Cognitive differences in horses performing locomotor versus oral stereotypic behaviour

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ABSTRACT

Preliminary investigations reveal altered learning patterns in horses performing oral stereotypic behaviour which coincide with differential functioning of the basal ganglia group of brain structures. However, no studies to date have investigated similar differences in the equine locomotor stereotypy phenotype. The aim of this investigation was to employ behavioural probes shown previously to reveal basal ganglia dysfunction to initialise the neurologic studies of locomotor stereotypy and to compare cognitive and neural aspects of the locomotor and oral stereotypy phenotype.

Spontaneous blink rate (SBR- number of full left eye-ball occlusions by the eye-lid in 30 minutes) and behavioural initiation rate (BIR- Behavioural transitions in 30 minutes) were conducted utilising a sample of crib-biting (n=8), weaving (n=8) and stereotypy free (n=8) animals. Horses were observed within their home box for SBR and BIR, with this being repeated three times over three consecutive days. All horses then completed an extinction learning paradigm featuring sensory specific satiety to dissect appetitive and habitual response patterns. Animals were initially shaped to press an A4 sized conditioned stimulus (CS) card mounted on an operant device for a food reward (5g pelleted feed). The extinction schedule was then split into two separate tasks. Task 1 required animals to conduct 20 operant responses (OR) followed by sensory devaluation (1kg freely available feed), whilst Task 2 required 40 OR prior to the devaluation phase. Following reward devaluation horses were subjected to an extinction phase where responses to the CS card were not rewarded.

Crib-biting horses demonstrated significantly lower SBR than control (p<0.05) and weaving (p<0.01) animals, though BIR was significantly increased for crib-biters (p<0.01) and weavers (p<0.05) compared to control equivalents. Both crib-biting and weaving groups acquired the initial operant response significantly faster than controls (p<0.001) and thus displayed accelerated learning. Moreover, crib-biting horses performed significantly more operant responses during extinction phase 1 and 2 compared to weaving (p<0.001 and p<0.01 respectively) and control animals (p<0.001 and p<0.001 respectively). Finally, crib-biting horses required significantly more trials to reach total extinction criterion compared to control (p<0.001) and weaving (p<0.01) equivalents.

These findings agree with previous studies in that crib-biting horses displayed a bias towards habitual response patterns, even in the context of minimal training. This tendency corroborates previous post-mortem evidence of up-regulated ventral and down-regulated dorsomedial activity in the striatum group of brain structures. On the other hand, weaving animals were resistant to over-training and failed to display habitual responding, at any stage of the investigation. This, when taken alongside increased BIR and faster rate of learning is suggestive of enhanced ventral striatal activity but a normal functioning dorsal striatum.

Highlights

* Behavioural probes were used as indirect measures of striatal function for stereotypy performing horses
* Crib-biting horses exhibit evidence of enhanced ventral but decreased dorsal striatal activity
* Weaving animals show accelerated learning, perhaps due to enhanced ventral striatal activity
* Crib-biting horses are prone to habitual responding whilst weaving animals are not
* Crib-biting horses may be at increased risk of overtraining

Key words: Striatum, Habit, Behaviour, Dopamine, Stereotypy, Equine

**1 Introduction**

Stereotypic behaviours are repetitive, invariant (Pell & McGreevy 1999; McBride & Hemmings, 2005) and idiosyncratic (Parker *et al.*, 2009) induced by frustration, repeated attempts to cope or central nervous system (CNS) dysfunction (McBride & Hemmings, 2009), and are often utilised as an indicator of poor welfare status (Mills *et al.,* 2002; Cooper & Albentosa, 2005; Wickens & Heleski, 2010). Crib-biting (CB) is an oral stereotypy whereby the animal grasps a solid object in the incisor teeth, and emits a grunting sound (Nicol *et al.,* 2002; Moeller *et al.,* 2008) with an estimated 2.4-8.3% of the equine population exhibiting the behaviour (Wickens & Heleski, 2010). Weaving on the other hand is a locomotor stereotypy characterised by lateral movement of the head accompanied by contralateral shifting of weight on the forelimbs, with approximately 3-10% of horses performing this behaviour in the equine population (Cooper *et al.,* 2000; McBride & Hemmings, 2009).

