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UNIVERSITY OF SOUTHAMPTON
FACULTY OF MEDICINE, HEALTH AND LIFE
SCIENCES

SCHOOL OF PSYCHOLOGY

Behavioural Correlates of the Equine Stereotypy Phenotype

By

Matthew Oliver Parker

Thesis for the degree of Doctor of Philosophy

September 2008

UNIVERSITY OF SOUTHAMPTON
ABSTRACT
FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES
SCHOOL OF PSYCHOLOGY
Doctor of Philosophy

BEHAVIOURAL CORRELATES OF THE EQUINE STEREOTYPY
PHENOTYPE

By
Matthew Oliver Parker

Stereotypic behaviour is characterised as repetitive, topographically invariant, rigid behaviour patterns, often displayed by captive, domestic and laboratory animals. The cause of these behaviours is thought primarily to be related to impoverished or species-atypical living environments, but recent research has outlined a neural phenotype, relating to basal ganglia dopamine physiology, which is common to several species. One species displaying the neural and associated behavioural phenotypes is the Horse. The purpose of this thesis was to explore, through behavioural assays, the behavioural phenotypes associated with endogenous basal ganglia dysfunction as a neural feature of equine oral stereotypy.

In the first study, the behavioural effects of one aspect of the neural phenotype – downregulation of dopamine transmission in the nigrostriatal pathways – were examined. Animals with the medial aspect of the dorsal striatum lesioned have been shown to exhibit learning differences in spatial navigation procedures. Employing two dry-maze procedures, stereotypic and control horses were required to locate food in different locations. No specific differences between the groups were found, but the stereotypic group were found to be less exploratory and tended to adopt ‘habitual’ response patterns.

In the next studies, the behavioural effects of a further feature of the neural phenotype – upregulation of mesoaccumbens dopamine transmission – were examined. Chronic amphetamine exposure leads to a similar neural phenotype, and this offered an interesting hypothesis in the context of the endogenous change reported in stereotypic horses. A Pavlovian to Instrumental Transfer task was initially employed, in which horses were first trained in a Pavlovian procedure, and subsequently trained to perform an instrumental response, reinforced by the same food as in the Pavlovian phase. In the transfer phase, the Pavlovian conditioned stimulus was introduced in the context of the instrumental responding. It was predicted that response rates would increase during the transfer phase; however, the results were inconclusive. In the third study, further behavioural effects of increased mesoaccumbens dopamine were tested by employing an instrumental choice procedure. Stereotypic horses and controls were trained to choose between two mutually-exclusive schedules of reinforcement, one associated with a short delay to gain food and one with a longer delay. Chronic amphetamine exposure leads to a decrease in sensitivity to delay, and it was demonstrated that stereotypic horses showed similar patterns, failing to choose the shorter schedule.

Finally, increased dopamine transmission is known to enhance the rate at which learning shifts from planned-action to habit, and this was examined in stereotypic horses using a ‘place-response’ preparation. Evidence was found to support this hypothesis in two variations of this procedure. The latter findings suggested an imbalance of the constituent cells that form the striatum: the striosome and matrix components. Taking this in conjunction with the other findings, this imbalance may hold the key to identifying the aetiology of stereotypic behaviour.


Declaration of authorship

I, Matthew Oliver Parker declare that the thesis entitled Behavioural Correlates of the Equine Stereotypy Phenotype and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was completed wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- parts of this work have been published as:

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Conference Proceedings Arising from this Thesis

- Data from Chapter 4, Experiment 2 were presented orally at the Association for Behaviour Analysis International Convention, May 2006, Hyatt Regency, Atlanta, USA.
- Data from Chapter 4, Experiment 2 was presented in poster format at the University of Southampton Faculty of Medicine, Health, and Life Sciences annual postgraduate conference, June 2007.
- Data from Chapter 2 were presented orally at the Association for Behaviour Analysis International Convention, May 2008, Chicago Hilton, Chicago, USA
- Oral presentation summarising data from all of the experimental chapters was given at the University of Southampton Faculty of Medicine, Health, and Life Sciences annual postgraduate conference, June 2008.

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List of Equations

$$\Delta V_n = \alpha\beta(\lambda - V_{n-1})$$

Rescorla-Wagner Theory (1972)

$$\frac{R_1}{R_2} = \frac{r_1}{r_2}$$

Matching law (Herrnstein, 1961)

$$\left(\frac{R_1}{R_2}\right) = b\left(\frac{r_1}{r_2}\right)^a$$

Generalised Matching law (Baum, 1974)

$$\Delta \omega_{i,j} = r a_i a_j$$

Hebb's rule (1949)

$$\frac{d\omega_i(t)}{dt} = \phi(\mathbf{c}(t)) d_i(t) - \epsilon \omega_i(t),$$

BCM model (Beinstock, Cooper, & Munro, 1982)

$$\left(\frac{R_1}{R_2}\right) = b\left(\frac{r_1}{r_2}\right)^{a_1} \left(\frac{M_1}{M_2}\right)^{a_2}$$

Generalised Matching with reinforcer magnitude function (Davison, 1983)

$$\left(\frac{R_1}{R_2}\right) = b\left(\frac{r_{i1}}{r_{i2}}\right)^{a_1} \left(\frac{r_{t1}}{r_{t2}}\right)^{a_2}$$

Generalised Matching for concurrent chains (Davison, 1983)

$$\left(\frac{R_1}{R_2}\right) = b\left(\frac{r_{i1}}{r_{i2}}\right)^{a_1} \left(\frac{r_{t1}}{r_{t2}}\right)^{a_2 \left(\frac{T_i}{T_i}\right)}$$

Contextual Choice Model (Grace, 1994)

$$\log\left(\frac{R_1}{R_2}\right) = \log b + a \log\left(\frac{r_1}{r_2}\right)$$

Logarithmic version of Generalised Matching

Glossary of Terms

Neurobiology

5-HT	Serotonin
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
DA	Dopamine
GCRs	Glucocorticoid receptors
LTD	Long-term depression
LTP	Long-term potentiation

Anatomy

CPu	Caudate Putamen
DLS	Dorsolateral striatum
DMS	Dorsomedial striatum
eGP	External globus pallidus
iGP	Internal globus pallidus
NAcc	Nucleus accumbens
PfC	Prefrontal cortex
SN	Substantia nigra pars compacta
VTA	Ventral tegmental area

Learning

CR	Conditioned response
CS	Conditioned stimulus
R-O	Response-outcome learning
RPE	Reward prediction error
S^D	Discriminative stimulus
S-R	Stimulus-response learning
UR	Unconditioned response
US	Unconditioned stimulus

Miscellaneous

5-CSRTT	Five-choice serial reaction time task
AMPH	Amphetamine
ANOVA	Analysis of Variance
CRF	Continuous reinforcement schedule
ESP	Equine Stereotypy Phenotype
FI	Fixed interval schedule of reinforcement
fMRI	Functional magnetic resonance imaging
FR	Fixed ratio schedule of reinforcement
PIT	Pavlovian-to-instrumental transfer
PET	Positron emission tomography
VI	Variable interval schedule of reinforcement
VR	Variable ratio schedule of reinforcement

Literature Review

Overview

Spontaneous stereotypic behaviour is conceptualised as topographically invariant, repetitive, rigid behaviour patterns, typically thought to be an artefact of captivity (Mason & Rushen, 2006). Within the horse stereotypic behaviours are commonly reported, probably owing to the high numbers of domesticated animals maintained under restrictive conditions (Waters, Nicol, & French, 2002). Recent research has highlighted a neural phenotype, with altered dopamine (DA) transmission within specific areas in the striatum being cited as a mediating aetiological factor (McBride & Hemmings, 2005). However, the behavioural phenotypes associated with these reported neurobiological perturbations are yet to be determined. In addition, there is some debate within the literature as to the nature of the dysfunction. Despite broad agreement that the basal ganglia structures (more specifically, the striatum) are key to the understanding of stereotypy (e.g., Cooper & Dourish, 1990), theories have yet to establish its neurobiological specifics. At present, it appears that stress (related to inappropriate environmental conditions) may be a key environmental factor in the development of the Phenotype (Cabib & Bonaventura, 1997), with some inbred strains of domestic animals developing behavioural sensitisation as a result. If this is the case, then animals displaying

characteristics of the Phenotype (i.e., stereotypic behaviour) may be expected to show similar signs of sensitisation.

The primary goal of this thesis is to examine some of the behavioural processes associated with endogenous basal ganglia dysfunction in the context of the Equine Stereotypy Phenotype (ESP). Despite evidence for a neural phenotype associated with oral stereotypies in the horse, the effects on behaviour and learning of these perturbations are somewhat less clear. In addition, the following investigations will help to educate us about the extent to which a model of endogenous sensitisation (the ESP) may be similar to basal ganglia alterations associated with pharmacological manipulation. Clearly without corroborating neurobiological evidence from the subjects tested here, the findings will be purely correlatory, and any conclusions that can be drawn from this series of investigations will need to be treated with a degree of caution. However, I hope that the data generated from the studies may pave the way for further comparative research, which will add more validity to the models we propose.

In the first chapter, I will introduce the concept of stereotypy, concentrating particularly on the domestic equine population. In order to apply context to the subsequent empirical chapters, I will provide an overview of the basic processes and neurobiology of animal learning. I will consider current theories regarding the neurobiology of stereotypy: specifically theories linking stereotypy to perturbations in midbrain and limbic DA innervation of the striatum. Finally, based on the evidence currently available, I will propose a model with operationally defined and testable hypotheses describing the ESP.

Stereotypies in Captive Animals

Stereotypic behaviour patterns are characteristic of captive or domestic animal species and humans with certain psychiatric disturbances, learning disabilities or pervasive developmental disorders (APA, 2002; Castellanos, Ritchie, Marsh, & Rapoport, 1996), but can also be reliably induced using DA agonists (e.g.,

amphetamine [AMPH]: Cho, Melega, Kuczenski, Segal, & Schmitz, 1999; Dickson, Lang, Hinton, & Kelley, 1994; Inglis, Allen, Whitelaw, Latimer, Brace, & Winn, 1994; Kabai, Liker, & Csillag, 1999; and quinpirole: Ben-Pazi, Szechtman, & Eilam, 2001). They are rarely seen in feral or semi-feral populations, suggesting that their development is an artefact of the captive environment (Mason & Latham, 2004; Wiepkema, 1983; Young, 2003). It has been proposed that sub-standard living conditions, particularly regarding incongruity from the species' feral environment, may account for the attenuation of species-specific behaviours and in addition, the development of stereotypic behaviour (e.g., Parker, Goodwin, Redhead, & Mitchell, 2006; Shepherdson, Mellen, & Hutchins, 1998; Young, 2003). On account of this, spontaneous stereotypy (i.e., environmentally induced) in captive animals is often considered atypical and may be cited as an indicator of poor welfare (Mason & Latham, 2004; Wiepkema, 1983).

Stabled horses have been shown to display a range of oral and locomotory stereotypic behaviours, the most common of which is crib-biting (McGreevy, 2004; Waters, Nicol, & French, 2002). Crib-biting is an oral stereotypy, described as the horse grasping an object between the upper and lower incisor teeth, inhaling air into the oesophagus, and contiguously emitting an audible 'grunt' (McGreevy, 2004; McGreevy & Nicol, 2008).

The presence of stereotypies in domestic animals is certainly not a recent phenomenon. For example, there are records dating back to the 17th Century which mention crib-biting in horses (Sollysel, 1696), and crib-biting has been subject to veterinary research since the early 19th Century (Holmes, 1839). Stereotypy was first reported within the zoo community in the 1930s at Edinburgh Zoo, where the repetitive pacing behaviour of tigers (*Panthera tigris*) was considered problematic on account of unpopularity with visitors (cf. Young, 2003). After that, many zoological parks often attempted to *reduce* stereotypic behaviour through various means, such as environmental enrichment (see Shepherdson et al., 1998). However, reducing these behaviours by altering the environment may make performing the behaviours physically difficult, but do little to address the underlying causal factors relating to the development or exhibition of the

stereotypy (Carlstead, 1996; Shepherdson et al., 1998). Therefore, the animal welfare is not improved unless the modifications are behaviourally relevant and allow highly motivated species-specific behaviour to be exhibited (e.g., Goodwin, Davidson, & Harris, 2002; Thorne, Goodwin, Kennedy, Davidson, & Harris, 2005).

Epidemiology

A recent review of data by Mason and Latham (2004) suggested that over 85,000,000 captive animals worldwide may be classified as stereotypic. Of these, the majority were farm animals, with confined sows (91.5% of the captive population), poultry (82.6% of the captive population) and zoo carnivores (82% of the captive population) being the most significantly affected. Also severely affected are zoo-housed elephants (47% of the captive population) and many laboratory animals (e.g., 50% of laboratory mice). Domesticated animals are also affected, especially animals involved in regular contact with humans. For example, Waters et al. (2002) suggested that between 18% and 35% of stabled horses display stereotypic behaviour. However, we recently carried out a cross-cultural survey of breeders, and our data suggested that these numbers may be decreasing (Parker, Goodwin, & Redhead, 2008). Notwithstanding this, these figures suggest that stereotypy is widespread throughout the captive and domestic animal population.

Stereotypy: links to Animal Welfare

Stereotypy being cited as an indicator of poor welfare has been vehemently debated in the scientific literature. Much early research in the field was driven by the theory that stereotypy was a coping mechanism for adverse environmental conditions (e.g., Wechsler, 1995). For example, Wiepkema, von Hellemond, Roessingh, and Romberg (1987) found that veal calves (which will also have experienced significant weaning trauma) performing oral stereotypies had a lower prevalence of gastric ulceration than those that did not, suggesting that stereotypy may serve somehow to reduce sympathetic nervous system activity, thereby reducing the risk of developing ulcers. In addition, McBride and Cuddeford (2001)

examined the serum-cortisol levels (a marker for stress) of horses that were prevented from performing an oral stereotypy (crib-biting). They demonstrated that crib-biting horses prevented from performing the behaviour had significantly higher serum cortisol levels than controls. This suggested performing stereotypy may serve to reduce hypothalamic adreno-cortical axis (HPA) activity. However, the horses were prevented from crib-biting by using a cribbing-collar (Figure 1.1). These have been shown to be highly aversive devices, possibly increasing the basal stress levels of the horse (McGreevy, 2004; McGreevy & Nicol, 1998).



Figure 1.1. Horse wearing a cribbing-collar. The collar works by asserting pressure on the throat, oesophagus and poll during crib-biting, hence making the activity aversive (Source: www.frogpool.com).

Cooper and Nicol (1991) carried out a place-preference study using caged bank voles (*Clethrionomys glareolus*) to investigate the coping hypothesis. They offered the voles a choice between an enriched environment and a barren environment. The voles spent significantly more time in the enriched environment as well as performing significantly more stereotypy in the barren environment. They suggested that this offered support for the coping hypothesis, citing that when in the barren environment, the voles performed the behaviour to cope with the adversity. However, this may have been an over-simplistic evaluation of the results. For example, there is no evidence that the voles were not more aroused by peripheral stimuli in the enriched environment, leading to a reduction in stereotypic responding. The results also showed that many of the voles would spend time in the enriched environment, and then return to the barren environment. If this

environment was causing increased stress, why did they choose to return? Although this study is important as it shows that enriched environments may increase 'normal' (i.e., non-stereotypic) responding, it fails clearly to demonstrate that barren environments are inherently 'stressful'.

The coping hypothesis has been specifically criticised in a number of ways, not only on account of its blatant circularity, but also in terms of a lack of plausible corroborating evidence. For example, Nicol, Davidson, Harris, Waters, and Wilson (2002) examined the links between crib-biting and gastric inflammation and ulceration in young horses. The crib-biting horses had significantly higher levels of ulceration than the non-crib-biters, suggesting that crib-biting is unlikely to be a coping mechanism. In addition, McGreevy and Nicol (1998) examined the physiological effects of preventing crib-biting in horses. While crib-biters were found to have higher β -endorphin levels than non-crib-biters, there was no significant rise in serum cortisol levels of crib-biters prevented from crib-biting. This suggested that crib-biting did not serve to reduce HPA activity.

Carlstead, Brown, and Seidensticker (1993) examined the behaviour and HPA activity in Asian leopard cats (*Felis begalensis*) following a change in environment. The change reduced exploratory behaviour and increased defensive behaviours (e.g., hiding), but they found no evidence for any relationship between stereotypic pacing and HPA activity, suggesting again that the functionality of stereotypic behaviour may not be related to coping with environmental adversity. More recently, Bachmann, Bernasconi, Herrmann, Weishaupt, and Stauffacher (2003) investigated stress reactivity in crib-biting and non-crib-biting horses. Subjects were subjected to an acute stressor (a bucket associated previously with palatable food was presented in an inaccessible location). Bachmann et al. (2003) measured both HPA activity (i.e., serum cortisol levels) and autonomic activity. Interestingly, they discovered that rates of crib-biting significantly reduced during the stressor. In addition, heart-rate variability (HRV) analysis, a physiological measure of autonomic nervous system activity, revealed that crib-biters had higher vagal tone than non-crib-biters did, and lower sympathetic tone, suggesting an increased sympathetic stress-response in the crib-biters. This suggests first that

crib-biting does not serve to reduce stress (as predicted by the coping hypothesis) and second, that crib-biters may be more stress-sensitive than non-crib-biters. This notion of stress-sensitivity and stereotypy will be returned to later in this review.

Würbel, Freire, and Nicol (1998) examined the coping hypothesis in laboratory mice using a stereotypy prevention procedure. The coping hypothesis would predict that following a period of prevention, animals would exhibit post-inhibitory rebound – that is, their levels of stereotypic behaviour would rise above baseline levels following reinstatement of stereotypy facility. Following prevention, stereotypy levels returned to baseline levels, but did not exceed this, suggesting that periods of prevention did not exacerbate stereotypy. These findings cast further doubt over the validity of the coping hypothesis (but see McGreevy & Nicol, 1997), and suggest that stereotypy may be the result of other factors.

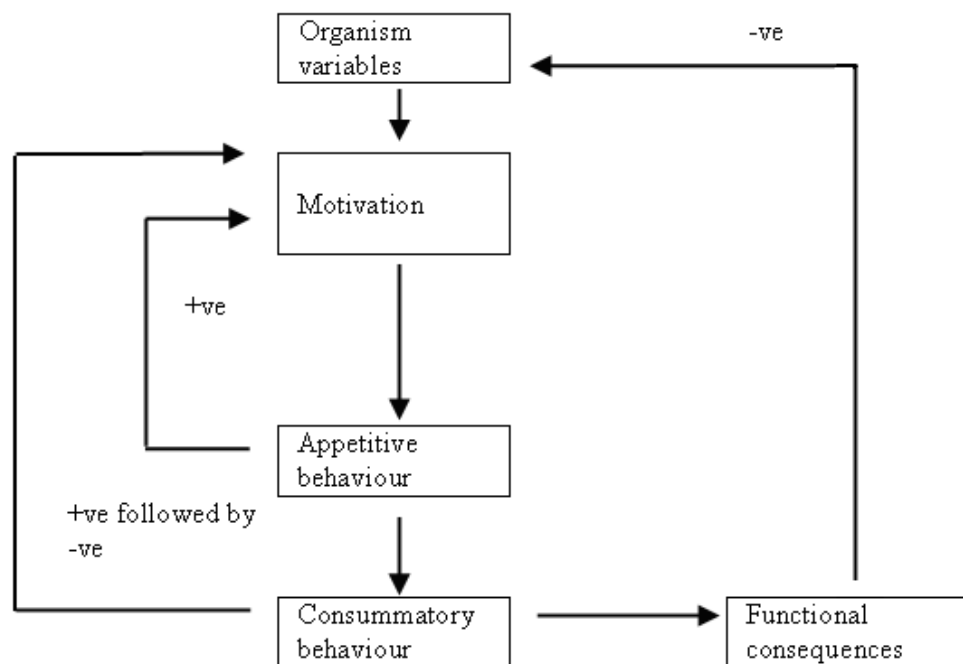


Figure 1.2. Schematic illustration of Hughes & Duncan (1988) model of motivation. Adapted from McBride & Hemmings (2004). Reproduced with permission.

Hughes and Duncan (1988) proposed an alternative theory of stereotypy (see Figure 1.2 for a schematic of the Model). Essentially, because stereotypies are

often seen in the presence of reward-predicting conditioned stimuli (CSs), Hughes and Duncan suggested that they may be the result of captive animals' restricted foraging opportunities. This would lead to disruption of negative feedback between consummatory behaviour and motivation, thus leaving the animal in a perpetuating, heightened motivation state. To explain, the animal's motivational state is dictated by internal, organism-level variables (such as reduced glucose levels). Typically this will lead to appetitive behaviours (e.g., focal search, foraging, etc.), and will terminate in consummatory behaviour. The consummatory phase is subsequently met with functional consequences (e.g., re-elevation of glucose levels) and hence provides negative feedback to motivational levels. Hughes and Duncan (1988) postulated that owing to the captive animals' limited opportunities to perform species-typical consummatory behaviours (e.g., the stabled horse has limited grazing opportunities) there is a lack of functional consequences and no negative feedback on motivation. Therefore, the animal remains in a heightened appetitive state and performs persistent and repeated behaviours.

Hughes and Duncan's (1988) model is not only intriguing, but it is steeped in biological plausibility. However, one of the difficulties with this approach is that it accounts only for stereotypies that are related to foraging and feeding. This may be problematic for the model in terms of its validity as a universal account of the aetiology of stereotypic behaviour, as there are some forms of stereotypy (e.g., weaving in the horse) that are difficult to link to foraging directly (McGreevey, 2004).

To summarise, stereotypy is prevalent in the captive animal population. It is generally thought to be related to stress caused by impoverished environments and/or lack of facility for the animal to express species-typical behaviour. Although early theories of stereotypy suggested that its function may be to reduce stress and to enable the animal to cope with adverse environmental situations, evidence to support this has been inconsistent. The Hughes and Duncan (1988) model of stereotypy offered an alternative account, suggesting that foraging-related

stereotypies are appetitive in origin, arising from a lack of negative feedback between consummatory behaviours and motivation.

As mentioned earlier, stereotypies can be reliably induced following the administration of some drugs, particularly DA agonists. Because of this, many researchers have posited that spontaneous stereotypies may be mediated by neurobiological factors. Later in this review, current neurobiological approaches to understanding stereotypy will be considered, particularly relating to links between stress and neural sensitisation. Behavioural sensitisation is closely linked to learning processes and motivation. Thus, initially we will consider basic learning processes in animals and their neurobiological underpinnings.

Animal Learning: Basic Processes

It may be laid down as a rule, that, if any two mental states be called up together, or in succession, with due frequency and vividness, the subsequent production of the one of them will suffice to call up the other, and that whether we desire it or not. (Huxley, 1872, p 306).

An animal must be able to predict contingencies within its environment in order successfully to forage, to avoid predation and to reproduce. Animal learning can be described in terms of two associative processes: Pavlovian conditioning, where two stimuli in the environment become paired, and instrumental conditioning, which examines the control of behaviour as a result of consequences. Associative learning therefore requires that two or more previously unpaired events become paired, and because of this, the animal's behaviour will observably change.

Associative learning

In Pavlovian conditioning, if a neutral stimulus is contingently presented with a biologically relevant unconditioned stimulus (US) which elicits an unconditioned response (UR), the two stimuli will become associated. The neutral stimulus then becomes a CS, and will elicit (independent of the US) a conditioned response (CR).

For example, if a bell (a neutral stimulus) is consistently paired with food (US), the bell will become a CS and elicit a US-preparatory response (CR) such as salivation. Pavlovian conditioning explains how animals learn about relationships between stimuli outside of the direct control of the animal – essentially, it can be described as a passive learning system (Pavlov, 1927). In instrumental conditioning however, animals learn the active (instrumental) relationship between responses and their outcomes.

There are three key components of instrumental learning. The organism is presented with a discriminative stimulus (S^D), which has no immediate control over behaviour. The organism then performs a response and this response is met with a consequence. The consequence will either strengthen (reinforce) or weaken (punish) responses, in the context of the S^D . Therefore responses can be said to be under the control of the S^D and the probability of the response being repeated is based on the nature of the consequence (Skinner, 1953).

Reinforcers (events that strengthen behaviour) can be delivered each time a response is emitted (discrete-trial procedure), or based on a pre-determined schedule (free-operant procedure). The key difference between the two procedures is that in discrete trial procedures, the dependent variable is the response *latency*, whereas in free-operant procedures, it is response *rate*. Within the laboratory setting, free-operant procedures can be employed to study how the behaviour of organisms is maintained by different schedules of reinforcement (Staddon & Cerutti, 2003). For example, free-operant procedures can be arranged by time elapsed during a trial (fixed interval [FI] or variable interval [VI] schedules) or based on the number of responses during a trial (fixed ratio [FR] or variable ratio [VR] schedules). In an FI n s schedule, reinforcement is delivered following the first response after n s has timed out. However, in a VI n s schedule, reinforcement would be delivered after an average of n s (across a number of trials). Similarly, in an FR n schedule, the subject must make n responses between each reinforcer, and in a VR n schedule, an average of n responses must be made between each reinforcer.

Response rates within reinforcement schedules depend on the rate and magnitude of the reinforcement (Grace, Mclean, & Nevin, 2003). For example, ratio schedules maintain a higher rate of responding than interval schedules, owing to the former requiring a greater number of responses to gain the same number of reinforcers (Ferster & Skinner, 1957; see Figure 1.3). VR and VI schedules are found to produce ‘steady-state’ response rates, whereas animals under FI and FR schedules tend to display longer post-reinforcement pauses. In addition, studies have shown that animals will respond faster on a VR schedule than on a VI schedule, even if the overall rate of reinforcement is the same across a set time period (Catania, Matthews, Silverman, & Yohalem, 1977; Matthews, Shimoff, Catania, & Sagvolden, 1977).

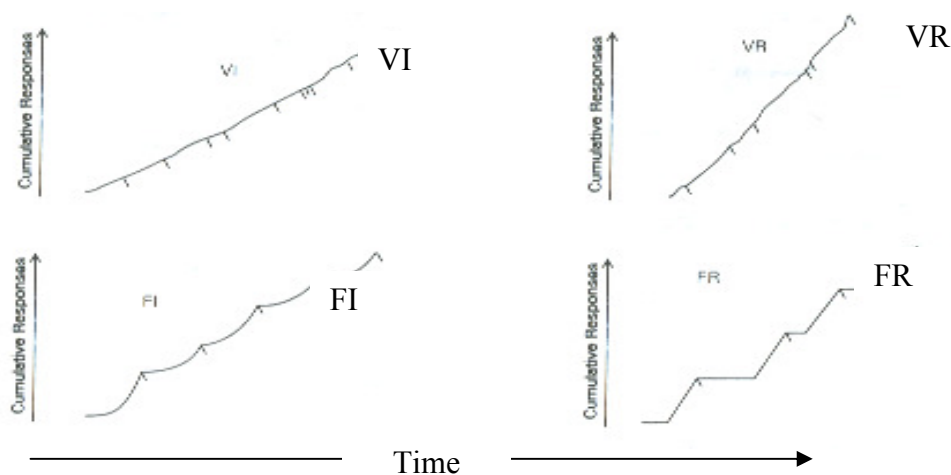


Figure 1.3. Speculative cumulative records showing typical response patterns under different schedules of reinforcement. VI = variable interval; FI = fixed interval; VR = variable ratio; FR = fixed ratio. Source: Mazur, 2001.

The rate of forming associations (known as acquisition) is related to the sensory or appetitive salience of the CS (or S_D) and US (or outcome) respectively (Rescorla & Wagner, 1972; but see Gallistel, Balsam, & Fairhurst, 2004). In the laboratory, acquisition is operationalised as the proportion of trials that the CS results in the CR. After a number of CS-US pairings, subjects reach an asymptotic rate of responding, according to the linear operator

$$\Delta V_n = \alpha\beta(\lambda - V_{n-1}) \quad [1.1]$$

where V represents the associative strength on a given trial, n , $\alpha\beta$ represents the salience of the CS and US respectively and λ represents the asymptotic strength of the association (Rescorla & Wagner, 1972). If having reached asymptote the CS is consistently non-reinforced (i.e., the US is withheld) the associative strength (V) will decrease. During this process, known as extinction, the value of V decreases according to the model described in Equation 1.1. Theories of associative learning suggest that extinction occurs as the result of an opposing inhibitory association between the CS and US. Evidence for this comes from experiments examining the effects of reintroducing the US following extinction. Typically, subjects quickly re-establish asymptotic associative strength (termed spontaneous recovery), suggesting that inhibitory CS-US associations are weaker than excitatory (e.g., Konorski, 1948).

There are several basic phenomena common to both forms of associative learning. For example in Pavlovian conditioning, the relationship between the CS and the US needs to be established in order for the CR to develop. Historically, temporal contiguity was thought to be sufficient to establish conditioning. However, in order for associations to be formed, the US must be contingent upon the CS: in other words, the probability (p) of the US given CS+ must be greater than p_{US} given no CS (Rescorla, 1966). This can be examined experimentally by giving the same number of CSs and USs within a set of trials, but varying the probabilities that the CS will predict the US. Contingencies can be either positive (i.e., $p_{US} | CS > p_{US} | \text{no CS}$), or negative (i.e., $p_{US} | CS < p_{US} | \text{no CS}$), or zero (i.e., $p_{US} | CS = p_{US} | \text{no CS}$), and either high-positive (e.g., $p_{US} | CS = 0.8$, $p_{US} | \text{no CS} = 0$) or low-positive (e.g., $p_{US} | CS = 0.05$, $p_{US} | \text{no CS} = 0$). Similarly in instrumental conditioning, the relationship between the response and reinforcement needs to be established in order to establish the instrumental response. This has been evaluated in discrete-trial procedures (e.g., Neffinger & Gibbon, 1975), but raises an interesting problem when examining contingency under free-operant procedures, in terms of maintaining response-reinforcer contiguity. Hammond (1980) organised a free-operant preparation that allowed a

systematic manipulation of local reinforcer probabilities which were not affected by response rate, therefore allowing for the maintenance of response-reinforcer contiguity. Thirsty rats were trained to press a lever for water on a second-by-second basis, with each response being weighted with a contingency-linked probability of reinforcement. For example, in the high positive contingency condition, $p \text{ reinforcer} | \text{response} = 0.2$, $p \text{ reinforcer} | \text{no response} = 0$; in the moderate positive contingency condition, $p \text{ reinforcer} | \text{response} = 0.05$, $p \text{ reinforcer} | \text{no response} = 0$; in the zero contingency condition, $p \text{ reinforcer} | \text{response} = 0.05$, $p \text{ reinforcer} | \text{no response} = 0.05$. Therefore, in a given trial in the high positive contingency condition, there was a 20% chance of receiving a reinforcer if the rat made a response and a 0% chance given no response. In the moderate positive contingency condition, there was a 5% chance of receiving a reinforcer if the rat made a response and a 0% chance given no response, and in the zero contingency condition in a given trial, there was a 5% chance of receiving a reinforcer given a response and a 5% chance of receiving a reinforcer given no response. Hammond found that the rates of lever-pressing declined linearly, according to the changes in contingency. Decline in lever presses during the zero contingency condition can clearly not be attributed to extinction, as the probability of reinforcement in the zero contingency condition was the same as in the previous moderate positive contingency condition (i.e., $p \text{ reinforcer} | \text{response} = 0.05$).

Finally, the presentation of Pavlovian CSs during instrumental responding has been found to increase instrumental response rate. This effect is commonly referred to as Pavlovian to instrumental transfer (PIT). Typically in a PIT procedure, the animal will first be trained in Stage 1 to associate a CS with a reinforcer. In Stage 2, the animal will be trained to perform an instrumental response in order to access to the same reinforcer (i.e., from Stage 1). In the test phase, carried out in extinction, the animal is placed in the operant chamber in the presence of the lever, and occasionally the CS is presented. Typically, the animal's response rate increases during the CS presentations from baseline, and this is referred to as the PIT effect (Hall, Parkinson, Connor, Dickinson, & Everitt, 2001;

Rescorla & Solomon, 1967). The associated potentiation of the instrumental response is directly related to the outcome associated with the initial CS-US pairing. This has been demonstrated by devaluation studies. For example, Colwill and Rescorla (1988) trained rats to associate a light with sucrose, and a tone with food pellets. Following this, they were trained to push a lever for food, and pull a chain for sucrose. In the test phase, the rats were occasionally exposed to each of the CSs independently, whilst in the presence of both the lever and the chain. Responding on each increased in the presence of the associated CS differentially, showing that the PIT effect was response-specific.

Instrumental Choice

The analysis of steady-state responding (i.e., responses under VI or VR schedules; see above) to examine choice between different reinforcer-alternatives has been widely and variously examined in the empirical literature. The ability to examine reinforcer choice is essential to increase understanding of motivational processes and the relative strength of reinforcers (Davison & Kirkwood, 1968). In order to examine choice, typically a pair of VI schedules is arranged concurrently on two separate response keys. Subjects are exposed to the schedules until response stability is reached. Following this, the schedules are changed, and the animals are re-trained to stability. Steady-state responding under these conditions gives insight into motivation, based on the relative value of the schedules. To illustrate, Herrnstein (1961) arranged concurrent VI VI schedules on two separate response keys (see Figure 1.4).

Figure 1.4 illustrates a VI 60 s VI 30 s schedule arrangement in a concurrent schedules procedure. The subject is presented with the two concurrently available schedules of reinforcement and is able to choose between the two alternatives. Responses are occasionally reinforced with access to food, according to the allocated VI schedule. For example, if the subject was to press the right key in Figure 1.4, it would receive food according to a VI 30 s schedule, but if it were to press the left key, food would be delivered according to a VI 60 s schedule. Choice

in such a procedure is operationalised the number of responses the subject makes to each alternative once steady-state responding has been reached.

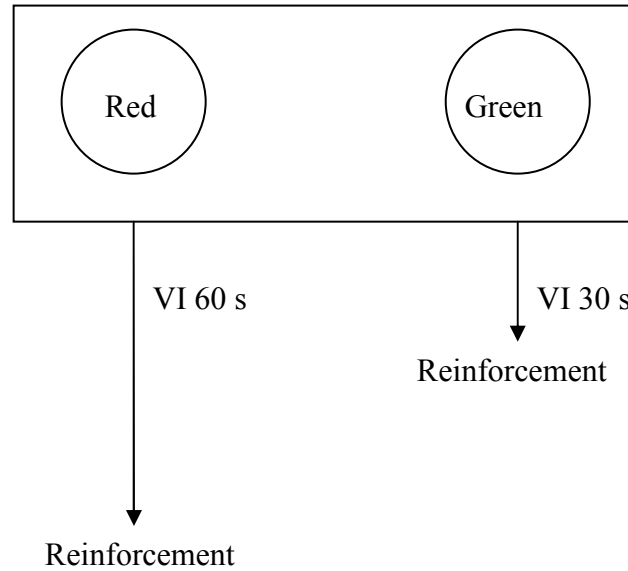


Figure 1.4. Schematic illustration of a typical concurrent schedules trial, arranged on a VI 60 s VI 30 s schedule.

Figure 1.5 displays Herrnstein's results. As the graph illustrates, the proportion of time spent engaged in responding matches the proportion of reinforcers gained from that response key. He found that given a number of different VI VI arrangements, pigeons' response ratios matched the relative ratios of reinforcement on two alternatives according to the following equation:

$$\frac{R_1}{R_2} = \frac{r_1}{r_2} \quad [1.2]$$

where R_1 and R_2 represent the response ratio on each alternative, and r_1 and r_2 represent the ratio of reinforcement. He also found that when this model was fitted to the existing data, it accounted for 92% of the variance of concurrent schedules data sets (Herrnstein, 1961).

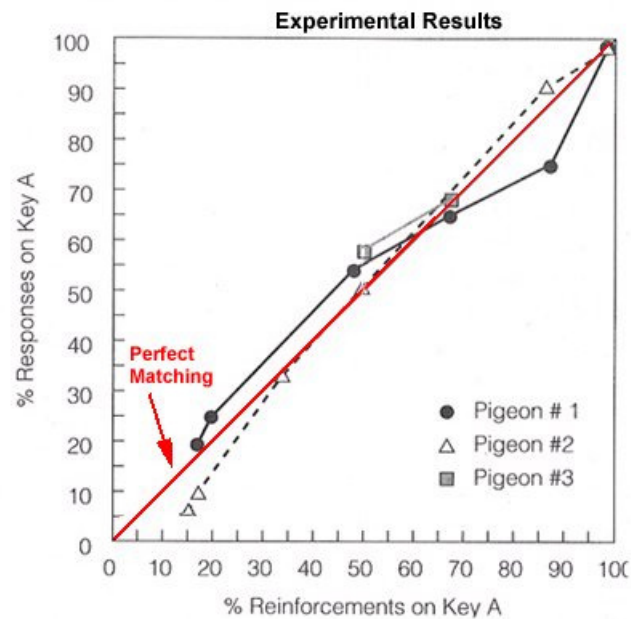


Figure 1.5. Results from Herrnstein (1961) showing the Matching Law. Adapted from Herrnstein (1961).

Despite the wide applicability of Equation 1.2, Baum (1974) described two situations in which it fails to account for the variability data: *bias* for a particular alternative and *undermatching*. In some situations, subjects' relative response ratios have been found to be less than the relative reinforcement ratio. Fantino, Squires, Delbuck, and Peterson (1972) referred to this phenomenon as 'undermatching'. For example, in a concurrent VI VI schedule design, suppose the left alternative was put on a VI 8 s schedule, and the right on a VI 16 s schedule. Assuming the subject did not have a bias for a particular alternative, Equation 1.2 would predict that the relative response ratio to the two alternatives would match the relative reinforcement ratio (i.e., the subject would make twice as many responses to the left key). However, in some cases, response rates have been seen to show less preference for the shorter alternative over a number of trials. For example, the subject may only make 60% of their responses on the left key. The reasons for this could be that the subject was not sufficiently motivated to respond, the average length of the immediacy ratios was very high (Baum, 1974) or that

there was an insufficient changeover delay (see below). This would be referred to as undermatching, and would not be accounted for by Equation 1.2. Overmatching, conversely, refers to a situation in which the subjects prefer the richer alternative to an extent greater than that predicted by Equation 1.2. For example, in a 2:1 ratio discrimination, a subject may respond to the shorter alternative 90% of the time. Overmatching is often due to either the alternative being FI FI rather than VI VI schedules (Gibbon, Church, Fairhurst, & Kacelnik, 1988) or that an excessively long changeover delay was used (see below).

