horses, in that the sensorimotor circuitry was controlling the behaviour, characterised by accelerated S-R learning. Consequently it would be most interesting considering the extinction task data presented here to observe the way in which weaving and CB horses perform within the cross maze to further determine which of the striatal circuits are dominant.

Future Study 3 – Genetic Probes

Determining whether there is a genetic component to behaviour sensitisation would be beneficial in attempting to manage stereotypic behaviour. For example, should a genetic link be found then the breeder would ensure that any form of stress, be it weaning stress or social isolation stress would be minimised to prevent the development of stereotypic behaviour. This would be particularly beneficial if genetic mutations were found on genes which code for dopamine receptors within the basal ganglia for weaving horses as has been previously observed for crib-biting horses (Hendry & Hemmings, 2012). In particular, it would be interesting to determine if the loci of this genetic mutation differs between the crib-biting and the weaving horses. This is particularly interesting as a genetic predisposition to sensitivity of cocaine has been suggested in rodents (Haile *et al.*, 2001) whereby the D1 type receptor has been highlighted as essential in the locomotive effect of cocaine in the NAcc (Xu *et al.*, 1994). If the same genetic alterations could be observed in weaving horses then there would be far more concrete evidence of altered ventral striatal circuitry resulting in increased levels of extracellular dopamine being causal of weaving behaviour,

particularly acting of the D1 receptors which would explain the increased rate of behaviour initiation due to activation of the direct pathway as well as explain the observed normal functioning of the dorsal striatum without significantly altered SBR.

Future Study 4 – Development of a Stereotypy Screening Process

As stereotypic behaviour is suggested to have a genetic component it would be most useful to identify gene targets which would identify whether the horse is likely to develop CB or weaving behaviour. Once this is established behavioural probes could be utilised to determine whether over time these alter in response to a) increased dopamine release b) decreased dopamine receptor density and c) dominance of the sensorimotor circuits. Initially, before any stereotypic behaviour has become part of the behavioural repertoire of the horse SBR, behavioural initiation and performance within the extinction paradigm would be recorded in a group of young horses. Stereotypic behaviour would then be environmentally induced within the sample of horses and the same parameters would be recorded again at this early stage within stereotypic behaviour development. Finally, following 2-3 years of exhibiting stereotypic behaviour SBR, behaviour initiation and performance within the extinction paradigm would be recorded once more. The findings of such a study would be able to determine whether these behavioural probes could be utilised as a form of screening for susceptibility to stereotypic behaviour that could be used within the equine industry with ease. Furthermore, the results would also be able to determine whether such parameters change over time which at this point is unknown within the horse.

5.9 Applications to Industry

The behavioural probes utilised during this study provided excellent ways of determining altered basal ganglia circuitry. Furthermore as they are relatively straight forward and non-invasive methods these could simply be applied by the lay-person for example when looking to buy a horse, or to see whether a young

horse has potential to develop stereotypic behaviours. In particular the behaviour initiation probe would give a potential buyer a clear indication of whether or not that horse is likely to either currently be stereotypic or liable to become stereotypic. In combination with SBR attempts could be made to identify the type of stereotypic behaviour the horse performs or is likely to perform following chronic stress. If the horse demonstrates high levels of behaviour initiation combined with a 'normal' blink rate then the horse has potential to be exhibit weaving behaviour, though increased behaviour initiation combined with a reduced blink rate then the horse has potential to exhibit crib-biting behaviour. Utilising the SBR is not as concrete as perhaps the behavioural initiation data, as the behavioural initiation tended not to demonstrate as much range as the SBR data, and of course individual differences have potential to impact this also. Furthermore these alterations in SBR and behavioural initiation may be post-sensitisation which provides further merit to the final study proposed within section 5.8.

The data presented here agrees with data previously described in that crib-biting horses demonstrate enhanced ventral to dorsal in circuitry activation. Indeed the extinction paradigm used here clearly demonstrated the enhanced rate of habit formation with crib-biting horses. This would be of great interest to horse trainers and riding instructors when handling crib-biting horses. For example in dressage, if a crib-biting horse responds habitually to a command and it will be very difficult, if not impossible, to alter the way they have learned to perform that behaviour. In contrast the weaving horses will still demonstrate flexibility within their learning behaviour, though this is potentially only up to a point, as future research may indeed demonstrate that weavers do demonstrate enhanced habit formation, though at a slower rate when compared to crib-biting horses.