Multiple causal factors have been suggested with regards to crib-biting although this is generally a post-prandial response (McBride & Hemmings, 2004), sometimes linked to pain induced by ulceration of the gastric mucosa (Nicol *et al.,* 2002; Hemmings *et al.,* 2007; Moeller *et al.,* 2008). In contrast, weaving is thought to stem from frustration associated with deprivation of social and locomotor activities (Pell & McGreevy, 1999; McAfee *et al.,* 2002) as well as an anticipatory response to feed (Cooper *et al.,* 2005).

When investigating aetiology of stereotypy from a neural perspective, research efforts to date have centred on a trio of anatomically connected but functionally distinct forebrain nuclei known collectively as the striatum (Carlson, 2001). The dorsal striatum is comprised of the caudatus and putamen, two structures implicated in associative learning, and habitual response patterns that result from over-training (see Canales (2005) for review). As they continue ventrally, the caudatus and putamen give way to the nucleus accumbens (NA) (Nolte, 2001), the function of which is generally associated with motivation and reward processes associated with the early stages of learning a new task (see Graybiel (2008) for review). Furthermore, the striatum receives direct innervation from midbrain neurons which project dopamine onto dense populations of D1 and D2 receptors expressed across all three striatal sub-regions (Haber *et al.,* 2000). Previous work centring on the striatum of crib-biting horses revealed up-regulation of D1 and D2 dopamine receptors in the ventral striatum and accompanying down-regulation of D1 receptor populations in the dorsally positioned caudate nucleus (McBride & Hemmings, 2005). This is similar to that found in chronically stressed rodents, thus highlighting the potential effect of stress on striatal functioning, particularly of the ventral striatum (Cabib *et al.,* 1998; Cabib, 2006; Dias-Ferreira *et al.,* 2009). Therefore it seems plausible that stress is linked to the receptor based changes recorded in the crib-biting horse (McBride & Hemmings, 2009). To date no similar neural investigations have been performed on horses prone to weaving.

Alongside stereotypy, these neural changes manifest in other aspects of the animals behavioural phenotype, for example during learning. Indeed, accelerated habit learning is observed in CB horses (Hemmings *et al.,* 2007; Parker *et al.,* 2009). The alterations outlined above create an accelerated shift of ventral to dorsal activity in the striatum region of the brain, causing a bias towards habitual responding (Dias-Ferreira *et al.,* 2009). Despite these findings, the weaving horse thus far has been overlooked in terms of striatal functioning, with no studies to date investigating the neural mechanisms of locomotor stereotypy. Whilst repetition of McBride and Hemmings’ (2005) work utilising weaving horses would provide details on receptor populations in weaving animals, radio-ligand binding methods are both invasive and expensive. However behavioural probes, which investigate underlying cognitive processes using simple behavioural tests, provide a proven, non-invasive means of determining striatal functioning in a variety of species including rodents (Garner & Mason, 2002; Kaminer *et al.,* 2011), birds (Garner *et al.,* 2003), humans (Chen *et al.,* 1996; Roebel & MacLean, 2007), non-human primate subjects (Karson, 1983; Taylor *et al.,* 1999) as well as limited data within the horse (Hemmings *et al.,* 2007; Parker *et al.,* 2009).