It is important at this stage to differentiate between bias and undermatching. Bias (i.e., a position preference) could be shown for r_1 or r_2 . Undermatching, however, represents a deviation from matching in the form of undifferentiated preference for either alternative (Fantino et al., 1972). In Herrnstein's (1961) study, it was found that if pigeons were able to switch between the two alternatives during a trial with no penalty, the relative response rates moved towards indifference (undermatching). Herrnstein therefore introduced a changeover delay (COD). To explain if, while a pigeon was responding on key 1 (VI 20 s), a reinforcer became available on key 2 (VI 10 s) and the pigeon switched to key 2, the pigeon would immediately be reinforced. However, if a COD is used a switch from key 1 to key 2 would require that pecks on key 2 would not be reinforced until the COD had elapsed. Therefore, an insufficient COD may result in undermatching (Baum, 1974; Silberberg & Fantino, 1970). Conversely, a long COD may lead to overmatching – a relative response ratio that exceeds the relative reinforcement ratio (Baum, 1982). In the light of this, Baum (1974) extended Equation 1.2 to incorporate these parameters. The following model forms the exponential regression equation:

$$\left(\frac{R_1}{R_2} \right) = b \left(\frac{r_1}{r_2} \right)^a \quad [1.3]$$

where b represents bias, and a is a scaling parameter related to sensitivity to reinforcement (i.e., log response ratio) as a function of logarithmic¹ reinforcer ratio. The parameter a accounts for undermatching and overmatching in matching experiments. In an example of classical matching (i.e., relative reinforcement ratio is equal to relative response ratio), b (the slope) = 0, and a (the intercept) = 1.

Baum (1974) argued that data from any given typical concurrent schedules procedure conformed to the predictions of Equation 1.3. Indeed, cross-species analysis has found Equation 1.3 to explain in excess of 95% of the variance of concurrent schedules performance in many species including primates (Lau & Glimcher, 2005), horses (Dougherty & Lewis, 1992), pigs (Pedersen, Holm, Jensen, & Jrgensen, 2005) and cows (Matthews & Temple, 1979).

Because matching shows that animals are making specific choices about which alternative to choose in a given context, it suggests learning about the subjectivity of the schedules is taking place. This approach therefore offers the opportunity to examine some features of stereotypy that will be outlined later in this review.

Response-Outcome and Stimulus-Response Learning

Finally, it is important to consider the nature of associations learned in Pavlovian and instrumental conditioning. Precisely, is the nature of the association a link between the stimulus and the response (stimulus-response learning; S-R), or the response and the outcome (response-outcome learning; R-O)? Clearly one of the difficulties with a S-R approach to learning, is that it suggests that learning is predominantly procedural, in that the actor will simply associate a response with a specific outcome. Subsequently it does not allow the actor to construct a causal relationship between the contextually appropriate response and outcome (Balleine & Dickinson, 1998). From this perspective, it would be predicted that the response would be maintained, regardless of alterations in either the subjective value of the

¹ The response data is often in the form of a geometric progression, and therefore the logarithmic version is used in order to produce an arithmetic progression and to avoid ceiling effects (Baum, 1974)

outcome, or indeed changes in the reinforcement contingency. Conversely a R-O approach would suggest that the organism is formulating some kind of 'internal' representation of future consequences.

Colwill and Rescorla (1988) trained rats to associate a light (CS₁) and a tone (CS₂) with two mutually exclusive USs (sucrose and food pellets respectively). In the second phase (in the absence of the CSs), the rats were differentially trained to press a lever for sucrose and to pull a chain for pellets. In the test phase, the CSs were presented independently, in the presence of the lever and chain. Results showed that during CS₁ presentation, responses on the lever increased, but there were fewer chain-pulls. Likewise, during CS₂ presentation, chain-pulls increased, but there were fewer lever presses. This suggests that the rats formed a representation of the reinforcer specifically associated with the CSs, providing evidence for contextually appropriate responding, and further for an R-O model of learning. However, there is evidence for an S-R approach from devaluation studies (e.g., Faure, Haberland, Condé & Massioui, 2005).

Typically, a subject is trained to press a lever in the context of a S^D to receive a food reinforcer. In addition, they are trained to pull a chain to receive a different reinforcer (e.g., sugar-water). Subsequent to training, one of the reinforcers (e.g., the sugar-water) is devalued by pairing it with an injection that causes nausea. The instrumental procedure is then reinstated, and results show that chain-pulls decrease. What is striking, however, is that despite devaluation, chain-pulling is never completely eliminated: in fact the level of post-devaluation chain-pulling is correlated with the amount of training the animal receives. In addition, the devalued reinforcer is rarely ingested, suggesting that following extensive training, the presence of the S^D alone retains motivational salience and maintains its capability to initiate the response (despite the reduced value of the reinforcer).

This anomaly has led to the theory that instrumental responding may initially be R-O based; however, after extensive training the control of the responses may become more S-R based (Balleine & Dickinson, 1998). This can be seen clearly when interval as opposed to ratio schedules of reinforcement are used. Specifically, if reinforcement is delivered according to ratio schedules, the

probability of reinforcement is contingent on responses. However, if reinforcement is delivered according to interval schedules, the probability of responses being reinforced is less apparent. Consequently, the animal may develop more habitual response patterns – steady state responding (also behavioural momentum: see Nevin & Grace, 2000 for a review) – in order to maximise reinforcement potential (Staddon & Cerruti, 2003). The evidence for both R-O and S-R associations in instrumental learning has led to the development of two-process theories of instrumental learning (e.g., see Rescorla, 1971; Rescorla & Solomon, 1967; or more recently, Yin & Knowlton, 2006). R-O learning is therefore sensitive to both reinforcer devaluation and changes in contingency (contingency degradation), whereas S-R action is impervious to both. The progression to this latter stage of learning is therefore often referred to as ‘habit formation’ (Yin & Knowlton, 2006), and this will be explored in more detail in the next section of this chapter.

This section has outlined some of the basic processes of animal learning. The following section will place this in the context of neurobiology, introducing theories regarding the neural basis of learning and memory. It will also introduce theory and research regarding the neural basis of motivation and reinforcement, and the neural basis of choice. Current theories of stereotypy suggest specific differences in the neurobiology of affected animals as compared to controls. In order to understand these potential differences, it is important first to consider basic concepts of the neurobiology of learning.

Neural Mechanisms of Learning

It has long been accepted that learning involves neuronal plasticity, resulting from changes at the synaptic level. One early example of this in the literature came from Pavlov (1927), who initially suggested that during associative learning sensory neurons (i.e., associated with the CS) become connected to motor neurons (i.e., associated with the CR), forming a basic neural network. However, there was little corroborating neurobiological evidence. Later, Donald Hebb (1949) argued that

learning was the result of networks of neuronal connections, in which a number of synapses change (synaptic plasticity) to form complex assemblies. This led to many theories of neuronal plasticity and its role in learning.

Initially, Hebb (1949) suggested that when cell i (pre-synaptic neuron) repeatedly and persistently fires in close proximity to cell j (post-synaptic neuron), synaptic efficacy will increase. Therefore,

$$\Delta \omega_{i,j} = r a_i a_j \quad [1.4]$$

where the weight (ω) of the connection between neurons i and j change as a result of learning rate (r) times the activation level (a) of each neuron.

Hebb's theory is somewhat over-simplistic and is certainly mathematically unstable, as he did not suggest, for example, any means by which synaptic efficacy could reduce. One theory that solves some of the difficulties with Hebb's model was proposed by Bienenstock, Cooper, and Munro (1982) and concentrates on synaptic plasticity in the oculomotor system. Within the Hebbian framework, the weight of the synapse increases with, input times the activation of the postsynaptic response times the learning rate (see Equation 1.4). However, in the Bienenstock et al. (1982) model a presynaptic output threshold is incorporated, and this overcomes some of the problems of mathematical instability within the Hebbian framework. Thus, Equation 1.4 becomes

$$\frac{d\omega_i(t)}{dt} = \phi(c(t)) d_i(t) - \epsilon \omega_i(t) , \quad [1.5]$$

where d is the input current of synapse i , ϕ is a postsynaptic activation function, c is the presynaptic output level and ϵ represents a time (t) decay function of all synapses in the network.

Experimental evidence for Hebbian theory and its associated revisions are various. Perhaps the most commonly cited came from Bliss and Lømo (1973), who found that if afferent cells in the perforant pathway (which innervates the

hippocampus) were electrically stimulated, this led to long lasting (or even permanent) increases in activity in hippocampal cell synaptic transmission, demonstrating Hebbian learning at the cellular level. This process, referred to as long-term potentiation (LTP), has also been found to occur *in vitro* in hippocampal tissue, as well as in other neural structures (e.g., limbic structures, prefrontal cortex, striatum, etc.), further illustrating its contribution to learning. Conversely, long-term depression (LTD) refers to a decrease in synaptic transmission, usually caused by repeated, low-frequency stimulation of afferent cells (Ito, 1989) or repeated incongruent pairings of pre- and post-synaptic cells (Lin, Way, & Gean, 1993; Sajikumar & Frey, 2004).

LTP results from increases in presynaptic glutamatergic activity. It has been posited that DAergic activity may activate glutamate release at the synapse and mediate LTP and LTD. Li, Cullen, Anwyl, and Rowan (2003) facilitated hippocampal LTP by placing rats in novel contexts. Inactivation of DA D1-like² receptors with SCH23390 blocked LTP whereas activation of D1-like receptors (using SCH38393) facilitated LTP. These findings suggest that hippocampal LTP is DA-dependent. In addition, Sajikumar and Frey (2004) demonstrated that, in rat hippocampal slices *in vitro*, DA challenge affected potentiation in a dose-dependent manner. Specifically, at higher doses, D1-like receptor antagonist SCH23390 induced delayed-onset LTP, but at lower doses it induced delayed-onset LTD. There is currently little evidence for the role of D2-like² receptors in hippocampal LTP or LTD, but their role in striatal LTP and LTD is well established and will be discussed later. Bliss and Lønmø (1973) were unsure as to whether LTP and LTD are reflective of the neural basis of learning and memory. However, many have cited qualitative similarities between the processes of learning, and LTP and LTD (i.e., fast induction, long-lasting change) as evidence that this constitutes the neural basis of learning and memory (Martinez, Barea-Rodriguez, & Derrick, 1998).

² DA D1 and D5 receptor subtypes are referred to 'D1-like', and DA D2, D3, & D4 receptors are referred to as 'D2-like'; they are categorised together owing to their G-protein activation status. For further information, see Nicola, Surmier, & Malenka (2000).

Neural Basis of Reinforcement: The role of DA D1 and D2 like receptors.

Olds and Milner (1954) first identified the brain's reinforcement centres as a result of their work with intercranial self-stimulation (ISS) of the rat medial forebrain (septum, hippocampus, amygdala, hypothalamus, nucleus accumbens and cingulate gyrus). They found that rats would press a lever thousands of times per hour to receive stimulation of these areas, suggesting the stimulation is inherently instrumental. However, if the electrical stimulation was turned off, the rats would stop pressing almost immediately. This was an unanticipated finding as such high response rates are typically associated with increased latency to extinguish responding. In addition, it was found that if rats were removed from the experimental chamber, they would not resume lever pressing without first being primed by the researchers. Olds and Milner (1954) suggested therefore that the rats were directly stimulating the brain's reward centres. Subsequently research has highlighted key brain regions and pathways in the neurobiology of reinforcement, encompassing both the appetitive and consummatory phases.

Extensive research has been carried out examining the role of DA in reward; primarily the effects of DA agents (selective DA agonists and antagonists) on instrumental behaviour. For example, Aberman, Ward, and Salamone (1998) trained rats to press a lever for food reinforcement on a CRF schedule. In condition one, the rats were given no treatment, and placed on a single-response-incremented progressive ratio (PR) schedule (i.e., trial 1 = 1 response for reinforcement; trial 2 = 2 responses for reinforcement; trial 3 = 3 responses for reinforcement;...trial n = n responses for reinforcement). In subsequent conditions, rats were administered increasing doses of the DA D1-like receptor antagonist SCH23390, and the DA D2-like receptor antagonist Haloperidol and again exposed to a PR schedule. Responses decreased under both conditions in a dose-response manner, suggesting that both D1-like and D2-like receptors play a role in the motivation for reinforcement (but see below for an outline of the physiological properties of D1-like and D2-like receptors within the striatum).

The specific role of D1-like and D2-like receptors in reinforcement is a matter of some debate, with some suggesting that the roles may be functionally dissimilar. For example, Sutton and Beninger (1999) reviewed studies examining D1-like receptors and their role in reinforcement. They suggested that the D1-like receptors may play a phasic role in the appetitive stage of reinforcement, by acting to predict reward, and mediate search locomotion. However, they argued that D2-like receptors are responsible for tonic DA activity, mediating responses to currently available reinforcers. D2-like receptors are therefore responsible for maintaining instrumental responding, and may be responsible for perseveration of responding. Further, studies employing the D2-like receptor agonist quinpirole have demonstrated increases in perseveration of instrumental responses (Kurylo, 2004).

Neural Mechanisms of Instrumental Choice. D1-like and D2-like receptors have been examined in terms of their roles in sensitivity to reinforcement in free-operant procedures. Bratcher, Farmer-Dougan, Dougan, Heidenreich, and Garris (2005) gave rats increasing doses of specific D1-like (SKF38393) and D2-like (quinpirole) agonists and examined their sensitivity to reinforcement under concurrent schedules of reinforcement. Sensitivity was measured according to the generalised matching law (Equation 1.3; Baum, 1974). They found that there was a dose-dependent change in reinforcer sensitivity to the D1-like agonist, but not to the D2-like agonist. This suggests that D1-like receptors are more likely to mediate appetitive/ searching behaviour (Sutton & Beninger, 1999).

O'Doherty, Dayan, Schultz, Deichmann, Friston, and Dolan (2004) investigated reinforcer choice under a matching paradigm based on the two-process theory of instrumental learning (Balleine & Dickinson, 1998). They discussed instrumental learning in terms of an 'actor-critic' model, where the actor is a constant reference system that allows the organism to maintain steady state responding (similar to S-R learning) and maximises reward potential based on previous reinforcement history. The critic component learns by experience to predict future reward (similar to R-O learning). O'Doherty et al. (2004) speculated

that these systems may be mechanistically mediated by phasic firing of DA neurons in the striatum. Specifically, the actor component appears to be related to the dorsal, and the critic component to the ventral striatal DA afferents. They investigated this using fMRI technology with human participants carrying out instrumental (to examine reward prediction) and Pavlovian (as a control condition) conditioning procedures. The instrumental task involved two conditions. In Condition 1, participants were shown two stimuli, one associated with a high probability (60%) of receiving a fruit juice reward, and the other with a low probability (30%). In Condition 2, participants were shown two different stimuli, this time associated with the same probability (i.e., 60% vs. 30%) of receiving a neutral liquid. The Pavlovian task employed the same design; however, participants did not choose the alternative. They were instead asked to indicate which choice the computer had made. O'Doherty et al. (2004) hypothesised that if the ventral striatum was mediating the critic component, reward prediction error (RPE) in both tasks would be correlated with DA firing in this area of the striatum. Similarly, if the dorsal striatum was mediating the actor component, RPE in the instrumental task would be stronger in the instrumental task. Their results confirmed the hypothesis, suggesting that the dorsal and ventral afferents of the striatum were dissociable, and in addition, that the ventral stream corresponded to the critic component, and the dorsal stream to the actor component of instrumental learning.

During trial-and-error learning, DA neurons provide an RPE signal to the organism, which assists in choice making. In terms of the previously described actor-critic model, the RPE is fed back to the actor component by the critic component in order to mediate future responses. Morris, Nevet, Arkadir, Vaadia, and Bergman (2006) examined the RPE feedback mechanism in primates using a probability-based matching paradigm. Morris et al. (2006) showed monkeys four different stimuli, each associated with a different probability of reward (0.25, 0.5, 0.75 and 1 respectively). Monkeys were initially trained using a Pavlovian procedure, where single stimuli, each with its related reward probability were displayed and reward delivered according to the weighted probabilities. However,

dispersed amongst the Pavlovian trials were instrumental choice trials, where the monkeys were shown two of the stimuli concurrently, and had to choose to which to respond after 2-sec. Midbrain DA firing was measured during choice and Pavlovian conditioning trials. During the Pavlovian conditioning trials (i.e., when no choice had to be made) midbrain DA firing was correlated with RPE for each of the stimuli. In the instrumental choice trials, monkeys' choices were analogous to the matching law (i.e., the probability of choosing the richer reward matched the probability of that reward being delivered in a given trial). However, during the instrumental choice trials, they found that immediately upon presentation of the two stimuli, DA firing related directly to the *forthcoming* choice. This suggested that although the monkeys had not yet completed their choice response, DA firing related to the probability of the reward (i.e., the critic system) was signalling the actor system to make the choice – the monkeys had already made a decision prior to making the response.

Finally, within the context of two-alternative choice based on differential reinforcement schedules, an animal must have some mechanism with which to recognise the differences in the elapsed time between response and reinforcer. To explain, in a concurrent schedules set-up (e.g., see Figure 1.4) if the left alternative is set to VI 30 s and the right to VI 60 s, the animal must be able to differentiate between these latencies in order to choose the richer alternative. Wei-Min, Pitts, Hughes, McLean, and Grace (2008) found that doses of AMPH attenuated pigeons' sensitivity to the delay of reinforcement, with exposed subjects failing to recognise the differences in concurrent schedules. This suggested that high levels of extra-cellular DA may minimise the critical evaluation of the delay.

To summarise, DA activity has been shown to play a key role in the neurobiology of reward and reinforcer choice. The general consensus in the behaviour neuroscience literature is that the striatum plays a significant role in learning, and in the next section, the striatum will be explored in more detail.

Striatum

Anatomy and Physiology. The corpus striatum forms the main neural gateway to the basal ganglia. Anatomically, it comprises the dorsomedial (caudate nucleus; DMS) and dorsolateral (putamen; DLS) regions, and the NAcc (ventral). Research has established that the three are both structurally and functionally dissociable, and some such evidence is outlined below. The striatum receives inputs from most cortical regions and comprises extensive afferents and efferents. The dorsal (including DMS and DLS) striatum, for example, is extensively innervated by excitatory glutamatergic neurons from the sensorimotor cortex, whereas the major limbic and PFC afferent innervations terminate in the NAcc (Geredman, Partridge, Lupica, & Lovinger, 2003). The major striatal efferent projects, via the globus pallidus, to the ventral lateral thalamus, the region from which global cortical functioning is mediated, forming a cortico-striatal-thalamo-cortical loop (Parent & Hazrati, 1995). The dorsal pathway (nigrostriatal pathway) originates at the substantia nigra pars compacta (SN) and projects to the DLS via the DMS and the ventral pathway (mesocorticolimbic pathway) originates in the ventral tegmental area (VTA) and projects to the prefrontal cortex (PFC) via the nucleus accumbens (NAcc). Lesions of the mesocorticolimbic pathway have been found to attenuate ISS (e.g., Fibiger, LePiane, Jacobovic, & Phillips, 1987), highlighting its role in the neurobiology of reward.

In terms of its cytoarchitecture, the striatum comprises predominantly inhibitory GABAergic Medium Spiny Neurons (MSNs), which are responsible for the initiation of movement, as well as fine and gross motor control. MSNs have extensively branching dendritic trees and receive extensive cortical, medullary and limbic input. There are two types of MSNs, striosome and matrix cells, which differ in physiological constitution (Canales, 2005). Striosomes are opioid-receptor enriched, whereas the surrounding matrix cells are rich in acetylcholine. The striosome cells are responsible for a third of striatal GABAergic efferent (Canales & Graybiel, 2000), extensively innervating the nigrostriatal-pathway DA cells.

In the light of this, DA has been argued to be integral to striatal synaptic activity, with striatal synapse excitability largely modulated by mid-brain DA innervation (Centozne, Piccioni, Gubellini, Bernardi, & Calabresi, 2001; Gerdeman et al., 2003). Two dissociable groups of striatal efferents are categorised according to whether they innervate the internal (iGP), or external globus pallidus (eGP). The group that innervates the iGP is often referred to as the ‘direct’ pathway and is rich in GABAergic neurons which increase motor activity through expression of D1-like receptors, whereas the group that innervates the eGP is referred to as the ‘indirect’ pathway; this inhibits movement and is rich in D2-like receptors (Nicola et al., 2000). Hence, GABAergic striosome cells innervate nigrostriatal DA receptors, and mediate DA activity in the direct and indirect pathways (Canales, 2005).

D1-like receptors are excitatory, increasing extracellular cyclic adenosine monophosphate (cAMP) concentration and are crucial for striatal LTP and LTD, whereas D2-like receptors are inhibitory, inducing phosphodiesterase activity which breaks down cAMP, and are crucial for dorsal striatum LTD, but not LTP (Calabresi, Maj, Pisani, Mercuri, & Bernardi, 1992; Geredman et al., 2003; also, see above). This suggests that the excitatory, direct pathway and the inhibitory indirect pathway both mediate LTD, but only the excitatory direct pathway is involved with striatal LTP. Given this, it would be expected that selective blockade of D1-like receptors and D2-like receptors should attenuate LTP, but blockade of D1-like receptors should have no effect on striatal LTD, and this is supported by the existing evidence (see Gerdeman et al., 2003 for a review).

LTP in the ventral striatum (NAcc) appears to be dependent on the developmental stage of the animal. To explain, hippocampal LTP in rodents is known to peak early in development (Bliss & Lømmø, 1973). Schramm, Egli, and Winder (2002) examined the developmental factors associated with NAcc LTP in young (3-weeks of age) and adult (6-20 weeks of age) mice. They found that LTP-evoked glutamatergic synaptic transmission is more likely to occur in the NAcc of the younger mice. They discussed their findings in relation to drugs of abuse and the effects of the age of initial exposure (i.e., humans exposed to drugs for the first

time as younger adults/adolescents are more likely to become dependent than older adults). However, this may have important implications in the current context (stereotypy development). To explain, we will see later that one of the key features of the proposed stereotypy phenotype is early exposure to ‘stressful’ environmental conditions. This may help to explain why the development of stereotypy in older animals is less common than in younger animals (Mason & Rushen, 2006).

Behavioural Functions. It is generally accepted that the ventral striatum, in particular the NAcc, is key in the mediation of appetitive states in Pavlovian learning. A large body of research has linked clearly the NAcc to the incentive value of Pavlovian CSs (see de Borchgrave, Rawlins, Dickinson, & Balleine, 2002, for a comprehensive review). For example, enhancement of NAcc firing by the introduction of DA agonists (e.g., AMPH) increases PIT, even in the absence of primary reinforcement (Wyvell & Berridge, 2000), and NAcc lesioned animals attenuate responding to reward-predictive CSs, even following DA agonist administration (Cardinal, Parkinson, Hall, & Everitt, 2002). In addition, the NAcc has been linked to instrumental learning. For example, NAcc lesions impair training on instrumental procedures, with subjects failing to learn R-O contingencies (de Borchgrave et al., 2002). It should be noted, however, that both the ventral and the DMS mediate CS-CR-US associations, whereas the DLS mediates S^D control over CRs (Everitt & Robbins, 2005; Yin & Knowlton, 2006). Subsequently, the DMS is often referred to as the ‘associative striatum’ and the DLS as the ‘sensorimotor striatum’ (Yin, Ostlund, Knowlton, & Balleine, 2005). Evidence for this comes from DLS lesioned animals which, during instrumental procedures where an S^D is used, show a marked acquisition deficit (see Faure et al., 2005 for a review). More specifically, Yin et al. (2005) examined instrumental performance in rats following pre-training only and pre-and post-training exotoxic lesions of the anterior and posterior DMS. R-O learning was operationalised as rats’ sensitivity to systematic outcome-devaluation (Colwill & Rescorla, 1988) and contingency degradation (Balleine & Dickinson, 1998). Pre- and post-training posterior DMS lesions were found to eliminate the behavioural effects of outcome

devaluation and contingency degradation. However, pre- and post-training anterior DMS lesions had no effect on either. These results suggested that the posterior, but not anterior DMS may be crucial to R-O learning (Yin et al., 2005).

Faure et al. (2005) carried out a devaluation study with overtraining, using rats with specific DLS lesions. Rats took part in a cued instrumental conditioning procedure, where the S^D (a tone or a light) was paired with an associated operant (a lever press or a chain pull) which was subsequently reinforced with either food or sucrose solution. Following extensive training, instrumental responses were examined after satiety-specific devaluation of both reinforcers individually. In the control animals, it was found that the lever press was insensitive to devaluation but the chain pull was not. However, in the lesioned animals, both the chain-pull and the lever press were sensitive to devaluation. First, this suggests that there may be a response-milieu specific effect on the development of habit learning. Specifically, it is probable that species-atypical responses (i.e., a chain-pull) remain under R-O control, at least for an extended period, owing to the extensive motor processing involved in making the response. Second, it shows that in the lesioned animals, there was no evidence of a move from R-O to S-R strategy, suggesting that the DLS is essential in the shift from R-O to habit formation (S-R).

There is compelling evidence from 'place-response' learning tasks that the DLS is involved in the latter stages of learning (i.e., S-R learning; Yin & Knowlton, 2006). Packard and McGaugh (1996) trained rats in a standard cross-maze (Figure 1.6) surrounded by a variety of allocentric contextual cues (i.e., pictures on the wall, the experimenter's position, the door of the laboratory, etc.). The rats were initially placed in the south arm of the maze, the east arm was baited with a food pellet, the west arm was left empty and access to the north arm was blocked. The rats were trained for a period of 16 days, with four trials per day. On the eighth and 16th days, the rats were exposed to a single probe trial. During these trials, the rats' position was moved from the south arm to the north arm of the maze and access to the south arm was blocked (see Figure 1.6). The key manipulation was that one group of rats, immediately prior to the probe trials, had the DLS chemically

inactivated (using 0.5 μ l lidocaine injection), while the other group received a saline injection.

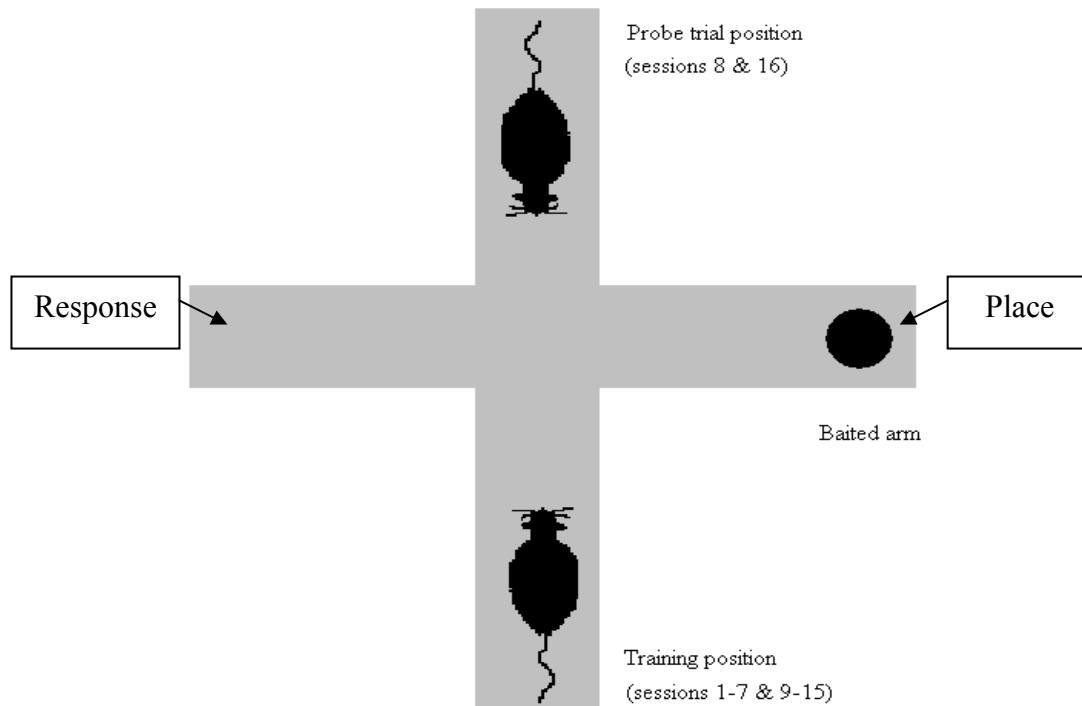


Figure 1.6. Procedural diagram for place/response test in rats using a cross-maze.

The results for both groups showed that on the first probe trial the rats chose the east arm (place strategy). This meant that the rats had formed some kind of representation of the actual location of the baited arm in relation to surrounding contextual cues. However, in the second probe trial, the saline group chose the east arm of the maze, but the lidocaine group chose the west arm. The results suggested that with overtraining, the saline group moved from a place strategy (i.e., using contextual cues) to a response strategy (i.e., always turning right to get food); however, the lidocaine group continued to use a place strategy. It can be concluded from this that the DLS is central to the development of habit formation, and further, that there is a shift to habitual responding as a result of overtraining.

Yin and Knowlton (2006) proposed that there was a hierarchy of the three systems within the striatum. Initially, there is the limbic network, which

incorporates the ventral striatum (nucleus accumbens), with excitatory input from the pre-frontal cortex; there is also an associative network, which incorporates the DMS, with excitatory input from the pre-frontal and parietal cortices; finally, the sensorimotor network, which incorporates the DLS, with excitatory input from the sensorimotor cortex. During habit formation, there is a shift through the hierarchy, from initial Pavlovian processes, through R-O judgements, finally to automated, habitual responses, driven by S_D .

Chronic or prolonged exposure to DA agonists such as AMPH has been shown to enhance habit formation. For example, Nelson and Kilcross (2006) trained rats to perform an instrumental response on a VR schedule of reinforcement. Following modest training, the reward was devalued and half of the subjects were administered with AMPH micro-injection. Typically, with short periods of training, it would be predicted that subjects' response rates would attenuate following the devaluation. However, the AMPH group were found to be insensitive to the devaluation procedure, suggesting that they had moved from R-O to S-R learning. The authors suggested that this illustrated that AMPH exposure results in enhanced shift from goal-directed (R-O) to habit-based (S-R) behaviour.

A neurobiological explanation for this shift is that chronic AMPH exposure leads to alterations in the cytochemistry of the striatum. As discussed earlier, R-O learning pertains to the DMS and S-R (habit) to the DLS (Yin & Knowton, 2006). Jedynak, Uslaner, Esteban, and Robinson (2007) repeatedly injected rats with 6mg/kg AMPH over a 20-day period. They reasoned that because control of behaviour shifts from DMS to DLS, it would be expected that there may be an increase in dendritic spines of DA receptor cells. This is indeed what they found, and they suggested that this may be due to a decrease in excitatory glutamatergic innervation to the DMS and concurrent increase in the DLS. In addition, Robinson and Kolb (2004) found that rats exposed to AMPH or cocaine, either via self- or experimenter-administration, showed perturbations in mesoaccumbens DA physiology. Specifically, they found persistent increases in dendritic spine density in the NAcc. These persistent alterations in neurophysiology following chronic AMPH exposure also mediate long-lasting and persistent alterations behavioural

responses to a single AMPH challenge. This process (behavioural sensitisation) will be discussed in the next section.

These enduring, AMPH-induced alterations in striatum physiology appear to be mediated in turn by adaptations in the striosome and matrix MSNs. Immediate early-gene induction assays have been employed to examine the associated behavioural phenotypes. For example, Canales and Graybiel (2000) administered rats chronically with AMPH, cocaine (a DA reuptake inhibitor) and the selective DA D1 and D2 receptor agonist, apomorphine. They carried out immunohistochemical analysis of striosomal activation in the DMS and DLS. They discovered that chronic AMPH, cocaine and apomorphine increased striosomal predominance in the DLS, although the apomorphine increase appeared only at higher doses. They also found that AMPH increased striosomal predominance in the DMS, but not the other drug-treatments. The results indicated that chronic DA agonist exposure may alter gene transcription factors through upregulation of striosome cells and downregulation of matrix components of the striatum, leading to relative increases in striosome afferent pathways from the basal ganglia. In addition they found strong correlations between the gene induction results described and behavioural phenotypes associated with chronic DA-exposure (i.e., increases in stereotypy).

To summarise, the striatum is structurally and functionally heterogeneous, with the ventral (NAcc) pertaining to the initial processing of information relating to reward-predictive (Pavlovian) cues, the DMS mediating R-O (planned action) and the DLS mediating S-R (habitual) learning. Advances in immunohistochemistry procedures have allowed for a systematic evaluation of the neurobiology of the striatum at the cellular level. Changes in the balance of activity in the basal ganglia between striosome and matrix cells, following chronic DA agonist exposure, result in upregulation of the indirect and concurrent downregulation of the direct pathways. This change appears to be related to a shift from R-O learning to S-R (habit) learning, and is facilitated by DA agonists. The next section will describe some of the behavioural features of chronic DA agonist

exposure and start to think about the conditions required for endogenous change in midbrain DA physiology.

Behavioural sensitisation

It is well established that psychostimulants activate DA neurotransmission (Robinson & Berridge, 1993). In addition, subsequent re-exposure to these drugs produces long-term enhanced locomotor activity in response to DA agonists, as compared to pre-exposure levels (see Vezina, 2004 for a review). This process, known as *sensitisation*, results in long-term elevated levels of extra-cellular DA and hence, enhanced facilitation of DA neurotransmission following subsequent AMPH exposure (Hu, Koeltzow, Cooper, Robertson, White, & Vezina, 2002). The neurophysiological process by which sensitisation occurs is a matter of some debate (Vezina, 2004); however, it seems fairly clear that it is mediated by the mesoaccumbens DA pathway (i.e., VTA to NAcc). Locomotor sensitisation can be reliably induced, for example, by infusing the VTA, but not other fields of the mesoaccumbens pathway (e.g., the NAcc), with AMPH (Gerdeman et al., 2003). In addition, prior AMPH infusion in the VTA facilitates enhanced DA neurotransmission in the NAcc in response to AMPH challenge, and this is necessarily mediated by NAcc D1-like receptors (Vezina, 1996). To illustrate, Hu et al. (2002) injected the VTA of rats with AMPH at three-day intervals. *In vivo* electrophysiological analysis showed that this treatment increased the sensitivity of NAcc neurons to the inhibitory effects of D1-like receptor activation.

Chronic stress has a similar effect on locomotor sensitisation to AMPH, a line of enquiry that has implications for the development of a model of stereotypy linked to stress-induced sensitisation. Herman, Stinus, and Le Moal (1984) exposed adult rats to repeated foot-shocks over a 10-day period. Following the final shock session, the rats showed sensitised locomotor response to AMPH challenge as compared to controls. They found that despite initial increases in cortico-limbic DA activity following shock sessions, levels returned to normal after 24-hours. In addition, apomorphine (a selective DA D2-receptor agonist)

exposure had similar hypolocomotor effects on control and treatment subjects. Taken together, these results suggested that chronic stress increased postsynaptic sensitivity to DA. More recently, Pacchioni, Gioino, Assis, and Cancela (2002) exposed rats to a single restraint-stress procedure. They found that the preparation was sufficient both to increase locomotor sensitisation and striatal DA output. This showed first that extreme levels of acute stress may be sufficient to induce sensitisation, but also that this effect is seen at both the behavioural and neurochemical levels.

There is also evidence that the effects of stress on sensitisation of the DA system may be genotype specific. Certain in-bred strains of mice are disproportionately prone to develop stereotypic behaviours and show (given stressful environmental conditions) signs of sensitisation. Cabib and Bonaventura (1997) exposed mice from both the C57BL/6 (C57) and from the DBA/2 (DBA) strains to chronic food restriction for nine-days. They found that mice from the DBA strain developed stereotyped behaviour patterns following the food restriction treatment, but not mice from the C57 strain. In addition, they found that DBA, but not C57 mice showed sensitisation to the locomotor effects of AMPH challenge. This suggested a genotype \times environment (stress) interaction leads to stress-induced behavioural sensitisation. The same inbred strain of mice (DBA) has been found to have altered mesoaccumbens DA pathway characteristics (Cabib, Giardino, Calza, Zanni, Mele, & Puglisi-Allegra, 1998). Specifically, following chronic stress, they show similar neurophysiological features to those associated with chronic AMPH-induced behavioural sensitisation (see above). These changes, termed stress-induced sensitisation, are characterised by upregulation of D1-like and D2-like receptors in the NAcc and concurrent downregulation of inhibitory D2-like receptors in the VTA, suggesting facilitated neurotransmission in the mesoaccumbens DA pathway in these animals (Cabib et al., 1998), an effect seen following chronic AMPH exposure.

In summary, this section first outlined some of the features associated with the neurobiology of learning. It then considered in detail the role of the striatum and outlined the neurobiological underpinnings of habit formation. Finally, it

considered the phenomenon of behavioural sensitisation, the effect of repeated drug or stress challenge on behavioural output in response to AMPH, and began to illustrate the possible role of this in the development of stereotypic behaviour.

The final section of this review will propose a theory of stereotypy based on dysfunction of the fundamental neurobiological mechanisms discussed, and it will suggest ways in which this may be operationalised for empirical study.

Neurobiological Approaches to Stereotypy

In the first section, we introduced stereotypy in its historical context. We also saw how simple physiological explanations of the functionality of stereotypy (related to sympathetic and HPA activity) fail adequately to explain the development of the behaviour. Drawing from research discussed in the previous sections on learning and its neurobiological underpinnings, it is now possible to examine in detail some of the neurobiological correlates of stereotypy.

Although the environmental predictors of stereotypy are relatively clear, the underlying neuropsychological correlates remain less so. Many of the neurobiological theories of stereotypy have stemmed from studies using pharmacological manipulations of stereotypic behaviour (e.g., Presti, Mikes, & Lewis, 2003). Typically, the studies have found that specific DA agonists (e.g., AMPH) induce stereotypy, or specific DA antagonists (e.g., haloperidol) reduce pharmacologically or environmentally induced stereotypy. However, there is a functional dissociation between the neurobiological mechanisms mediating pharmacologically- and environmentally-induced stereotypy (see Presti, Gibney, & Lewis, 2004). There is also a substantial body of evidence which suggests that environmentally-induced stereotypy has a key neuropsychological component, but the specific aetiology is a matter of some debate, as are the neuroarchitectural and neurochemical components.