Ultimately, these findings suggest implications that are relevant to the equine industry in both the training aspect as described above, but also in terms of welfare. Horse establishments are known to attempt to alleviate the symptoms of stereotypic behaviours (McBride & Hemmings, 2009), despite the fact that in

scientific literature stereotypy is suggested to fulfil a coping function (Garner *et al.,* 2003a). In addition, some establishments remove stereotypy performing animals from the yard as many lay horse people believe stereotypy can be copied and effectively learned from neighbouring horses, despite there being no evidence within scientific literature to support this (Albright *et al.,* 2009). However, these methods of preventing or limiting stereotypic behaviours do not acknowledge previously reported alterations in neural physiology of the crib-biting horse (McBride & Hemmings, 2005). The data reported here suggest that for crib-biting animals, the crib-biting response is a habitual one, and thus attempting to alleviate the response could not only increase the stress of the animal (Hemmings *et al.,* 2004) but also does not consider the changes of the neural physiology previously reported and further suggested here through non-invasive means. Furthermore, these non-invasive behavioural probes also suggest weaving animals demonstrate significantly altered striatal dopamine physiology and as such are in a highly motivated state, a concept not previously explored within the weaving horse. Thus, it is somewhat evident that prevention techniques which alleviate the behaviour post emergence are only attempting to alleviate the symptoms observed i.e. the unwanted 'vice' rather than addressing the stress causing issue which is likely to result from poor management at an early stage of life. As a result, it is important for the lay horse owning populace to understand that high welfare standards, through appropriate management regimes, are required immediately from birth, particularly at weaning (), to prevent the occurrence stereotypic behaviours. In addition, once stereotypy performance is part of the behavioural repertoire of an animal, complete reversal may not be possible due to the changes within neural physiology (though whether these occur prior to or post stereotypy performance has not been reported within scientific literature and requires further investigation). However appropriate management regimes i.e. reducing stress and therefore increasing welfare standards may reduce the stereotypic response but is unlikely to cease it altogether.

Chapter 6: Final Conclusions

Previous invasive methods indicate that altered mesoaccumbens dopaminergic physiology is causal of stereotypic behaviour in both rodents and horses. The selected behavioural probes provided a new insight into the dopaminergic pathways within crib-biting and weaving horses. Crib-biting horses demonstrate increased sensitivity to dopamine which results in a highly motivated state, hence the emergence of the crib-biting response. Initially then, the crib-biting horse is trapped in the appetitive stage of the Hughes and Duncan (1988) model. However, as a result of atrophy of the associative pathway, in combination with hypertrophy of the sensorimotor pathway, this behaviour becomes habitual at a much faster rate than for control horses. Therefore crib-biting horses' exhibit altered dorsal striatal pathway which allows the emergence of the prolonged crib-biting response. In contrast, the data collected from the weaving horses suggests that the neural pathways behind the weaving response differ to that resulting in the crib-biting response. Indeed these data suggest that the weaving response occurs due to altered ventral striatal circuitry. The increase in phasic dopamine release (determined by the trend for increased SBR) results in a highly motivated state, thus allows an increased activation of the direct pathway accounting for the increase in rate of behaviour initiation. Therefore the weaving horse is trapped in the appetitive stage of the Hughes and Duncan (1988) model, but as there is no hypertrophy of the dorsal circuitry this behaviour does not become habitual. This is an area that requires further research to fully understand the differing neuro-aetiologies of CB and weaving behaviour proposed here. This is the first known report of differences between striatal physiologies between two different stereotypic behaviours within a single animal species.

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Figshare (2014) *Sagittal view of subcortical structures with segmentation examples from FreeSurfer of Caudate (light blue), Putamen (hot pink), Thalamus (green), Globus Pallidus (dark blue), Nucleus Accumbens (light brown), Amygdala (turquoise), and Hippocampus (yellow)* [online]. Available from: https://www.google.co.uk/search?q=mortgage+calculator&ie=utf-8&oe=utf-8&rls=org.mozilla:en-US:official&client=firefox-a&channel=fflb&gws_rd=cr&ei=P6nuUq4q4tPsBumZgLgJ#channel=fflb&q=barclays+mortgage+calculator&rls=org.mozilla:en-US:official. [Accessed 19 September 2014].

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Table 3 Horse Specification (TB = Thoroughbred, WB = Warmblood).