For example, spontaneous blink rate (SBR), an indication of dopamine physiology in the striatum (Karson, 1983; Taylor *et al.,* 1999; Kaminer *et al.,* 2011), has been utilised to determine striatal functioning in stereotypy performing humans, with reduced SBR being attributed to increased postsynaptic receptor sensitivity (Roebel & MacLean, 2007). Furthermore, patients with dopamine depletion due to Parkinson’s Disease demonstrate reduced SBR, though this is attributed to lower dopamine availability as opposed to postsynaptic receptor sensitivity (Karson, 1983). In contrast, patients with schizophrenia exhibit significantly increased SBR as a result of elevated dopamine availability associated with this condition (Karson, 1983; Mackert *et al.,* 1991). Additionally, dopamine agonists (i.e. elevated dopamine) and antagonists (i.e. reduced dopamine) reliably increases or decreases SBR respectively (Karson; 1983; Chen *et al.,* 1996; Kaminer *et al.,* 2011). Considering the findings of McBride and Hemmings (2005), it is of value to conduct SBR assessment in a sample of stereotypy performing horses (oral and locomotor) to further investigate altered striatal functioning as a factor in stereotypic motor output.

Furthermore, Garner and Mason (2002) observed significantly increased behaviour initiation (BIR) (i.e. the number of behavioural transitions performed in a given period) in bank voles performing bar-mouthing, suggesting disinhibition of the striatonigral pathway of the dorsal striatum in these animals. This allows dominance of the striatopalladial pathway resulting in a significant increase in the number of behavioural ‘switches’ conducted (Garner & Mason, 2002; Garner, 2006). Considering the alterations in dopamine physiology observed in the CB horse (McBride & Hemmings, 2005) and the fact that dopamine agonists increase the rate of behaviour initiation (Garner & Mason, 2002; Garner, 2006), measurement of this phenomenon will be utilised to further elucidate the neural pathways recruited within stereotypy performing animals.

Finally, extinction learning tasks have been utilised previously to investigate the neural basis of equine stereotypy (Hemmings *et al.,* 2007). However traditional extinction paradigms such as that employed by Hemmings *et al.* (2007) have been criticised as they fail to dissect perseverance related to goal directed (i.e. directed at feeding) versus true habitual responding (see Yin and Knowlton (2006) for review). As such, this study utilised a two phase devaluation-extinction paradigm featuring sensory specific satiety, which effectively removes feeding motivation therefore allowing accurate discrimination between appetitive and habitual responding (Dias-Ferreira *et al.,* 2009) to further investigate behavioural differences in a sample population of crib-biting, weaving and stereotypy free control horses.

Following on from our previous post-mortem work that revealed significant alterations to basal ganglia physiology in the crib-biting horse (McBride & Hemmings, 2005) we hypothesised that both stereotypy performing cohorts would demonstrate a bias towards perseverative responding under extinction conditions, combined with SBR and BIR recordings that reflect altered functioning of the dopamine system.

**2 Methods**

*2.1 Sample population characteristics and management*

Eight control, eight crib-biting and eight weaving horses of various breeds, age and sex (Table 1) were recruited for the study. In order to recruit suitable numbers of stereotypy performing horses, animals from eight diverse establishments were utilised. These ranged from smaller yards of 10 animals up to larger establishments of 50, although work requirements of all three groups were similar (all were used for so called Sport Horse disciplines such as Polo). All animals were fed to meet individual dietary requirements for maintenance according to the National Research Council (NRC, 2007). Furthermore, all observations and the extinction paradigm were conducted within the horses home box (3.65mx3.65m) with access to forage and water. Each had eye-contact with fellow conspecifics which were not part of the study to remove the potential influence of isolation stress on learning and dopamine transmission. No experimental procedures were conducted within an hour before or after the horses usual feeding time and at a quiet time of day (mid-morning to mid-afternoon) to minimise the impact this may have on parameters such as dopamine release. Some horses had preventative devices such as crib-biting straps and weave bars fitted, however these were removed 10 minutes prior to experiment commencement. The introduction of this period allowed time for post-prevention normalisation and habituation to observer presence.

Screening process to confirm behavioural phenotype and stereotypy rate

In order to confirm stereotypy performing / stereotypy free status of the studied groups, an owner questionnaire and detailed owner interview was initially conducted. This also enabled the study team to determine the presence of other abnormal behaviours e.g. door-kicking, . Following this initial screening phase, stereotypy rate was quantified in response to feed delivery, as equine stereotypy has been recorded to occur primarily in this context (see McBride and Hemmings 2004 for review).