Limbic-striatum efferent

One predominating argument is that the hippocampus may play an important role in the early development of stereotypy. For example, if animals experience chronic stress early in life (a suggested environmental cause of stereotypy, often associated with abrupt weaning; Parker et al., 2008; Waters et al., 2002), this can have a profound and deleterious effect on the structure and function of hippocampal neurons (Fenoglio, Brunson & Baram, 2006; Karten, Olariu & Cameron, 2005). Specifically, granulate cells within the dentate gyrus of the hippocampus are distinct from other neurons, as they continue to develop into adulthood (neurogenesis). However, early chronic stress has been found, in rats, to retard or even attenuate neurogenesis (Karten et al., 2005). In addition, mice that have been reared in an enriched environment have been found to have a richer distribution of hippocampal pyramidal cells (Kempermann, Khun & Gage, 1997). These cytoarchitectural alterations have been found to produce specific behavioural alterations. For example, rats that experienced maternal deprivation in early life show reduced performance on tasks known to involve the hippocampus (e.g., morris watermaze: Hout, Plotky, Lenox & McNamara, 2002; declarative memory tasks: Clark, Zola & Squire, 2000). In addition to their role in learning, glucocorticoid receptors (GCRs) in the hippocampus are thought to be related to the efficacy of the mammalian stress-response (e.g., see Gunnar & Quevedo, 2007, for a review) and the early epigenetic development of GCRs are mediated by specific interactions with the mother (e.g., Weaver et al., 2001; Weaver et al., 2004).

The hippocampus extensively innervates the striatum (e.g., Schaub, Schmelzeis & Mittleman, 1997) and it has been suggested that the hippocampus modulates particularly ventral striatum DA activity (White, Whitaker & White, 2006). It is therefore likely that hippocampal dysregulation may have a modulatory effect on ventral striatum DA activity. Goto and O'Donnell (2002) examined the effects of neonatal ventral hippocampus lesions on DA activity in the rat ventral striatum. They found that lesioned rats showed a marked increase in NAcc DA

activity, possibly mediated by perturbations of ventral VTA afferents. Given this, it is possible that hippocampal dysfunction may modulate neurophysiological morphosis in the striatum.

During the course of this thesis, we will be concentrating predominantly on the role of the striatum in the aetiology and pathogenesis of stereotypy, and its associated behavioural features. In the light of this, the role of the hippocampus will not be discussed at any length hereafter. However, this remains an important line of enquiry, and future research may be designed specifically to look at the role of hippocampal neurogenesis in the aetiology of spontaneous stereotypy.

The role of the basal ganglia in stereotypy

Recent research has highlighted the possibility that the specific site of neurobiological dysfunction in stereotypic animals may be the striatum. For example, during associative learning tasks, animals with lesions to the dorsal striatum (i.e., CPu) have been shown to have an increased latency to extinguish CRs in the absence of USs. It has been proposed that the reason for this lies in the inhibitory pathway of the dorsal striatum. The striatum is known to have two distinct pathways: direct, which mediates purposive (e.g., appetitive) behaviour, and the indirect pathway, which inhibits rival or competing responses, perhaps by focussing attention on the current task (Garner, Mason & Smith, 2003; also see above for a more detailed review of the pathways). Lesions to the indirect pathway result in general behavioural 'disinhibition' (see Garner et al., 2003 for a review). Some researchers have equated this to stereotypy, suggesting that the perseverative response patterns seen in stereotypy animals are suggestive of disinhibited response sequences exhibited by CPu-lesioned subjects.

Garner et al. (2003) performed a battery of tests on stereotypic caged songbirds designed to examine basal ganglia functioning. The tests used were standard extinction paradigms (using instrumental procedures, and a quasi-naturalistic food-caching design) and sequential responding in a gambling task. The authors reported that stereotypy was positively correlated with latency to

extinguish CRs in the extinction procedures and ‘predictable’ response patterns (as opposed to maximising income) on the gambling task. They suggested that this was evidence for a direct relationship between rate of stereotypy and behavioural disinhibition. The extinction findings have recently been extended to include non-laboratory species (e.g. horses, Hemmings, McBride & Hale, 2007; and bears, Vickery & Mason, 2005), suggesting that stereotypy may be the result of a decrease in activity of the indirect and an increase in the direct pathway, with stereotypic animals perhaps being caught in a negative feedback ‘loop’ of unresolved, highly motivated response patterns (see Hughes & Duncan, 1988). However, this hypothesis is rather at odds with the more recent striosome work discussed earlier (Canales, 2005). In fact, it may be that the increase in perseveration is the result of imbalances between striosome and matrix output from the striatum, causing a decrease in DA activity in the direct and concurrent increase in the indirect pathway. Indeed, Canales and Graybiel (2000) found a direct correlation between DA agonist-induced stereotypy and the above cytochemical measures of striatal function.

As discussed earlier, Cabib et al. (1998) found that DBA (but not C57) mice showed signs of neural sensitisation following chronic stress both behaviourally (in terms of their sensitised locomotor response to AMPH challenge and increased spontaneous [drug-free] stereotypy) and their neurophysiology (upregulation of the mesoaccumbens DA pathway). In the light of this, McBride and Hemmings (2005) examined the striatal DA receptor profiles of crib-biting horses. The theoretical basis for their work stemmed from several lines of evidence. The first was that stereotypies are the result of highly motivated appetitive behaviours that are restricted in their consummation, owing to chronic environmental constraint (Hughes & Duncan, 1988). This is particularly pertinent to crib-biting horses, as many lines of enquiry have shown that restricted feeding is a key risk factor in the development of the behaviour (e.g., Parker et al., 2008; Waters et al., 2002). The second is that the mesoaccumbens pathway (specifically, the NAcc) is activated during appetitive behaviours (Ikemoto & Panskepp, 1999; Robinson & Berridge, 1993). Finally, there is evidence that crib-biting in horses may have a genetic

component (Vecchiotti & Galanti, 1986). Based on work in mice by Cabib and Bonaventura (1997), McBride and Hemmings (2005) argued that as a result of the interaction between the chronic stress of unresolved appetitive motivation and genotype (n.b., the latter was never tested directly in their study), the mesoaccumbens DA pathway would become sensitised, owing to downregulation of inhibitory D2-like receptors and upregulation of excitatory D1-like receptors. They found that the crib-biting horses had higher D1-like and D2-like receptor densities in the ventral striatum (NAcc) as compared to non-crib-biting horses suggesting, based on the synaptic plasticity (LTP and LTD) characteristics of the mesoaccumbens pathways, facilitated DA neurotransmission (neural sensitisation).

McBride and Hemmings also found that crib-biting horses had a reduction in excitatory D1-like receptor density in the DMS (caudatus), suggesting downregulation of DA transmissions in the nigrostriatal pathway within the horse. The NAcc results supported previous findings in stress-induced stereotypy in an inbred strain of mice (Cabib et al., 1998), but the DMS results were not, suggesting this may be an artefact of chronic developmental course of environmentally induced stereotypy or may be specific to the ESP. Persistent AMPH exposure causes a decrease in dendritic spine density in the DMS, and concurrent increase in the DLS in rats, and this appears to be mediated by decreased corticostriatal glutamatergic afferent (Jedynak et al., 2007). The DMS differences in the ESP may therefore be the result of sustained increased extracellular mesoaccumbens DA, as a result of the stress-induced alterations in DA physiology reported, leading to control of behaviour moving from DMS to DLS. It is possible that the lack of significant difference in this region reported by Cabib et al. (1998) reflect the relatively short exposure time to stress in their subjects as opposed to much more sustained exposure in the horses (McBride & Hemmings, 2005). However, note that McBride and Hemmings did not find any increase in DA receptor densities in the DLS, casting some doubt over this account.

Contrary to Garner et al's (2003) hypothesis relating to dysfunction of inhibitory pathways in the dorsal striatum, McBride and Hemmings' (2005) data suggested that perhaps, owing to chronic stress and/or unresolved appetitive

motivation (for example, lack of wild-type foraging facility) stress-induced behavioural sensitisation results in the stereotypic animal remaining in an enduring, heightened appetitive state (McBride & Hemmings, 2005). This would relate to sustained neuronal firing in the VTA-NAcc of stereotypic animals and hence upregulation of the mesoaccumbens pathway and a move in behavioural control from DMS to DLS.

As I introduced in an earlier section of this review, LTP in the NAcc appears to be developmentally regulated (Schamm et al., 2002). Specifically, NMDA receptor-dependent LTP is more regular in the striatum of younger, as opposed to older, mice. If this feature of developmental neurophysiology is common to other higher vertebrates (e.g., horses), it may be expected, in the context of the ESP, that early experiences may be particularly pertinent to the pathogenesis of the condition. Indeed, evidence suggests that early stressful experiences may contribute significant risk factors in the development of stereotypy in horses (e.g., Parker et al., 2008; Waters et al., 2001) and further, research with other species has suggested that, once established in early life, stereotypies become emancipated in the behavioural repertoire and are less susceptible to the effects of enrichment and other stereotypy-reducing techniques (Cooper, Ödberg, & Nicol, 1996; Mason, 1991; McGreevey, 2004).

Stress-induced behavioural sensitisation. Learning strategy shifts from R-O (i.e., DMS) to S-R (i.e., DLS) during instrumental procedures (Yin & Knowton, 2006). This shift in behavioural control (after overtraining) results in an increased propensity to respond, not only in the absence of USs, but also if the US has been devalued (Colwill & Rescorla, 1988). Therefore, in the light of the stress-induced sensitisation theory of stereotypy, the increased latency to extinction in the stereotypy animals (e.g., Hemmings et al., 2007; Garner et al., 2002) may be the result of an early shift from ventral and DMS to DLS control, resulting in the early formation of persistent CRs even in the absence of overtraining. During instrumental learning, DA depletion in the DMS striatum impairs the shift from R-O to S-R habit learning, without affecting instrumental performance (Faure et al.,

2005). In addition, single-unit recording studies have shown that there is an increase in firing of tonically active neurons in the striatum during overtraining, illustrating the importance of DA in the shift from R-O to S-R learning (Aosaki, Tsubokawa, Ishida, Wantanabe, Graybiel, & Kimura, 1994). Finally, evidence has shown that pharmacologically-induced behavioural sensitisation leads to an accelerated shift from R-O to S-R (habit) learning (Nelson & Killcross, 2006).

I argue that extinction deficits demonstrated by Garner et al. (2003) and Hemmings et al. (2007) could be explained exclusively in terms of the phenotype. Specifically, the finding that stereotypy animals have an increased latency to extinguish CRs is similar to what would be expected after overtraining. Because this effect appeared to be related to the frequency of occurrence and duration of stereotypic behaviour, it could be argued that DA receptor density in the ventral striatum may increase as a function of proportion of time budget engaged in stereotypy, resulting in increased afferent dorsal DA transmission from the NAcc to the caudate (Faure et al., 2005; McBride & Hemmings, 2005). In turn, this general increased striatal DA may lead to an accelerated shift during learning from goal-directed R-O strategies to S^D mediated S-R habit (e.g., Nelson & Killcross, 2006), resulting in perseveration during extinction trials.

To summarise, there is extensive evidence for the role of dysfunctional DA physiology in stereotypy and, theoretically, the striatum has been implicated as being the key neural structure in this dysfunction. In particular, it seems that upregulation of the mesoaccumbens pathway may be illustrative of endogenous neural and behavioural sensitisation. The final section of this review will propose a paradigm shift in the study of stereotypic behaviour to examine, based primarily on existing lesion and pharmacological studies of the different regions of the striatum, behavioural correlates of the ESP. Based on the evidence outlined above, it is possible that the striatum in general may be integral to the understanding of stereotypy, perhaps with different behavioural affectations associated with ventral and dorsal regions. An integrative model of the neurobiology of stereotypy will now be introduced with clearly operationalised variables and testable hypotheses.

Striatum Dysfunction and the Equine Stereotypy Phenotype

In this review, the neurobiology of learning and its relation to spontaneous stereotypy has been examined in detail. The striatum appears to play a modulatory role in some of the associated characteristics of animals displaying stereotypy, although there is some discrepancy as to the exact nature of this. Evidence for heterogeneous dysfunction in the basal ganglia is clearly defined, and it seems that the anomalous response patterns seen during instrumental procedures in stereotypic animals (e.g., Garner et al., 2003; Hemmings et al., 2007) may be the result of an enhanced feedback loop within the dorsal striatum, accompanied by a persistent enhanced appetitive state. As suggested by McBride and Hemmings (2005) the extinction findings may be the result of enhanced DA neurotransmission in the ventral aspects of the striatum, with the dorsal striatum (particularly the DLS) becoming more important in the latter stages of stereotypy.

Figure 1.7 shows a schematic of the aetiology of stereotypy, according to the evidence outlined in this review. Early-life stress, including particularly restricted feeding/foraging opportunities (but also social isolation during early development and/or environmental deprivation), interacts with genotype to lead to increases in mesoaccumbens DA transmission and stress-induced behavioural sensitisation, as well as decreases in transmission in the nigrostriatal pathway. The former has been shown to lead to increased locomotor response to AMPH challenge and also to increased behavioural stereotypy. The model would be expected to result in reduced sensitivity to delay, increased rate at which learning is shifted dorsally in the striatum, resulting in difficulties in some learning tasks (also mediated perhaps through decrease in DMS DA activity) and accelerated DLS-mediated S-R learning, and finally increased sensitivity to cues that predict reward. The latter may be expected to result in difficulties in maintaining R-O learning during complex cognitive tasks.

The clear differences in striatal DA receptor density found in crib-biting horses justify the model for this species, yet corroboratory behavioural evidence for this is currently lacking. If the hypotheses generated from the model are supported

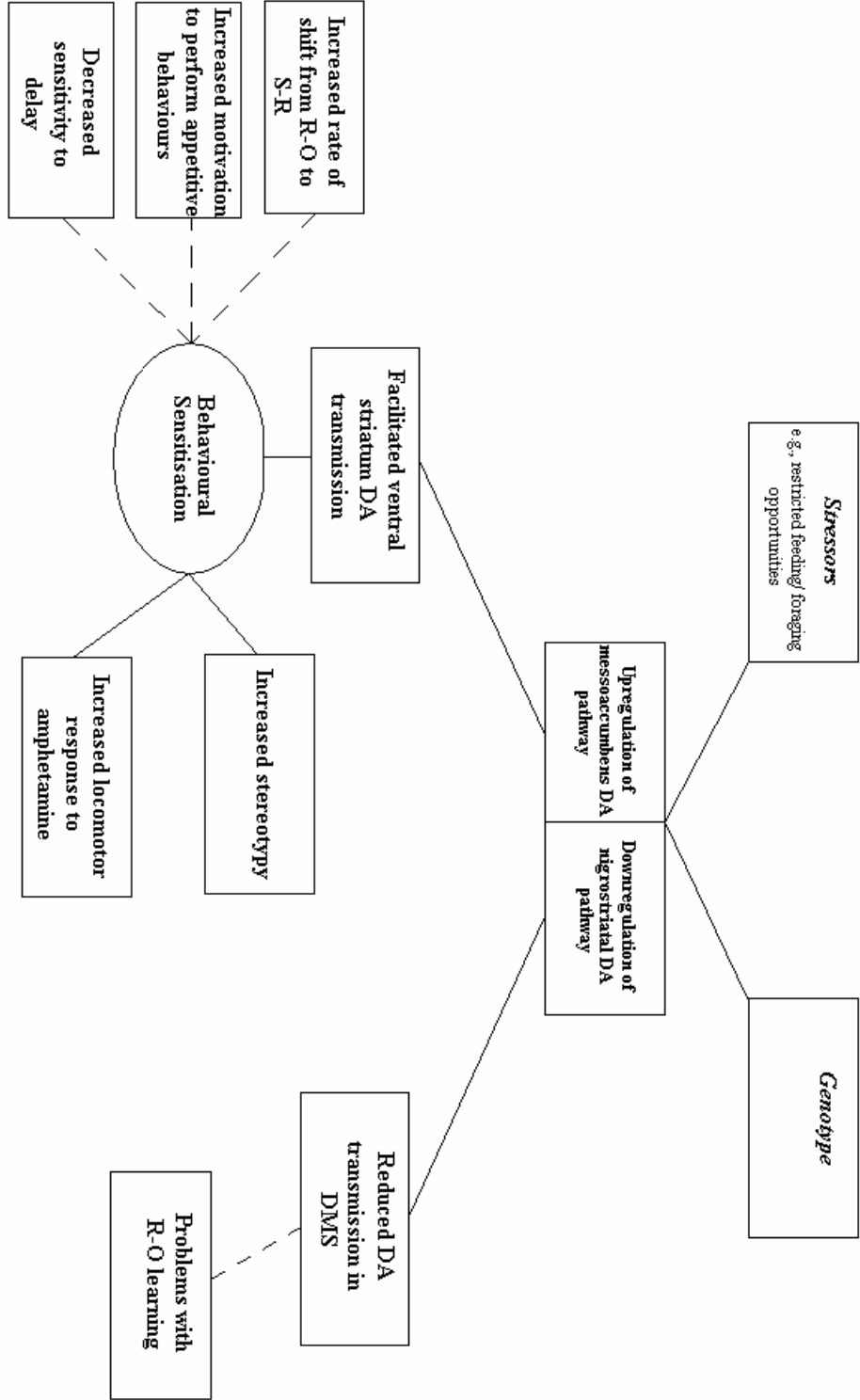


Figure 1.7. Schematic illustration of the stress-induced sensitisation model of stereotypy development. Note: Solid lines indicate features supported by existing evidence; dashed lines indicate features to be examined in the thesis. DA = dopamine; R-O = response – outcome; S-R = stimulus-response.

in this population, owing to the abundance of evidence from other species, further research may be justified in order to assess the model's external-validity. We are offered a number of operationally defined and testable hypotheses, incorporating a battery of rigorously designed behavioural assays. First, it is possible to examine specifically the behavioural correlates of dorsal striatum dysfunction specifically by employing spatial navigation paradigms. For example, DMS lesions are known to affect various aspects of navigation, such as acquisition time and search strategies (Devan et al., 1996). In addition, increased motivation to perform appetitive behaviours could be examined in the context of sensitivity of to Pavlovian cues by placing crib-biting horses in a PIT procedure (Wyvell & Berridge, 2001). It may be expected that unregulated mesoaccumbens DA systems would result in a heightened PIT in the crib-biting horses.

Behavioural sensitisation following AMPH exposure leads to animals showing a reduction in sensitivity to delay in instrumental procedures (Wei-min et al., 2008). We suggest that stress-induced sensitisation may lead to similar behavioural changes. In order to examine this, crib-biting horses could initially be trained in a free-operant, reinforcer-delay paradigm (concurrent chain schedules) where it is possible to assess choice by examining relative response ratios according to different relative reinforcement schedules on two-choice alternatives. In addition, crib-biting horses could be trained in a discrete trial, 'place/response' t-maze paradigm, where subjects are trained always to 'turn left' for food at a choice point. After a number of training trials, the horses would be introduced to the maze from the opposite side to training. If they adopt a response strategy, this would suggest that they are using S-R learning, whereas if they turned right (i.e., where the food was in the training trials) this would suggest R-O learning. Based on the stress-induced behavioural sensitisation hypothesis, we would expect this shift in crib-biting horses at an earlier stage than non-crib-biting counterparts (Jedynak et al., 2007; Nelson & Kilcross, 2006).

One of the features of the Model yet to be examined is the role of striosome and matrix-cell imbalance within the context of endogenous neurophysiological change. This is undoubtedly a crucial component of any aetiological formulation

of spontaneous stereotypy, and will need to be examined more closely in the future. For now, I have left this component out of the Model for simplification, concentrating on aspects of stereotypy development that are testable in the context of behavioural methodologies. However, it may be possible to make inferences regarding this feature of the Model if we find differences in the phenotype relating particularly to R-O – S-R learning. If, as we predict, the ESP causes an accelerated shift to S-R learning, this may be indicative of overrepresentation of striosome cells in the output pathways in the basal ganglia.

During the course of this thesis, I will examine each of these hypotheses using crib-biting horses, using the Model as a framework. The results from these studies will not only provide valuable insight into the behavioural correlates of the neurobiological factors associated with equine stereotypy, but it will also go some way into the further systematic development of a more general stereotypy phenotype.

Spatial Learning

Analysis of Spatial Learning Strategies in Crib-Biting Horses

In the first experimental chapter of this thesis, I explored the behavioural effects of the reported reduction of DA receptors found within the DMS of horses displaying the behavioural ESP (McBride & Hemmings, 2005). The DMS was found to be structurally intact within the brains of crib-biting horses; however, it is fairly well established that DA efferents to the DMS is a crucial mediator of its role in learning (e.g., Balliène & Dickinson, 1998; Yin & Knowlton, 2006). Thus, a reduction in DA receptor cells in this region of the striatum may be illustrative of reduced functionality and may theoretically be equated to lesions of this region.

The DMS is thought to mediate R-O learning (Yin & Knowlton, 2006). Early in the learning process, evaluation of reinforcers and reward processing appears to be mediated predominantly by DA activity in the mesoaccumbens pathway (e.g., Everitt & Robbins, 2005; Yin & Knowlton, 2006). The planning of actions and responses relating to the reinforcers and their associated cues is mediated, in turn, by mesolimbic innervation of the DMS (Yin & Knowlton,

2006). This has been demonstrated experimentally in the context of outcome devaluation. In devaluation procedures, typically the subject is trained for either a short (e.g., 10-days) or an extensive (e.g., 30-days) period on an instrumental procedure. Following training the reinforcer is devalued, and the animal is re-introduced to the apparatus. Typically, following modest periods of training, the animal will be sensitive to the devaluation and attenuate, or significantly reduce, instrumental responding. After lengthy periods, however, the subject will typically continue to respond at pre-treatment levels. This procedure demonstrates that after extensive training (overtraining), learning shifts from planned action (R-O) where the animal has some kind of ‘cognitive’ representation of the link between the S^D , the response and the reinforcer, to more habitual response patterns (S-R), where the animal is responding in a more automated fashion, driven by the S^D (Balleine & Dickinson, 1998).

Yin et al. (2005) trained rats in a standard instrumental procedure. Following training, the reinforcer was devalued (LiCl) and the rats’ DMS were exotoxically lesioned. Typically, following modest periods of training, rats will be sensitive to the devaluation procedure, and instrumental responses in the learnt context will attenuate. However, they found that DMS lesioned rats showed insensitivity to the devaluation procedure, continuing to respond at similar rates (Yin et al., 2005). This suggested that the DMS was crucial for R-O learning. Lesions of the DLS, however, have the opposite effect; that is, they render the animal sensitive to outcome devaluation, even following extensive periods of training (Yin et al., 2005).

DMS lesions in rats have also been seen to affect guidance-based, allocentric spatial navigation. For example, Devan, McDonald, and White (1999) lesioned the DMS and DLS of rats and examined their performance in a series of Watermaze tasks. In Stage 1, they trained rats to locate a hidden platform in the NW quadrant of the Watermaze. In Stage 2, they trained rats to locate a visible platform located in various locations in a different pool. In Stage 3, they initially re-trained rats in the original pool with the hidden platform in the NW quadrant, followed by four trials with the visible platform in the SE quadrant. They found

that rats with pre-training lesions of the DMS, but not DLS, displayed longer escape latencies during the early stages of Stage 1 and Stage 2, as well as prolonged Thigmotaxis (tendency to stay close to the wall of the maze) in both stages. In the final stage, the DLS and control groups both showed no cue competition, choosing the visible and hidden platforms equally often. However, the DMS lesion group showed a significant increase in choices for the visible platform, showing this group responded preferentially to local, as opposed to distal spatial cues.

Devan et al's (1999) findings suggest that the DMS may be crucial for allocentric spatial navigation tasks. The reason for this appears to be related to the role of the DMS in integrating R-O and S-R learning, possibly mediated by limbic innervation of the striatum (Devan et al., 1999; O'Keefe & Nadel, 1978). Spatial learning is known to be mediated by hippocampal afferents to the striatum (O'Keefe & Nadel, 1978), and it has been suggested that the DMS acts to integrate limbic input and subsequent response output in the context of spatial learning (Devan et al., 1999).

The following experiments were designed to examine the effects on allocentric spatial navigation of possible DMS dysregulation associated with the ESP (McBride & Hemmings, 2005). Initially, we designed a simple circular field-maze, with a number of available local cues, in order to examine food approach latency and search strategy in crib-biting and non-crib-biting horses. In the second experiment, we examined spatial learning in a rectangular maze, this time examining competition between learning about distal and local cues.

Experiment 1

Crib-biting horses have reduced densities of D1-like and D2-like receptors in the DMS (McBride & Hemmings, 2005), but the specific behavioural effects of this feature of the ESP are yet to be established. In order to examine the behavioural correlates of the proposed DMS dysfunction in crib-biting horses, we designed a discrete trial spatial navigation procedure. The horses were assessed for crib-

biting status, and subsequently introduced to a round arena with food located in one of seven identical buckets, all placed around the circumference of the arena. Initially, food approach latency was recorded for each trial-block, as well as their tendency for Thigmotaxis (operationalised as proportion of route within 2m of the perimeter, as well as total route length), were measured as a function of crib-biting status. Thigmotaxis in early training trials is common in many species. In this context, the optimum strategy would be first to explore the perimeter (i.e., as this allows the animal to search all of the buckets for food) but in later trials, adopt a more direct route (O'Keefe & Nadel, 1978). Based on previous evidence from other species (Devan et al., 1999), we expected crib-biting horses to show increased food approach latency and increased latency to adopt a more direct route to the food through the training trials (i.e., an indication of Thigmotaxis).

Method

Participants

Sixteen horses (N = 8 crib-biters and N = 8 non-crib-biters) of various breeds were recruited for the study. Each of the crib-biters was matched as far as possible to each control, with each control coming from the same institution. Each of the groups consisted of three mature geldings and five mares (ages of horses was often unknown precisely by owners, therefore this information is excluded).

Apparatus

A circular arena (Figure 2.1) was constructed using plastic stakes (1m high) and nylon tape in a dressage arena or similar (e.g., sand arena, exercise yard, etc.). The arena was 10 m in diameter, and around the circle were located four closable entrance gates, A, B, C, and D. Around the perimeter of the arena were located seven identical black plastic buckets, one of which was baited trial-by-trial with a

small handful (approximately 5g) of pelleted feed (Spiller's Hi-Fibre cubes). The buckets were spaced approximately evenly apart between the entrance gates. For half of the participants (i.e., $n = 4$ crib-biters and $n = 4$ controls) the baited bucket was located as in Figure 4.1a, and for the other half (i.e., $n = 4$ crib-biters and $n = 4$ controls) the baited bucket was located as in Figure 2.1b.

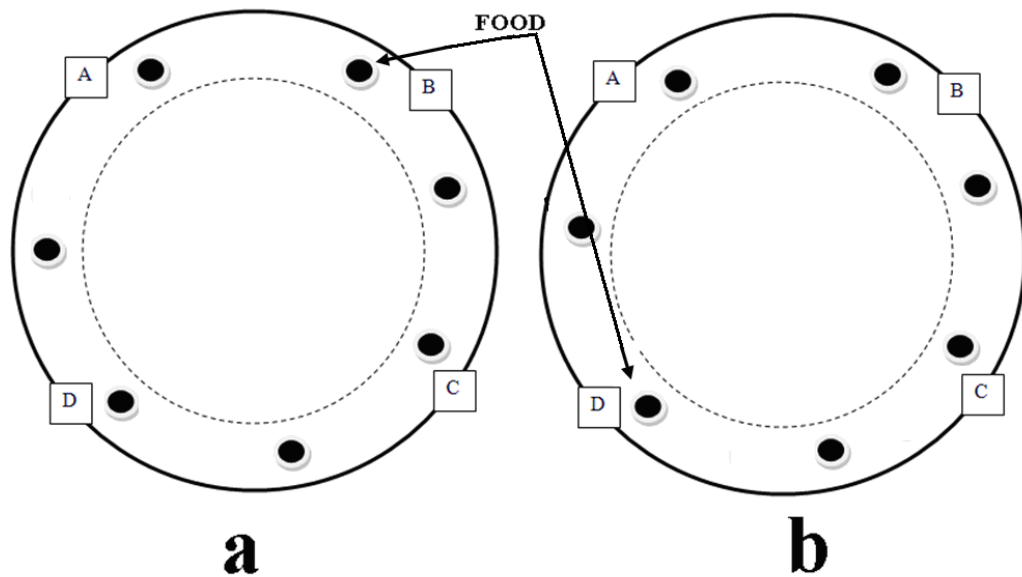


Figure 2.1. Design of circular arena ($D = 10$ m). The arena was constructed using plastic stakes and nylon tape. For half of the participants, the food was located as depicted in maze 'a', and for the other half, it was located as depicted in maze 'b'. The boxes A, B, C, and D represent the entrance gates in the maze. The order of entry was counterbalanced across participants and across trials.

Procedure

In order to confirm the crib-biting status of the horses, all subjects were given two handfuls of concentrate feed and observed for five-min. Crib-biting is known to be most prominent *post prandium*, and this has been found to be an effective measure for inducing the behaviour (McBride & Hemmings, 2005). All horses were handled by someone used to handling horses during the trials. This was to ensure that the horses remained calm during testing and for health and safety

reasons. Before commencing data collection, all horses were introduced to the arena without any food in the buckets. They were led around the external perimeter and the inside of the arena. One of the buckets (see Figure 2.1) was then baited with a small amount of the pelleted food. Each horse took part in 20 reinforced trials, split into five blocks of four. There was a 30-second break between each block. During the trials, the horse was led to one of the entrance gates and released into the arena, after which the gate was closed while the horse located and consumed the food. The order of entrances was counterbalanced across participants and across trials, pseudo-randomly. Once the horse had located and consumed the food, the handler entered the arena and led the horse out to the perimeter. The time taken to locate the food in each trial was recorded, as was the route that the horse took. The latter was achieved by recording the route taken on a scaled-down illustration of the maze (Appendix A).

The dependent measures were food approach latency in each block of four trials and Thigmotaxis, the latter operationalised as time spent during each trial within 2m of the perimeter of the arena. Owing to the lay-out of the maze and organisation of the buckets, during each trial-block two of the trials would be likely to indicate apparent Thigmotaxis in all participants. To explain, in Figure 2.1a, for example, the baited bucket lies immediately proximate to entrance 'B' as well as in relatively close proximity to entrances 'A' and 'C' (i.e., relative to 'D'). For this reason, only trials that commenced from entrances 'D' were used for analysis (this changed across subjects according to the counterbalance schedule).

Results and Discussion

All raw data was entered into SPSS[®] 15 for Windows for analysis. Statistical analyses are reported with respect to an α level of 0.05. All horses were observed prior to commencing data collection. It was confirmed that all of the crib-biters performed crib-biting and that none of the control group performed that, or any other form of stereotypic behaviour. Owing to an administrative error, paths taken in the periphery of the arena for three of the horses (1001-1003) were lost

and therefore omitted from analysis. However, the food approach latency data was available for these animals.

Figure 2.2 displays the mean food approach latencies for each group, as a function of trial-block. As is clear, both groups learned to locate the goal box at similar rates, with subjects taking longer in early trials, but quickly learning the location of the baited bucket. All food approach latency data was logarithmically transformed (base-10) prior to analysis, to normalise the distribution. A mixed-design, 2×2 analysis of variance (ANOVA) on the food approach latency data, with group as the between-subjects and trial-block as the within-subjects factor revealed a significant main effect for trial-block, $F(4, 56) = 21.87$. There was no significant main effect for group, nor was there a group \times trial-block interaction ($F_s < 1$). Post-hoc analyses, corrected for multiple tests, showed food approach latency significantly decreased after Block 1 (all $p_s < 0.01$), but no other significant differences between blocks.

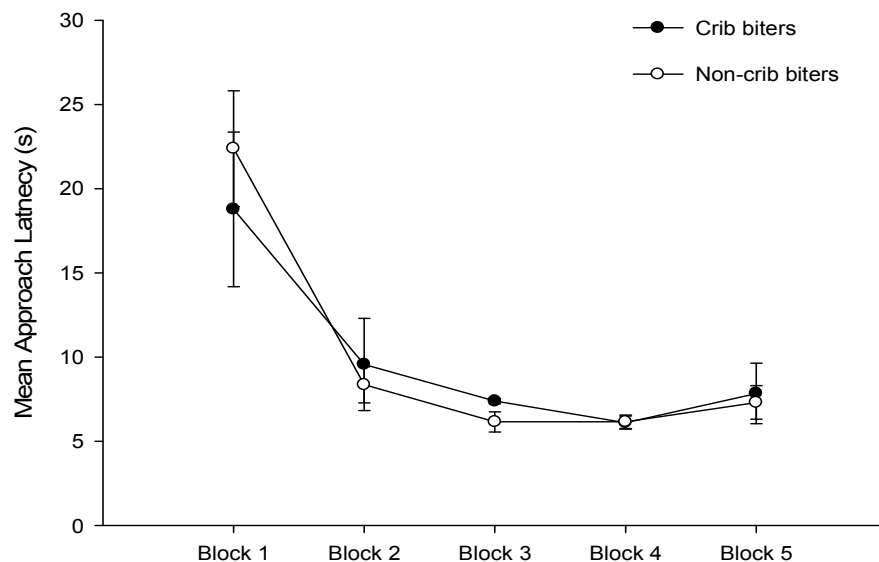


Figure 2.2. Mean food approach latencies (sec; \pm SE) of crib-biters and non-crib-biters as a function of trial-block. Each trial block consisted of four-trials.

Figure 2.3 illustrates the proportion of time spent in the periphery of the arena in both groups as a function of trial-block. The crib-biters showed similar

proportions across blocks whereas the non-crib-biters appear to have decreased across the initial trials, but increased again in the final session. All data were arcsine-root³ transformed prior to analysis to normalise the distribution of proportions. A mixed design 2×2 ANOVA on the peripheral arena data, with group as the between-subjects and Trial-Block as the within-subjects factor, revealed no significant main effect for Trial-Block and no significant group \times Trial-Block interaction, $F_s < 1$. However, there was a significant main effect for group, with the crib-biters spending significantly more time in the outer perimeter of the arena, $F(1, 11) = 4.99$. Post-hoc, between-subjects t-tests revealed a significant effect for group in Trial-Block 4, $t(11) = 4.01$. There was a group effect found in Trial-Block 3 and this approached significance, $t(11) = 1.98$, $p = 0.07$. No other significant differences were found between the groups (all $p_s > 0.1$).

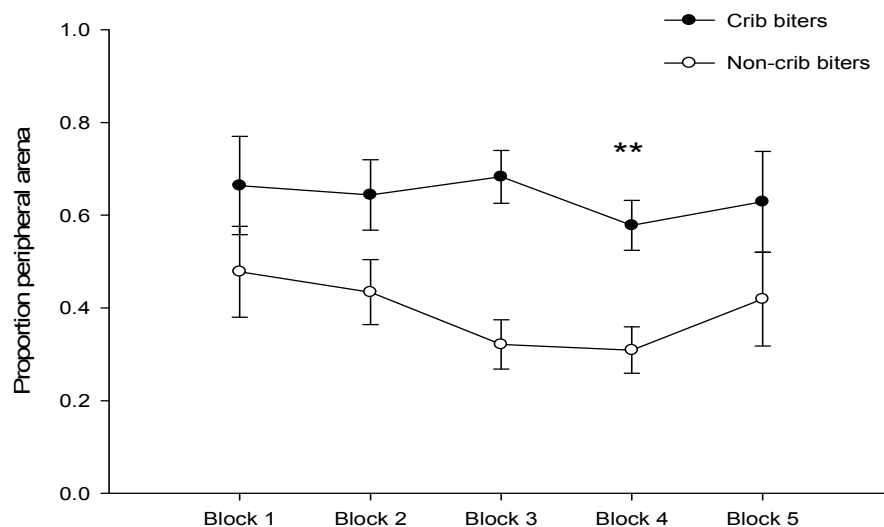


Figure 2.3. Mean proportion (\pm SE) of time spent in the peripheral area of the arena for crib-biters and non-crib-biters as a function of trial-block. Means relate only to trials in each block commencing from non-proximal entrance points (see above for explanation). *Note:* ** $p < 0.01$.

On initial analysis of the route data, we observed that the crib-biters appeared to be following repetitive routes during trials, regardless of the location

³ For proportion data, arcsine-root transformations are a suitable method for normalising the distribution owing to the natural internal skew of proportion scales.

of the baited bucket. Specifically (see Figure 2.1a), if the horse started its journey at entrance ‘A’, its most efficient strategy would be to turn left to access the bucket, while on entering at C it would be best to turn right. Thus within a set of four trials a horse should turn in both directions 50% of the time. However, we observed that the crib-biters, rather than switching, would persistently move in the same direction, often resulting in their following an apparently less-effective route. In order to examine this more closely, each set of four trials was assessed as to whether the horses persistently turned left or right. In each block of four trials, the horse would be expected to turn left and right twice (i.e., 50% each). We developed an index of direction as the proportion of trials in which the horse persevered in the same direction. To explain, if in a block of four trials the horse turned the same way three or more times (e.g., LLRL, RRLR or any other similar combination), this would receive an index of 75%. Similarly, if the horses turned the same way twice (e.g., LLRR, RRLL or similar), they received an index of 50%.