Horse	Age (Years)	Sex	Breed	Turnout (hours/day)	Exercise (At rest/Light/Medium/Competition)
Control 1	19	Gelding	TB x Hanoverian	9.5	At Rest
Control 2	12	Gelding	TB x Argentinian Polo Pony	9.5	At Rest
Control 3	32	Gelding	Argentinian Polo Pony	9.5	At Rest
Control 4	22	Gelding	Argentinian Polo Pony	9.5	Light
Control 5	12	Mare	Argentinian Polo Pony	9.5	Light
Control 6	15	Mare	Argentinian Polo Pony	9.5	Light
Control 7	7	Gelding	Irish Sport Horse	24	Medium
Control 8	20	Gelding	Welsh Section A	9.5	Light
Crib-biter 1	5	Gelding	Argentinian Polo Pony	12	Competition
Crib-biter 2	4	Mare	Argentinian Polo Pony	12	Competition
Crib-biter 3	14	Gelding	Argentinian Polo Pony	12	Competition
Crib-biter 4	8	Mare	Argentinian Polo Pony	12	Competition
Crib-biter 5	25	Gelding	Argentinian Polo Pony	11	Medium
Crib-biter 6	20	Gelding	TB	24	Light
Crib-biter 7	19	Gelding	Welsh Sec A	12	Light
Crib-biter 8	18	Gelding	ISHxTB	0	Medium
Weaver 1	16	Gelding	TB	24	Medium
Weaver 2	18	Gelding	WB	24	Light
Weaver 3	14	Mare	TB x Hanoverian	24	At Rest
Weaver 4	12	Mare	Cob	24	Medium
Weaver 5	9	Gelding	Belgian WB	12	Medium
Weaver 6	6	Mare	Connemara x TB	24	Light
Weaver 7	11	Gelding	Irish Draught	24	At Rest
Weaver 8	14	Gelding	TB	24	Light

Appendix B: Spontaneous Blink Rate Pilot Study

Aim of the Spontaneous Blink Rate Pilot Study

The pilot study was conducted on the 09/04/2012 at Fosse Hill farm as part of the Royal Agricultural University, Cirencester. This pilot study was conducted on one of the control horses prior to data collection. No data collected from this pilot study were utilised for statistical analyses. The aims of this pilot study were to:

- Test the method adapted from unpublished data conducted on horses (Issaoui & Hemmings, 2011) and published data conducted on humans (Chen *et al.*, 1996);
- Examine whether the prepared data collection sheet would be adequate for recording data;
- Identify and resolve any potential issues that may arise during the course of the pilot study to proactively prevent them during data collection.

Methodology

The horse was brought in from the field and tethered within the stable utilising a head collar and lead rope for safety, due to the close proximity of observing the horse within the stable. The horse was then habituated to the observers presence for 10 minutes. Immediately following habituation, each blink (as defined by Karson (1983)) of the left eye was recorded utilising a mechanical counter for 10 minutes, as measured by a stopwatch. Once complete, the number of blinks conducted was recorded on the data sheet and the horse was released within the stable.

Results

The method adapted from previous authors (Chen *et al.,* 1996; Issaoui & Hemmings, 2011) allowed accurate collection of SBR in the horse. The pilot study highlighted that some full blinks occur in quick succession, whilst in other

cases a full blink would be followed by half blinks or winks. The data collection sheet prepared was more than adequate to accurately record SBR.

Conclusions

As a result of the pilot study, all further data collected from this point was more likely to be an accurate representation of blink rate presented by the horse, as the observer was alerted to the quick succession and variety of blinks the horse exhibits prior to data collection. When the first aim of the pilot study is considered, it was found that the method chosen to record SBR is suitable in this case. Furthermore, the data collection sheet produced was ideal for recording blink rate, and the use of the mechanical counter was invaluable in being able to quickly and accurately record blink rate. No significant issues were raised during the course of the pilot study, thus no amendments to the proposed method were required.

Appendix C: Behavioural Initiation Pilot Study

Aim of the Behavioural Initiation Pilot Study

The pilot study was conducted on the 09/04/2013 at Fosse Hill farm as part of the Royal Agricultural University, Cirencester. The behavioural initiation pilot study utilised a control horse prior to data collection. No data from this pilot study were subjected to statistical analyses. The aims of this pilot study were to:

- Test whether the method adapted from Garner and Mason (2002) was suitable for use on the horse;
- Determine whether the pre-prepared data collection sheet was suitable for use to allow recording of behavioural initiation;
- Identify and resolve any potential issues that may arise during the course of the pilot study proactively prevent them during true data collection.

Methodology

The horse was brought in from the field and released within their usual stable and habituated to the observers' presence for 10 minutes. Following habituation, a stopwatch was utilised to time the 10 minute study period. During this time, each new initiation of behaviour was recorded as per Garner and Mason (2002), utilising a mechanical counter. All behaviours were pre-defined by an ethogram described by McDonnell (2003). Please note, the behaviours conducted were not recorded, only the initiation of each behaviour. Following the 10 minute pilot study the number of behaviours initiated was recorded on the pre-prepared data collection sheet.

Results

It became apparent that in line with Garner and Mason (2002) it was not necessary to consider the movement between two behaviours as a new initiation, for example walking between ingestion behaviour to drinking

behaviour. The pilot study highlighted that the mechanical counter was ideal to count behavioural initiations quickly and accurately. In addition, the pre-prepared data collection sheet was suitable to allow the recording of behavioural initiation.