 For the crib-biting group crib-biting responses (each incident of grasping followed by audible grunt) were recorded for 15 minutes before and 15 minutes after feeding 5g of commercially available pelleted feed (Spillers High Fibre Cubes™). On the other hand, as weaving is thought to be an anticipatory response performed in the context of feed predictive conditioned stimuli (CS), the arrival of the feed was cued by the shaking of a feed bucket 15 minutes prior to being given access to its contents (5gms Spillers High Fibre Cubes™). As all horses were regularly fed pelleted feed in buckets, they were deemed conditioned to the rattling sound produced by bucket shaking. Individual ‘weaves’ were then counted (each ‘left-right-left’ or ‘right-left-right’ shifting of the head) for 15 minutes post CS and for a further 15 minutes post-delivery of feed.

It should be noted that the feed predictive CS was not applied the crib-biting horses as this stereotypy is proven to be primarily post-prandial (Clegg *et al.,* 2008). For both groups, stereotypy rate recording was repeated three times over consecutive days to enable calculation of a representative mean.

*2.2 Spontaneous Blink Rate*

Horses were loosely tethered using a head-collar and a lead-rope for ease of observation. Following habituation each full spontaneous blink (defined by Karson (1983) as the bilateral paroxysmal brief repetitive eye closures occurring continuously) was recorded utilising a mechanical counter for 30 minutes. This was repeated until three observations had been conducted to achieve a mean SBR. Considering the anatomy of the horse, true bilateral eye closures would be difficult to measure thus only the left eye was observed for all horses.

*2.3 Behavioural Initiation Rate*

The animals were habituated as described above. Following habituation, every behaviour initiation was recorded with a mechanical counter for 30 minutes. All behaviours were defined by a pre-determined ethogram (McDonnell, 2003), though after Garner and Mason (2002) only number of behavioural transitions were recorded, not type of behaviour. Each bout of behaviour was recorded as a new initiation irrespective of the previous behaviour, for example the sequence ‘Feeding-Grooming-Feeding-Drinking-Standing at Rest’ was recorded as four initiations (Garner & Mason, 2002). This was repeated until the observation had been completed three times to achieve mean rate of behavioural initiation for each sample group.

*2.4 Extinction Paradigm*

This protocol was adapted from previous extinction paradigms using rodent and horse models incorporating a two-phase devaluation-extinction task using sensory specific satiety as a method of removing feeding motivation. The extinction paradigm was divided into eight distinct phases. Crib-biting straps and weaving bars were removed prior to the extinction task. An operant device was constructed (L122cmxW60cm) utilising chipboard, allowing a surface for the CS card to be adhered to. A tube was passed through a hole through to the front of the board and secured, via which the feed reward was delivered into a rubber feed bowl also adhered to the front of the board. Similarly, a door viewing lens was utilised so the observer could observe operant responses whilst out of view of the horse to minimise observer effect. Resizable hooks attached to the reverse surface allowed the operant device to be hung over the stable door, allowing all extinction paradigm assessments to be undertaken in the horse’s home box.

*2.4.1 Shaping Phase*

Initially, the horse was loosely tethered and habituated to the operant device by placing 5.00g of high fibre pelleted feed into the feeding bucket. The habituation process was considered complete when the horse consumed the food within the bucket. Successive approximation was then utilised to shape the horse to associate the CS card with food reward. Each time the horse made muzzle contact with the CS card 5.00g of feed was rewarded via the feeding tube. This process was repeated in 10 minute trials, with a two minute break between each trial, until the horse made 10 successive muzzle contacts with the CS card whilst the observer was present. This process was repeated in the same manner with the observer positioned behind the operant device in such a way that the observer was no longer visible to the horse. Learning criterion for completion of the shaping phase was set at ten successive operant responses to the CS card when presented on the operant device with no human contact. The number of trials to reach learning criterion was recorded. Completion of the shaping phase was immediately followed by a two minute break in the absence of the operant device.