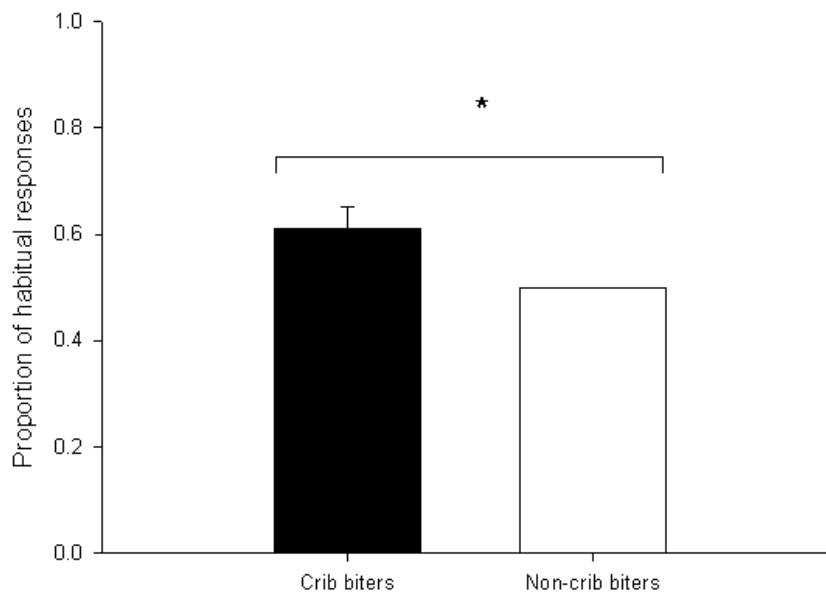


Figure 2.4. Mean (\pm SE) proportion of habitual responses during the final two trial-blocks (eight trials). The optimum strategy would be to turn left/right 50% of the time in each block. *Note:* * $p < 0.05$.

We were interested in whether the horses were using the best strategy or whether they were adopting a habitual pattern (e.g., always turning right/left). It

would be expected that if they were learning the most appropriate strategy, they would persist with one direction initially, but would adopt a best-strategy approach in later trial blocks. We only analysed the data from the final two trial blocks, to ensure that both groups had sufficient opportunity to learn the location of the baited bucket. The data are displayed in Figure 2.4. As is clear from the graph, the non-crib-biters turned right/left equally in the final two trial blocks, but the crib-biters were more likely to persist habitually in one direction. An independent samples t-test (equal variances not assumed) revealed that this difference was significant, $t(5) = 2.74$.

The aim of the present study was to examine behavioural effects of DMS dysfunction in the ESP. It was predicted that crib-biting horses would show increased approach latency and increased time spent in the peripheral of the arena during the early stages of training in a circular maze. Although we found no evidence to support the first hypothesis, we did find support for the second, in that crib-biters spent significantly more time in the outer perimeter of the arena during testing. However, neither group showed any significant decrease in time spent in the peripheral area across the trial-blocks. In addition, we found that crib-biters tended to follow habitual routes, even in the latter stages of learning, whereas non-crib-biters learnt to adopt the most efficient strategy.

Rats with DMS lesions show retarded acquisition rates in the Morris Watermaze in the context of both hidden and visible platforms (Devan et al., 1999). Devan et al. (1999) posited that this increased acquisition was closely correlated with increased peripheral pool time (Thigmotaxis) in the lesioned sample. Thigmotaxis is often seen in many species during early spatial-learning trials. During initial exploration of a novel space animals typically remain close to, or even in contact with the perimeter (Barnett, 1968). Claimed to be essential in the early stages of learning about a novel environment, Thigmotaxis allows the organism to form a 'cognitive map' by outlining the boundaries of the space (O'Keefe & Nadel, 1978). It is also seen as a 'fear' response, allowing the organism to explore the space from the relative safety of the wall (Barnett, 1968), and because of this Thigmotaxis is thought to be related to anxiety. This is

reflected in pharmacological studies where, for example, prolonged Thigmotaxis is observed in rats in response to administration of anxiogenic agents (e.g., dexAMPH: Simon, Dupuis, & Costentin, 1994) and is reduced with anxiolitics (e.g., phenobarbitol: Treit & Fundytus, 1988).

It may be that the DMS lesioned rats, as well as the crib-biting horses in the present study, were showing increased anxiety and maintaining proximity to the relative ‘safety’ of the perimeter. However, Devan et al. (1999) also tested DMS lesioned rats in an open-field arena (i.e., a dry arena). In this novel context, the rats were tested seven days after the water-maze for Thigmotaxis. They were placed in the centre of the dry arena for five-min and their searching behaviour examined. They found no group differences in Thigmotaxis in this context, suggesting that anxiety may not explain the increased rates in the DMS lesioned group.

The DMS is essential for the integration of responses and associated outcomes (R-O learning; Yin & Knowton, 2006). It could be that animals with dysfunction of this region may find this integration problematic. Increased levels of peripheral-maze time could be explained in this context, as it may be indicative of a failure to integrate local (i.e., the buckets) and distal (i.e., peripheral global cues around the arena) allocentric spatial cues, with the crib-biters relying more on local cues. Whishaw, Mittleman, Bunch, and Dunnett (1987) found that rats with DMS lesions were able to navigate to a goal using distal and local cues where necessary, but were more likely to utilise a taxon (i.e., local cue-based) strategy if this is available. In the present study, this may explain why the crib-biting horses spent more time in the outer perimeter of the arena. To explain, buckets (dummy and baited) were placed around the perimeter of the arena during trials. It may be that the crib-biters were systematically searching the buckets, which were acting as local cues, with the non-crib-biter group using distal spatial cues more effectively to locate the baited bucket, thereby following more direct routes. If this study were to be repeated, it may be prudent to remove the buckets in a probe trial to examine where the animals searched.

This is further supported by our findings relating to horses' habitual responses. In the final two trial-blocks, crib-biters were found to turn the same way, habitually, regardless of the optimum strategy. The optimum strategy within the context of our maze was to turn left/right 50% of the time. However, the crib-biters failed to do this, tending to turn the same way significantly more often. This suggests that instead of the crib-biters using the available distal spatial cues to locate the baited bucket, they were relying primarily on local cues (i.e., the buckets). This indicates that DMS dysfunction associated with the ESP (McBride & Hemmings, 2005) may interrupt the integration of local and distal cues which aid optimum navigation (Devan et al., 1999). DMS lesions have been found to result in the early formation of S-R habits (see Yin & Knowton, 2006, for a review). The reason for this appears to be related to the role of the DMS in the integration of R-O judgements made during task learning (Devan, Goad, & Petrie, 1996). The increased habitual responses in late trials by the crib-biters may reflect this. In order to examine this in more detail, we designed a second allocentric spatial navigation experiment. This time, we reasoned that the addition of a salient local cue would disrupt place learning in crib-biting horses.

Experiment 2

The results of Experiment 1 showed that there was little difference between crib-biters and non-crib-biters in terms of their food approach latencies in a circular maze task. However, crib-biters did show increased time spent in the periphery of the arena, suggesting they were systematically searching the buckets around the perimeter. We suggested that this may be indicative of the crib-biters failing to integrate local (i.e., the buckets) and global (i.e., global peripheral cues) owing to DMS dysfunction. Therefore, in Experiment 2, we examined specifically local and distal cue competition in crib-biting and non-crib-biting horses.

All subjects were trained to locate food in one of two visually discriminable containers within a rectangular arena, split into four arbitrary

quadrants (see Figure 2.5). In Phase 1, all horses were trained to locate the baited bucket (CS+) which was always located in the NE of the arena (the decoy bucket [CS-] was located in the SW of the arena). In Phase 2, horses were introduced to the arena empty (CS₀); in Phase 3 the locations of CS+ and CS- were switched (e.g., CS+ = SW, CS- = NE [CS-CS+]); in Phase 4, the horses were introduced to the arena with 2 × CS+, located in NE and SW respectively. Cue integration was operationalised as time spent in the quadrant where CS+ was previously located during the test phases. If crib-biters display enhanced local-cue-biased responding and fail to integrate local and distal cues as we expect, it could be predicted that they would spend equal time in NE and SW during Phase 2 and Phase 4, whereas the non-crib-biters may be expected to prefer the NE quadrant in both instances. However, in Phase 3, we would expect the crib-biters to spend more time in the incorrect quadrant, whereas the non-crib-biters should spend equal time in the two (NE & SW) quadrants.

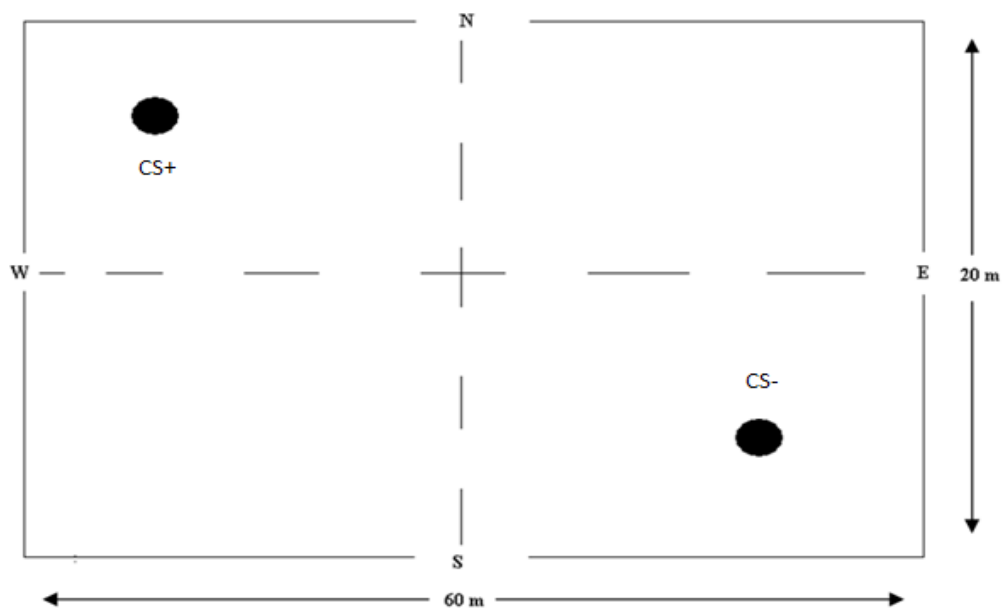


Figure 2.5. Schematic of the arenas. The dimensions were 20m x 60m. The location of the baited bucket (CS+) was counterbalanced between the NW and SE across participants, as was the location of the decoy bucket (CS-; see Figure 2.6).

Method

Subjects

Nine mature horses (N = 5 crib-biters and N = 4⁴ controls) from two different institutions were recruited for this study. There were two crib-biting geldings and three crib-biting mares, and two non-crib-biting geldings and two non-crib-biting mares. The precise ages of the horses were not available, owing to diverse backgrounds and acquisitions, therefore these data were omitted. All lived in captivity, and were regularly used for riding. All of the subjects used for the study were kept predominantly in a field with other horses. However, they were periodically kept in stables (stalls) during winter months to avoid deleterious effects on grazing. At the time of the study, all of the horses were living in the field exclusively. In addition, matched control horses were selected from the same establishment as the crib-biters to control for extraneous variability.

Apparatus

The trials were carried out in rectangular arenas measuring 20m x 60m. One arena was located in a disused barn, and the floor was concrete covered with a layer of sand. The second arena was the same dimensions, but located outdoors. Surrounding both arenas were located a large number of distal cues, including trees, out-buildings and hedgerows for the outdoor arena and hay-bales, doorways and pillars in the indoor arena. Each of the arenas were divided arbitrarily into quadrants along the horizontal (20m width-wise) and vertical (60m length-wise) (see Figure 2.5). Two plastic buckets, one yellow (Figure 2.6a) and one black (Figure 2.6b), were used in the study and were located either in the NW or SE of the arena. Horses are dichromats and should be able to visually discriminate between yellow and black objects based on colour alone (McGreevy 2004). The location of CS+ was counterbalanced between the yellow and black buckets and across locations, across subject-pairs. The food used in the training trials

⁴ Nb. We initially started with n = 5 control horses, but one of the subjects became unwell during the course of the experiment, and was unable to continue.

comprised of a handful (approx 5g) of pelleted horse feed (Spiller's Hi-Fibre cubes).



Figure 2.6. Food was located in one of the buckets. Bucket ‘a’ was yellow and measured 70cm diameter; Bucket ‘b’ was black and measured 30cm diameter. The location of the food was counterbalanced between the buckets across pairs of subjects.

Procedure

Initially, all horses were assessed for crib-biting status (as described in Experiment 1). Before commencing training, each subject was walked around the perimeter of the arena wearing a head-collar and lead-rope, to habituate them to the experimental set-up. This was always carried out by a naïve third party who was used to dealing with the subject. In all subjects, for the first trial, the leader led the horse to CS+ with a lead-rope and ensured that the horse consumed the food. Each horse then took part in nine⁵ further training trials. Before each trial, the leader took the horse to one of the four start-points (i.e., i.e., N, S, E, W; see

⁵ In Experiment 1, the animals took part in 20 training trials. However, we noted that all of the horses acquired the location of the food very quickly, and we felt that 10 trials would be sufficient for this experiment.

Figure 2.5). During each training trial the subject was released from its lead-rope, facing the wall, and allowed to explore the arena freely. Following location of CS+, the subject was allowed to consume the food, the lead rope was re-attached, and the horse was led out of the arena. The horse remained outside the arena with the Leader for 30-sec and was then led back into the arena and to the next start-point. The order of start points was randomised across trials.

Following training, the subjects took part in three test-trials in extinction. The order of these trials was counterbalanced across subjects, and each was interspersed with a re-training trial. Subjects were released from either the E or W start-points for all test-trials (see Figure 2.5). The proportion of time subjects spent in the correct quadrant (i.e., the previous location of the baited bucket) was examined for a period of 45-seconds. For Test-Trial 1, the horse was introduced to the arena with no buckets (CS₀) present. For Test-Trial 2, the buckets were switched (CS-CS+; i.e., in Figure 2.5, CS+ would be placed in the SE corner, and CS- in the NW). For Test-Trial 3, the arena contained 2 × CS+.

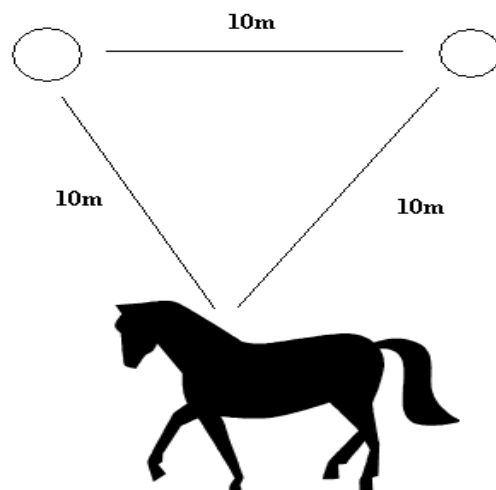


Figure 2.7. Schematic illustration of conditioned discrimination trial.

In order to examine whether the horses could differentiate between the buckets, we carried out a conditioned discrimination trial at the end of testing. During this, the two empty buckets were placed 10m apart at one end of the arena (n.b., this was always the opposite end to the previous location of CS+). The

subject was released approximately 10m from the buckets and we noted which bucket was approached (Figure 2.7).

Results and Discussion

All horses were observed prior to commencing data collection. It was confirmed that all of the crib-biters performed crib-biting and no other stereotypic behaviours, and further that none of the control group performed crib-biting, or any other form of stereotypic behaviour. All raw data was entered into SPSS® 15 for Windows for analysis. Prior to analysis, food approach latency data were log (base-10) transformed and proportion data arcsine-root transformed to normalise the distribution. All statistical analyses are reported in respect to a Type I error rate 0.05.

Training data are displayed in Figure 2.8 and 2.9. Figure 2.8 pertains to food approach latency and Figure 2.9 to the proportion of deviation (error) from the optimal route for both groups across the nine training-trials. The latter was calculated by measuring the total route taken by the horse from the start point to the baited bucket, and dividing this by the distance from the start point to the bucket. Both of these measures were examined using mixed design 2 (group) \times 9 (trial) ANOVAs. The latter was included to remove the effect of individual differences in motility speed of the subjects. As is clear from Figure 2.8, the variance in the approach latencies for both groups is relatively large (i.e., as compared to Figure 2.9), and there were no significant main effects with respect to approach latency for trial or group, $F_s < 1$, nor was there a significant group \times trial interaction, $F(8, 56) = 1.34$.

However, with respect to error (see Figure 2.9), there was a significant main effect for trial, $F(8, 56) = 5.14$, and a significant trial \times group interaction, $F(8, 56) = 2.70$. There was also a trend towards a main effect for group, but this fell short of significance, $F(1, 7) = 4.33$, $p = 0.07$. Post-hoc analyses, corrected for multiple tests, revealed that subjects made a lower proportion of error in Trial

1 as compared to trials 2-9, (all $ps < 0.05$). It seems that in this context the crib-biters quickly learnt a direct route to the food, whereas the non-crib-biters took significantly longer (5 – 7 trials) to learn the location of the food.

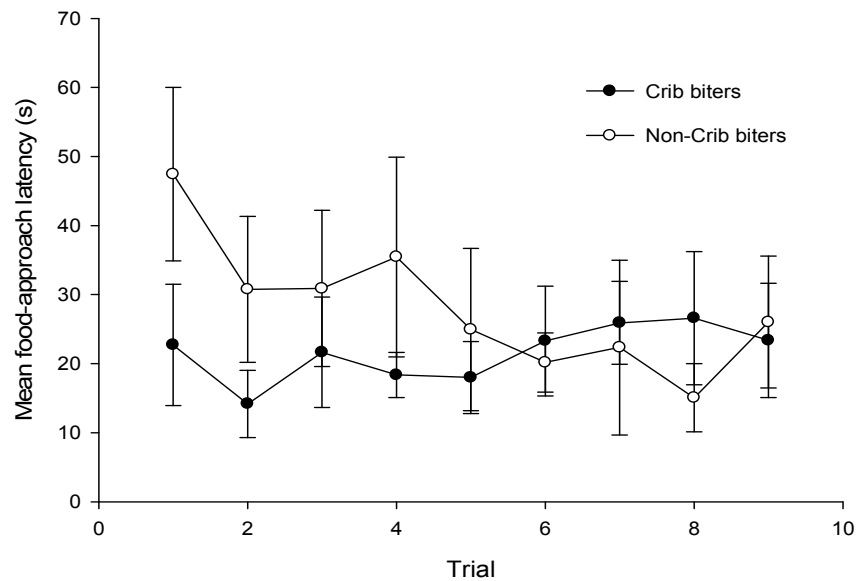


Figure 2.8. Mean (\pm SEM) approach latencies (s) of crib-biters and non-crib-biters in open field maze.

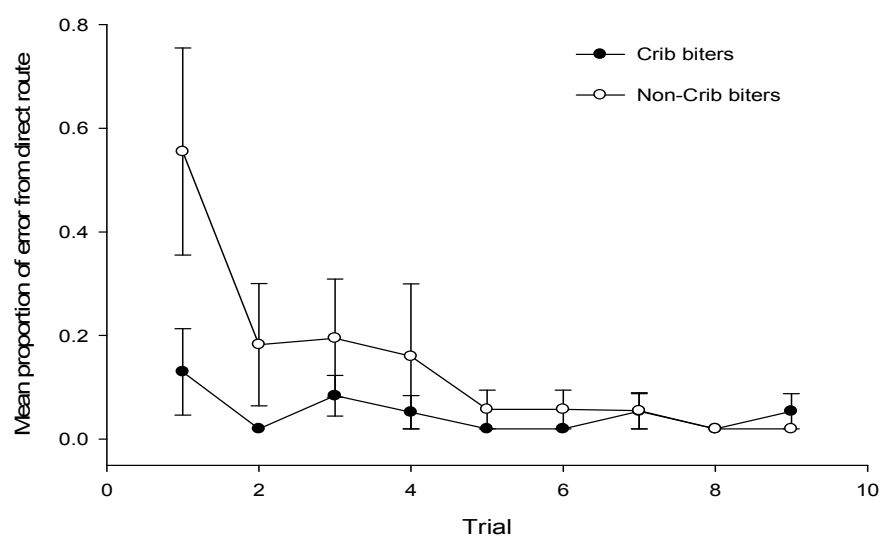


Figure 2.9. Mean (\pm SEM) proportion of error from optimal route of crib-biters and non-crib-biters.

During the three probe trials, use of place cues (as opposed to local cues) was operationalised as time spent in the previously reinforced quadrant of the arena (i.e., previous location of CS+). Figure 2.10 illustrates the proportion of time spent in each quadrant during the probe trials for each group. A mixed design 2 x 3 ANOVA, with crib-biting status as the factor and proportion of time spent in the correct quadrant in each of the three probe trials as the dependent measure, revealed no significant main effect for trial or group, nor was there an interaction (all F s < 1). In addition, a 2 x 3 ANOVA on the latency to approach the area of the arena where CS+ had been previously located between the groups (see Figure 2.11) revealed no significant main effects for group or trial, nor was there a group x trial interaction (all F s < 1).

One-sample t -tests, with the test value set at 0.25, revealed that the crib-biters spent significantly more time than would be predicted by chance in the correct quadrant for CS₀, $t(4) = 2.77$, and for 2 x CS+, $t(4) = 4.03$ conditions.

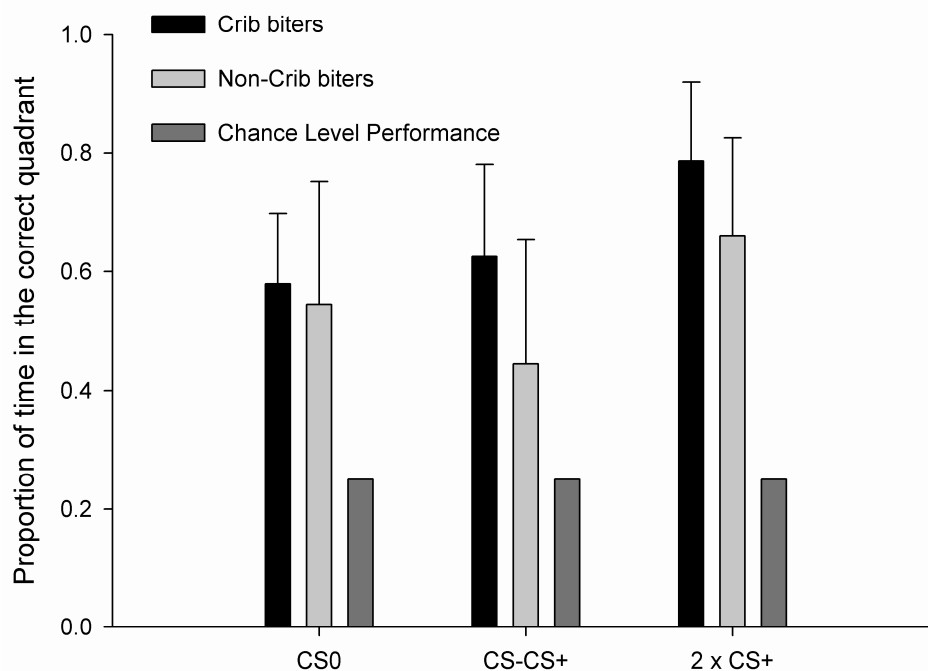


Figure 2.10. Mean (\pm SEM) proportion of time spent in the quadrant where food had been previously located (correct quadrant) during three probe trials. Chance Level Performance was set at 0.25.

They also spent more time than would be predicted by chance in the correct quadrant in the CS-CS+ condition, and this approached significance, $t(4) = 2.39$, $p = .07$. However, for the non-crib-biters, there was no significant difference from chance in the CS₀, $t(3) = 1.43$, and for the CS-CS+, $t < 1$, conditions. The non-crib-biters spent more time than would be predicted by chance in the correct quadrant in the 2 x CS+ condition, but this fell marginally short of significance, $t(3) = 2.50$, $p = 0.08$.

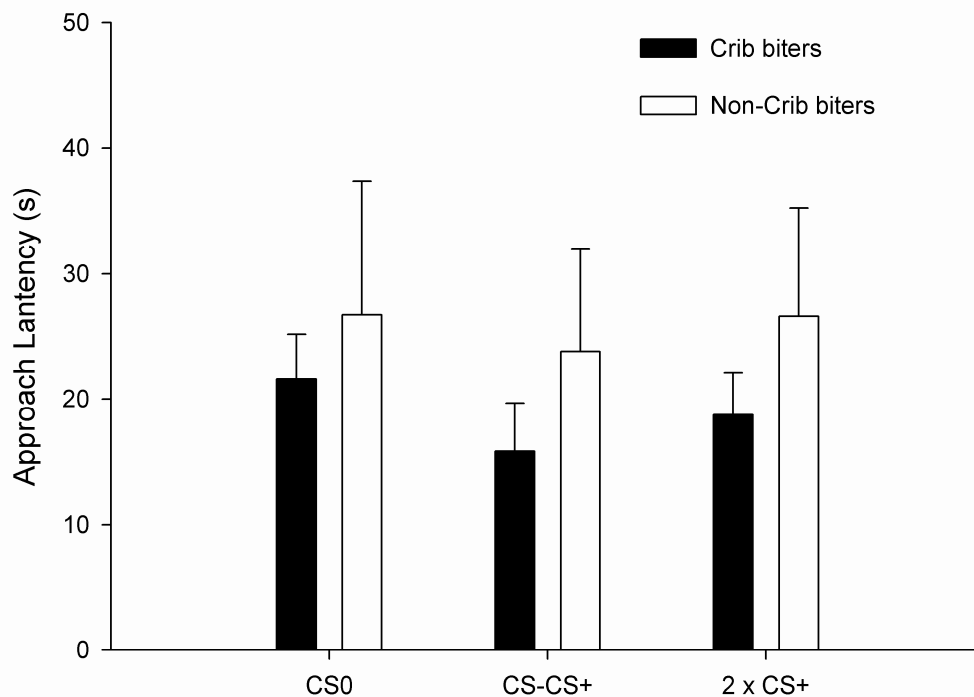


Figure 2.11. Mean (\pm SEM) approach latencies (s) during probe trials.

Following the final probe trial, all of the subjects were subjected to a single conditioned discrimination trial. Figure 2.12 displays the frequency that each bucket was chosen by members of each group. Although the crib-biters were

marginally more likely to choose CS+ and non-crib-biters were more likely to choose CS-, a chi-squared test revealed that there was no significant difference between the groups in terms of their preference for either bucket during the trial, $\chi^2(1) = 0.53$.

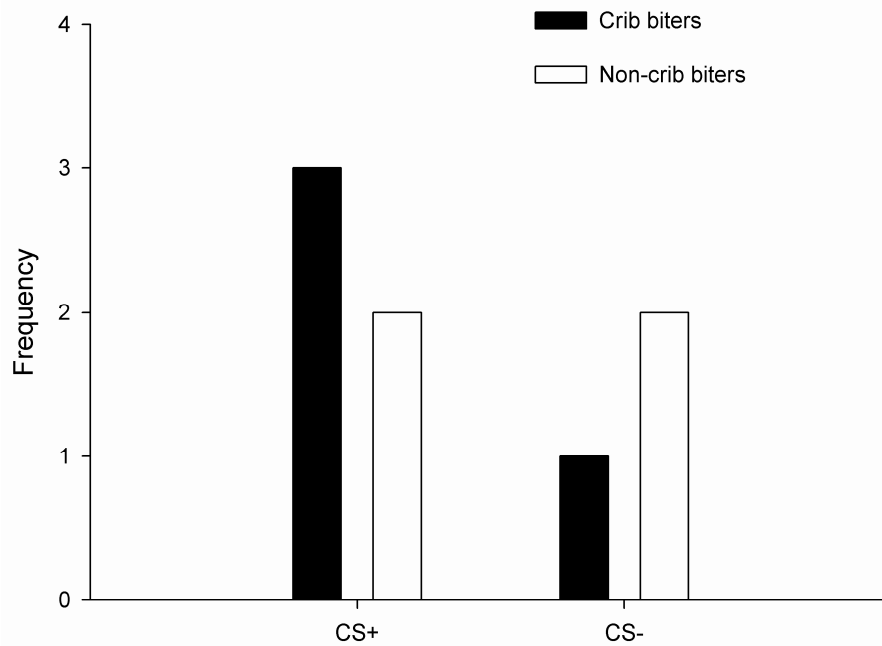


Figure 2.12. Frequency of crib-biters' and non-crib-biters' choices of CS+ and CS- in a post-training conditioned discrimination trial.

Results from the current study were somewhat unexpected, given the results from Experiment 1. It was predicted that crib-biters, with dysregulation of the DMS, would fail to integrate local and distal cues effectively, thereby displaying different search strategies during probe trials in a simple allocentric place-learning experiment. However, crib-biters were faster to learn the location of CS+ during training and spent more time (as compared to chance-level performance) in the correct quadrant during the probe trials than non-crib-biting counterparts. Results from the conditioned discrimination trial suggested that

crib-biters were marginally more likely to choose CS+ over CS-; however, the results were not significant. Possibly increasing the sample size for future attempts at this procedure may uncover any small group differences. During the training trials, crib-biters may have been more focussed on CS+. This could be a reflection of neurobiological features of the ESP (McBride & Hemmings, 2005). Specifically, animals with facilitated DA transmission in the mesoaccumbens DA pathways may be expected to display heightened reward-sensitivity in the early stages of learning, illustrated in enhanced reward-specific response levels (Wyvell & Berridge, 2001).

An alternative explanation may be that the crib-biters were less likely to explore the maze in the early stages of training and in the subsequent probe trials. Figure 2.13 displays routes taken by two non-crib-biters and two crib-biters during early training trials (Trial 2 in each case). As is clear, the non-crib-biters in the example showed a higher rate of exploration of the arena, whereas the crib-biters took a more direct route to CS+.

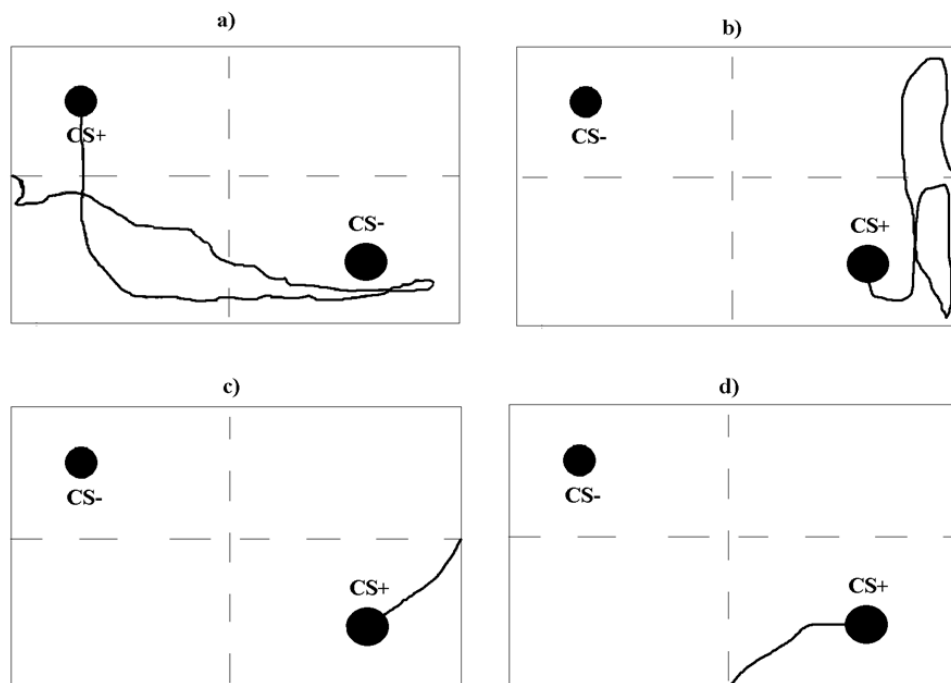


Figure 2.13. Search strategies during Trial 2 for two non-crib-biters (a & b) and two crib-biters (c & d).

Similar patterns were observed in the probe trials. Figure 2.14 displays the routes taken by two crib-biters and two non-crib-biters during the CS₀ Probe Trial. Despite there being no group difference in approach latency during the probe trials, the crib-biters spent significantly more time than would be expected by chance in the previously reinforced quadrant during the probe trials, whereas the non-crib-biters did not. As is clear from Figure 2.14, the crib-biters approached the goal, and remained in the direct vicinity of the previous location of CS⁺, whereas the non-crib-biters, during the 45-second window, explored different quadrants (previously non-reinforced).

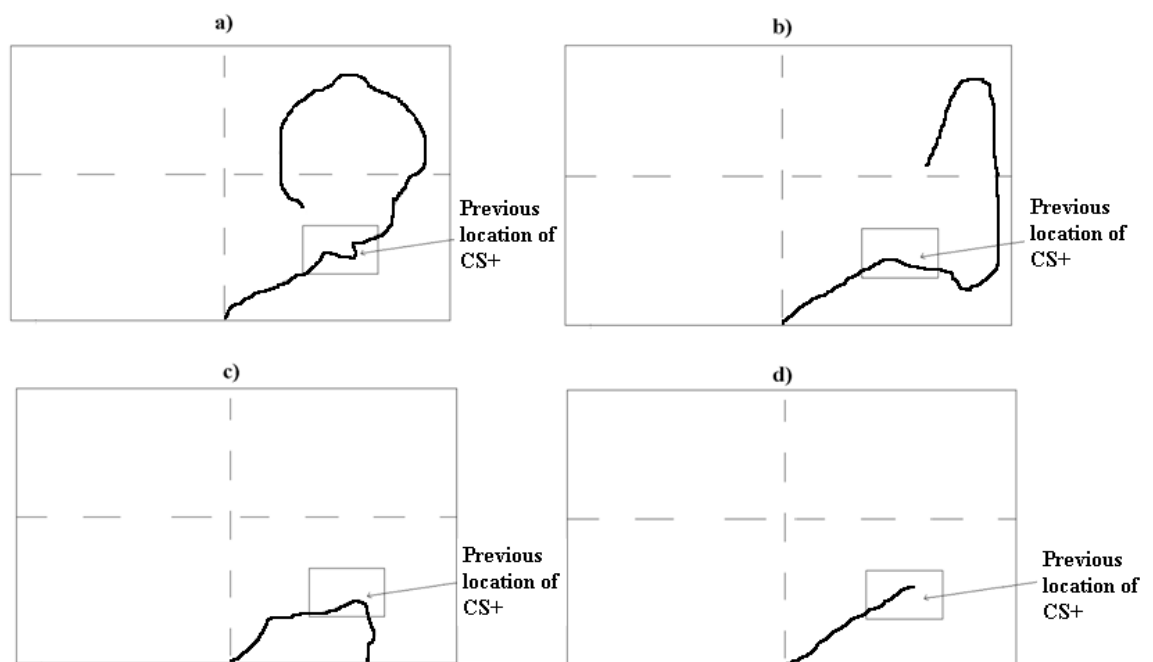


Figure 2.14. Search strategies during CS₀ probe trials for two of the non-crib-biters (a & b) and two of the crib-biters (c & d).

General Discussion and Conclusions

The aim of the first two experiments was to investigate allocentric navigation strategies in crib-biting horses. Animals with DMS lesions perform differently to controls in such procedures (Devan et al., 1999). Therefore it was predicted that crib-biters, with reduced DA D1 receptor subtypes in the DMS (McBride & Hemmings, 2005), would show: 1) retarded acquisition as inferred from slower food approach latencies and 2) increased time spent in the outer perimeter of the arenas (Thigmotaxis). In Experiment 1, we found no support for the first hypothesis, with both groups' food approach latencies being similar. However, we found that crib-biters spent significantly more time in the outer-most perimeter of the arena, suggesting that this group may have failed to integrate local and global cues effectively; a reported feature of DMS lesions (Devan et al., 1999). In Experiment 2, we examined this hypothesis in detail, by placing two distinctive buckets in different quadrants of a large arena. We reasoned that if crib-biting horses were unable to integrate local and global cues effectively, in probe trials they would show a place-learning failure, but we found no direct support for this hypothesis.

We did, however, find a number of interesting differences between the groups in both experiments. In Experiment 1, for example, we found that the crib-biters were more likely to adopt habitual response patterns. The non-crib-biters, in the final two trial-blocks, all chose the optimum strategy: they turned right and left equally often. However, the crib-biters appeared to adopt a more habitual pattern, in that they were more likely to turn the same way repeatedly, despite this strategy often increasing route-length. DMS lesions cause an increase in DLS-mediated S-R learning and a decrease in DMS-mediated R-O learning (Yin & Knowlton, 2004). It is possible that the current findings are indicative of enhanced habit formation, owing to the downregulation of DA activity in the medial aspects of the dorsal striatum. Enhanced habit formation has been linked to imbalance of striosome and matrix cells in the striatum. Together, striosome and matrix cells are responsible for R-O learning. However, striosome predominance has been linked to an increase in stimulus-guided (S-R) learning (and a decrease in R-O learning). Chronic AMPH exposure leads to an

accelerated shift from R-O to S-R learning in rats (Nelson & Killcross, 2006). It appears that the reason for this is that DA agonist exposure causes concurrent overactivation of DA D1 and D2 receptors. This concurrent activation has been shown to increase striosome predominance in the striatum (and silence matrix cells; Capper-Loup et al., 2002). One of the Key features of the ESP is the upregulation of D1 and D2 receptors in the NAcc. Owing to the LTP characteristics of the striatum (e.g., Jedynak et al., 2007), this is indicative of facilitated DA throughput. Given this, it may be expected that crib-biting horses may be found to show striosome predominance and concurrent matrix silencing. This is yet to be established empirically, however, it raises an interesting question for future research. In addition, it raises the possibility that enhanced habit-formation may also be a feature of the ESP, and this will be explored later in this thesis.

Because there was no difference between the groups in the probe trials in Experiment 2, we must assume that crib-biters are equally capable of learning using global allocentric cues, and hence there is no apparent difference in cue competition. Although we found no specific evidence that crib-biting horses fail to integrate global and local cues effectively, as was demonstrated in DMS lesioned animals (Devan et al., 1997), we did discover some differences in the navigation strategies of crib-biters and controls during training, and during the probe trials. Strikingly, we found that the crib-biters learnt to locate the food in the large arena (Experiment 2) at a slightly faster rate than the controls, at least in early trials. The precise reasons for this are somewhat unclear. However, a qualitative analysis of the route-strategies taken by both groups in the early acquisition trials (e.g., see Figure 2.13 for an example) appeared to show that the non-crib-biters were more exploratory than the crib-biters. Similar patterns were observed in the probe trials (e.g., see figures 2.10 and 2.14). Once they had searched the expected spatial location of the food, the crib-biters tended to remain in the same quadrant. However, the non-crib-biters, once they had ascertained that the food was absent, began to search beyond the quadrant.