Conclusions

As a consequence of the pilot study data collected for statistical analyses had increased reliability, as the observer had a greater understanding of what constituted the start point of individual behaviours. In addition, by not recording the movement between behaviours as a new behaviour initiation ensured behavioural initiation was not over-represented which would have resulted in un-reliable and inaccurate results. Furthermore, both the method and the data collection sheet were found to be sufficient for determining and recording behaviour initiation within the horse, having been successfully adapted from a bank vole study (Garner & Mason, 2002). Consequently, no amendments to the method were actioned prior to data collection.

Appendix D: Extinction Task Pilot Study

Aim of the Extinction Task Pilot Study

The pilot study was conducted on 27/05/2013 at Fosse Hill Farm as part of the Royal Agricultural University, Cirencester. The horse utilised for the pilot study was not part of the sample population for data collection, consequently any results collected were disregarded and not included for statistical analysis. The aims of the pilot study were to:

- Test the suitability of the device utilised in previous extinction tasks (Issaoui & Hemmings, 2011);
- Test the method adapted from previous extinction tasks (Hemmings *et al.*, 2007; Dias-Ferreira *et al.*, 2009; Issaoui & Hemmings, 2011) to determine suitability for this adaptation of the extinction task;
- Ensure horses were comfortable with the operant device to minimise safety issues;
- Construct a data collection table to allow ease of recording data;
- Identify potential problems which would impact data collection.

Methodology

The pilot study was undertaken within the horses' usual stable to minimise the effects of external stimuli which could potentially distract the horse from the extinction task. The horse was tied up on a long (1m) lead rope to attempt to maintain the horses' attention on the task. Initially the horse was habituated to the operant device (Fig. 23) by placing 5g of SPILLERS® High Fibre Cubes into the feed bowl of the device. The triangle CS card was then presented next to the operant device. Upon muzzle contact of the CS card, 5g of the high fibre cubes were deposited via the feeding tube into the feed bowl at fixed ratio 1. Successive approximation was then utilised so the horse associated the food reward with the operant device rather than the handler. Learning criterion for the shaping phase was set at 10 successive muzzle contacts of the CS card placed

on the operant device whilst the handler was no longer visible to the horse. This shaping phase was conducted in ten minute trials with a two minute break between each trial, as indicated with a stopwatch. Response acquisition was recorded as number of trials taken to reach learning criterion. This was then recorded on the pre-prepared data collection sheet.

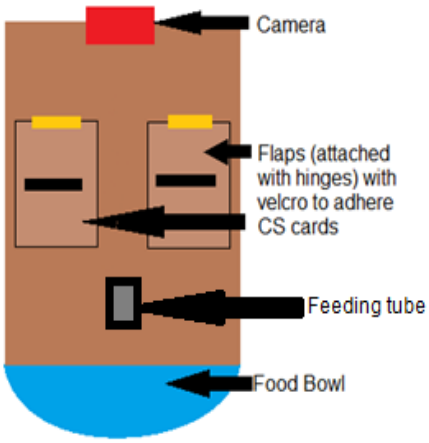

Figure 23: The operant device. Only the left flap was utilised as one CS card was presented.

The horse was allowed a two minute break after shaping before task 1 commenced. The successful completion for task 1 was set at 20 successive muzzle contacts with the CS card with reward being attained at fixed ratio 1. Following the 20th successful muzzle contact the device was removed from the stable door and the horse allowed a two minute break. Devaluation 1 then commenced, whereby the horse was allowed free access to 1kg of SPILLERS® High Fibre Cubes for five minutes in the absence of the operant device. Once five minutes had passed the remaining feed was weighed utilising digital weighing scales, and recorded on the pre-prepared data collection sheet. The horse then underwent extinction 1 for a ten minute trial. The operant device was presented to the horse, though this time muzzle contact with the CS card did not yield a food reward. The number of operant responses attempted during this ten minute trial was recorded on the data collection sheet, as was the latency of approach to the first operant response, as measured with a stopwatch. A two minute break followed extinction 1.

This process was subsequently repeated in the same manner for task 2, devaluation 2 and extinction 2. The only difference was between task 1 and task 2 in that completion criterion for task 2 was set at 40 successive operant responses. Following extinction 2 the device was removed for two minutes then re-presented under extinction. The latency of approach to first operant response and number of operant responses during the trial was recorded on the data collection sheet as before. The number of trials taken to reach total extinction (set at two consecutive trials with no response to the CS card) was recorded. Once total extinction criterion had been met the device was removed and the horse was returned to the field.