*2.4.2 Task Phase 1*

The presentation of the operant device signified the start of the trial. Each operant response to the CS card was rewarded with 5.00g of high fibre pelleted feed (Fixed ratio 1). Completion criterion for Task 1 was set at 20 successive operant responses of the CS card. Following the 20th operant response, the operant device was removed from the stable door and the horse allowed a two minute break prior to Devaluation Phase 1.

*2.4.3 Devaluation Phase 1*

The horse was allowed *ad lib* access to 1000g of high fibre pelleted feed in a black bucket for five minutes in the absence of the operant device, as pilot studies indicated this is a sufficient amount to reduce feeding motivation. Following Devaluation 1 the bucket was removed from the stable and the horse allowed a two minute break prior to the next phase commencing. During this time the remaining pellets were weighed on electronic scales with the resultant mass being recorded to the nearest gram.

*2.4.4 Extinction Phase 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 feed administration. The latency of approach (LA) to first operant response was recorded as a measure of motivation. The total number of operant responses performed during the 10 minute trial was also recorded. Following the 10 minute trial the operant device was removed from the stable door and the horse allowed a two minute break.

*2.4.5 Task Phase 2*

Task 2 was undertaken in the same manner as Task 1, though completion criterion was set at 40 successive operant responses of the CS card. Following the 40th operant response, the operant device was removed from the stable door and the horse allowed a two minute break.

*2.4.6 Devaluation Phase 2*

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

*2.4.7 Extinction Phase 2*

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

*2.4.8 Total Extinction of the Operant Response*

Following Extinction 2, the operant device was presented to the horse with no reward being achieved. Total extinction criterion was set at two discrete 10 minute trials with no operant responses to the CS card, with a two minute break between each trial to avoid the effects of spontaneous recovery. Latency of approach to first operant response and number of operant responses during each trial was recorded. Once extinction criterion had been met, the total number of trials taken to reach extinction criterion was recorded. For all data collection procedures given above, blind testing was not possible as stereotypy performance often continues within experimental settings.

2.5 Statistical Analysis

The Statistical Package for Social Sciences (SPSS) v.20.0 was utilised for all statistical analyses. A test for normalcy was conducted prior to the completion of parametric statistical tests.

*2.5.1 Rate of Stereotypy Pre- and Post-Feed*

Mean rate of stereotypy was calculated for pre- and post-feed with data subsequently analysed with a paired samples t-test.

*2.5.2 Spontaneous Blink Rate*

Mean SBR was calculated for the crib-biting, weaving and control animals using mean SBR from each individual horse (n=8). A one-way analysis of variance (ANOVA) was conducted with a post-hoc least significant difference (LSD) to look for significant differences between the three sample groups.

*2.5.3 Rate of Behavioural Initiation*

Mean rate of behavioural initiation was calculated for the crib-biting, weaving and control horses using mean behavioural initiation data from each individual horse (n=8). A one-way ANOVA was conducted with post-hoc LSD analyses to probe for differences between the three sample groups.

*2.5.4 Extinction Paradigm*

A series of one-way ANOVAs were conducted to test for the following between crib-biting, weaving and control animals:

* Total number of trials taken to reach learning criterion;
* Total number of trials taken to reach total extinction criterion;
* Total number of operant responses conducted during Extinction 1;
* Latency of approach to first operant response during Extinction 1;
* Total number of operant responses conducted during Extinction 2;
* Latency of approach to first operant response during Extinction 2;
* Feed remaining following Devaluation 1;
* Feed remaining following Devaluation 2.

A paired samples t-test was applied to total number of operant responses conducted during Extinction 1 compared to Extinction 2 data for the crib-biting, weaving and control sample groups.