A possible explanation for this difference may lie in the underlying ventral striatum perturbations associated with the ESP. Crib-biting horses have increases in DA D1 and D2 receptors in the NAcc, suggesting facilitated DA transmission in the mesoaccumbens pathway (McBride & Hemmings, 2005). An increase in DA D1 and D2 receptor activity, as induced by AMPH exposure, has been found to enhance the salience of reward-predictive cues (e.g., Wyvell & Berridge, 2001). It could be argued that the reason the crib-biters were less exploratory during training and during the probe trials was that they had an enhanced sensitivity to the food reward cue (i.e., the bucket) therefore ignored peripheral stimuli. This ‘narrowing’ of search behaviour seems plausible in the light of the behavioural phenotype of repetitive, topographically invariant response patterns. These findings may also be interesting in relation normal patch-foraging behaviour which perhaps the non-crib-biters were showing. Goodwin et al. (2002) found that recently grazed patches are not immediately revisited as feed resources are likely to have been reduced or eliminated; hence increasing the motivation to move between patches. This may explain some of the group differences in exploratory behaviour in this study.

In summary, the first two experiments were designed to examine the behavioural effects of DMS dysfunction, as a feature of the ESP. We found some evidence that crib-biters began to adopt a habitual response pattern during Experiment 1, but also that crib-biters were less likely to explore the maze to the same extent as controls in the Experiment 2. We suggested that this narrowing of the behavioural repertoire may be indicative of facilitated DA transmission in the ventral striatum. This would be expected to result in an increase of the phenomenological salience of reward-predictive cues. In the next chapter, we will explore this hypothesis in more detail.

Sensitisation and Instrumental Responding

Experiment 3: Rates of Instrumental Responding in the Presence of a Pavlovian Cue in Crib-Biting and non Crib-Biting Horses

In the previous chapter, we found that crib-biters seemed less exploratory than non-crib-biters. It was unclear precisely how this could be explained in terms of the ESP; however, we suggested that it might be the result of facilitated DA transmission in the NAcc. Learning is mediated by both Pavlovian and instrumental contingencies. In Pavlovian conditioning, repeated pairings of a previously neutral stimulus (CS; e.g., a bell) with a biologically relevant stimulus (US; e.g., food) leads to an anticipatory response to the CS (CR). However, in instrumental conditioning, the animal learns that a specific action (e.g., a lever press) will lead to an outcome (e.g., food). If contingent delivery of the outcome following the response increases the probability that the response will be performed subsequently, the outcome can be said to have reinforced the response. Essentially, the difference between the two types of learning is the extent to which the animal is in control of the contingencies. In Pavlovian conditioning, the contingencies are independent of the animal's behaviour, whereas in instrumental conditioning, the contingencies are dependent on the animal's response. Despite the differences between the two types of learning, motivational characteristics relating to the

incentive salience of the US (or reinforcer) may be mediated by the same mechanisms, and research has shown that Pavlovian CSs can impact upon instrumental responding. Pavlovian-to-Instrumental Transfer (PIT) preparations are able to demonstrate this effect (Rescorla & Solomon, 1967).

Typically in a PIT procedure (summarised in Chapter 1 of this thesis), the animal will first be trained in Stage 1 to associate a CS (e.g., a light) with a US (e.g., sucrose solution). In Stage 2, the animal will be trained to perform an instrumental response (e.g., a lever press) in order to access to the same US (i.e., the sucrose). In the test phase, carried out in extinction, the animal is placed in the operant chamber in the presence of the lever, and occasionally the CS is presented. Typically, the animal's response rate increases during the CS presentations from baseline, and this is referred to as the PIT effect (Hall, Parkinson, Connor, Dickinson, & Everitt, 2001; Rescorla & Solomon, 1967).

The reasons related to the ability for a Pavlovian CS to increase instrumental responding are generally not well understood; however, it may be related to increased incentive motivation, an effect mediated by NAcc DA activity (see Everitt & Robbins, 2005 for a review). For example, lesions of the NAcc core (but not the shell region) abolish the PIT effect (Hall et al., 2001), as does DA antagonist administration (Dickinson, Smith, & Mirenowicz, 2000). In addition, DA agonist micro-injections into the NAcc core enhance the PIT effect (Wyvell & Berridge, 2001). This final observation was particularly interesting as there were no increases found in general levels of responding in extinction for the DA agonist group, whereas the presence of the CS increased responding by 100% compared to controls (Wyvell & Berridge, 2001). It seems likely, therefore, that mesoaccumbens DA transmission may hold the key to the transfer effect.

One of the most striking neurobiological features of the ESP is that crib-biting horses have upregulation of mesoaccumbens DA transmission (McBride & Hemmings, 2005), as inferred from increased densities of DA D1 and D2 receptors in the NAcc, an effect similar to that seen in chronically stressed DBA (but not C57) mice (Cabib et al., 1996). These findings provide evidence for stress-induced neural phenotype in these animals (see Chapter 1 of this thesis for a detailed

discussion of these features). The subsequent facilitated DA transmission in the mesoaccumbens pathway associated with the Phenotype may be expected to increase the PIT effect, described earlier, in a similar fashion to intra-accumbens DA agonist injection.

In the current study, we trained crib-biting and non-crib-biting control horses first to associate a CS with food delivery. Following this, we trained them to press a muzzle-plate for access to the same food reinforcer. In the test phase, we placed both groups in extinction and occasionally presented the CS from the Pavlovian training trials. Based on previous evidence from pharmacological manipulations (Wyvell & Berridge, 2001) and the neural features of the ESP (McBride & Hemmings, 2005) we predicted that the increase in response rate (compared to baseline) of the crib-biters would be relatively higher than controls during the transfer phase. Some theories of the aetiology of stereotypic behaviour have cited imbalance of the direct and indirect pathways of the basal ganglia as a causal factor (e.g., Garner et al., 2003). Lesions of the indirect pathway, for example, lead to disinhibition and perseveration in motor responding, and lesioned animals have been shown to persevere with an instrumental response during extinction. If this theory is correct, we would expect extinction latency in the crib-biting horses to be more than the controls.

Method

Subjects

Four horses⁶ (mares; n = 2 crib-biters [1001, 1002]; n = 2 controls [1003, 1006]) were used for the study. Three of the horses were experimentally naïve, but one had previous experience with instrumental procedures (1002). All subjects were

⁶ N.b. We began training with six horses (n = 3 crib biters and n = 3 controls) but two were unable to complete the training, owing to health-related problems, and had to be eliminated from the study.

managed in the same way, with 12hr turnout/12hr stabling. All horses were in regular work within a riding-school. During the time spent in the stable, all horses were given 8kg hay/day in addition to their regular feed (concentrate).

Materials and Apparatus

The procedure was run using a custom-built device, mounted on the stable wall (see Appendix B for a photograph of the device). During the Pavlovian phase, we used a tone CS (3.0 KHz; 77 db). For the instrumental phase, there was one response plate (6" × 3" steel), behind which was located a roller-actuator micro-switch. The specified operation force for the switch was 100g (~1N). However, the actual force required to actuate the switch would vary with the position at which force was applied to the actuation plate. For example, if the horse pressed directly where the roller touched the plate then the force required would be 100g. If the horse pressed at the bottom of the plate then the actual force required could be closer to 50g (~0.5N), due to the additional lever action of the switch. Each depression of either switch was programmed to log a response. The apparatus was designed around a Mitsubishi Alpha programmable logic chip (1998). VI intervals and CS timings were generated, and response data logged, via a C++ Builder programme. Data were logged into a spreadsheet via a computer in a room adjacent to the testing stable. Reinforcers in both phases were delivered via a stepper motor-controlled conveyor belt, located behind the food hopper (out of sight of, and inaccessible to, the subjects). The device was mounted on the stable wall, with the food receptor trough located below (see Figure 3.1).

It was not considered necessary to deprive the subjects of food prior to the study. Horses are naturally trickle feeders, eating for between 16 H and 18 H in a typical day (Goodwin, Davidson, & Harris, 2002; Harris, 1999; Tyler, 1972). For this reason, their motivation to acquire food would be sufficiently high regardless of a period of pre-trial deprivation. In addition, the reinforcers used (pelleted feed) have been shown empirically to be a highly palatable substrate (Goodwin et al.,

2002; Ninomiya, Mitsumasu, Aoyama, & Kusunose, 2007). Therefore, subjects were reinforced with approximately 15g of Dodson and Horrell Pasture Nuts[®].

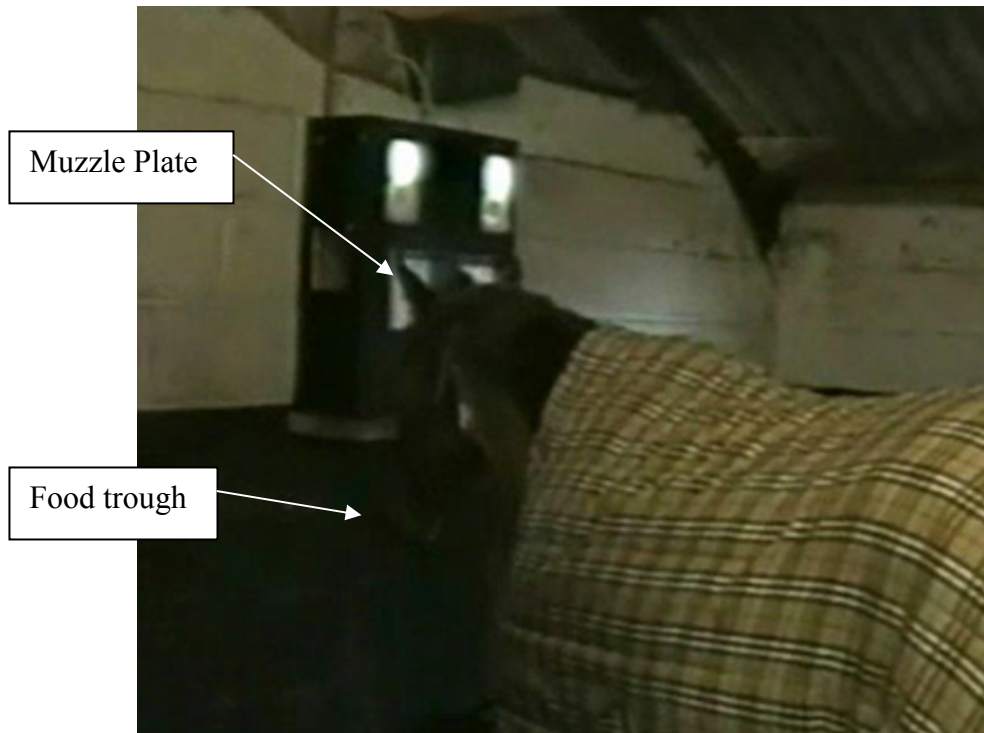


Figure 3.1. Apparatus mounted on stable wall.

Procedure

Pavlovian Training. The response-plate was concealed during the Pavlovian phase to avoid the animals developing (or extinguishing) button pressing prior to instrumental training. All animals were trained for eight 45-min sessions, during which they received a total of 104 CS-US pairings. The sessions were run during the week (i.e., Monday-Friday) on consecutive days. During each of the first six sessions (days 1-6), subjects were exposed to 15 CS presentations (10-sec duration, VI 180-sec ITI), and during the final two sessions (days 7-8) they were exposed to seven CS presentations (10-sec duration, VI 360-sec ITI). Approach to the food-

receptor trough was assessed during the final two-sessions (i.e., $p(\text{approach} \mid \text{CS}) - p(\text{approach} \mid \sim \text{CS})$) to ensure the horses had formed the association.

Instrumental Training. All instrumental training sessions were run for 45-min. In the initial two sessions (days 9-10), the horses were shaped by a process of successive approximations to press the response plate for food. All horses were successfully trained to press the plate within this period. For the next five sessions (days 11-15), horses were trained to press the response plate for a food reinforcer first on a CRF schedule, then on incrementally increasing VI schedules across sessions (i.e., VI 4-sec, VI 16-sec, VI 32-sec, VI 32-sec) to encourage steady-state responding.

Extinction Phases. On day 16, following the final session of instrumental training, the horses were given one 45-min session in extinction (with the response plate available) to habituate them to the extinction condition. During the first 15-min of the session, food was made available on a VI 32-sec schedule. Following this, the extinction phase was immediately initiated and ran for 30-min. Response data was taken during this phase for all of the subjects. On the final day (i.e., day 17) the transfer phase was run, during which the CS (10-sec duration) was presented five times ($\text{ITI} = \text{VI } 360\text{-sec}$). Again, this session ran for 45-min, with the first 15-min on a VI 32-sec schedule followed immediately by the 30-min transfer session. Transfer was assessed by calculating a change score for each subject, by the number of responses during the CS presentation (10-sec) and the 20-seconds following it, as compared to the 30-second bin immediately prior to the CS (taken as baseline).

Results

Pre-screening of all of the subjects confirmed that the crib-biters performed the behaviour, and that the non-crib-biters did not. During the Pavlovian phase, all of the horses were observed to have formed an association between the CS and US.

However, owing to differences in responsivity to the CS, it was not possible to formulate a specific dependent measure as is typical in previous PIT studies (e.g., Wyvell & Berridge, 2001). To explain, two of the horses (1002 and 1006) stood in close proximity to the device throughout the sessions. Behavioural observations confirmed that the association had been made, however, as both of the horses visibly changed their behaviour during the CS presentation. For example, both were seen to be attentive (i.e., ears forward and erect; McGreevey, 2004) during the course of the buzzer. The other two horses (1001 and 1003) both were seen to approach the apparatus during the CS presentations.

Instrumental Training

During the course of instrumental training, all of the horses retained a relatively steady state of responding. Figure 3.2 displays the mean responses per minute for each of the subjects during training.

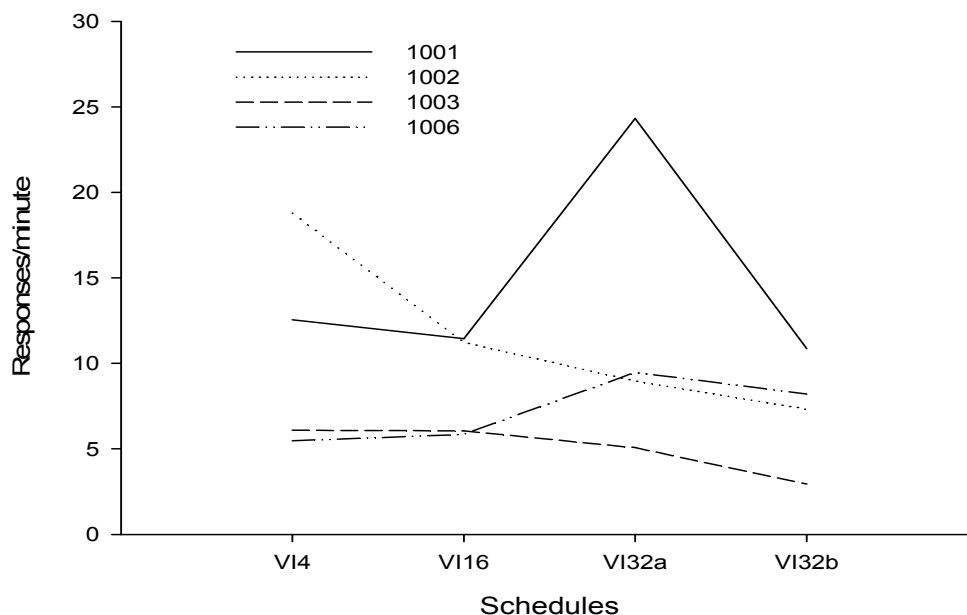


Figure 3.2. Mean responses/minute for each subject during instrumental training under each of the four VI schedules (1001 & 1002 = crib-biters; 1003 & 1006 = non-crib-biters).

The mean response rates per minute for each horse across schedules were: 1001 = 14.8; 1002 = 11.6; 1003 = 5.1; 1006 = 7.2. Owing to the limited sample size, inferential statistics are not appropriate to analyse this data any further, but it is clear from this and from the graph, that the crib biters generally had higher response rates than their non-crib biting counterparts.

Extinction Sessions

During the first extinction session, all subjects were seen to extinguish responding over the course of the 30-min session (see Figure 3.3). Again, owing to the sample size, inferential statistics were not appropriate; however, there appears to be little difference in extinction rates between subjects, or between groups.

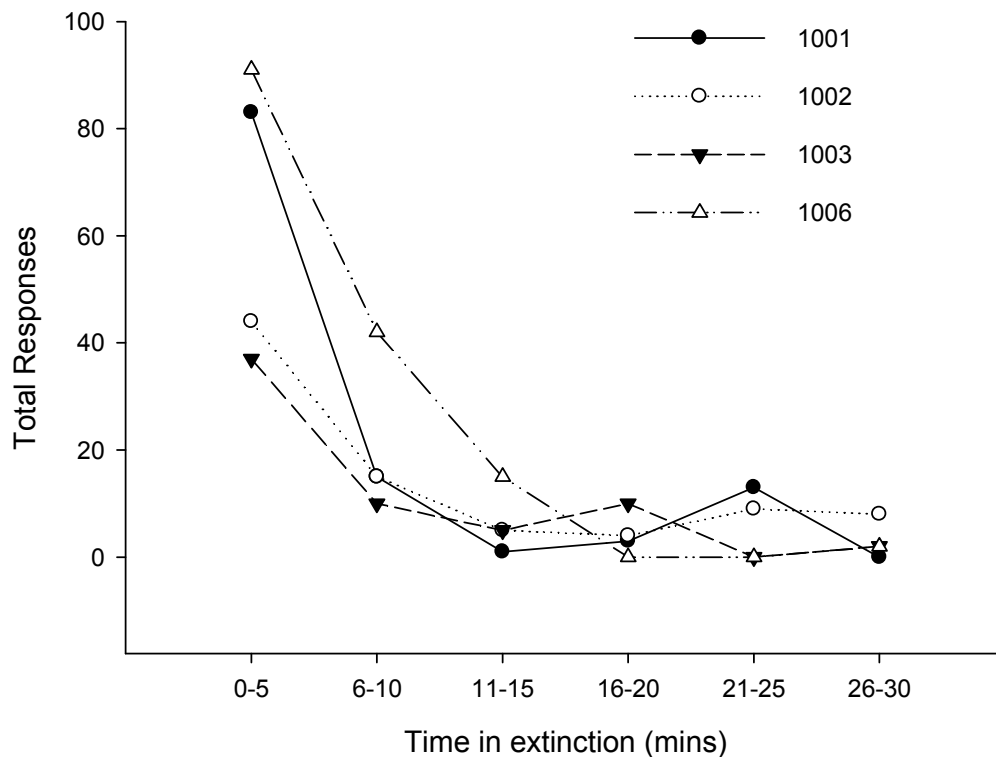


Figure 3.3. Total responses/ 5-min interval during 30 minute extinction session (1001 & 1002 = crib-biters; 1003 & 1006 = non-crib-biters).

Total response rates during the transfer session are displayed in Figure 3.4. It is clear that all subjects showed spontaneous recovery following the first extinction session and the reintroduction of the VI 32-sec schedule, although the overall response rates were a little lower than in the initial extinction session. Again, there appears to be little difference between subjects' and group response rates. However, subject 1002 (crib-biter) appeared to make marginally more responses during the final stages of this session than the other subjects.

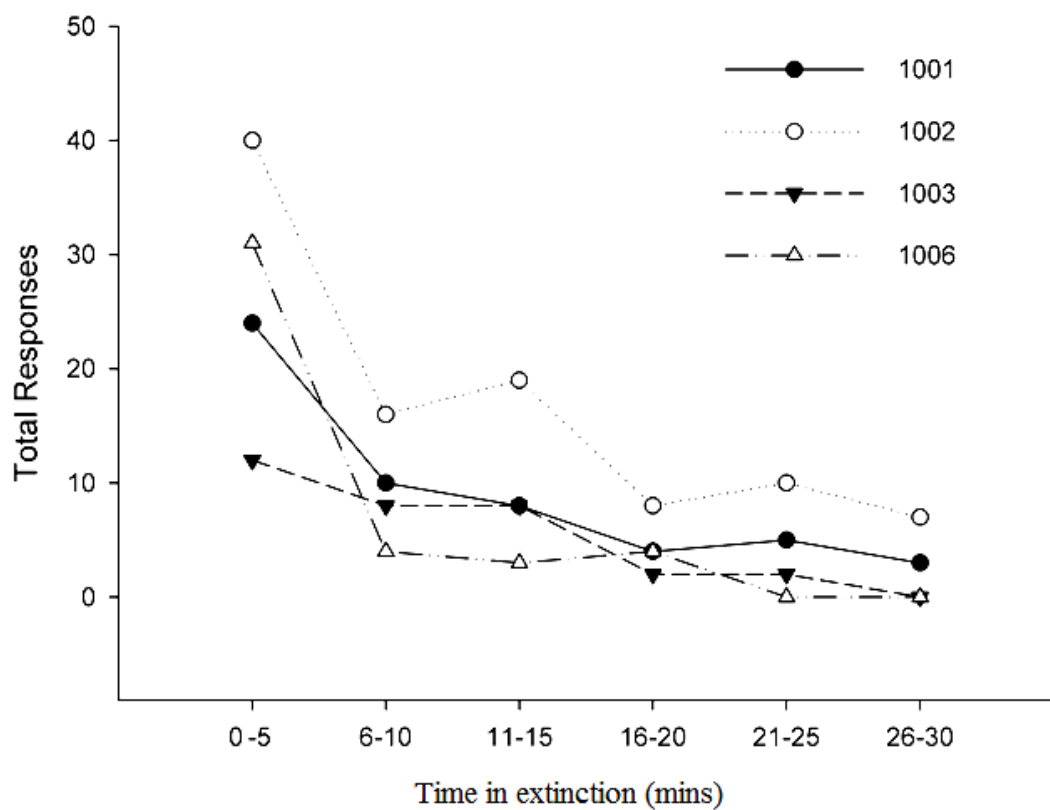


Figure 3.4. Total responses during transfer session (1001 & 1002 = crib-biters; 1003 & 1006 = non-crib-biters).

Transfer

Finally, we considered evidence for transfer in all of the subjects. This was examined in two ways: first by comparing the total responses of each animal during CS presentation to baseline, and second by examining total responses/minute for

each horse during this session to examine dynamic change. Figure 3.5 displays the transfer data from each animal. In the graph, the x-axes relate the five CS presentations, and the y-axes to the increase in responses above baseline (i.e., the 30-secs preceding the CS presentation) made by each subject during the CS presentation (and the 20-secs following). According to the graphs, it appears that subjects 1001 and 1002 (the crib-biters) did make marginally more responses during the CS presentation than the non-crib-biters. 1001 showed some evidence of transfer during CS1 and CS3, and 1002 in CS1 and CS3. Of the non-crib-biters, subject 1003 showed some evidence of transfer during CS3, but not during any of the other CS presentations; subject 1006 showed no evidence of transfer whatsoever. However, again we were unable to verify any reliable difference between the groups owing to the small sample size.

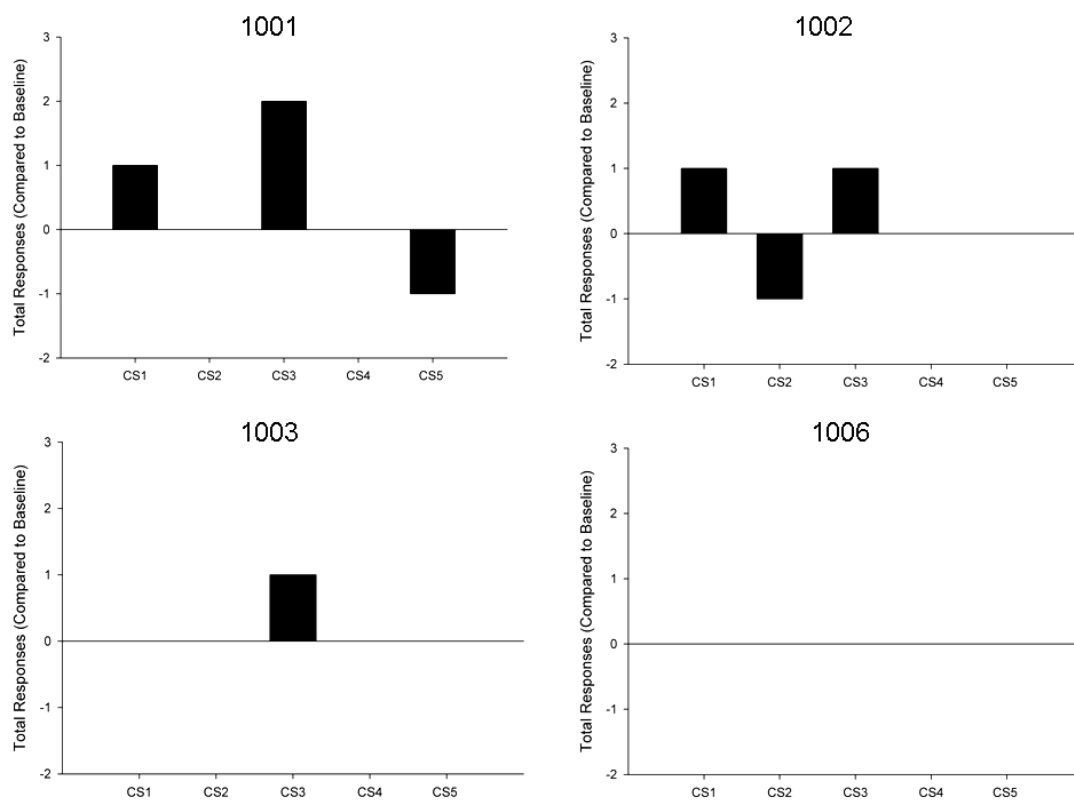


Figure 3.5. Difference in responses during CS period as compared to baseline in each subject (1001 & 1002 = crib-biters; 1003 & 1006 = non-crib-biters).

Figure 3.6 displays the total responses per minute period for each of the subjects. It would be expected that if the subjects were experiencing PIT, response rates would increase immediately after CS presentation and quickly decay post-exposure. This appears to be the case in the crib-biters, but it is not so clear in the non-crib-biters. For example, subject 1001 (crib-biter) responses increased in the min following CSs 1 and 3, and in subject 1002, responses increased in the minute following CSs 1 and 2. For the non-crib-biters, however, there is no evidence for post-CS increases at any stage of the session. Again, without inferential statistics, it would be unwise to draw any conclusions from these observations, and any inferences relating to the hypotheses should be treated with extreme caution.

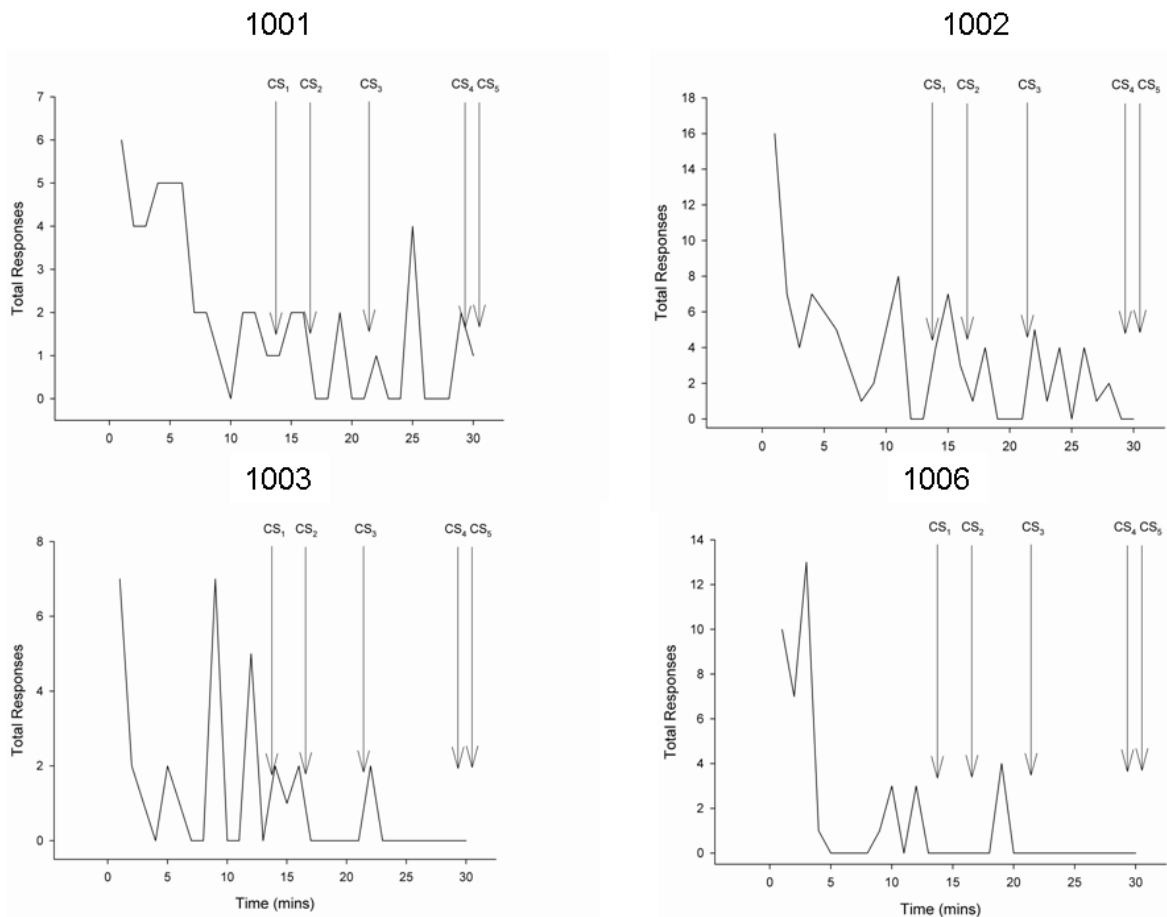


Figure 3.6. Responses/minute during the transfer session for each of the four horses. (1001 & 1002 = crib-biters; 1003 & 1006 = non-crib-biters).

Discussion

In the previous chapter (Chapter 2) we saw that crib-biters were less exploratory in their search strategies in the context of a spatial maze. I argued that this may be indicative of increased cue salience, as a result of upregulation of the mesoaccumbens DA pathway. Thus, the aim of the current experiment was to examine whether this feature of the ESP (McBride & Hemmings, 2005) increased an instrumental response in the presence of a Pavlovian cue. The neural phenotype suggests sensitisation of dopaminergic systems similar to that seen following chronic AMPH administration. The results of the current study were inconclusive, owing primarily to the low sample size. However, there was some suggestion that crib-biting horses showed an increase in PIT. In addition, we found no evidence that the crib biters in our sample showed increased extinction latency, as suggested by previous studies (Garner et al., 2003; Hemmings et al., 2007).

Considering the latter finding first, some theories of stereotypy have suggested that imbalance between the direct and indirect pathways of the basal ganglia may lead to increase in response-perseveration, as demonstrated by increases in latency to extinguish responding in an operant context (Garner et al., 2003; Hemmings et al., 2007). Our findings, however, failed to support this, with the crib biters and non-crib-biters extinguishing at similar rates. Despite our low sample size, it is notable that none of the crib-biters showed any signs of increased extinction latency. A possible reason for this anomaly could be related to methodological differences. For example, there is no mention in either of the papers cited above of the total number of training trials to which the subjects were exposed. In addition, it is unclear if the number of training trials was held constant across subjects. In the present study, subjects were trained on the operant procedure for a total of 225-min (over 5-days), received in excess of 800 reinforcers and were trained to respond according to a VI schedule of reinforcement. If the above theory of stereotypy (i.e., Garner et al., 2003) was correct we would have expected long extinction latencies in the crib-biters.

However, while the non-crib-biters attenuated responding completely during the course of the extinction trials, the two crib-biters did appear occasionally to respond in the latter stages particularly of the second session (i.e., the transfer session). The reason for this may be that the salient cue (i.e., the CS presentation) primed responding in the crib-biters. This would fit with a model whereby enhanced habit formation (S-R learning) was an associated behavioural phenotype (see Chapter 1; *Figure 1.7*).

We found some limited support for the notion that crib-biting horses may show heightened sensitivity to a Pavlovian cue. This behavioural phenotype would be predicted by the original model of stereotypy proposed (*Figure 1.7*) as upregulation of DA transmission in the mesoaccumbens pathway would be expected to result in increases in extracellular DA, which has been found to increase the incentive salience of reward-predictive cues (Wyvell & Berridge, 2001). However, the results of this study should be treated in terms of a pilot investigation, as the sample size was very limited.

Thus far, we have demonstrated that crib-biting horses learn differently within spatial navigation procedures. However, the findings were somewhat confusing, and did not accurately support the hypotheses generated by the model outlined in Chapter 1 (*Figure 1.7*). We found that crib-biters seemed to display reduced exploratory behaviour. We argued that this may be mediated by upregulation of the mesoaccumbens pathway, suggesting that the neural phenotype may increase sensitivity for reward-predictive cues. We therefore carried out a PIT procedure to examine this in detail, but owing to a low sample size, we were unable to confirm or reject this hypothesis. Upregulation of the mesoaccumbens pathway is an important feature of the phenotype, and we felt that in the light of the disappointing findings of this study, a further investigation of this would be prudent. There is evidence, for example, that increased midbrain DA activity leads to differences in learning in the context of instrumental choice. The following set of studies therefore was designed to test this in crib-biting horses.

Instrumental Choice

Impaired Instrumental Choice in Crib-Biting Horses⁷

Concurrent schedule preparations examine subjects' choices between unconditioned reinforcers (Herrnstein, 1961). In other words, given the S^D (e.g., a red and a green light) the final response on a chosen alternative is reinforced, according to a VI schedule. However, it is possible to assess choice for stimuli that *signal* primary reinforcement (conditioned reinforcers) using a two-phase choice paradigm. During this procedure (concurrent-chain schedules), choice during initial-links (choice-phase 1) is a measure of the relative value of two, mutually exclusive terminal-links (choice-phase 2). This procedure is empirically intriguing, as steady state responding and peak-choice in the initial-links phase is dependent on a variety of variables, particularly the total durations of initial- and terminal-links (Grace, Berg, & Kyonka, 2006). Figure 4.1 illustrates a schematic of such a procedure.

⁷ Data from this chapter are published as: Parker, M., Redhead, E. S., Goodwin, D., & McBride, S. D. (2008). Impaired instrumental choice in crib-biting horses (*Equus caballus*). *Behavioural Brain Research*, 191, 137-140.

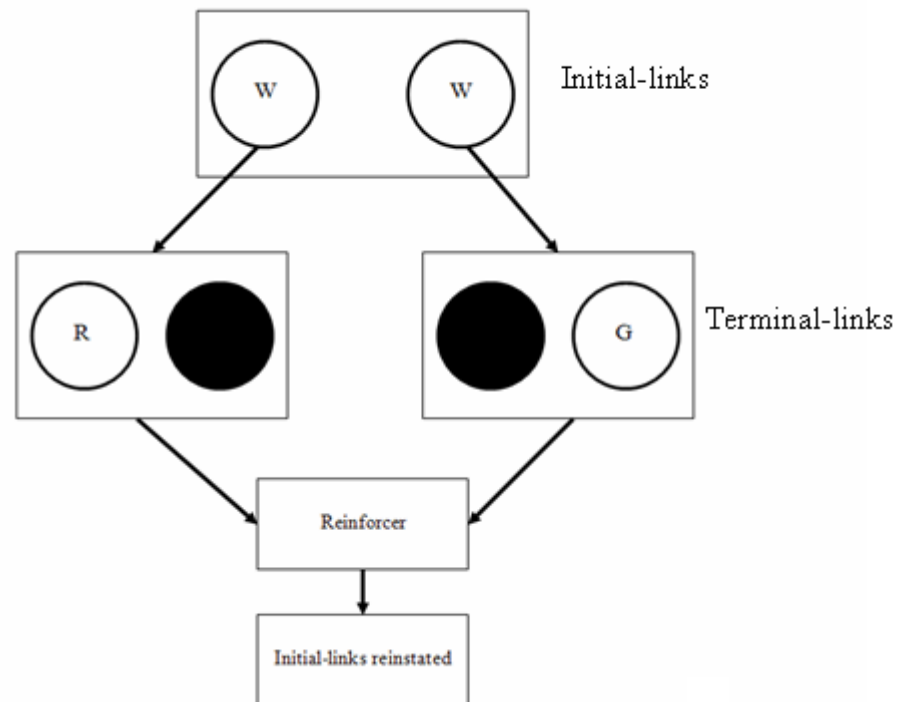


Figure 4.1. Schematic illustration of a concurrent-chain trial. Subjects can respond to either key during concurrent VI VI initial-links. Responding on the alternatives is reinforced with entry into one of two terminal-links, where one key is illuminated red or green, and the other becomes dark and inoperative. The terminal-link alternatives are reinforced with food on differential FI FI or VI VI schedules. Following reinforcement, the initial-links are reinstated. W = white light; G = green light; R = red light.

Typically, in the initial-links, the subject is presented with two white lights, each associated with concurrent VI VI schedules (e.g., VI 10 s VI 10 s). Responses during the initial-links produce entry to one of two, mutually exclusive terminal-links, signalled by either a red (left) or a green (right) light, and the darkening of the white lights. Subsequently, responding during terminal-links produces reinforcement on a predetermined schedule. For example, the left terminal-link may be FI 10 s and the right may be FI 20 s. As in concurrent schedules procedures, subjects' relative response rates in initial-links have been found to match relative terminal-link ratios (Fantino, 1969). However, the extent of the matching is dependent on certain contextual variables. First, known as the 'initial-link effect', terminal-link sensitivity is inversely related to initial-link VI

length (Fantino, 1969). Therefore, as the duration of initial-links increases, the sensitivity to terminal-link reinforcement rate decreases. Second, known as the ‘terminal-link effect’, terminal-link sensitivity increases if: a) terminal-links are FI schedules (Fantino & Royalty, 1987) and, b) if terminal-link schedules (VI or FI) are multiplied by the same factor (Williams & Fantino, 1978). In a recent review Mazur (2001) stated that these phenomena must be accounted for in models of concurrent-chain performance. Producing a quantitative, parameter invariant model of choice under concurrent-chain schedules has proved very problematic. Davison (1983) suggested that owing to the interaction of initial- and terminal-link schedules, the search for such a model may be fruitless. However, various models accounting for concurrent-chain choice have been proposed, many including up to three free parameters.