Results

The pilot study highlighted a number of key issues with the operant device that had been utilised for a previous unpublished extinction paradigm (Issaoui & Hemmings, 2011). These issues included:

- Instability of the device when positioned on the stable door;
- The feed bowl was both too deep and positioned too highly on the operant device to allow the horse to consume the food reward;
- The camera was unable to be suitably adhered to the operant device, thus resulting in continual interruptions throughout the extinction task;
- The presence of two flaps on the operant device was deemed unnecessary and inconvenient.

Whilst the data collection sheet was found to be useful in recording data from the parameters, it was determined the use of a mechanical counter would be beneficial to count number of operant responses instead of relying on memory.

Conclusions

Following the pilot study a number of alterations were made to allow the smooth running of data collection. The method adapted from previous extinction paradigms (Hemmings *et al.*, 2007; Dias-Ferreira *et al.*, 2009; Issaoui &

Hemmings, 2011) proved successful, particularly as the method was altered from that of previous extinction tasks which took place over a two day period (Hemmings *et al.*, 2007; Issaoui & Hemmings, 2011). Instead, extinction occurred immediately after learning the task. This was found to be sufficient enough for the horse to have successfully learnt the task as well as to measure extinction and rate of habit formation. Furthermore, this relatively short shaping phase in comparison to previous studies such as Hemmings et al (2007) and Issaoui and Hemmings (2011) reduced the likelihood of spontaneous recovery in response to the CS card, increasing the reliability of the results.

However, the pilot study highlighted that the operant device was not suitable for this adaptation of the extinction paradigm. Thus, a new operant device was designed and constructed with the specific aims and method of this extinction paradigm being considered. In addition, whilst the data collection sheet was suitable for recording the data collected, it was decided to implement a mechanical counter to tally the number of operant responses during extinction 1 and 2. These changes were subsequently implemented prior to data collection.

Appendix E: Raw Data Tables

Spontaneous Blink Rate

Table 4 Raw data collected from the 30 minute Spontaneous Blink Rate (SBR) observations for each horse.

Horse	SBR Day 1	SBR Day 2	SBR Day 3	Mean SBR/horse
Control 1	312	310	222	281
Control 2	354	321	444	373
Control 3	379	367	360	369
Control 4	556	585	572	571
Control 5	483	494	374	450
Control 6	473	478	489	480
Control 7	655	764	571	663
Control 8	393	372	452	406
Crib-biter 1	379	412	407	399
Crib-biter 2	309	402	414	375
Crib-biter 3	386	332	268	329
Crib-biter 4	312	345	384	347
Crib-biter 5	283	354	365	334
Crib-biter 6	373	515	411	433
Crib-biter 7	230	260	327	272
Crib-biter 8	191	235	212	213
Weaver 1	527	753	662	647
Weaver 2	323	542	630	498
Weaver 3	554	551	605	570
Weaver 4	290	493	469	417
Weaver 5	555	520	651	575
Weaver 6	349	443	435	409
Weaver 7	801	637	810	749
Weaver 8	391	454	580	475

Behavioural Initiation

Table 5 Raw data collected from 30 minute Behaviour Initiation (BI) observations for each horse.

Horse	BI Day 1	BI Day 2	BI Day 3	Mean BI/horse
Control 1	22	19	24	22
Control 2	28	21	21	23
Control 3	15	13	2	10
Control 4	16	22	14	17
Control 5	19	6	4	10
Control 6	9	10	10	10
Control 7	17	17	16	17
Control 8	3	3	3	3
Crib-biter 1	42	79	70	64
Crib-biter 2	193	266	223	227
Crib-biter 3	77	366	78	174
Crib-biter 4	91	88	73	84
Crib-biter 5	72	106	110	96
Crib-biter 6	84	141	142	122
Crib-biter 7	86	58	42	62
Crib-biter 8	308	364	401	358
Weaver 1	43	131	63	79
Weaver 2	235	195	200	210
Weaver 3	103	74	58	78
Weaver 4	35	40	42	39
Weaver 5	97	128	19	81
Weaver 6	53	84	125	87
Weaver 7	82	44	79	68
Weaver 8	63	96	54	71

Stereotypy Rate Pre-/Post Feed

Table 6 Raw data collected from the 15 minute pre-feed and 15 minute post-feed observations for each horse.