**3 Results**

*3.1 Rate of Stereotypy*

The paired samples t-test confirmed that crib-biting horses performed significantly more crib-biting responses post-feed (104.83±14.04 S.E.M) when compared to crib-biting responses pre-feed (39.29±10.19 S.E.M) (t7=6.442, p<0.001). However, the rate of weaving response was not significantly different when comparing pre- (58.33±19.09 S.E.M) and post-feeding (52.25±22.76 S.E.M) weaving responses (t7=0.956, p=0.371). No other forms of abnormal behaviour i.e. door kicking, pawing were observed in either of the stereotypy or stereotypy-free cohorts at any time during experimental procedures.

*3.2 Spontaneous Blink Rate*

The post-hoc LSD highlighted that the SBR for the crib-biting horses was significantly lower when compared to the control (p=0.047) and the weaving (p=0.001) animals. No significant difference was observed for the SBR between the control and weaving samples (p=0.091) (Fig. 1).

*3.3 Behavioural Initiation Rate*

Crib-biting (p=0.001) and weaving (p=0.003) horses performed significantly more behaviour initiations when compared with control animals, however no significant difference was identified between crib-biting and weaving animals (p=0.087) (Fig. 2).

*3.4 Extinction Paradigm*

*3.4.1 Trials Taken to Reach Learning Criterion*

Control horses required significantly more trials to reach learning criterion compared with the crib-biting (p<0.001) and weaving animals (p<0.001), however no significant difference was identified between the crib-biting and weaving horses (p=0.789) (Fig.3).

*3.4.2 Feed Remaining Following Devaluation Phase 1*

No significant differences were identified between the amount of feed remaining following Devaluation 1 between the control (645.50±205.98g±SD), crib-biting (526.88±306.70g±SD) or weaving (455.13±238.93g±SD) animals (F2,21=1.146, p=0.337).

*3.4.3 Operant Responses Conducted During Extinction Phase 1*

Crib-biting animals conducted significantly more operant responses when compared with both the control (p<0.001) and weaving (p<0.001) animals. No further differences were observed between these groups (p=0.276, see Fig. 4).

*3.4.4 Latency of Approach to First Operant Response during Extinction Phase 1*

Crib-biting (p=0.035) and weaving (p=0.026) horses demonstrated significantly decreased latency of approach to the first operant response than control animals, however no differences were identified between the crib-biting and weaving (p=0.887) animals (Fig. 5).

*3.4.5 Feed Remaining Following Devaluation Phase 2*

No significant difference (F2,21=0.616, p=0.550) was observed in the amount of feed remaining following Devaluation 2 for the control (695.88±223.45g±SD), crib-biting (575.25±263.95g±SD) or weaving (570.00±279.28g±SD) horses.

*3.4.6 Operant Responses Conducted During Extinction Phase 2*

Crib-biting horses demonstrated significantly more operant responses compared with both weaving (p=0.001) and control (p<0.001) animals. No further differences were identified between the three sample populations (p=0.547, see Fig. 4).

*3.4.7 Latency of Approach to First Operant Response during Extinction Phase 2*

There was no significant difference (F2,22=0.504, p=0.611) in the latency of approach to first operant response during Extinction 2 between the crib-biting (20.08±13.81s S.E.M), weaving (14.70±7.80s S.E.M) or control (29.90±10.15s S.E.M) animals.

*3.4.8 Trials Taken to Reach Total Extinction Criterion*

The crib-biting animals required significantly more trials to reach total extinction criterion when compared to the control (p<0.001) and weaving (p=0.009) horses. No additional significant findings were revealed in this particular analysis (Fig. 6).

*3.4.9 Number of Operant Responses Conducted during Extinction Phase 1 when Compared to Extinction Phase 2*

Crib-biting horses performed significantly less operant responses in Extinction 2 when compared with Extinction 1 (t7=4.365, p=0.003), however no difference was observed in number of operant responses between extinction trials for either the control (t7=1.629, p=0.147) or weaving (t7=1.528, p=0.170) horses.