Concurrent-chain performance can be explained in terms of an extension of generalised matching law (Baum, 1974). For example, Davison (1983) proposed that terminal-link functions in concurrent-chain are mathematically equivalent to changes in magnitude function in concurrent schedules. To explain, if reinforcer magnitude is varied in concurrent schedules (as well as immediacy ratio) this creates a new independent measure that can be incorporated into generalised matching law algebraically as thus:

$$\left(\frac{R_1}{R_2} \right) = b \left(\frac{r_1}{r_2} \right)^{a_1} \left(\frac{M_1}{M_2} \right)^{a_2} \quad [4.1]$$

where R_1 and R_2 represent the response ratio on each alternative, r_1 and r_2 represent the ratio of reinforcement, M_1 and M_2 represent magnitude of reinforcement on the two alternatives, b represents bias, a_1 is a scaling parameter related to sensitivity to reinforcement (i.e., log response ratio) and a_2 acts as a scaling parameter for magnitude of reinforcement. Therefore, if M_1 and M_2 were constant and varied (e.g., $M_1 = 0.5$ and $M_2 = 1$) and r_1 and r_2 were also varied, it would be expected that subjects would produce a biased matching relationship. Algebraically, the fit between (R_1/R_2) and (r_1/r_2) would have a slope b with the

intercept $(M_1/M_2)^{a_2}$ (Davison, 1983). In the same way, if M_1 and M_2 were constant and equal, Equation 4.1 reduces to generalised matching law.

In concurrent-chain schedules, Davison (1983) argued, the added independent variable is the effect on initial-link response ratios of terminal-link delays. Therefore, Equation 4.1 becomes:

$$\left(\frac{R_1}{R_2}\right) = b \left(\frac{r_{i1}}{r_{i2}}\right)^{a_1} \left(\frac{r_{t1}}{r_{t2}}\right)^{a_2}, \quad [4.2]$$

where r_{i1} and r_{i2} , and r_{t1} and r_{t2} represent initial- and terminal-link reinforcement ratios respectively. This way, as the terminal-link ratios approach zero, Equation 4.2 reduces to the generalised matching law.

A further extension of the generalised matching law was proposed by Grace (1994). The contextual choice model (CCM) is based on the assumption that context, as defined by the relative ratios of time spent in the initial- and terminal-links, is the most important factor associated with concurrent schedules performance (Grace, 1994). The model is illustrated as:

$$\left(\frac{R_1}{R_2}\right) = b \left(\frac{r_{i1}}{r_{i2}}\right)^{a_1} \left(\frac{r_{t1}}{r_{t2}}\right)^{a_2 \left(\frac{T_i}{T_t}\right)}, \quad [4.3]$$

the key feature of CCM being the contextual exponent (T_t / T_i) , which represents the ratio of time spent in the initial- and terminal-links.

Grace (1994) carried out a quantitative analysis of CCM, applying all known suitable data sets of concurrent-chain procedures. He found that the model accounted for an average of 91% of the variance of the data sets, using between two and three free parameters. However, previous models of choice within concurrent-chain schedules (e.g., Delay Reduction Theory; Squires & Fantino, 1971) had attempted to account for concurrent-chain choice with parameter-invariant models. Mazur (2001) found that if the same number of free parameters were used within other models of concurrent-chain choice, a similar amount of the variance of the same data sets could be explained. Despite this, CCM remains an excellent model of reinforcer choice within concurrent-chain schedules. It is the first model to incorporate a contextual exponent relating to

the interaction between initial- and terminal-link ratios and further, it reduces to generalised matching as the terminal-link length approaches zero.

The concurrent-chain preparation has been applied in the context of examining the effects of drugs on behaviour. Wei-Min, Pitts, Hughes, McLean, and Grace (2008) arranged VI VI initial-links with FI FI terminal-links, with one of the terminal-link FIs fixed at FI 8 s and the other changing between FI 4 s and FI 16 s unpredictably across sessions. They found that pigeons' relative response ratios matched relative terminal-link reinforcement ratios according to generalised matching. Pigeons were then administered with increasing doses of *d*-AMPH. They found that *d*-AMPH treatment reduced preference for the shorter of the terminal-link FI schedules, with terminal-link sensitivity (as suggested by generalised matching parameter estimates) reducing in a dose-response manner. This suggested that AMPH sensitisation may reduce sensitivity to delay.

The following set of experiments was designed to assess the behavioural correlates of the ESP in the context of concurrent-chain schedules in horses. Post-mortem analyses of crib-biting horses have revealed relative increases in both D1-like and D2-like receptor affinity in the ventral striatum, suggesting upregulation of the mesoaccumbens pathway (McBride & Hemmings, 2005). This is similar to the neural phenotype resulting from chronic stress found in inbred strains of laboratory mice (Cabib et al., 1998). It is possible therefore that the ESP may result in similar consequences to a pharmacologically-induced behavioural phenotype (i.e., by psychostimulants such as *d*-AMPH; Wei-Min et al., 2008). Specifically, crib-biting horses may be less sensitive to delay than their non-crib-biting counterparts. In the light of this, it was predicted that stereotypic horses may fail to match relative initial-link response ratios with relative terminal-link reinforcement schedules within a simple version of the concurrent-chain preparation. A comparison of the concurrent-chain performance of crib-biting and control horses was carried out over three half-hour learning sessions.

Data relating to horses' performance under concurrent-chain schedules has not previously been published. In the light of this, an initial experiment was

designed to examine in detail horses' performance using a concurrent-chain preparation, and further to ensure the preparation was suitable for the research hypotheses.

Experiment 4

This experiment aimed to examine the logistics and suitability of concurrent-chain schedules in horses. Subjects were trained for eight sessions before the start of the experiment; the subjects took part in three sessions; session duration (during training and during experimental trials) was determined by number of reinforcers gained; terminal-link schedules were VI VI in an attempt to reduce the chance of overmatching; each subject took part in terminal-link ratio-reversal phases (2:1: VI 20 s VI 10 s; 4:1: VI 24 s VI 6 s; 1:2: VI 10 s VI 20 s; and 1:4: VI 6 s VI 24 s).

In order to validate the procedure against existing concurrent-chain data, it would be expected that the data would fit an existing model of concurrent-chain choice (CCM; Grace, 2004). Although there have been many studies employing concurrent-chain schedules, none have used horses, and hence it is unknown whether the effects of temporal context (Tt/Ti) would hold for this species (R. Grace, personal communication). Therefore, the conditions were designed such that the exclusive terms of CCM were rendered superfluous. Specifically, equal initial-link schedules were used to guarantee equal exposure to the terminal-links (therefore keeping a_1 constant) and in addition, the Tt parameter was kept constant across conditions, while varying the immediacy ratio (Berg & Grace, 2004). Consequently, the 4:1 and 1:4 conditions were VI 24 s VI 6 s and VI 6 s VI 24 s, respectively, and the 2:1 and 1:2 conditions were VI 20 s VI 10 s and VI 10 s VI 20 s. This meant that all four sets of terminal-link schedules summed to 30-seconds. Because of this, and because the initial-links were held constant (*conc* VI 10 s VI 10 s), Tt/Ti and a_1 remained constant, and CCM reduced to the generalized matching law (Baum, 1974) for least squares curve fitting.

Method

Subjects

Three experimentally naïve horses were recruited from a local agricultural college (SN103, SN105, SN108). All three were male and of mixed breed. The subjects were regularly checked by veterinarians and were deemed in good general health. All subjects were housed in individual stables, and were regularly turned out into the paddock. Prior to the study, the subjects were fed a diet of concentrate feed (Dodson & Horrell[®] pasture nuts), alfalfa and grain, and all had *ad libitum* access to hay, or haylage, and water. The stables were lined with rubber matting, and subjects also had additional straw bedding.

Materials

Instrumental device. The instrumental device was as described in Experiment 3 (and see Appendix B), but with various alterations. This time, two lights (1 and 2) were comprised of four multi-function LEDs, which could either be off, red, green⁸, or white. In addition, we now incorporated two 6" × 3" steel response plates. Each depression of either switch was programmed to log a response. Schedules were generated and response data logged via a C++ Builder programme, and data were logged into a spreadsheet via a computer in a room adjacent to the testing stable. Reinforcers were delivered via a stepper motor-controlled conveyor belt, located behind the food hopper (out of sight of the subjects). The device was mounted on the stable wall, with the food receptor trough being located below.

Reinforcers. It was not considered necessary to deprive the subjects of food prior to the study. Horses are naturally trickle feeders, eating for between 16 H

⁸ Research has suggested that horses are able to discriminate between red and green stimuli (Smith & Goldman, 1999).

and 18 H in a typical day (Goodwin, Davidson, & Harris, 2002; Harris, 1999; Tyler, 1972). For this reason, their motivation to acquire food would be sufficiently high regardless of a period of pre-trial deprivation. In addition, the reinforcers used (pelleted feed) have been shown empirically to be a highly palatable substrate (Goodwin et al., 2002; Ninomiya, Mitsumasu, Aoyama, & Kusunose, 2007). Therefore, subjects were reinforced with approximately 15g of Dodson and Horrell Pasture Nuts[®].

Procedure

Training. All training sessions lasted 30-min and were carried out in a single, uninhabited stable. Initially, the horses were reinforced by successive approximations to the response keys. This process was found to take between one and three sessions for individual horses. Following shaping, the two white lights were illuminated continuously, and responses were reinforced according to a CRF schedule. In subsequent sessions, responses were reinforced on a progressive ratio (PR) schedule. This was set at concurrent (*conc*) FR-1 FR-1, *conc* FR-2 FR-2, *conc* FR-4 FR-4 and *conc* FR-8 FR-8. Once the horses responded for a minimum of 10-min within *conc* FR-8 FR-8 schedules, they were deemed ready for the final phase of training. In the final phase, trials started with the red and the green lights illuminated concurrently. On each of the alternatives, 40 reinforcers were available according to a CRF schedule. Once the reinforcers from one of the alternatives were expended, the corresponding light became darkened and inoperative. The other alternative remained active until all of the 40 reinforcers had been depleted. Each horse was trained on this for eight sessions (i.e., 40 reinforcers per alternative, per session). All of the training data was logged and the patterns of responding analysed.

Concurrent-chain Procedure. In each trial, both of the white lights were illuminated at the start. This constituted the initial-link. During this time, the subjects were free to respond on which ever response plate they chose. The

initial-links were arranged on *conc* VI 10-s VI 10-s schedule for all horses. Seventy-two intervals were used according to the arithmetic progression $a, a + b, a + 2b, \dots a + 35b$; where $a = b/2$, presented in random order. When the initial-link VI had elapsed, the last response made during the phase determined which terminal-link was entered. For example, if the last response was on the left, then the red terminal-link was entered. Terminal-link entry was signalled by the left white light changing to red, and the right light becoming darkened and inoperative. Similarly, if the last response was on the right, the green terminal-link was entered. Terminal-link entry was signalled by the right white light changing to green, and the left light becoming darkened and inoperative.

Table 4.1.

Terminal-Link Arrangements for Experiment 4. Initial-Links conc. VI 10-s VI 10-s. Each Terminal-Link Schedule was run for 3-Sessions Consisting of 72-Terminal-link Entries.

Horse ID	1	2	3	4
SN103	VI 6 s VI 24 s	VI 20 s VI 10 s	VI 10 s VI 20 s	VI 24 s VI 6 s
SN105	VI 20 s VI 10 s	VI 6 s VI 24 s	VI 24 s VI 6 s	VI 10 s VI 20 s
SN108	VI 10 s VI 20 s	VI 2 s VI 6 s	VI 6 s VI 24 s	VI 20 s VI 10 s

Once the terminal-link VI schedule had elapsed, the subsequent response on the corresponding response plate was reinforced with a delivery of 15g of the pelleted feed into the food receptor trough. The following initial-link phase was entered when the horse made a response on either plate. For the terminal-links phases, schedules were arranged as shown in Table 4.1. Again, 72-VI schedules were used for the terminal-links, according to the arithmetic progression $a, a + b, a + 2b, \dots a + 71b$; where $a = b/2$. These intervals were presented in random order. Each subject completed three sessions on consecutive days. All sessions ended after 72-terminal-link entries, or 60-min had elapsed: whichever the sooner.

Data Analysis. Training data was analysed to ensure that the subjects were reliably pressing both alternatives prior to the concurrent-chain procedure. This was achieved by examining the number of errors made during the training procedure, as well as the patterns of responding to each alternative.

All concurrent-chain data were log (base-10) transformed prior to analysis to normalise the distribution. The dependent measure was the relative response rate in the initial-links with the within-subjects independent measure the various immediacy ratios in the terminal-links. Data from each schedule for each subject was plotted against relative reinforcement and conditioned reinforcement rate (as predicted by generalised matching law; Baum, 1974) in log-log form and a least squares regression was carried out using the simple logarithmic form of the generalised matching law:

$$\log\left(\frac{R_1}{R_2}\right) = \log b + a \log\left(\frac{r_1}{r_2}\right) \quad [4.4]$$

Parameter values for b and a for each subject were estimated for individual subjects and for all of the subjects together. Finally, the variance accounted for (VAC) by the fitted line was calculated. All raw data was entered into SPSS® 15 for Windows for analysis.

Results and Discussion

Training.

All horses were trained until their relative response rates on the two alternatives were approximately equal over 80 concurrently available reinforcers. Figure 4.2 displays the normalised relative response rates to the two alternatives across the training trials. Table 4.2 illustrates the number of training sessions and the mean normalised response ratios on each alternative across the training sessions. Normalised response ratios equal to '0' would indicate no preference for either

alternative. The training results illustrate that all of the horses were sampling both alternatives with similar frequency.

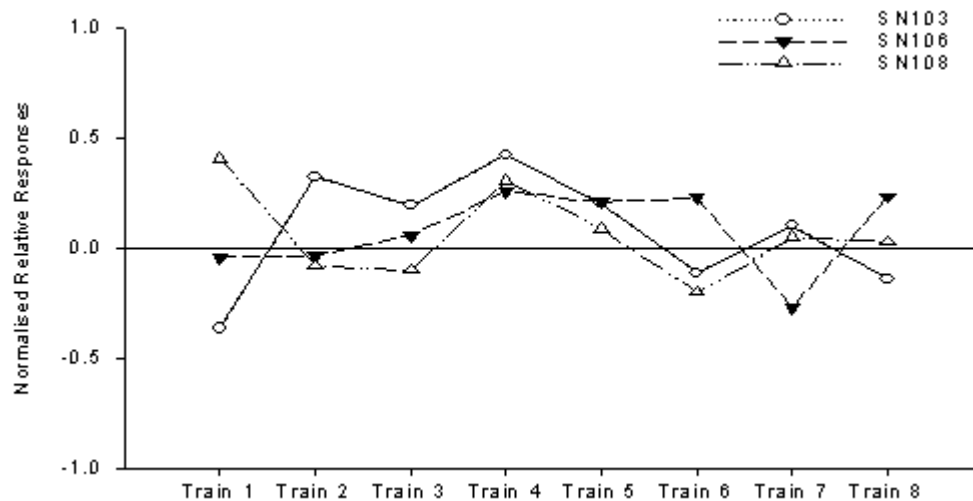


Figure 4.2. Normalised relative response rates on both alternatives during training.

Table 4.2

Mean Logarithmic Relative Response Ratios on Two Alternatives in Training Trials.

Horse ID	Number of training sessions	Mean Log Response ratios (SD)
SN103	8	0.08 (0.26)
SN105	8	0.08 (0.19)
SN108	8	0.06 (0.21)

Analysis of Concurrent-chain Performance.

Initially, the mean proportion of initial-link responses by the three subjects for the shorter terminal-link was calculated across the three sessions. Figure 4.3 suggests that preference for the shorter initial-link/terminal-link cycle appeared to increase

across the three sessions. This was confirmed with a one-factor within-subjects ANOVA, which showed that preference for the shorter terminal-link increased significantly across the three sessions, $F(2,22) = 13.895$, $p < 0.01$. Post-hoc repeated measures t-tests revealed an increase in preference between sessions 1 and 2, $t(11) = -4.21$, $p < 0.01$, and between sessions 1 and 3, $t(11) = -4.22$, $p < 0.01$. However, the increase in preference between sessions 2 and 3 fell short of significance, $t(11) = -1.77$, $p = 0.11$.

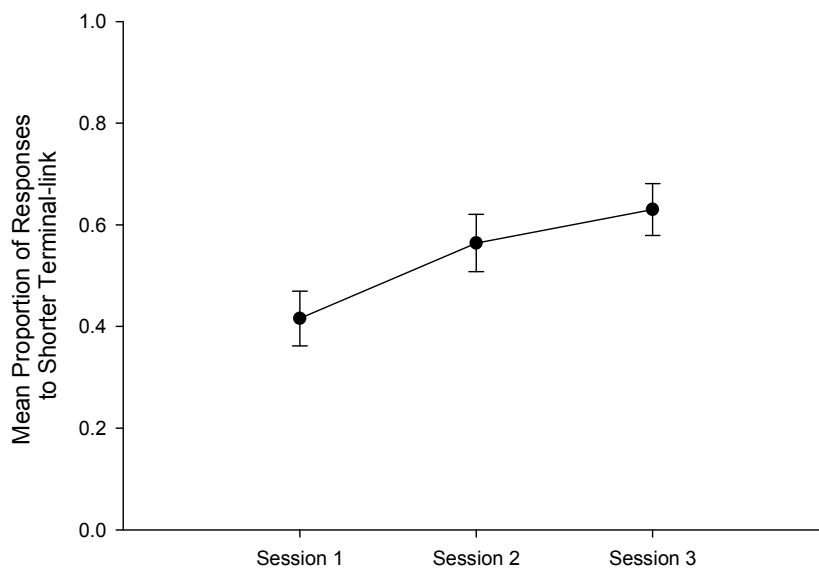


Figure 4.3. Mean (\pm SEM) proportion of responses on the shorter initial-link across three 1-hour sessions.

Table 4.3

Estimated Parameters and Regression Statistics for Horses Under Concurrent-chain schedules, Averaged Across Subjects. (b = bias; a = reinforcer sensitivity; VAC = Variance Accounted for by Generalised Matching Law (Baum, 1974), m = slope; b' = intercept, SDm = standard deviation of the slope, SDb' = standard deviation of the intercept, SEy = standard error of fit).

b	a	VAC	m	b'	SDm	SDb'	SEy
1.08	0.61	95	1.00	0	0.16	0.05	0.05

Table 4.3 displays the parameter estimates (b and a), slopes, intercept and VAC by Generalised Matching law (Baum, 1974) for the final 72-reinforcer session across the three subjects. This suggests that the subjects learned to match relative response rate with relative rate of reinforcement according to the generalised matching law during three sessions. Figure 4.4 illustrates the normalised data for the three subjects across the four immediacy ratios.

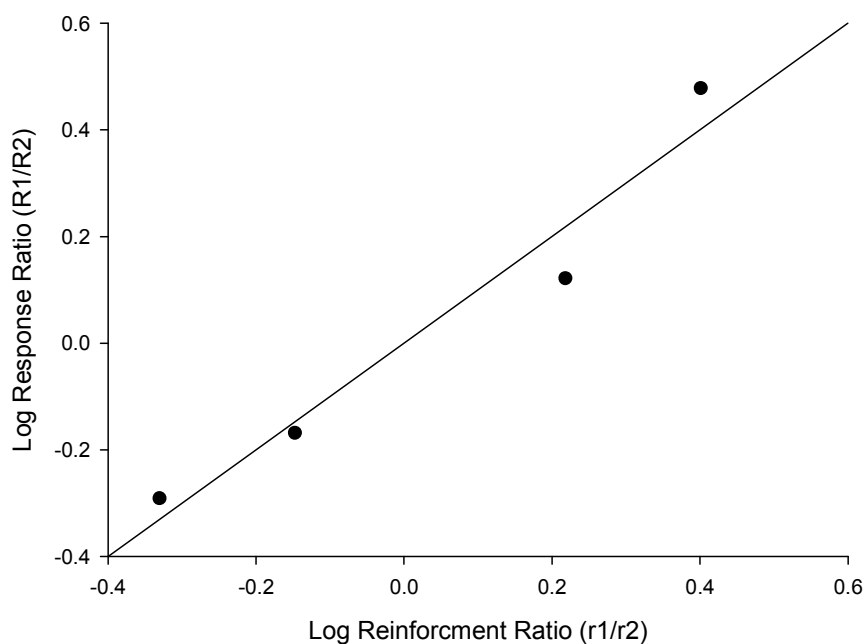


Figure 4.4. Normalised response ratios as a function of reinforcement ratio for all subjects under four terminal-link VI VI schedules.

The results of this experiment showed that horses were able, after three exposure sessions to VI VI terminal-link schedules, to match relative response rate to relative rate of reinforcement in concurrent chain. In addition, we showed that the Generalised Matching law (Baum, 1974) described terminal-link preference well in horses. This study has a number of implications. First, it further extends the generality of the matching law to incorporate horses (*Equus caballus*). Second, it shows that concurrent-chain schedules appear to be a suitable preparation for examining conditioned reinforcer choice in horses. Finally, it confirms that the reinforcer used (Dodson & Horrell pasture nuts) was

an appropriate substrate to maintain instrumental responding under reinforcement schedules. Based on the results of this study, it is now possible to examine the response ratios of crib-biting horses under concurrent-chain schedules.

Experiment 5

Crib-biting horses have been found to have upregulation of the mesoaccumbens DA pathway, as inferred from a relative increase in D1-like and D2-like receptor affinity in the ventral striatum (McBride & Hemmings, 2005), and this neural phenotype is similar to that which is produced by chronic stress in inbred strains of laboratory mice (Cabib et al., 1998). Despite a number of behavioural effects commonly seen in stereotypic animals being linked to more general basal ganglia dysfunction (e.g., extinction deficits, perseverative response patterns; Garner et al., 2003; Hemmings et al., 2007) a sufficiently rigorous analysis of stereotypic animals' performance in paradigms designed specifically to examine the above neural phenotype is currently lacking (McBride & Hemmings, 2005).

AMPH-infusion increases subjects' sensitivity to large rewards in discrete-trial preparations. Specifically, if a subject is offered the choice between a large-delayed or a smaller-immediate reward, typically it will choose the larger reward as a linear decreasing function of delay (e.g., Rachlin, 1978), and if the subject is administered with DA agonist (e.g., AMPH) the effects of the size of reward are increase in a dose-response manner (Wade, deWit,, & Richards, 2000). It is also fairly clear that this process is mediated by the NAcc, as lesions of this area are found to increase impulsive choice in such preparations (Cardinal et al., 2001). One of the hypothesised reasons for this effect is that the drugs decrease the sensitivity to delay, rather than increase the salience of the reward (Pitts & Febbo, 2004), and choice procedures such as concurrent schedules or concurrent chain schedules offer an opportunity to test this hypothesis (Wei-min et al., 2008).

Concurrent-chain schedules for example, may be a useful tool for examining the extent to which the ESP affects sensitivity to reinforcer delay (Wei-min et al., 2008). Crib-biting horses and non-crib-biting controls were exposed to three training sessions with *conc* VI VI initial-links and *conc* FI FI terminal-links. The results from Experiment 4 (with VI VI terminal-links) have indicated that this number of sessions should be sufficient for horses to learn to discriminate between a rich and lean alternative. Given the neural ESP (i.e., increased extracellular DA in the striatum), it would be expected that crib-biting horses would be less sensitive to the delay of reinforcement in the terminal-link schedules to controls. In addition, if this is directly related to a reduction in delay sensitivity, it would be expected that there would be no difference in overall response rates between the groups.

Method

Subjects

Eight horses: three non-crib-biting geldings (SN101, SN107, SN109), one non-crib-biting mare (SN106), two crib-biting geldings (EC201 and OC202) and two crib-biting mares (SN102 and QC204) were recruited. The subjects were regularly checked by veterinarians and were deemed in good general health. All subjects were housed in individual stables and were regularly given access to the paddock. Prior to the study, the subjects were fed a diet of concentrate feed, alfalfa and grain, and all had *ad libitum* access to hay and water. The stables were lined with rubber matting and all subjects had straw bedding.

Materials

The instrumental device and reinforcers used were as in Experiment 4.

Procedure

In order to confirm the crib-biting status of the horses, all subjects were given limited access to concentrate food and observed for five-min. Subjects were exposed to equal initial- and unequal terminal-link schedules to assess ability to select the more valuable terminal-link conditioned reinforcer. Shaping and training procedure was as in Experiment 4.

The concurrent-chain procedure was the same as in Experiment 4. However, in the current experiment various methodological changes were made. First, a two-second ITI was used. Second, an incorrect response during terminal-link schedules (i.e., after the VI schedule had timed out) was punished with the termination of the schedule, and the instatement of the ITI. Finally, FI FI schedules were used in the terminal-links (as opposed to VI VI) with the schedules set at FI 10 s FI 20 s (i.e., 2:1 ratio). The above changes were employed in order to ensure the terminal-link schedules were clearly differentiable over relatively few exposures. Previous research has shown that subjects show more extreme preference for FI over VI terminal-link schedules (Gibbon et al., 1988). The position of the FI 10-sec terminal-link was counterbalanced across the eight participants. In total each subject took part in three 30-min choice sessions. In order to analyse the data, the dependent measure was the proportion of on-target responses (i.e., responses to the shorter initial-link terminal-link chain) during initial-links and the independent factors were group (i.e., crib-biters and non-crib-biters) and session (i.e., 1, 2, & 3).

Results and Discussion

Behavioural observations confirmed that the crib-biting horses all performed crib-biting regularly. In addition, it was confirmed that none of the control group performed crib-biting. No members of the control group or crib-biting group were observed to perform any other type of stereotypy during the pre-trial observations. All raw data was entered into SPSS[®] 15 for Windows for analysis.

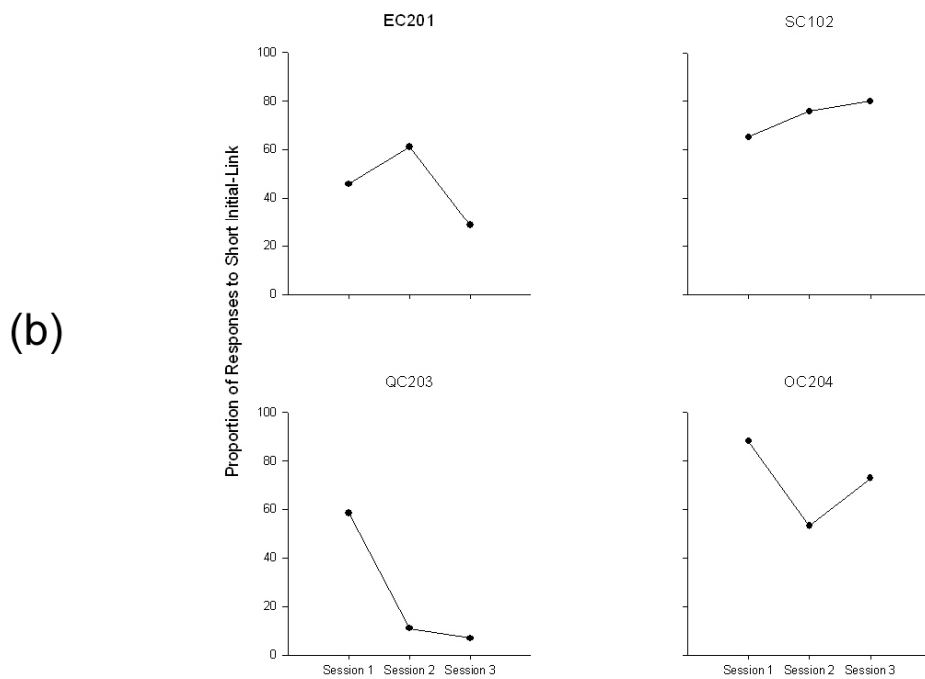
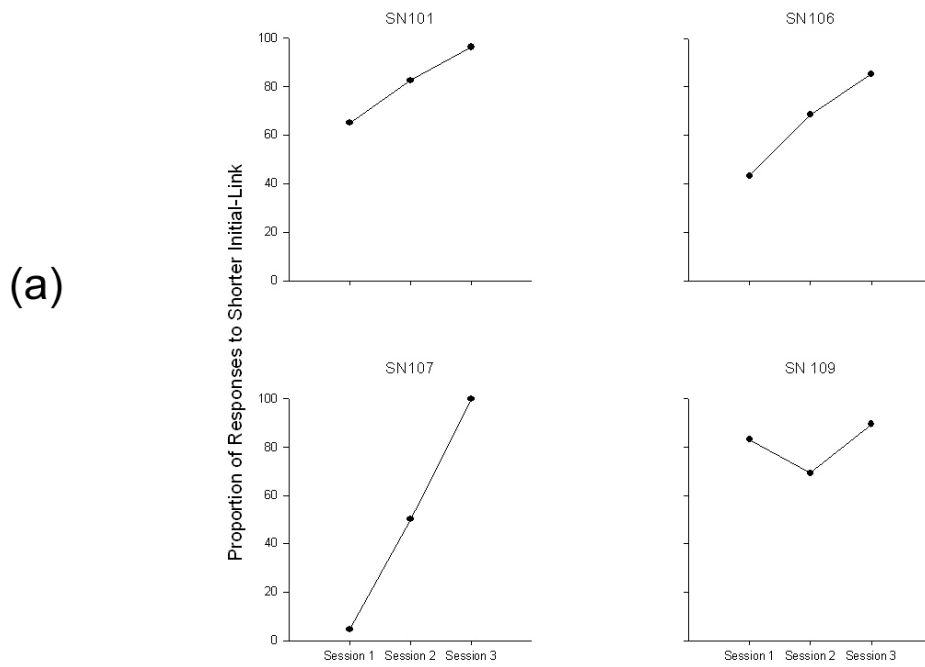


Figure 4.5. Individual horses' data under FI 10 FI 20 terminal-link schedules. Non-crib-biters' data are displayed in 'a' and crib-biters' data in 'b'.

Shaping took marginally longer for the crib-biters ($M = 45$ mins, $SD = 17.3$) than for the non-crib-biters ($M = 37.5$ mins, $SD = 15$) but this difference was not significant, $t(6) < 1$. We also examined the overall response-rates in the initial and terminal-links between groups. The non-crib-biters ($M = 474.9$, $SD = 339.6$) made marginally more responses on average than the crib-biters ($M = 313.6$, $SD = 250.9$) but this difference was not significant, $t(22) = 1.3$, $p = 0.2$.

Preference for the shorter terminal-link schedule for individual horses in the three sessions is illustrated in Figure 4.5. As is clear, generally the non-crib-biter group increased preference for the shorter terminal-link chain over the course of the three sessions (Figure 4.5a). However, in the crib-biters, horse EC201 showed what approaches indifference for the two alternatives, and QC203 appeared preferentially to choose the longer terminal-link FI schedule. The non-crib-biters all showed a marked preference for the shorter terminal-link schedule, with each reaching a relative response ratio $> 85\%$. However, in the crib-biters, horses SC102 and OC204 did appear to favour the shorter chain, but did not reach the same levels of preference across the three sessions, with SC102 reaching 80% and OC204 reaching 72% (Figure 4.5b).

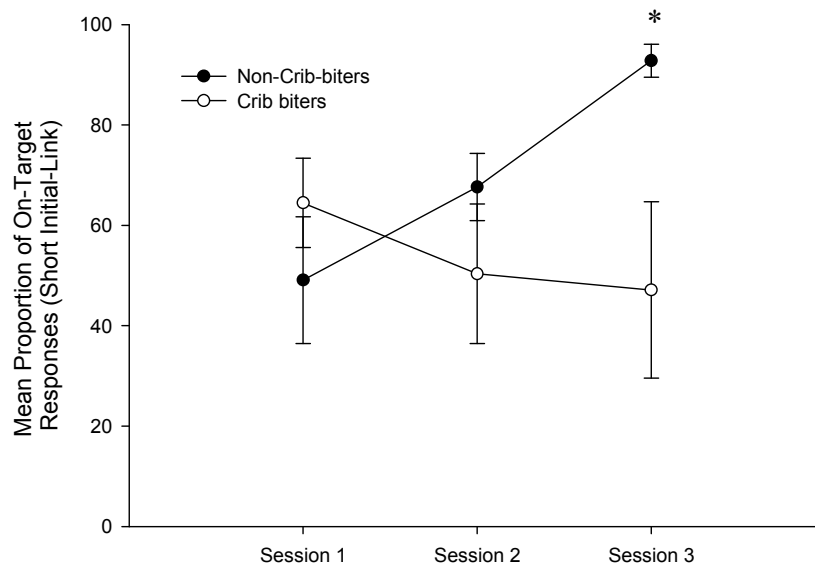


Figure 4.6. Mean (\pm SE) proportion of responses to the initial-link that led to the shorter terminal-link in crib-biters and controls. Note: * $p < 0.05$.

Figure 4.6 displays the collated mean proportion (\pm SE) of responses to the short initial-link for the crib-biters and non-crib-biters during the final third of each of the three half-hour experimental sessions. We only analysed data from this period to ensure that all of the subjects had reached steady-state responding in the session; this is common in choice procedures (e.g., Grace et al., 2006). As is clear, the non-crib-biters learned to choose the initial-link that led to the FI 10-sec terminal-link across the three sessions, where the crib-biters did not. Because of the low sample size and nature of the distribution, the data were not considered to be suitable for parametric analysis. Therefore, non-parametric tests were used, with group and trial block as between- and within-subjects independent measures respectively and relative response ratios in the initial-links phase as the dependent measure⁹. Owing to the low sample size, significance values were calculated using the ‘Exact Test’: this is more robust for small sample sizes compared to the default Asymptotic method, and it yields a lower probability of making a Type-II error (Siegel & Castellan, 1988). Freidman tests for multiple related samples were carried out for each of the groups. For the non-crib-biters, their responses for the initial-link related to the shorter terminal-link immediacy ratio increased across sessions, χ^2 ($N = 4$) = 6.5, Exact $p < 0.05$. However, for the crib-biters, there was no significant change in response allocation across trials, χ^2 ($N = 4$) = 0.5, Exact $p > 0.05$. Wilcoxon rank-sum tests were carried out between groups in all sessions. There was no significant group difference for sessions 1 or 2 (Exact $ps > 0.05$), however, non-crib-biters responded more to the initial-link associated with the shorter terminal-link immediacy ratio in Session 3, W_S (4, 4) = 10, $p < 0.05$.

Crib-biting status was found to affect concurrent-chain performance. Initially, there was no difference in responding between the groups in the first session, however, over the course of the three sessions, two of the crib-biters did not increase preference for the shorter chain, whereas all of the non-crib-biters preference increased as would be expected. This supports the hypothesis that

⁹ The data were also subjected to parametric analyses (2×2 ANOVAs), and the results obtained were identical. For a description of the parametric analyses see Appendix A.

owing to upregulation in DA activity in the mesoaccumbens pathway as associated with the ESP (Cabib et al., 1998; McBride & Hemmings, 2005) sensitivity to delay may decrease. In addition, it highlights individual differences in the behavioural phenotype, suggesting that the extent to which stress-induced neural perturbations affects behaviour and learning may be varied. Previous research has found that pharmacological sensitisation (e.g., Wei-Min et al., 2008) reduces pigeons' relative terminal-link response ratios to indifference without affecting overall response rate, and the current results suggest that the same may be true in some crib-biting horses.

General Discussion and Conclusions

Experiment 4 revealed initially, that horses were able to learn a complex free-operant procedure in a relatively short period of time. In addition, the response data has further extended the generality of the generalised matching law (Baum, 1974) to incorporate a different species (*Equus caballus*). The aim of Experiment 4 was to examine the effects of differences in striatum neurophysiology in the ESP, through an assessment of sensitivity to delay in the context of concurrent-chain choice. The results suggested that crib-biting horses' choices for the shorter terminal-link approached indifference.

Neurobiological evidence (McBride & Hemmings, 2005) has offered support for the theory that crib-biting is associated with similar basal ganglia dysfunction to that associated with chronic stress-induced sensitisation (Cabib et al., 1998), and the results of Experiment 5 offer initial support in the form of behavioural assay. We predicted that crib-biting horses would show similar behavioural characteristics relating to reduced sensitivity to delay as animals which have been administered with DA agonists. Although all of the non-crib-biting controls were able to match relative initial-link response rates to relative terminal-link reinforcement ratios (also, in Experiment 4, *all* of the subjects matched relative response rates to relative reinforcer ratios) two of the crib-biters in the current study were unable to do so. However, in the other two crib-biters,

their response allocation was relative to terminal-link immediacy ratios. This suggests that the behavioural phenotype described may vary between individuals. This assumption is supported by previous research examining basal ganglia dysfunction in laboratory rodents, with the difference perhaps being related to the severity of the stereotypy (Garner et al., 2003). Future research may examine this more closely.

If the reduction in relative response-rates in the crib-biting horses to the different immediacy ratios is, as we suggest, related to a reduced sensitivity to delay (Wei-Min et al., 2008), a possible mechanism for this behavioural phenotype may relate to DA D1 receptor activation. Matell, Berridge, and Aldridge (2006) carried out a temporal-structure analysis of stereotypic grooming sequences displayed by rats exposed to the selective DA D1 receptor agonist SKF81297. Within this analytic framework, it is possible to examine the entire stereotyped response-sequence phase-by-phase, specifically by analysing the temporal relations between the phases. They found that drug exposure significantly reduced the grooming phase-lengths, which was in turn correlated with a reduction in numbers of discrete responses within each phase. These results suggest that D1-receptors may mediate the animal's 'internal clock', thus regulating behavioural output temporally. However, over-activation of this receptor may speed-up the clock, thus shortening any perceived delay. This links well to the neural features of the ESP (McBride & Hemmings, 2005) and may be an explanation for the findings of Experiment 5. However, one of the difficulties with this interpretation is that crib-biting horses also were found to have increases in D2-like receptors in the ventral striatum. The effect of DA agonist manipulations of these receptor sub-types on phenomenological perception of delay is unclear (Matell et al., 2006).