Horse	Day 1Pre-Feed	Day 1 Post Feed	Day 2 Pre-Feed	Day 2 Post Feed	Day 3 Pre-Feed	Day 3 Post Feed	Mean Stereotypy Pre-Feed	Mean Stereotypy Post Feed
Crib-biter 1	9	104	27	132	20	154	19	130
Crib-biter 2	53	79	65	78	67	100	62	86
Crib-biter 3	64	155	101	148	12	155	59	153
Crib-biter 4	21	85	22	98	5	16	16	66
Crib-biter 5	16	92	11	67	24	86	17	82
Crib-biter 6	30	125	51	144	71	130	51	133
Crib-biter 7	11	44	3	40	2	54	5	46
Crib-biter 8	95	135	72	181	91	114	86	143
Weaver 1	36	5	29	4	22	0	29	3
Weaver 2	144	127	265	286	44	0	151	138
Weaver 3	104	109	164	215	93	139	120	154
Weaver 4	5	0	0	0	4	0	3	0
Weaver 5	15	1	79	45	8	23	34	23
Weaver 6	11	0	16	2	30	20	19	7
Weaver 7	144	181	0	38	114	40	86	86
Weaver 8	8	14	11	0	54	5	24	6

Extinction Paradigm

Table 7 Raw data collected from the extinction paradigm for each horse (LA=Latency to Approach; OR=Operant Response).

Horse	Trials to Learning Criterion	Leftovers Devaluation 1	LA to 1st OR Extinction 1	OR Extinction 1	Leftovers Devaluation 2	LA to 1st OR Extinction 2	OR Ext 2	Trials to Total Extinction
Control 1	9	252	63.02	8	210	30.76	4	3
Control 2	12	955	4.56	3	925	3.78	2	3
Control 3	6	577	6.54	3	590	3.64	7	3
Control 4	8	628	3.04	4	685	77.97	3	3
Control 5	5	693	120.47	4	699	28.86	2	3
Control 6	11	606	83.22	4	775	20.02	2	3
Control 7	6	837	2.91	4	820	67.75	2	3
Control 8	4	616	6.21	6	863	6.44	3	3
Crib-biter 1	2	408	2.55	30	353	6.55	17	4
Crib-biter 2	1	512	1.82	33	533	3.15	27	4
Crib-biter 3	3	981	2.09	25	908	4.32	13	4
Crib-biter 4	2	734	3.56	23	753	5.12	14	5
Crib-biter 5	1	355	1.62	30	378	2.1	31	6
Crib-biter 6	1	401	23.01	17	585	10.12	13	5
Crib-biter 7	1	814	3.35	12	902	12.96	4	4
Crib-biter 8	1	10	7.36	17	190	116.35	12	4
Weaver 1	1	462	2.9	6	550	2.28	12	4
Weaver 2	2	324	3.71	4	516	20.12	5	3
Weaver 3	4	497	7.1	8	953	3.83	7	3
Weaver 4	1	582	1.43	10	568	2.21	3	4
Weaver 5	1	359	1.5	21	392	2.34	10	5
Weaver 6	1	505	5.4	4	602	64.54	1	4
Weaver 7	1	36	3.48	5	71	22.27	1	3
Weaver 8	3	876	4.3	3	908	0	0	2

Appendix F: Effect of Sex on Behavioural Parameters

Effect of Sex on Rate of Stereotypy

Independent Samples T-Tests found that there was no significant difference (table 7) between mares and geldings when accounting for all horses (t_{14}=0.293, p=0.774), nor within the crib-biting group (t_6=0.20, p=0.985) or the weaving horses (t_6=0.416, p=0.692) for pre-feed data. Similarly no significant difference was found between mares and geldings when accounting for all horses (t_{14}=0.722, p=0.482), crib-biting horses (t_6=1.229, p=0.265) and weaving horses (t_6=-0.048, p=0.963).

Table 8 Mean rate/ 15 minutes of stereotypy performed both pre-feed and post-feed for all (n=16), crib-biting (n=8) and weaving horses (n=8) (NS= Not Significant).

	Pre-Feed			Post-Feed		
	Geldings	Mares	Significance Value	Geldings	Mares	Significance value
All Horses	51.00±42.79	44.00±21.44	NS	85.73±57.84	62.60±63.09	NS
Crib-biting Horses	39.50±30.96	39.00±32.53	NS	114.50±41.53	76.00±14.14	NS
Weaving Horses	64.80±54.26	47.33±63.44	NS	51.20±58.96	53.67±86.96	NS

Effect of Sex on SBR

An Independent Samples T-Test demonstrated that there was no significant difference of mean SBR between geldings and mares (Table 8) when taking into account all horses (t_{22}=0.235, p=0.816), control horses only (t_6=-0.198, p=0.850), crib-biting horses only (t_6=-0.513, p=0.626) or weaving horses only (t_6=1.601, p=0.160).

Table 9 Mean spontaneous blink rate for geldings and mares during the 30 minute observation period for all (n=24), control (n=8), crib-biting (n=8) and weaving horses (n=8) (NS = Not Significant).