**4 Discussion**

*4.1 Spontaneous Blink Rate and Behavioural Initiation*

The CB horses demonstrated significantly reduced SBR compared to control equivalents, which, based on previous human work (Roebel & MacLean, 2007) indicates dopamine receptor sensitisation in CB animals. This notion is strongly supported by ligand binding experiments which revealed increased D1 dopamine receptor density and affinity in the striatum of horses that have performed oral stereotypy (McBride & Hemmings, 2005). SBR of weaving animals did not differ significantly from controls, suggesting that weaving horses do not exhibit the type of striatal differences observed in the CB population.

Crib-biting horses performed approximately 11 times the behaviour initiations when compared to control animals. From a neural mechanisms perspective, Garner (2006) previously suggested that increased BIR in a given time period occurred due to enhanced activity of the D1 rich, movement increasing pathway projecting from the rodent striatum. Given the physiological evidence for sensitisation of equine D1 receptor mechanisms in our previous work (McBride & Hemmings, 2005), the dramatic increase in BIR reported here is hardly surprising. Along similar lines weaving horses performed around 6 times the behaviour initiations, still significantly more than control horses whilst remaining statistically similar to the crib-biting group. This suggests that a degree of direct pathway sensitisation is also associated with the locomotor stereotypy phenotype. When considering the precise pattern of striatal activity, stereotypy induction by psychostimulants involves a ventral mediated hyper-locomotive phase at lower doses of drug, followed by dorsally dominated stereotypic output as dosage elevation progresses (Robbins & Sahaikian, 1983). As such, given that the morphology of weaving puts this behaviour into the locomotor category, it might be tentatively implied that the neural differences occurring in weaving animals are centred on the ventral striatum.

In summary as both CB and weaving animals demonstrated significantly increased BIR, it is possible this simple behavioural probe could in the future be utilised as a measure of stereotypy susceptibility, leading to screening paradigms for young horses at risk of stereotypy development. Of course, validation of a screening methodology would depend on the development of a genetic tool for identification of susceptible animals. This process is ongoing in our laboratory.

*4.3 Operant response acquisition*

Stereotypy performing groups attained the operant response significantly faster compared to controls. In support of this finding anecdotal accounts from experts in equitation support the notion of hastened learning ability in stereotypy performing horses. Indeed, some trainers favour crib-biting horses due to their perceived high intelligence levels (personal observation). Furthermore Nagy *et al.* (2009) reported a significant positive relationship between crib-biting status and trainability. In other species (i.e. humans) elevated striatal DA has been shown to increase the rate of learning in tasks featuring positive feedback (Frank *et al.,* 2004) such as that employed here. Early in task acquisition, activity of the ventral striatum dominates (See Yin and Knowlton (2006) for review), and so elevated activity of this structure in the stereotypy groups studied could well explain increased rates of task acquisition. Whereas ventral striatal sensitisation has been previously reported for crib-biters (McBride & Hemmings, 2005) the presence of increased rate of learning for both stereotypy populations constitutes an additional line of evidence for ventral striatal changes in the weaving group. In addition, latency to approach was reduced in both stereotypy cohorts and combined with BI findings of this study, provides growing evidence of hyper-locomotive tendencies being a feature of the stereotypy phenotype. Once again drawing support from previous rodent data, hyper-locomotion is a commonly recorded phenomenon stemming from increased striatal dopamine release centred on ventral sub-regions (i.e. the nucleus accumbens, see Cabib (1993) for review). Finally, previous authors have suggested that stereotypy induced by restrictive environments will retain topographic characteristics of frustrated highly motivated behaviours. As such, the tendency of the weaving group to manifest a locomotor rather than an oral stereotypy suggests a motivational bias towards movement and therefore increased ventral striatal activity.