Research utilising post-training devaluation has shown that behavioural sensitisation using pharmacological agents facilitates an accelerated dorsal shift from R-O (DMS-mediated) to S-R (DLS-mediated) learning. Specifically, Nelson and Killcross (2006) found AMPH-sensitised rats were not affected in terms of initial establishment of goal-directed actions; however, they were found

to persist to respond in order to access a devalued substrate after relatively few training sessions – an effect typically associated with over-training. This suggested that the sensitised group shifted faster from R-O to S-R strategy. Given the present results, increased levels of endogenous ventral striatal DA (as proposed in the ESP; McBride & Hemmings, 2005) appears to result in endogenous sensitisation of the striatum. It is therefore reasonable to predict that crib-biting horses would also show an accelerated shift from R-O to S-R learning.

We found some limited evidence that this may be occurring in crib-biting horses, with these subjects appearing to adopt a habitual pattern of navigation in Experiment 1. It could also be argued that the results of the present experiment (Experiment 5) were illustrative of the same process. It may be that, given the present results, increased synaptic transmission within the ventral striatum as well as decreased transmission in the DMS (McBride & Hemmings, 2005) act to facilitate an accelerated shift from R-O to S-R- based processes in crib-biting horses, compared with controls, within the context of the three sessions. Such a model of stereotypy would further elucidate findings of Garner et al. (2002) and Hemmings et al. (2007), who found respectively that stereotypic caged songbirds and crib-biting horses showed significant perseveration of instrumental responses during extinction procedures as compared to controls. Enhancement of habit formation, owing to chronic or prolonged DA agonist exposure, causes a decrease in dendritic spines of DA receptor cells in the DMS and a significant increase in the DLS (Jedynak et al., 2007). The former but not the latter (as inferred from D1-like and D2-like receptor B_{\max} values) has been observed in the ESP (McBride & Hemmings, 2005) and may suggest differences in environmental as opposed to psychostimulant-induced neurophysiological changes. In the final set of experiments in this thesis, this proposal will be examined in more detail.

Habit Formation

Differential place and response learning in horses displaying an oral stereotypy¹⁰

In previous chapters, we saw that crib-biting horses differ from controls in the way that they choose between delayed rewards in a free-operant choice paradigm and navigate in a spatial environment. This provided some preliminary behavioural correlates the ESP, with upregulation of the mesoaccumbens pathway, as well as downregulation of the nigrostriatal pathway of this group (McBride & Hemmings, 2005).

Stress-induced alterations of CNS DA physiology have been suggested to play a role in the development of stereotypies. For example, chronic stress during early development of DBA-2 mice is associated with a variety of behavioural phenotypes, including sensitisation to the locomotor effects of AMPH and

¹⁰ Data from this chapter are in press as: Parker, M., McBride, S. D., Redhead, E. S., & Goodwin, D. Differential place and response learning in horses displaying an oral stereotypy *Behavioural Brain Research*.

increased stereotypy (Cabib et al., 1998). A neural phenotype was also demonstrated in chronically stressed DBA mice, which were found to have upregulation of the mesoaccumbens DA pathway. This aspect of the neural phenotype has recently been replicated in crib-biting horses (McBride & Hemmings, 2005).

Nelson and Killcross (2006) examined the effects of AMPH-induced sensitisation, on the shift from planned-action (R-O) to habit (S-R) learning. Rats were trained in an operant chamber on a standard VR schedule. Following training, the reinforcer was devalued using LiCl. One group of rats was then exposed to AMPH and another group to saline solution. Both groups were then tested in extinction. They found that the AMPH sensitised group persisted in their operant responses, despite the devaluation, whereas the control group displayed significant response attenuation. They also found that pre-training AMPH-sensitised rats showed no difference in acquisition speed during training. These findings suggest that pharmacological sensitisation of the striatum accelerates the shift from R-O to S-R learning (Nelson & Killcross, 2006). It is therefore possible that the stress-induced sensitisation (as observed in crib-biting horses) may result in a similar behavioural phenotype. Specifically, it could be predicted that crib-biting horses would shift control from R-O to S-R learning at an enhanced rate as compared to non-crib-biting controls.

Using reinforcer devaluation would be practically unfeasible in horses, owing to the constitution of their digestive systems (McGreevey, 2004). However, an alternative method to examine the shift is a place/response task (reviewed in Packard & McGaugh, 1996). Typically, rats are trained in a two-alternative forced choice Tolman cross-maze (Tolman, 1938) to locate a food reward in one of two baited arms. For example, with the north-arm of the maze inaccessible, the east-arm (A+) is baited with food, and the west-arm (B-) is non-reinforced. The rats are placed into the south-arm, and required to locate the food. After a number of training trials, the rats are given probe trials in extinction, where the rats are placed in the north-arm; this time with the south-arm inaccessible. It is hypothesised that if rats are using S-R learning, they would turn

right (response strategy -- i.e., the same way they turned in the training trials). However, if they are using R-O learning they would be expected to turn left, having formed a representation of the spatial arrangement of the environment, and to make a decision based on available distal cues (place strategy). After a moderate period of training, rats will typically use the place strategy during probe trials, suggesting that they are using R-O learning. However, after lengthy periods of training (or overtraining) rats adopt an S-R strategy (Packard & McGaugh, 1996).

In the current study, horses were placed into a cross-maze and trained to locate food in one of the arms. The results of Experiments 1 and 2 of this thesis have demonstrated that crib-biting horses are able to use place-cues effectively. Initially, a pilot study was carried out using two non-crib-biting horses, in order to ascertain the number of trials typical horses take to adopt a S-R strategy. In the subsequent experiment, crib-biters and non-crib-biters were assessed in a place-response paradigm, using a matched-pairs design. After 20 and 40 training trials, the horses were given probe trials at the novel start point in extinction to examine if they used place or response strategy. It was predicted that the crib-biting group would show similar approach latency characteristics to controls, but adopt an S-R strategy at a faster rate than the control group.

Experiment 6

Previous research examining place-response learning has tended to train subjects for multiple sessions, typically over a number of days (e.g., Packard and McGaugh, 1996; Yin & Knowlton, 2004). As this procedure has never before been attempted with horses, it was not clear in the first instance the number of trials or the number of sessions required in order that the animals learn the location of the food. In the light of this, the aim of the first experiment was to examine the context in which a horse would shift from an R-O to an S-R strategy.

Initially, we decided to train the horses over a two-day period, with five four-trial sessions per day.

Method

Participants

Two non-crib-biting horses, P1 and P2, were recruited for the pilot study (one mare and one gelding, respectively). Both of the subjects were kept at pasture and were given additional regular access to forage (hay and haylage). Both were observed prior to the study to ensure that they did not show signs of crib-biting or any other stereotypic behaviour.

Apparatus

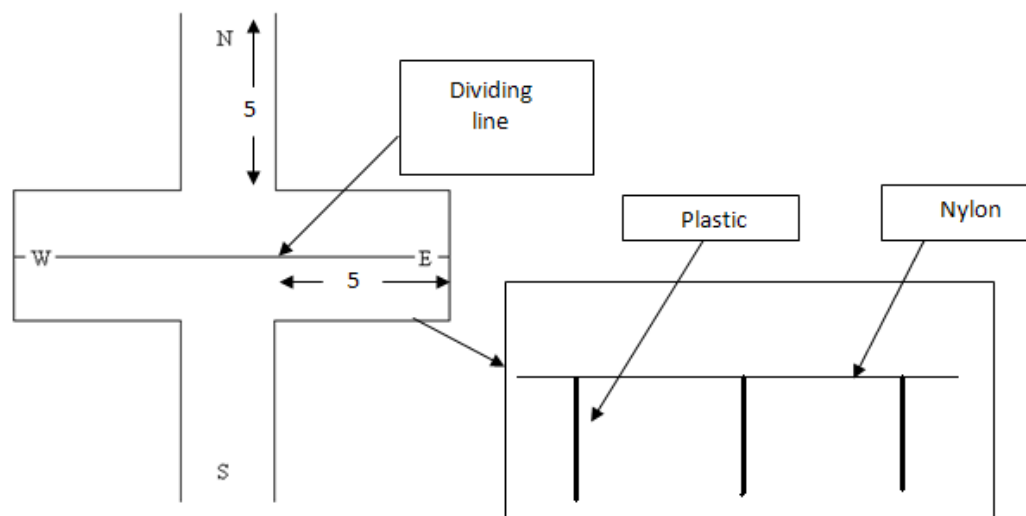


Figure 5.1. Illustration of the T-maze. During training trials, the horse was led into the maze in the (e.g.) south arm, and the baited bucket was located in the (e.g.) east arm, with the (e.g.) west arm containing an identical empty bucket. During probe trials, the horse was led in through the (e.g.) north arm, with both the (e.g.) east and west arms containing empty buckets. The dividing line in the centre was put in place to avoid the subjects following scent/tracks from the training trials.

The Cross-Maze (15m²) was constructed using plastic stakes (h = 60cm) and nylon tape (see Figure 3.1). Each arm of the maze measured 5m. Four identical black plastic buckets were located in each arm of the maze (two in the east arm, two in the west arm). The baited bucket contained approximately 5grms of hi-fibre concentrate (this has been confirmed in previous studies as being highly palatable substrate (Goodwin et al., 2002). The mazes were erected within the subjects' own establishments, in a dressage arena or similar. In all cases the mazes were surrounded with a variety of distal cues (e.g., trees, buildings, etc.). A stopwatch was used to measure approach latency to the buckets.

Procedure

In order to confirm the horses were not crib-biters, the subjects were given limited access to concentrate food and observed for five-min. Horses were initially habituated to the maze for approximately two-min. No food was available during the habituation period. For all trials, their owner, or someone who was familiar to the animal, led the horses into the maze. Following habituation, subjects were led to an external start point. This position was different for each subject (i.e., for P1 it was adjacent to the east arm, and for P2 to the west arm of the maze). Before being led into the maze in each trial, horses were led around the maze in either a clockwise or anticlockwise direction in a pseudorandom order for each trial in order to avoid a generalisation decrement in probe trials.

During the task, the horse was released from the start point (i.e., the South arm for P1 and the North arm for P2) and allowed freely to traverse the maze to locate the food. Once the food was located and consumed, the owner entered the maze and led the horse back to the start point. In the initial training trials, if the horse did not locate the food within a period of 60-seconds, the owner entered the maze and physically guided the horse to the baited bucket. For P1, this was repeated four times in each session, and there were five sessions carried out in the first day. Immediately following the final trial, the horse was exposed to a probe trial in extinction, during which it was released from the opposite side of the maze

(i.e., the North arm). The direction it chose at the choice point was noted. On day two, this procedure was repeated, with a probe trial again immediately following the final training trial.

Results and Discussion

Both subjects were observed and confirmed as horses that did not crib-bite. For pilot subject 1 (P1; mare), the training trials were split into two sessions of 20 trials, and one session of 12 trials. In each session, the last trial (i.e., trial 20) was a probe. Initially, we intended to run two sessions. However, P1 failed to adopt a response strategy after the two sessions therefore we ran a further, shortened session, to ascertain the point at which P1 shifted to a response strategy. For analysis, each session was further split into five arbitrary blocks of four-trials. Initially the proportion of correct trials in each block was plotted (see Figure 5.2).

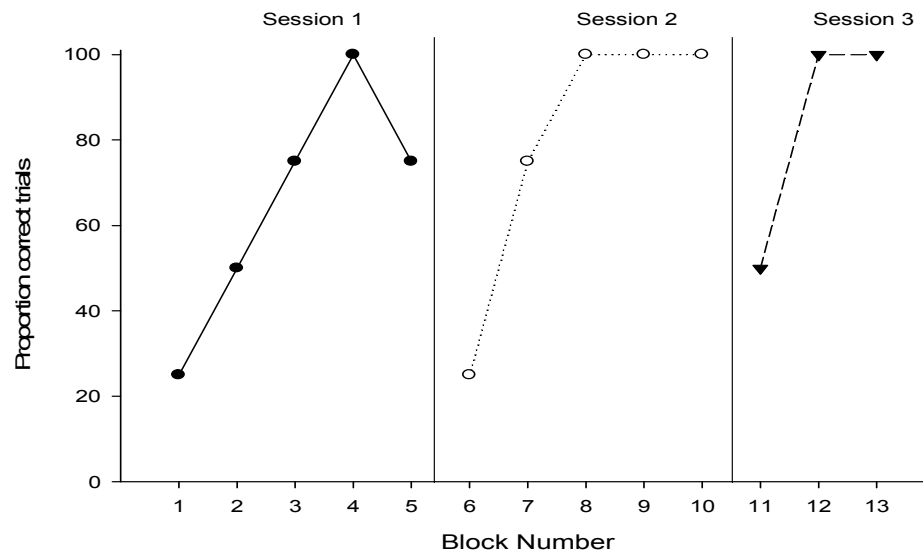


Figure 5.2. Proportion of correct trials in each block for P1. Sessions 1 & 2 lasted 20-trials, and session 3 lasted 12 trials.

As can be seen in Figure 5.2, during all sessions, P1 improved response accuracy through the blocks. During probe trials, P1 used place strategy during the first and second probe trial (i.e., after 20 and 40 training trials), but adopted a response strategy after the third probe trial (i.e., after 52 training trials). Sessions 1 and 2 were run on consecutive days. We reasoned that perhaps for horses, a longer ITI was not appropriate in this context, and this may have been the reason why P1 failed to adopt a response strategy after the 40th trial. Session 3 was run after Session 2, following a 45 min break. We therefore decided for the second pilot-subject, to run two consecutive sessions, separated by a 45 min break.

For P1, the trials in each session had been run consecutively, with no breaks in-between blocks. It was noted that P1's approach to the baited bucket was slowing during the course of the trials. In the light of this, it was decided that the training sessions would be formally split into five blocks of four trials, with a 2-min break between blocks. This would give the horse a brief rest, and would help to increase motivation for the next block. Food approach latency was also recorded for each trial.

Pilot subject 2 (P2; gelding) took part in two training sessions, with each separated into five blocks of four trials. As stated above, each session was separated by a 45 min break, during which the subject returned to its stable. For P2, the time taken to locate the food in the maze (approach latency ms) was collected, as well as the proportion of correct trials in each block. Probe trials were again run in the 20th trial of each session. Figure 5.3 displays the acquisition data for P2 across the two sessions.

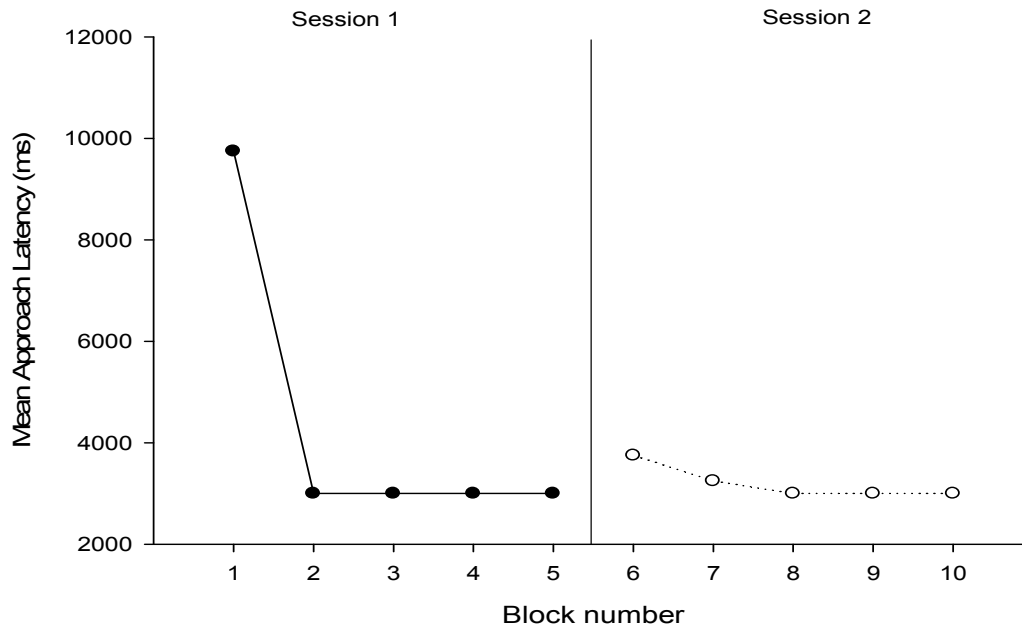


Figure 5.3. Mean approach latency (ms) across the ten training blocks for horse P2.

P2 learnt the discrimination quickly, and made no errors other than in Trial 1 (Block 1). For this reason, the error graph is not shown. In the first of the probe trials, P2 turned towards the previously reinforced arm of the maze, but for the second, towards the previously non-reinforced side. This suggested that P2 had used a place (R-O) strategy after 20-trials, but had adopted a response (S-R) strategy after 40-training trials.

The difference between P1 and P2 in terms of time to shift strategy may have been the result of the long retention interval between Session 1 and Session 2. This supposition is further supported by the number of errors made in Session 2 by P1, as compared to P2. The results of the pilot study suggested that 40-training trials may be a sufficient number to induce a shift from an R-O to an S-R strategy in horses, providing the training trials are carried out in one day, with a break between sessions.

Experiment 7

The results of the pilot study in Experiment 6 suggested that non-crib-biting horses may adopt an S-R strategy after around 40-training trials, provided that the trials are split into blocks, and that sessions are completed concurrently. Therefore, for Experiment 7, all subjects took part in two, 20-trial sessions, each separated formally into four-blocks of five-trials. In addition, both sessions were run in the same day. The aim of the following experiment was to assess the rate at which crib-biters adopt an S-R strategy as compared to non-crib-biting controls. Based on pharmacological sensitisation studies (Nelson & Killcross, 2006) and neural phenotype associated with crib-biters (McBride & Hemmings, 2005), it was expected that crib-biters would adopt an S-R strategy at a faster rate than controls.

Method

Subjects

Ten horses ($n = 3$ crib-biting and $n = 3$ non-crib-biting geldings, and $n = 2$ crib-biting and $n = 2$ non-crib-biting mares) were used for the study. The subjects were selected following an advertisement on an internet-based forum. For each crib-biter, a control subject was recruited from the same establishment that did not display crib-biting (in one case, two controls were selected from the same establishment). The subjects were matched as far as possible for age, and the age of the crib-biters ($M = 12.0$, $SD = 4.53$) and controls ($M = 12.8$, $SD = 2.59$) did not differ significantly, $t(8) = -0.34$. They were also matched as far as possible for breed and management routine. The latter was achieved by assuring that each control resided with a crib-biter, and in addition, were managed in the same way (i.e., turn-out regime, forage availability, work regime, etc.).

Apparatus

The materials were as in Experiment 6 (see Figure 5.1).

Procedure

The general procedure and habituation regime was as in Experiment 6. In order to confirm the crib-biting status of the horses, all subjects were given limited access to concentrate food and observed for five-min. For all learning trials, horses were led into the maze by the owner, who was in all cases blind to the aims and objectives of the experiment. The reasons for this were both to avoid experimenter bias and to ensure the horse remained calm during the procedure. Training trials were carried out in blocks of four, with a two-min break between each block. During the break, the horse stood with the owner by the start point. In total, each horse completed 20-trials in the first session. For each subject, the 20th trial was a probe trial, during which the horse was led to the opposite entry point to the training trials and released in the same manner by the owner. During the Probe Trial, neither bucket contained food.

Following the Probe Trial, the horses had a break from the procedure for 45 min. During this break, the subjects were returned to their normal environment (i.e., stable or field) and were given access to hay-forage. In the second session, the training trials were re-instated as in Session 1. In the second session, the horses completed 20-trials, with the 20th being a probe trial. The food-location-latency and proportion of correct trials in each block were collected for each subject.

With respect to data analysis, chi-squared tests were carried out with crib-biting status as the independent factor and place or response choice during each of the probe trials as the dependent variable. In addition, acquisition rate was assessed with food approach latency (ms) in each block as the dependent measure, block as the within-subjects factor, and crib-biting status as the between-subjects

factor. All statistical analyses were performed in respect to a Type I error rate of 0.05. Raw data were entered into SPSS[®] 15 for Windows for analysis.

Results and Discussion

Prior to data collection, all subjects were screened to assess crib-biting status. It was confirmed that all reported crib-biters performed crib-biting regularly and further that none of the non-crib-biting controls performed the behaviour. One member of the crib-biting group was also observed to perform box-walking.

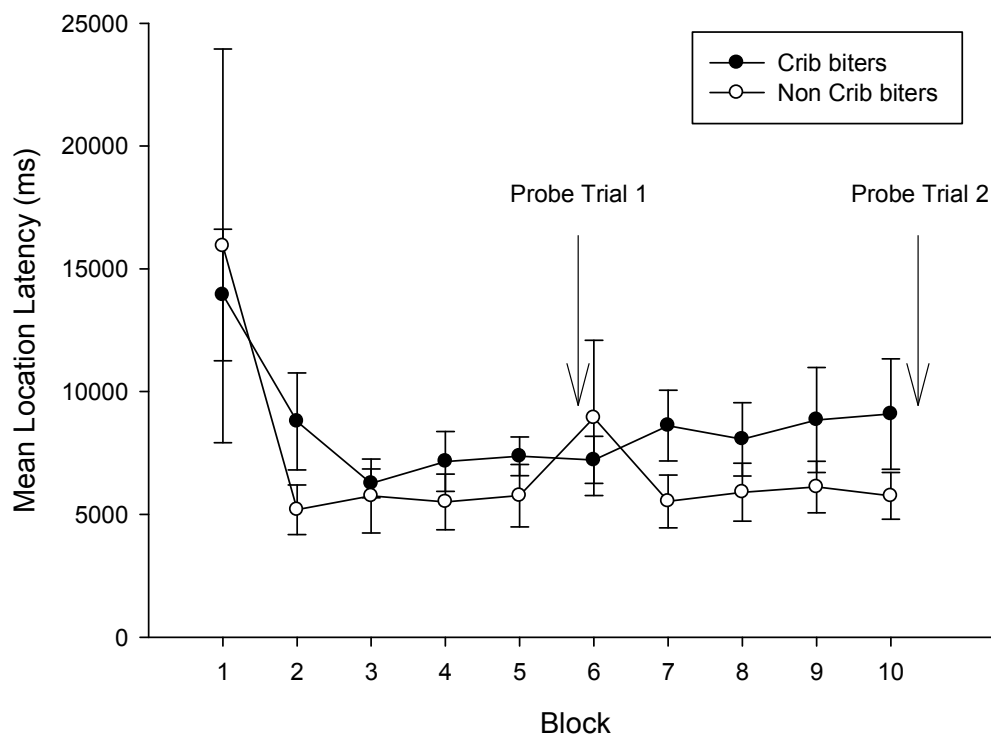


Figure 5.4. Mean (\pm SE) food location latency (ms) as a function of block (i.e., four trials) in crib-biting and non-crib-biting horses. Probe trials were conducted in the last trial of the fifth and tenth blocks.

In order to examine acquisition, a mixed design 2×10 ANOVA was carried out, with crib-biting status as the between subjects factor and block as the within subjects factor. There was a significant main effect for block, $F(9, 72) = 4.5$, but there was no main effect for group, nor for group \times block interaction, $F_s < 1$. This suggests that both groups learned to locate the food at similar rates, with the location latency typically reducing to stabilise after the first block of learning trials (see Figure 5.4). All horses experienced two probe trials in extinction, the first in Trial 20 and the second in Trial 40, and the number of horses that displayed place and response learning during these trials is illustrated in Figure 5.5.

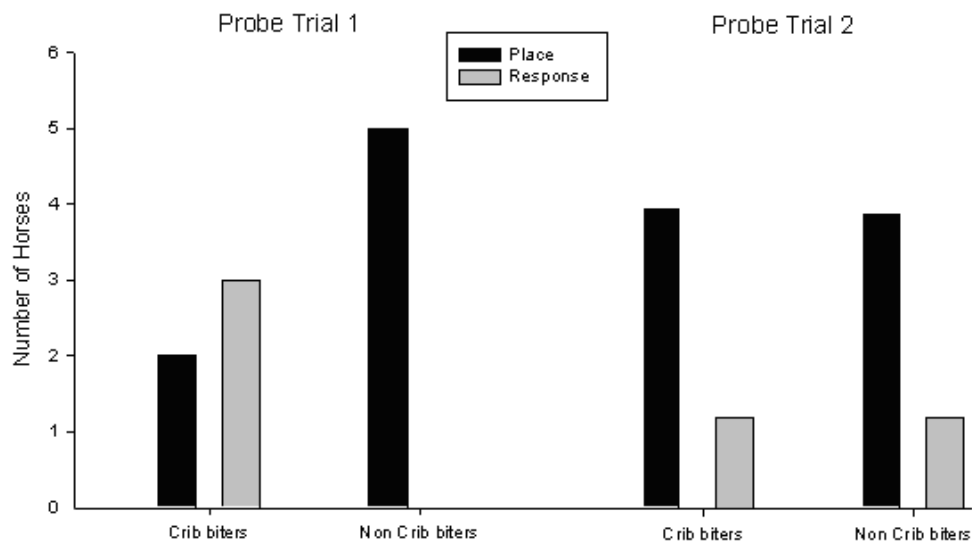


Figure 5.5. Number of horses in each group that showed place or response learning in the probe trials (i.e., learning trials 20 and 40)

In the first probe trial, there was a significant difference between the groups, with more of the crib-biters displaying response learning than non-crib-biters, $\chi^2(1) = 4.29$. However, in Probe Trial 2 there was no difference between the groups, with both primarily displaying place learning, $\chi^2(1) = 0$. These results were somewhat surprising as, based on the results of Experiment 6, it was expected that both groups would adopt response learning after 40 trials. The

results from Probe Trial 1 suggest that crib-biters adopt response learning at a faster rate than controls.

The explanation for the result in Probe Trial 2 may be that, for the non-crib-biters, 40 trials did not provide sufficient exposure to the maze to guarantee the adoption of response learning. Owing to time constraints and owner-convenience, the trials were carried out in one day, with approximately one-hour break between sessions. As we saw in Experiment 6, splitting the sessions up into separate days seemed to lead to the subject re-learning the maze and failing to adopt the response strategy. Perhaps if the sessions had been carried out over a number of days with, for example, one block per day for each participant, response learning may have been seen after 40 trials. Without extensive previous research with this species this is speculative, and is based on results from the existing rat literature (e.g., Packard & McGaugh, 1996).

An alternative explanation may be related to the cross maze design in the present study involving, essentially, two mazes: one ‘training’ and one ‘mirror’ maze. This is somewhat atypical for this procedure, with previous studies blocking access to the Probe Arm during training trials, with the ‘east’ and ‘west’ arms remaining the same. We employed the current methods to avoid the horses either following a scent at the choice point, or following an indented trail in the terrain. In laboratory based studies, this is not an issue, as the maze can be constructed of solid material and can be cleaned between trials to remove scent markings. It is possible that the horses in the current experiment had learnt during Probe Trial 1 that the mirror maze represented a different context to the learning trials. This would have been exacerbated by the Probe Trial being in extinction. The rapid learning rates in the acquisition trials (as well as results from Experiments 1 & 2 of this thesis) would support the suggestion that horses learn context relatively quickly. If this is the case, during the second probe trial, the horses may have recognised the different context, and the horses may have displayed conditioned discrimination of the mirror maze.

Another caveat was that during Probe Trial 1, although three of the crib-biters appeared to adopt an S-R strategy, two did not. This may present a problem

in the interpretation of the data. Because of the low sample size, the analyses are more open to Type I error (Siegel & Castellan, 1988). We therefore decided to carry out a further experiment, this time forcing the animals to use S-R learning. It would be expected that if the crib-biters shifted from R-O to S-R at an enhanced rate, they would learn this procedure at a faster rate than controls.

Experiment 8

The results of Experiment 7 suggested that crib-biting horses shift from place to response at a faster rate in an over-training paradigm. This group, owing to neurophysiological perturbations associated with the ESP (McBride & Hemmings, 2005), may display behavioural characteristics of sensitisation (Nelson & Killcross, 2006). However, results from the second probe trial in Experiment 7 were confusing as it was expected, based on results from the pilot study (Experiment 6) that the horses would have shifted to a response strategy after 40-trials. We reasoned that this anomaly may be due to horses learning a conditioned place-discrimination as a result of the first probe trial, but to be sure, we designed a second study, this time teaching the horses to use a response strategy.

Crib-biters and controls were placed into a similar cross maze as that described in experiments 6 and 7. This time however, they were trained in a discrete trial-format, that food was always located on the left. This was done by changing the point of entry into the maze from trial to trial, in an attempt to nullify the efficacy of using place-cues. Based on the results of Experiment 7, it was expected that the crib-biters would learn this discrimination at a faster rate than controls.

Method

Subjects

Four crib-biting mares, one non-crib-biting mare and three non-crib-biting geldings were selected for the study. All of the subjects came from the same institution. In previous studies in this thesis, we have attempted to match for gender. However, in this study, owing to the resources available at the time of the study, we were unable to do so. Having said this, we have found no evidence for gender differences in any of the previous studies, so this was not considered to be a confounding issue.

Apparatus

The general apparatus was similar to that described in experiments 6 and 7. However, in the current study, we did not create a ‘mirror maze’; the horse entered the apparatus from both ends (i.e., North and South).

Procedure

As in previous studies, all subjects were screened to assess their crib-biting status. Initially, subjects were led into the maze, directed into the reinforced arm and allowed to consume the food. Following this, the training trials commenced. Unlike experiments 6 and 7, the horses were not allowed to traverse the maze freely to locate the food. In these studies, incorrect initial choices could have been inadvertently maintained, as the trials always ended in reinforcement. Because of the relative simplicity of the original designs, this did not prove to be problematic, with all of the subjects quickly learning the spatial location of the food. However, because of the relative complexity of the current procedure, we

implemented a discrete-trial format, whereby incorrect responses (i.e., the horse choosing the non-reinforced arm) were never reinforced (the horse was immediately removed from the maze).

The order of entry (i.e., North [N] or South [S]) was arranged in random order (e.g., NNSSSNSSNNSSNS etc). The random order was generated through a simple algorithm written in Microsoft Excel. During trials, the arm directly opposite the entry-arm was blocked (i.e., if the horse entered via the South arm, the North arm was blocked). Food was always located either to the left or to the right, and this was counterbalanced across subjects. For example, if a 'turn-left' horse entered via the South arm, food would be located in the West arm, whereas if it entered via the North arm, food would be located in the East arm. Horses were trained for 40-trials, split into eight blocks of five trials. Between each block, horses had a break of 1-min. Training took approximately 45-min per animal.

Following training, each horse was exposed to two probe trials. During the probe trials, the subjects entered the maze via the East or West arms. This was carried out in extinction, with two empty buckets placed in the North and South arms respectively. The two probe trials were separated with four reinforced training trials, two via the North arm and two via the South arm. Again, the order of entry was randomised across subjects.

Owing to the format of the procedure (i.e., strict discrete-trial), location latency was deemed inappropriate as an operationalisation of learning in this context. Therefore, the dependent measure was the proportion of errors within each trial block. All statistical analyses were carried out in respect to a Type I error rate of 0.05.

Results and Discussion

Behavioural observations confirmed the crib-biting status of the subjects. In addition, none of the subjects (in either group) were observed to perform any

other type of stereotypy. Figure 5.6 displays the error rates of both groups as a function of Trial Block.

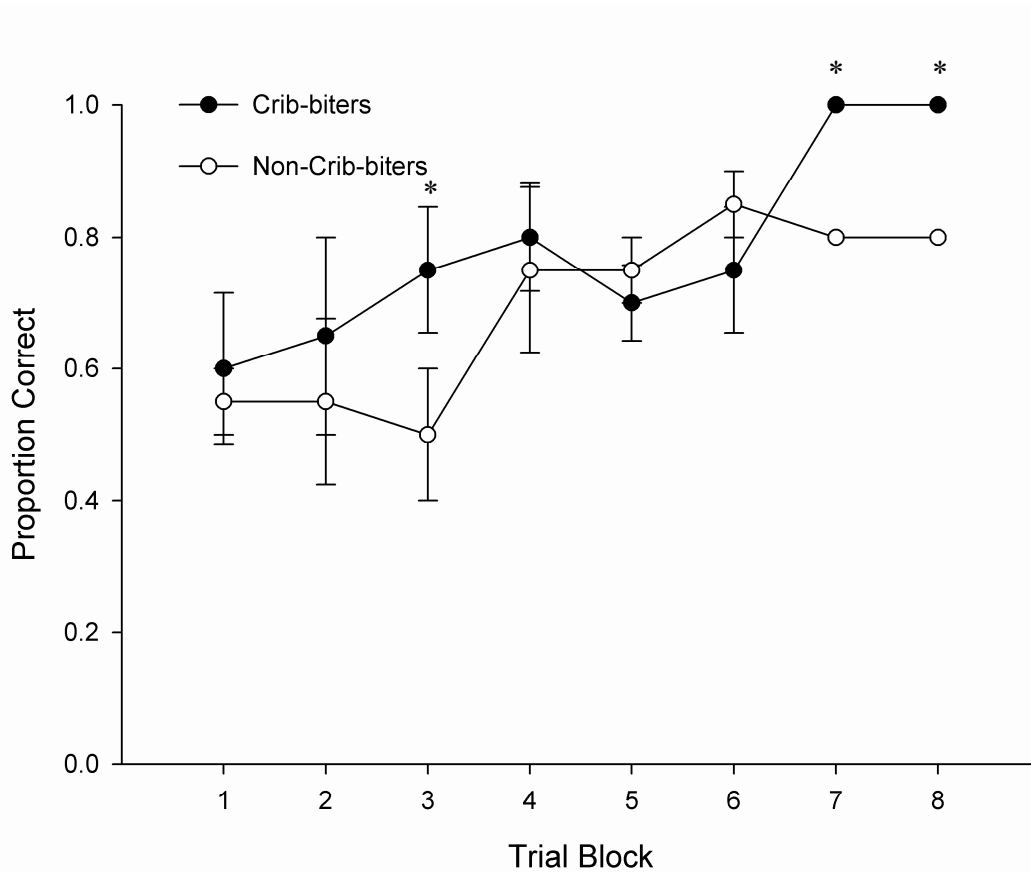


Figure 5.6. Mean (\pm SEM) proportion of correct trials in each trial block for the crib-biters and non-crib-biters. Note * $p < 0.05$.

As is clear from the graph, both groups appeared to learn the discrimination across the trials. Proportion data were arcsine-root transformed prior to analysis. The data were analysed using a mixed-design, 2×8 ANOVA, with crib-biting status as the between subjects and Trial Block as the within-subjects factor. We found a significant main effect for Trial Block, $F(7, 42) = 5.79$, and for status, $F(1, 6) = 10.12$, and a significant Trial-Block by status interaction, $F(7, 42) = 2.26$. Simple main-effects analyses (Keppel, 1973) revealed that the crib-biters made a

significantly lower proportion of errors than the non-crib-biters in the third, $F(1, 48) = 4$, seventh, $F(1, 48) = 10$, and eighth, $F(1, 48) = 10$, trial blocks.

Unfortunately, owing to an error, the empty buckets were not present during the probe trials for subjects CB1004 and NCB1004 and their data is therefore excluded from further analysis. Table 5.1 displays the data from the probe trials. Although this demonstrates that the crib-biters appeared to be more likely to turn the same way as during training, as the sample size was only $n = 3$ in each group, it was not considered that statistical analyses on the Probe Trial data would prove fruitful.

Table 5.1

Number of Crib-biters and non Crib-biters that Turned the Same Way as in Training Trials

	Probe Trial 1		Probe Trial 2	
	Same	Opposite	Same	Opposite
Crib-Biters (N)	2	1	2	1
Non Crib-Biters (N)	1	2	1	2

The results from the training trials demonstrated that the crib-biters learned the discrimination at a faster rate than controls, suggesting that they shifted to an S-R strategy at an enhanced rate in the context of this procedure. These findings offer further support to the stress-induced sensitisation model of the ESP (McBride & Hemmings, 2005) and go some way to validating the results of Experiment 7. Alternatively, the results may be explained by altered patch foraging behaviour – perhaps the crib-biters were more focussed on the highly palatable and frequently replenished food than the controls and hence learned the task faster. This would also fit with a theory of overall stimulus enhancement in this group, as suggested from the mesoaccumbens perturbations associated with the phenotype. One issue with this approach however may be that in the original study (Experiment 7) we found no significant differences in acquisition. It may

be preferable in future studies to remove this possible confound, perhaps by using other operationalisations of the shift – for example, contingency degradation (Balleine & Dickinson, 1998).

General Discussion and Conclusions

Initially, it was found in Experiment 6 that horses appear to shift their learning strategy from S-R to R-O after around 40-training trials. In Experiment 7, we examined the rate at which crib-biters shift their learning strategy from R-O to S-R. Based on previous neurophysiological profiling of these animals (McBride & Hemmings, 2005), it was predicted that the crib-biters would adopt an S-R strategy at a faster rate than matched-control horses. The results offered some initial support for the hypothesis, with crib-biters tending to adopt a response strategy earlier than controls. However, the results were inconclusive, as during the second probe-trial, the crib-biters appeared to revert to place learning. Finally, in Experiment 8, we found that crib-biters learnt a discrimination which required S-R learning at a faster rate than controls. Taken together, the results suggest that crib-biters do shift from R-O to S-R learning at a faster rate than controls, supporting the notion of stress-induced behavioural sensitisation in the ESP.

The explanation for the early shift to response learning in the crib-biters may be related to alterations in basal ganglia function. Specifically, inactivation of the DLS impairs response learning even after extensive learning (Packard & McGaugh, 1996) and inactivation of the hippocampus leads to a general decrease in ‘place’ choices and an increase during early training of ‘response’ choices. The latter finding is unsurprising, given extensive evidence that the hippocampus is crucial to spatial navigation (e.g., Good & Macphail, 1994; Nadel, 1994; Nadel & Hardt, 2004; Nadel & Macdonald, 1980; Okeefe & Dostrovs, 1971; Okeefe & Nadel, 1979). However, a complete lack of spatial awareness does not seem a likely interpretation of the present results based on the findings in Chapter 2 of this thesis relating to crib-biters’ place learning. Instead, crib-biting horses, with

postulated upregulation of the mesoaccumbens, and downregulation of the nigrostriatal pathways, appear to show a similar behavioural phenotype to AMPH-sensitised rats, whereby the shift from DMS-mediated R-O learning shifts to DLS-mediated S-R learning at an enhanced rate.