	Mean SBR			
	Geldings	Significance Value	Mares	Significance Value
All Horses	446.29±151.73	NS	435.43±73.96	NS
Control Horses	443.86±143.37	NS	465.00±21.21	NS
Crib-biting Horses	330.00±80.55	NS	361.00±19.80	NS
Weaving Horses	588.80±112.29	NS	465.33±90.73	NS

Effect of Sex on Behaviour Initiation

Independent Samples T-Test analysis determined there was no significant difference between geldings and mares (Table 9) when considering all horses (t_{22}=0.270, p=0.790), control horses (t_6=0.941, p=0.383), crib-biting horses (t_6=-0.106, p=0.919) or weaving horses (t_6=0.895, p=0.405).

Table 10 Mean rate of behaviour initiation per 30 minutes between geldings and mares for all horses (n=24), control horses (n=8), crib-biting horses (n=8) and weaving horses (n=8) (NS= Not Significant).

	Mean Rate of Behaviour Initiation			
	Geldings	Significance Value	Mares	Significance Value
All Horses	86.88±90.28	NS	76.43±74.24	NS
Control horses	15.33±7.61	NS	10±0.00	NS
Crib-biting horses	146.00±111.88	NS	155.50±101.12	NS
Weaving horses	101.80±60.73	NS	68.00±25.51	NS

Effect of Sex on Trials Taken to Reach Learning Criterion

Independent Samples T-Test analysis found no significant difference between mares and geldings (Table 10) when taking into account all horses (t_{22}=0.04, p=0.962), control horses (t_6=-0.198, p=0.850), crib-biting horses (t_6=0.000, p=1.000) or weaving horses (t_6=-0.442, p=0.674).

Table 11 Mean number of trials taken to reach learning criterion for geldings and mares considering all horses (n=24), control horses (n=8), crib-biting horses (n=8) and weaving horses (n=8) (NS = Not Significant).

	Mean Trials taken to Reach Learning Criterion			
	Geldings	Significance Value	Mares	Significance Value
All Horses	3.65±3.39	NS	3.57±3.64	NS
Control Horses	7.50±2.81	NS	8.00±4.24	NS
Crib-biting Horses	1.50±0.84	NS	1.50±0.71	NS
Weaving Horses	1.600±0.89	NS	2.00±1.73	NS

Effect of Sex on Feed Leftover Following Devaluation 1

There was no significant difference between the amount of feed leftover between geldings and mares (Table 11) following devaluation 1 when comparing all (t_{22}=-0.828, p=0.417), control (t_6=-0.029, p=0.978), crib-biting (t_6=-0.483, p=0.646) and weaving (t_6=-0.639, p=0.546) horses.

Table 12 Mean amount of feed leftover following devaluation 1 compared between geldings and mares considering all horses (n=24), control horses (n=8), crib-biting horses (n=8) and weaving horses (n=8) (NS = Not Significant).

	Amount of Feed Leftover Following Devaluation 1(g)			
	Geldings	Significance Value	Mares	Significance Value
All horses	523.00±298.66	NS	589.86±94.54	NS
Control horses	644.17±242.14	NS	649.50±64.52	NS
Crib-biting horses	494.83±349.05	NS	623.00±156.98	NS
Weaving horses	411.40±304.02	NS	528.00±46.93	NS

Effect of Sex on Number of Operant Responses Performed in Extinction 1

There was no significant difference found between mean number of operant responses made during extinction 1 and sex (Table 12) when comparing all horses (t_{22}=-0.139, p=0.890), control horses (t_6=0.455, p=0.665), crib-biting horses (t_6=-1.008, p=0.352) or weaving horses (t_6=0.101, p=0.923).

Table 13 Mean number of operant responses conducted during extinction 1 when comparing geldings and mares considering all horses (n=24), control horses (n=8), crib-biting horses (n=8) and weaving horses (n=8) (NS = Not Significant).

	Mean Number of Operant Responses Conducted during Extinction 1			
	Geldings	Significance Value	Mares	Significance Value
All horses	11.65±9.74	NS	12.29±11.35	NS
Control horses	4.67±1.97	NS	4.00±0.00	NS
Crib-biting horses	21.83±7.57	NS	28.00±7.07	NS
Weaving horses	7.80±7.46	NS	7.33±3.06	NS

Effect of Sex on Latency to Approach to First Operant Response during Extinction 1

An independent samples t-test determined that although there were no significant differences between geldings and mares and mean latency of approach to first operant response during extinction 1 (Table 13) when considering all horses (t_6=-1.244, p=0.257), crib-biting horses (t_6=0.643, p=0.544), and weaving horses (t_6=-1.061, p=0.330) there was a significant increase in LA for geldings when compared to mares for control horses (t_6=-4.407, p=0.005).