*4.4 Extinction Task 1*

During the first phase of the extinction task the CB horses performed significantly more operant responses compared to both control and weaving animals. From a striatal function perspective, repetition of the response leads to increasing dorsal striatal activity, until the putamen (dorso-lateral striatum) is the dominant mediator of behaviour output (Yin & Knowlton, 2006; Dias-Ferreira *et al.,* 2009). At this point the behaviour becomes habitual and resistant to reward devaluation. This suggests CB horses are demonstrating the accelerated shift from ventral to dorsal circuitry control, such as that previously observed in chronically stressed rodents (Dias-Ferreira *et al.,* 2009). Further examination of these rodents highlighted atrophy of the caudate and hypertrophy of the putamen, suggesting that the animals were relying on sensorimotor circuitry in the putamen to perform the task (Dias-Ferreira *et al.,* 2009). Similar reduction of caudate output has been observed in the CB horse (McBride & Hemmings, 2005; Hemmings *et al.,* 2007; Parker *et al.,* 2009), providing further merit to the possibility of accelerated habit formation within these animals.

Unlike the crib-biters, weaving horses did not perform significantly more operant responses compared to the control group, suggesting that the instrumental response is still resistant to reward devaluation. As such, the dorsal striatia of the weaving horse would appear to function in a similar manner to that of the control animals.

*4.5 Extinction Phase 2 and Total Extinction*

Crib-biting horses once again performed significantly more operant responses compared to control and weaving animals in both the second and total extinction phases thereby demonstrating persistent habitual response patterns compared to both of the other groups. Similarly, the weaving horses did not respond habitually despite the accruing number of repetitions, thus emphasising the neural differences between two different stereotypic behaviours within a single animal species. Therefore, CB horses demonstrate liability to form habitual behaviours compared to weaving horses, suggesting that the CB horse is at risk of overtraining whereas weaving horses are not. As such, training methods should be adapted to feature reduced repetitions. This will ensure crib-biting animals do not transit to the realms of habitual responding, thereby guarding against deleterious consequences of habit formation in the training and competition setting. In order to determine at what point a particular behaviour becomes habitual, further work utilising this paradigm would be required. For example, during this study the CB animals demonstrated habitual behaviours following only 20 repetitions, thus it would be beneficial to repeat this probe with 10 or less repetitions initially to examine rate of habituation in CB animals. This would therefore inform horse trainers at what point overtraining is likely to occur, so they could actively avoid overtraining in CB animals.

*4.6 Final Conclusions*

Crib-biting and weaving populations appear to show singularly unique differences in striatal function compared to stereotypy free controls based on the results from our chosen correlative measures of dopamine and the extinction paradigm. For crib-biters, sensitisation of the ventral circuitry in combination with atrophy of the dorsal circuitry highlighted by previous authors (McBride & Hemmings, 2005; Hemmings *et al.,* 2007; Parker *et al.,* 2009) and also suggested here, appears to result in accelerated habit formation, clearly observed during the two-phase extinction paradigm.

On the other hand, weaving behaviour is considered here to be related to altered ventral striatal physiology alone, resulting in a hyper-locomotive phenotype in the absence accelerated habit formation. Finally, due to the rigorous screening of stereotypy free status in the control group, we can be confident that the differences reported herein can be reliably attributed to the equine stereotypy phenotype. To our knowledge this this is the first indication of differential learning (and in turn brain function) between locomotor and oral stereotypy phenotypes of a single animal species.

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**Figure Captions**

Figure 1. The mean ±S.E.M spontaneous blink rate observed between Control, Crib-biting and Weaving horses. Values sharing superscripts are not significantly different.

Figure 2. The mean ±S.E.M behaviours initiated during the 30 minute observation period for Control, Crib-biting and Weaving horses. Values sharing superscripts are not significantly different.

Figure 3. The mean ±S.E.M number of trials taken for each group to reach learning criterion. Values sharing superscripts are not significantly different.

Figure 4. The mean ±S.E.M number of operant responses performed during Extinction 1 and Extinction 2 by the Control, Crib-biting and Weaving horses. Values sharing superscripts within the same extinction trial are not significantly different.

Figure 5. The mean ±S.E.M latency of approach to the first operant response during Extinction 1 for Control, Crib-biting and Weaving horses. Values sharing the same superscript are not significantly different.

Figure 6. The mean ±S.E.M number of trials taken for the Control, Crib-biting and Weaving horses to reach total extinction criterion. Values sharing superscripts are not significantly different.