Table 14: Mean latency of approach to first operant response during extinction 1 when comparing geldings and mares for all horses (n=24), control horses (n=8), crib-biting horses (n=8) and weaving horses (n=8) (NS = Not Significant).

	Mean Latency of Approach (s)			
	Geldings	Significance Value	Mares	Significance Value
All Horses	8.36±14.23	NA	31.86±49.04	NA
Control Horses	14.38±23.88*	0.005	101.85±26.34*	0.005
Crib-biting Horses	6.66±8.27	NA	2.69±1.23	NA
Weaving Horses	3.18±1.06	NA	4.46±2.91	NA

Effect of Sex on Feed Leftover Following Devaluation 2

No significant differences between geldings and mares for amount of feed remaining following devaluation 2 (Table 14) when comparing all horses (t_{22}=-1.047, p=0.306), control horses (t_6=-0.280, p=0.789), crib-biting horses (t_6=-0.393, p=0.708) or weaving horses (t_6=-1.095, p=0.315) were observed.

Table 15 Feed leftover following devaluation 2 when comparing geldings and mares considering all horses (n=24), control horses (n=8), crib-biting horses (n=8) and weaving horses (n=8) (NS = Not Significant).

	Feed Leftover Following Devaluation 2(g)			
	Geldings	Significance Value	Mares	Significance Value
All horses	579.18±281.45	NS	697.57±145.67	NS
Control horses	682.17±261.58	NS	737.00±53.74	NS
Crib-biting horses	552.67±300.41	NS	643.00±155.56	NS
Weaving horses	487.40±301.72	NS	707.67±213.14	NS

Effect of Sex on Number of Presses Performed during Extinction 2

There was no significant difference in the mean number of operant responses performed during extinction 2 between geldings and mares (Table 15) when comparing all horses (t_{22}=0.047, p=0.963), control horses (t_6=1.076, p=0.323), crib-biting horses (t_6=-0.751, p=0.481) and weaving horses (t_6=0.565, p=0.593).

Table 16 Mean number of operant responses conducted during extinction 2 when comparing geldings and mares for all horses (n=24), control horses (n=8), crib-biting horses (n=8) and weaving horses (n=8) (NS = Not Significant).

	Mean Number of Operant Responses during Extinction 2			
	Geldings	Significance Value	Mares	Significance Value
All horses	8.18±7.80	NS	8.00±9.52	NS
Control horses	3.50±1.87	NS	2.00±0.00	NS
Crib-biting horses	15.00±8.92	NS	20.50±9.19	NS
Weaving horses	5.60±5.32	NS	5.67±3.06	NS

Effect of Sex on Latency of Approach to First Operant Response during Extinction 2

No significant difference was found for latency of approach to first operant response during extinction 2 (Table 16) between geldings and mares for all horses (t_{22}=0.340, p=0.737), control horses (t_6=0.290, p=0.782), crib-biting horses (t_6=0.638, p=0.547) or weaving horses (t_6=-0.670, p=0.420).

Table 17 Latency of approach to first operant response during extinction 2 when comparing geldings and mares for all horses (n=24), control horses (n=8), crib-biting horses (n=8) and weaving horses (n=8) (NS = Not Significant).

	Latency of approach to first operant response during extinction 2			
	Geldings	Significance Value	Mares	Significance Value
All horses	22.93±33.10	NS	18.25±22.82	NS
Control horses	31.72±33.60	NS	24.44±6.25	NS
Crib-biting horses	25.40±44.73	NS	4.14±1.39	NS
Weaving horses	9.40±10.83	NS	23.53±35.53	NS

The Effect of Sex on Number of Trials Taken to Reach Total Extinction Criterion

There was no significant difference for mean number of trials taken to reach total extinction criterion between geldings and mares (Table 17) when considering all horses (t_{22}=-0.160, p=0.875), crib-biting horses (t_6=0.000, p=1.000) or weaving horses (t_6=-0.369, p=0.725). Please note that due to there being no difference between the means of the control geldings and mares an independent sample t-test could not be calculated.

Table 18 Mean number of trials taken to reach total extinction criterion when comparing geldings and mares considering all horses (n=24), control horses (n=8), crib-biting horses (n=8) and weaving horses (n=8) (NS = Not Significant).

	Mean number of trials taken to reach total extinction criterion			
	Geldings	Significance Value	Mares	Significance Value
All horses	3.65±1.00	NS	3.71±0.76	NS
Control horses	3.00±0.00	-	3.00±0.00	-
Crib-biting horses	4.50±0.84	NS	4.50±0.71	NS
Weaving horses	3.40±1.14	NS	3.67±0.58	NS