Canales and colleagues (e.g., Canales, 2005; Canales, Capper-Loup, Hu, Choe, Upadhyay, & Graybiel, 2002) have demonstrated that during the shift between R-O and S-R learning, there is a change in balance between matrix and striosome cell activation in the striatum. This behavioural and physiological alteration in functioning is enhanced by chronic AMPH exposure (Canales & Graybiel, 2000) and thus, it appears that the shift between R-O and S-R learning and the progressive imbalance of striosome and matrix cells are mediated through DAergic pathways. The behavioural data presented in this study suggest that crib-biting horses also have a similar imbalance of striosome and matrix cells.

In the light of the general understanding of the physiology of learning, which currently does not fully take into account the striosome/matrix differentiation, the link between the behavioural and physiological data of the oral stereotypy phenotype can also be explained in terms of structural differences in the striatum. There is a clear anatomical and functional dissociation within the dorsal striatum between the lateral (DLS) and medial (DMS) aspects, with the former pertaining to S-R, and the latter to R-O learning (Featherstone & McDonald, 2005; Yin & Knowlton, 2004; Yin & Knowlton, 2006). Yin and Knowlton (2006) proposed that there was a hierarchy of three systems within the striatum. The limbic network incorporates the ventral striatum (nucleus accumbens; NAC), with excitatory input from the pre-frontal cortex. The associative network incorporates the DMS, with excitatory input from the pre-frontal and parietal cortices. The sensorimotor network incorporates the DLS putamen, with excitatory input from the sensorimotor cortex. During habit formation, there is a shift through the hierarchy, from initial Pavlovian processes, through R-O judgements, finally to automated, habitual responses, driven by discriminative stimuli.

Chronic exposure to stimulant drugs (Cooper & Dourish, 1991; Robbins & Everitt, 2007) and chronic stress during early development (Cabib et al., 1998) result in heightened locomotor response to amphetamine, increased stereotypy and alterations in dopaminergic activity in the striatum. In the early stages of this process there is evidence for the involvement of the NAcc. Positively valenced unconditioned stimuli (USs; e.g., food, drugs, sex, etc.) enhance dopamine release in the NAcc (see Robbins & Everitt, 2007 for a review). During associative learning, dopamine activity in the ventral striatum correlates with reductions in reward-prediction error between the CS, US, and CR (Morris et al., 2006). During this process, information about future responding in the presence of a specific reward-predictive Pavlovian CS is stored in the dorsomedial striatum (DMS; Morris et al., 2006). This sequence can be equated to the transition to S-R learning where, in the latter stages of learning, the DLS exerts top-down control with DMS dopamine activity becoming subsidiary (Yin & Knowlton, 2006).

Dopamine efflux in the ventral striatum in the presence of Pavlovian CSs provides a feedback mechanism which modulates the activity of the DLS during the S-R stage of learning (Ahn & Phillips, 2007). Crib-biting horses have significantly higher D1 and D2 receptor subtypes in the NAcc and significantly lower D1 receptor subtypes in the DMS (McBride & Hemmings, 2005), therefore, the accelerated shift from R-O to S-R observed here may be explained by this alteration in DAergic cytoarchitecture. However, co-activation of DA D1 and D2 receptors is also a pre-requisite for striosome-to-matrix predominance (Capper-Loup et al., 2002). This suggests that, through the commonality of DA mediation, the latter is intrinsic to both environmentally-induced stereotypy development and the formation of habitual responding. As a caveat to this, it should be stated that striosome-to-matrix predominance may still be an artifact rather than a critical component of environmentally-induced stereotypy development. Further work elucidating the signal of striosome neurons may help clarify its role in the development of these behavioural phenomena.

To summarise, during two experiments examining place- and response-learning, crib-biting horses were found to shift from R-O to S-R at a faster rate

than non-crib-biting controls. These findings are an important step towards a more clear understanding of the behavioural processes associated with endogenous basal ganglia dysfunction and may have important implications for the development of conceptual aetiological models of spontaneous stereotypy. It may be that the early shift relates to overrepresentation of striosome cells in the striatum, as compared to matrix cells. Future research may attempt to explore this further, perhaps utilising early-gene assays or, at least in the first instance, Mu-opioid receptor activation. In addition, the shift could be further explored by a systematic *in vivo* examination of basal ganglia activity during the various stages of the learning process.

General Discussion

Purpose of thesis

The primary goal of this thesis is to examine some of the behavioural processes associated with a model of endogenous basal ganglia dysfunction in the context of the ESP. The model was developed based on research with in-bred strains of laboratory mice, where an interaction of genetic and environmental factors (particularly stress) results in behavioural sensitisation leading to a sensitised locomotor response to DA agonist challenge and alterations in midbrain DA physiology (Cabib & Bonaventura, 1996). The neural phenotype was found in horses displaying an oral stereotypy (McBride & Hemmings, 2005) suggesting similar stress-induced sensitisation in this group. However, the extant data regarding the effects of behaviour and learning of these differences was inconsistent.

A model of the aetiology of stereotypy was therefore proposed (Chapter 1; *Figure 1.7*) with a number of testable hypotheses. Behavioural sensitisation is associated with a decrease in sensitivity to delay (Wei-min et al., 2008), increased motivation to perform operant behaviours in the context of a Pavlovian cue

(Wyvell & Berridge, 2001) and an accelerated shift from R-O to S-R learning (Nelson & Killcross, 2006). In addition, downregulation of DMS DA transmission may lead to differences in cue-guided spatial navigation (Devan et al., 1999). We designed a series of experiments examining whether the neural phenotype associated with oral stereotypy in horses resulted in the same behavioural effects as those exhibited by animals sensitised through pharmacological agents.

Summary of findings

Spatial Learning

The first studies we carried out related to downregulation of DA transmission in the DMS. DMS lesions have been shown to affect spatial navigation strategies in rats (Devan et al., 1999). One of the neurobiological features of the ESP is a downregulation of DA transmission in the DMS (McBride & Hemmings, 2005), and we reasoned that crib-biting horses may show similar differences as the lesioned group. We therefore placed crib-biting and non-crib-biting horses into two spatial navigation procedures.

In Experiment 1, we designed a circular maze where the subjects were required to locate a food reward in one of a number of buckets placed around the perimeter. Devan et al. (1999) found that DMS lesioned rats showed increased location latency and increased Thigmotaxis (a tendency to remain close to the perimeter of the maze during search). We found no support for the first hypothesis, but we did find that crib-biters spent significantly more time than controls in proximity to the perimeter. It is generally well established within the Spatial Navigation literature that Thigmotaxis should reduce during the course of the learning process (e.g., see Devan et al., 1999), which was not the case in the present study. We therefore suggested that perhaps our results were illustrating difficulties in integrating local and global cues in the crib-biting horses, with this

group perhaps preferentially processing local (i.e., the buckets) over global (i.e., distal) cues, another possible effect of DMS dysfunction (Devan et al., 1996).

In order to test this, in Experiment 2 we designed a second spatial navigation experiment, this time using a decoy salient local cue and a number of distal cues. We reasoned that if the crib-biters had difficulties integrating local and distal cues, preferentially learning about the local cue, they would fail to learn about the location of a food reward in relation to distal cues when the local cue was removed. The results failed to show any difference between the groups, with both performing equally in the probe trials. However, a number of interesting findings came from the studies.

For example, during the circular maze study (Experiment 1) the crib-biters appeared to adopt a ‘habitual’ response pattern. To explain, in the round maze there were four possible entrances, the order of which was counterbalanced across trials. In each block of four trials, regardless of the location of the food, the most effective strategy would be to turn left twice, and right twice. However, when we analysed the route strategies from the final two trial-blocks, we found that while the non-crib-biters adopted the most effective strategy, the crib-biters appeared often to repeat previous responses. Increased extra-cellular mesoaccumbens DA is known to result in increased rate of habit formation (i.e., shift from R-O to S-R learning) and the findings of Experiment 1 may reflect this (Jedynak et al., 2007; Nelson & Killcross, 2006).

Crib-biters were also more likely to follow direct routes to the reinforced buckets in Experiment 2. To explain, during the learning trials, the non-crib-biters appeared to explore the arena prior to locating the food reward. Similar patterns were found in the probe trials, whereby the crib-biters tended to remain in the vicinity of the previously rewarded bucket, but the non-crib-biters tended to explore the arena. It is possible that features of the ESP caused the crib-biters to be more stimulus-focussed and less likely to perform patch foraging behaviour and exploratory behaviour. To explain, upregulation of the mesoaccumbens pathway may lead to an increase in sensitivity to reward-related cues (i.e., in this case, the relevant bucket) and this was supported by our results showing that crib-

biters were marginally more likely to choose the CS+ in a post-training conditioned discrimination trial. In addition, decreased DA transmission in the DMS and a faster shift to DLS control may have resulted in a fast shift to S-R learning. Based on these results, the Model outlined in Figure 1.7 could be adapted as illustrated in Figure 6.1.

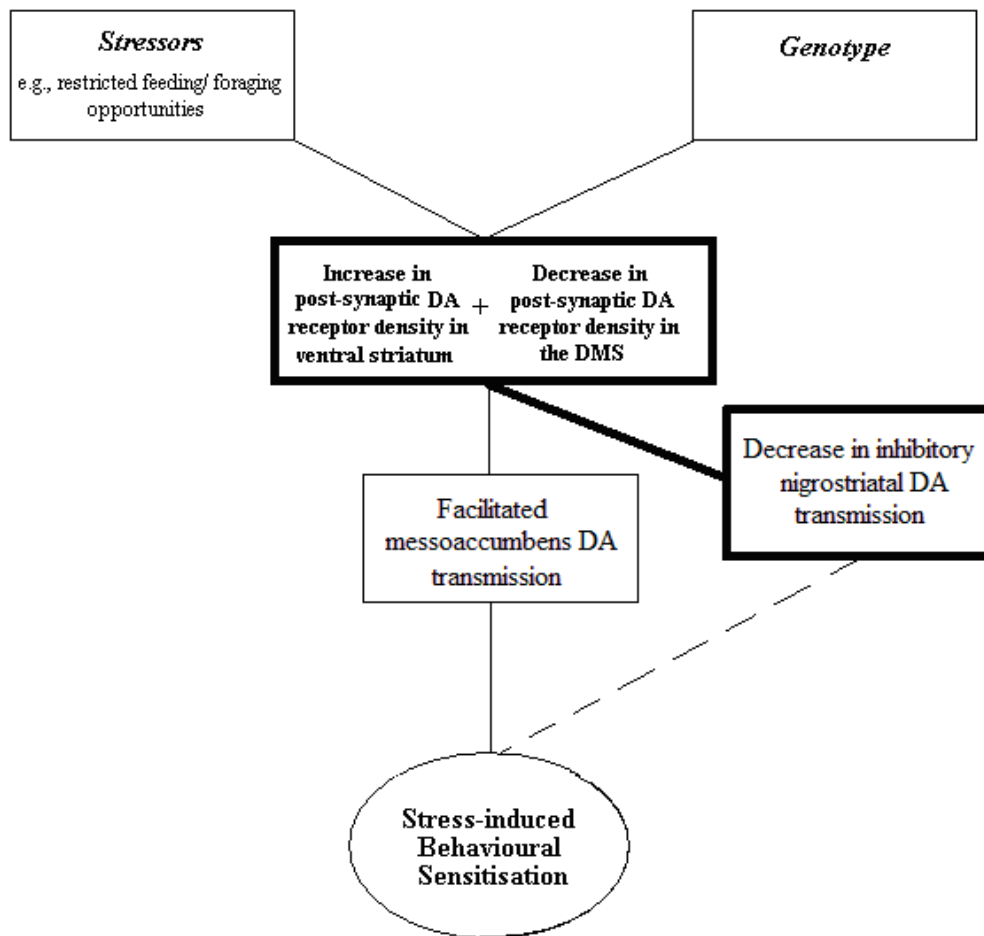


Figure 6.1. Schematic of stress-induced behavioural sensitisation model of stereotypy. n.b. solid lines indicate features supported by existing evidence, dashed lines indicate features not yet fully determined by research.

In this adaptation of the original model, we illustrate that no behavioural evidence was found to suggest that DMS dysfunction and upregulation of the mesoaccumbens pathway could be treated as mutually exclusive features of the Phenotype. Therefore it could be argued that increases in extracellular DA, as a

direct result of facilitated DA transmission in the mesoaccumbens pathway, lead to a general decrease in DMS control over behaviour, resulting in increased DLS control (habit). This theory is supported by existing accounts of neural plasticity during the process of pharmacological sensitisation (e.g., Jedynak et al., 2007) and could therefore be hypothetically implicated in stress-induced behavioural sensitisation.

Incentive Sensitisation

In the next experiment (Experiment 3) we wanted to examine in detail the behavioural effects of upregulation of mesoaccumbens DA reported in the ESP (McBride & Hemmings, 2005). AMPH-exposed rats were found, in the presence of a Pavlovian reward-cue, to show increased operant responses for the same reward (Wyvell & Berridge, 2001), suggesting that sensitisation leads to an increased incentive for reward in the presence of a CS. Facilitated DA transmission in the ESP could therefore be predicted to lead to similar increased incentive motivation.

We tested crib-biting and non-crib-biting horses in a PIT procedure, where subjects were first trained to associate a sound-CS with food delivery, and subsequently to make an operant response to receive the same US. In the test trials, the animals were put into extinction, and exposed to a number of CS presentations. It was hypothesised that the crib-biters would perform more responses during the CS presentation than the non-crib-biters, compared to baseline. Unfortunately, owing to logistical problems, our sample size in the study was limited to $N = 4$ ($n = 2$ crib-biters and $n = 2$ non-crib-biters) therefore our results were not suitable for statistical analysis. We uncovered trends within the data; namely, there appeared to be no difference in extinction rates between the groups, contrary to previous theories (e.g., Garner et al., 2003), and further, the crib-biters made marginally more responses during the transfer phases than the non-crib-biters. However, these results were far from conclusive and we suggest that the data should be treated in this instance as a pilot study. Further study of

the effects of increased endogenous NAcc DA (in the context of the ESP) on incentive motivation in the presence of a Pavlovian cue will be necessary before conclusions can be inferred. Therefore, the model proposed in Chapter 1 (Figure 1.7) cannot be altered relating to this aspect of the ESP at this point (Figure 6.2).

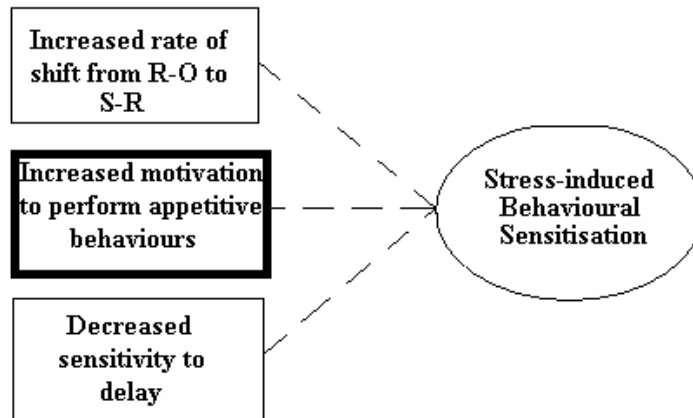


Figure 6.2. Schematic of behavioural correlates of stress-induced behavioural sensitisation. n.b. solid lines indicate features supported by existing evidence, dashed lines indicate features not yet determined by research.

Instrumental Choice

The third set of studies in the Thesis aimed to examine the behavioural effects of the ESP in relation to reinforcer choice. In concurrent chain choice, where subjects are required to choose between two alternatives associated with differential conditioned reinforcement schedules, AMPH infusion causes a decrease in sensitivity to delay in pigeons (Wei-min et al., 2008). Specifically, AMPH-infused subjects' choice for a conditioned reinforcer associated with a shorter, over a longer, delay, approached indifference. We therefore reasoned that crib-biting horses, with facilitated DA transmission in the mesoaccumbens pathway, may show similar effects when faced with the same experimental preparation. In Experiment 4, as horses' behaviour in these procedures had not previously been assessed, we carried out a pilot study to examine performance in relation to existing models of concurrent chain choice. Initial-links were held at

conc. VI 10 s VI 10 s, and the terminal-links were set at VI 6 s VI 24 s, VI 24 s VI 6 s, VI 10 s VI 20 s, VI 20 s VI 10 s. Each terminal-link schedule was run for 3-sessions of 72-trials. Relative response rates were log-log plotted against relative terminal-link reinforcement rates using two-free parameters. We found that the data fitted existing models of choice well (Generalised Matching Law; Baum, 1974), suggesting that horses match response to reinforcer rates in the context of concurrent chain choice.

In Experiment 5, we examined crib-biting and non-crib-biting horses' choice under concurrent chain schedules. This time, we used FI FI terminal-links to ensure high-sensitivity to the schedules in all animals (Gibbon et al., 1988). All horses were exposed to three half-hour sessions, with the initial-links set at *conc.* VI 10 s VI 10 s and the terminal-links at FI 10 s FI 20 s. There were no significant differences found in overall response rates between the groups. When the choice results were collated we found a marked and significant group difference, with the non-crib-biters matching response rates to relative rates of reinforcement, but the crib-biters failing to do so. While all of the non-crib-biters (as well as all of the subjects in Experiment 4) showed marked preference for the shorter initial-link terminal-link schedule over the three sessions, only two of the crib-biters did not. We suggested that these results supported the hypothesis that upregulation of the mesoaccumbens pathway associated with the ESP (McBride & Hemmings, 2005) may lead to a reduction in delay sensitivity. A reduction in delay sensitivity has not previously been discussed in the context of its relation to stereotypy. It is possible that this may constitute an adaptive mechanism to help the animal 'cope' with environmental adversity and requires further study in relation to endogenous neural dysfunction.

We conceded, however, that enhanced habit-formation in the crib-biting horses may be responsible for the findings. To explain, a decrease in nigrostriatal DA afferent to the DMS is known to lead to a faster shift from R-O to S-R learning (e.g., Jednyak et al., 2007). If the crib-biters were exhibiting this, it would be expected that a complex choice procedure would be problematic.

Therefore, it is not inconceivable that it is this feature of the Phenotype which is illustrated in the results, rather than decreased sensitivity to delay.

However, as illustrated in the individual horse data, two of the crib-biters did appear to be sensitive to the delay, suggesting that future research may be necessary before firm conclusions can be drawn. In spite of the anomalous findings from two of the crib-biters, the model may be cautiously updated to incorporate reduction in sensitivity to delay (Figure 6.3).

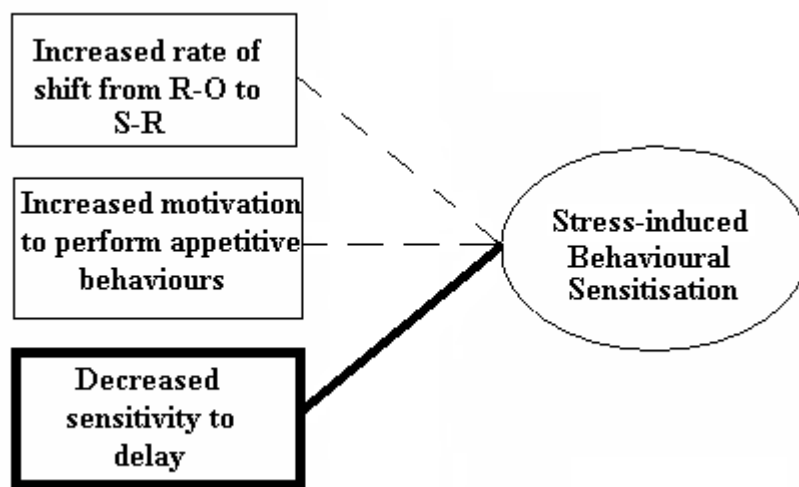


Figure 6.3. Schematic of behavioural correlates of stress-induced behavioural sensitisation. n.b. solid lines indicate features supported by existing evidence, dashed lines indicate features not yet determined by research.

In summary thus far, we found some evidence to support the notion that endogenous alterations in basal ganglia DA physiology, as inferred from the neural phenotype demonstrated in crib-biting horses, may produce similar behavioural effects to AMPH-induced sensitisation. One of the enduring facets of the studies reported above is that the results from experiments 1 and 4 could also be explained in terms of enhanced habit-formation. Specifically, enhanced levels of extracellular DA in the striatum has been found to be associated with an accelerated shift from planned action (R-O) to habit (S-R). In the final set of studies in the Thesis, we aimed to examine this question more specifically using a place-response procedure.

Habit Formation

In Experiment 6 we initially carried a pilot study to ascertain the number of trials in a place-response procedure horses typically require in order to shift from R-O (place) to S-R (response) learning, and we found that this appeared to be around 40-trials. In Experiment 7 we tested crib-biters and non-crib-biters in the procedure, with probe trials at trial-20 and trial-40. We hypothesised that non-crib-biters would, in the first probe trial, choose the ‘place’, but in the second probe trial, the ‘response’. However, we suggested that the crib-biters would choose the ‘response’ in both probe trials, owing to having shifted to an S-R strategy at a faster rate.

Initially, we found no differences in acquisition rate between the groups, with both crib-biters and non-crib-biters learning the location of the food at similar rates. In the first probe trial, we found that all of the non-crib-biters adopted a ‘place’ strategy, choosing the previously baited arm. In the crib-biters’ group, three of the subjects used a ‘response’ strategy (i.e., the visited the previously unbaited arm). This suggested that they had adopted an S-R strategy, whereas the non-crib-biters an R-O strategy. However, not all of the crib-biters chose this strategy, so the results should be treated with a degree of caution in this instance. In the second probe trial the results were somewhat unexpected, with four out of the five subjects in each group choosing the ‘place’ strategy. This was contrary to the expected outcome, with our hypothesis stating that all of the horses (i.e., in both groups) should have used a ‘response’ strategy. One of the possible reasons for this anomaly may have been methodological. To explain, to avoid problems with scent/ track-following in the probe trials, we constructed a ‘mirror maze’ for use in the probe trials (see Chapter 5; *Figure 5.1*). It may be that this was encoded as a separate maze by the animals which then caused a generalisation decrement during the second probe trial. Because of this unexpected result, we

carried out one further study using the t-maze, this time using a forced ‘response’ procedure.

Our final study, Experiment 8, aimed to deal with some of the issues raised in Experiment 7. If crib-biters were faster to shift between R-O and S-R learning, it could be hypothesised that in a procedure where subjects were forced to adopt a ‘response’ strategy, crib-biters would learn at a faster rate than non-crib-biters. In order to test this, we employed an adapted version of the place-response procedure where, during training trials, horses were forced always to turn the same way in order to locate the baited bucket. We found support for the hypothesis, with crib-biters learning to locate the food at a faster rate than controls. This may again be explained by altered patch foraging behaviour – perhaps the crib-biters were more focussed on the highly palatable and frequently replenished food than the controls; this would fit with a theory of overall stimulus enhancement in this group, as suggested from the mesoaccumbens perturbations associated with the phenotype. We also carried out a series of probe trials, where the horses were placed at a novel start point. We expected that if the horses had adopted a response strategy, they would choose the direction they had learnt during the training trials. Owing to a methodological error (the buckets were omitted from two of the animals’ probe trials), we were unable to find any conclusive results in the probe trials.

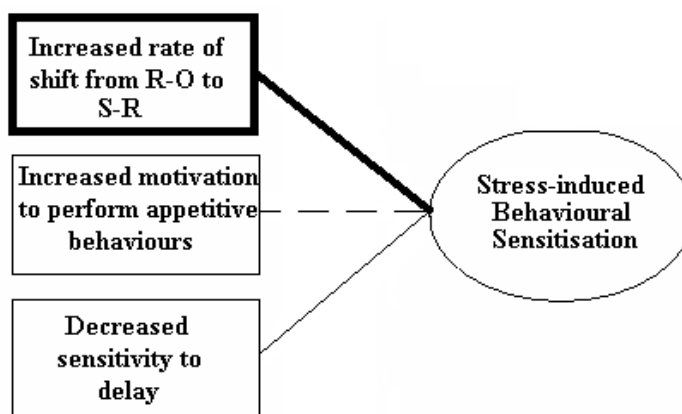


Figure 6.4. Schematic of behavioural correlates of stress-induced behavioural sensitisation. n.b. solid lines indicate features supported by existing evidence, dashed lines indicate features not yet determined by research.

Notwithstanding this, the results of experiments 7 and 8 support the notion that crib-biting horses appear to shift their learning from R-O to S-R at a faster rate than non-crib-biters. This suggests that stress-induced behavioural sensitisation associated with the ESP may produce similar behavioural effects to behavioural sensitisation resulting from DA agonist exposure. Based on the results of experiments 7 and 8, we are able to re-summarise the Model (Figure 6.4).

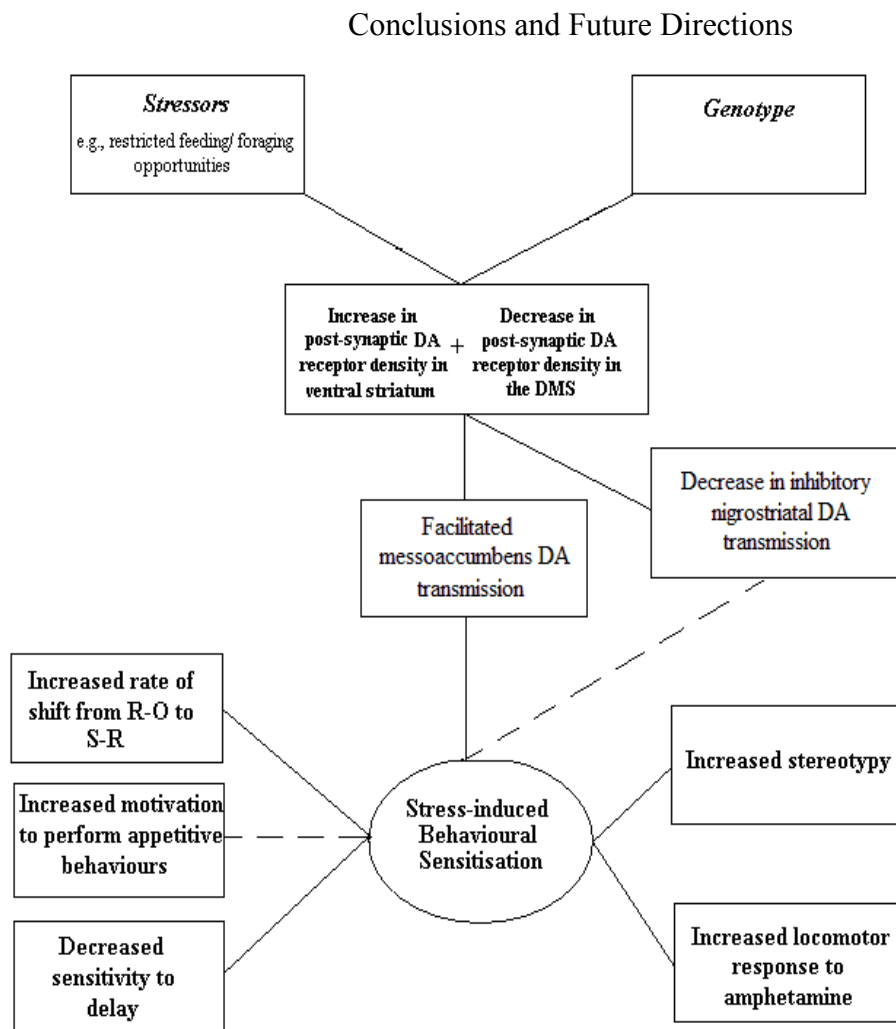


Figure 6.5. Schematic illustration of the aetiology and behavioural outcomes of stereotypy development in regard to the ESP. n.b. solid lines indicate features supported by existing evidence, dashed lines indicate features not yet determined by research.

The results of the studies reported in this thesis go some way to informing us of the behavioural effects of stress-induced sensitisation associated with the ESP. Based on our findings, the Model suggested in Chapter 1 (*Figure 1.7*) may now be re-presented with a number of changes (see *Figure 6.5* for a schematic).

In the current version of the model, genotype and stress interact initially to alter striatal DA physiology. Specifically, via an increase in post-synaptic DA receptors in the NAcc and a decrease in DA receptors in the DMS, mesoaccumbens DA transmission is increased, leading to higher endogenous extracellular DA levels in the ventral striatum, and a decrease in DA activity in the DMS. These two facets may interact to lead to behavioural sensitisation, and an increase in stereotypy, an increase in the locomotor response to AMPH, an accelerated shift from R-O to S-R learning and a decreased sensitivity to delay. However, the specific mediating role (if any) played by downregulation of the nigrostriatal pathway in the behavioural phenotype is yet to be firmly established. There is also some limited evidence for incentive sensitisation in stereotypic horses. Our findings relating to this feature of the Model were akin to a pilot study at this stage and will require further study before it can be confidently incorporated.

The processes by which striosome and matrix cell changes, as shown in chronic DA agonist work (e.g., Canales & Graybiel, 2000), affect the Model are yet to be empirically determined. Future research to examine this in the context of endogenous change is currently in progress. Initial observations suggest that within the ESP, there is an over-representation of striosome cells (as inferred from Mu-opioid-receptor Bmax and Kd values) in the VTA, ventral striatum and DMS (S. D. McBride, unpublished findings). Canner-Loup et al. (2002) found that concurrent activation of DA D1 and D2 receptors using DA agonists led to striosome predominance and increased behavioural stereotypy, following chronic DA agonist exposure. This is very interesting in the light of the ESP, as this may offer a clear aetiological explanation of stereotypy development. For example, the Model may be extended, cautiously, to incorporate striosome predominance

(Figure 6.6). A clear understanding of the process by which this change occurs will be a crucial next-step in a search for a substantive model of stereotype development, and indeed in the scholarly pursuit of clear functional and neurobiological underpinnings of mid-brain stress physiology.

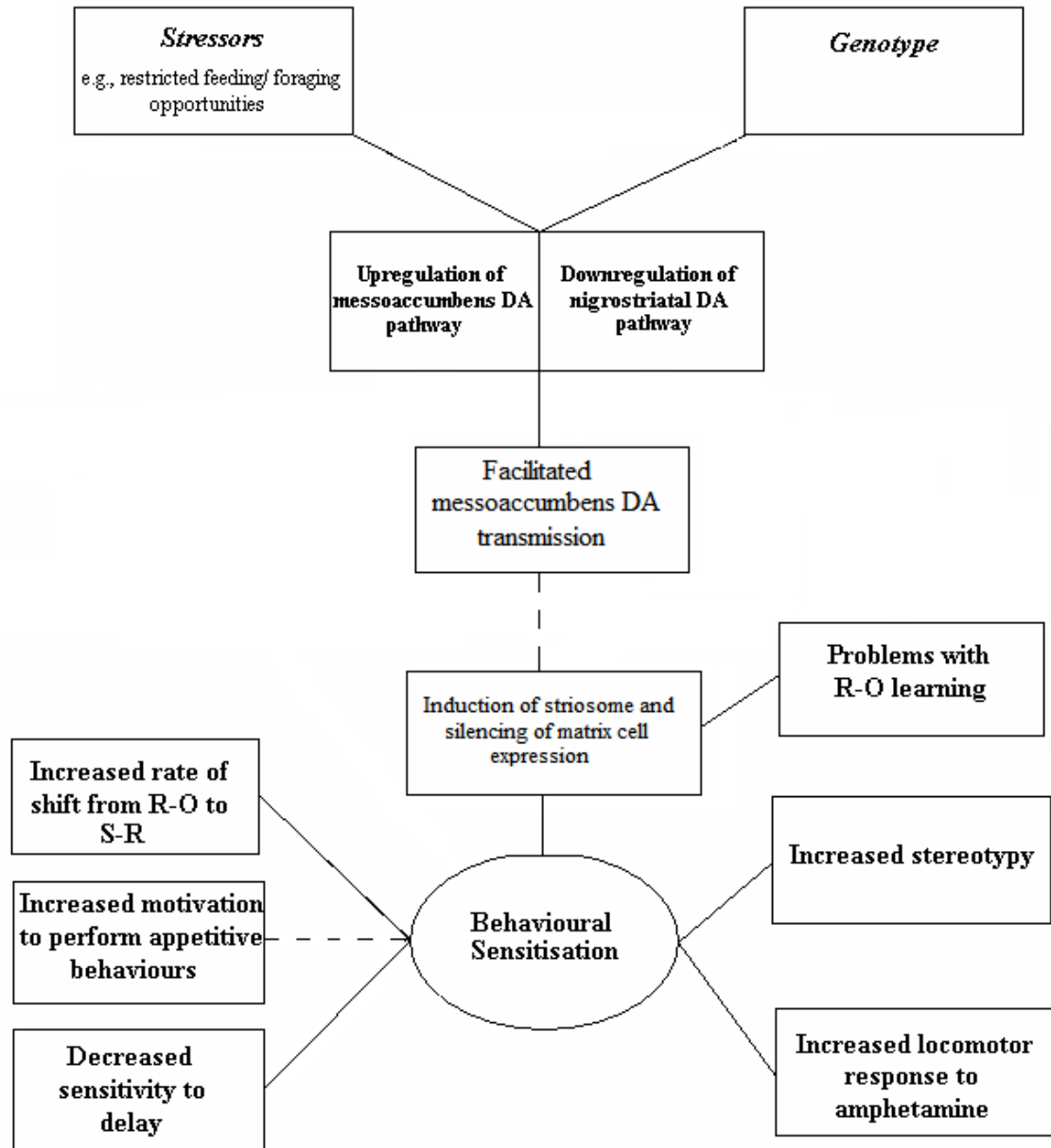


Figure 6.6. Schematic illustration of the aetiology of the ESP. Note: dashed lines indicate hypotheses yet to be explored, solid lines indicate findings either from this thesis or others' work.

To summarise, we have found corroborating behavioural evidence which complement extant neurobiological models of the ESP. These findings were very pleasing from a theoretical perspective, as it may help in the search for a clearly defined aetiological model of spontaneous stereotypy. In addition, the findings are interesting from a functional neuroanatomical perspective, as an endogenously produced, aberrant phenotype may be a useful tool for complementing existing neuropsychological research (e.g., MRI, PET, lesion, pharmacology) into the role of the basal-ganglia in learning. Future research with other species may help to further this as a valid behavioural methodology and elucidate some of the functional heterogeneity debates currently surrounding the striatum (e.g., Wickens, Budd, Hyland, & Arbthnott, 2007).

As a final thought, Stereotypic Behaviour is not only distressing for animals' owners/carers; it is also potentially damaging to the animal and is often cited as an indicator of reduced welfare. The present studies, and many before, have highlighted the potential deleterious effects on animals' learning and behaviour of failing to provide adequate, species-appropriate facility to perform naturalistic behaviours. If these changes are, as we suggest, altering endogenous neurophysiology, this may have important connotations for the validity and reliability of using animals in research where behaviour or learning is used as an outcome measure; particularly if there are issues surrounding the welfare of the animals, such as limited facility to express species-typical behaviour. Clearly the animals exploited for this thesis were horses, a species not typically used in laboratory research. However, the behavioural and neural phenotypes suggested are not limited to the horse (Cabib et al., 1996), and future research using more commonly used species may increase the model's external validity.

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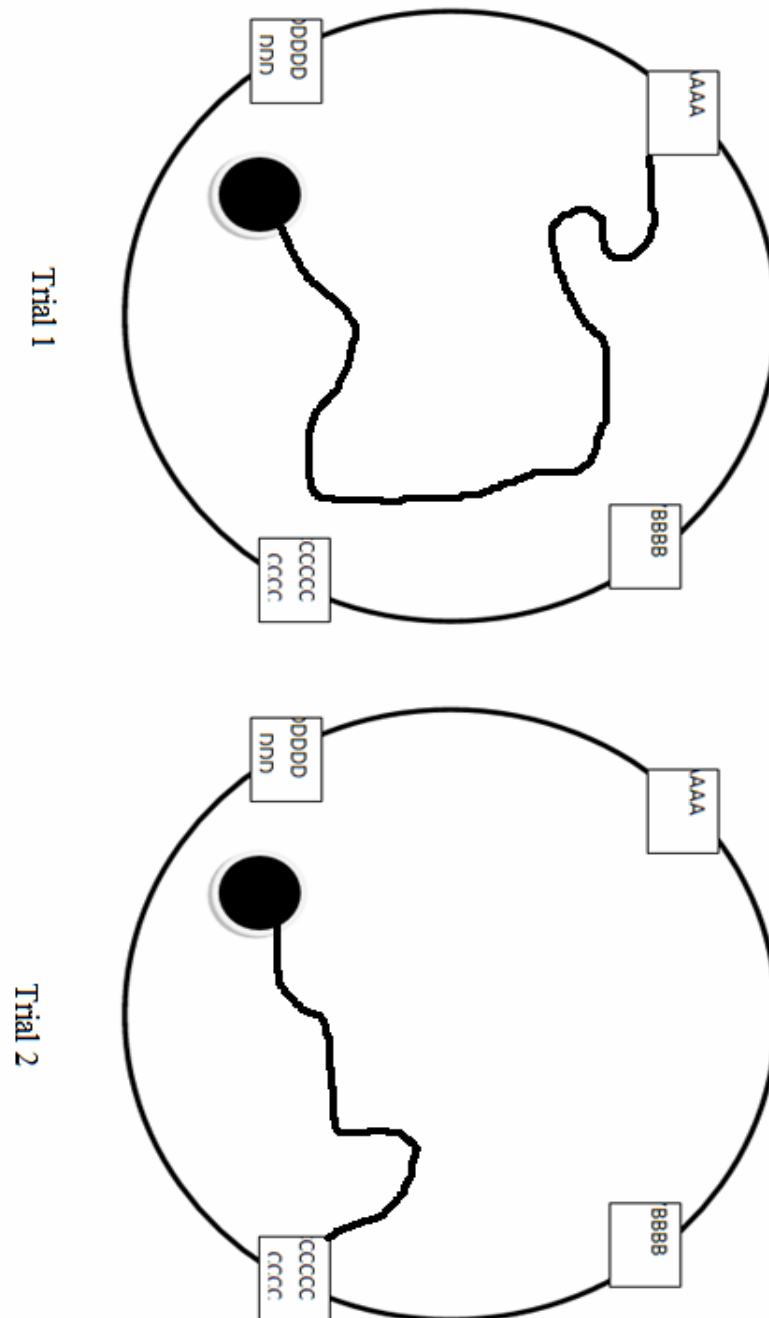
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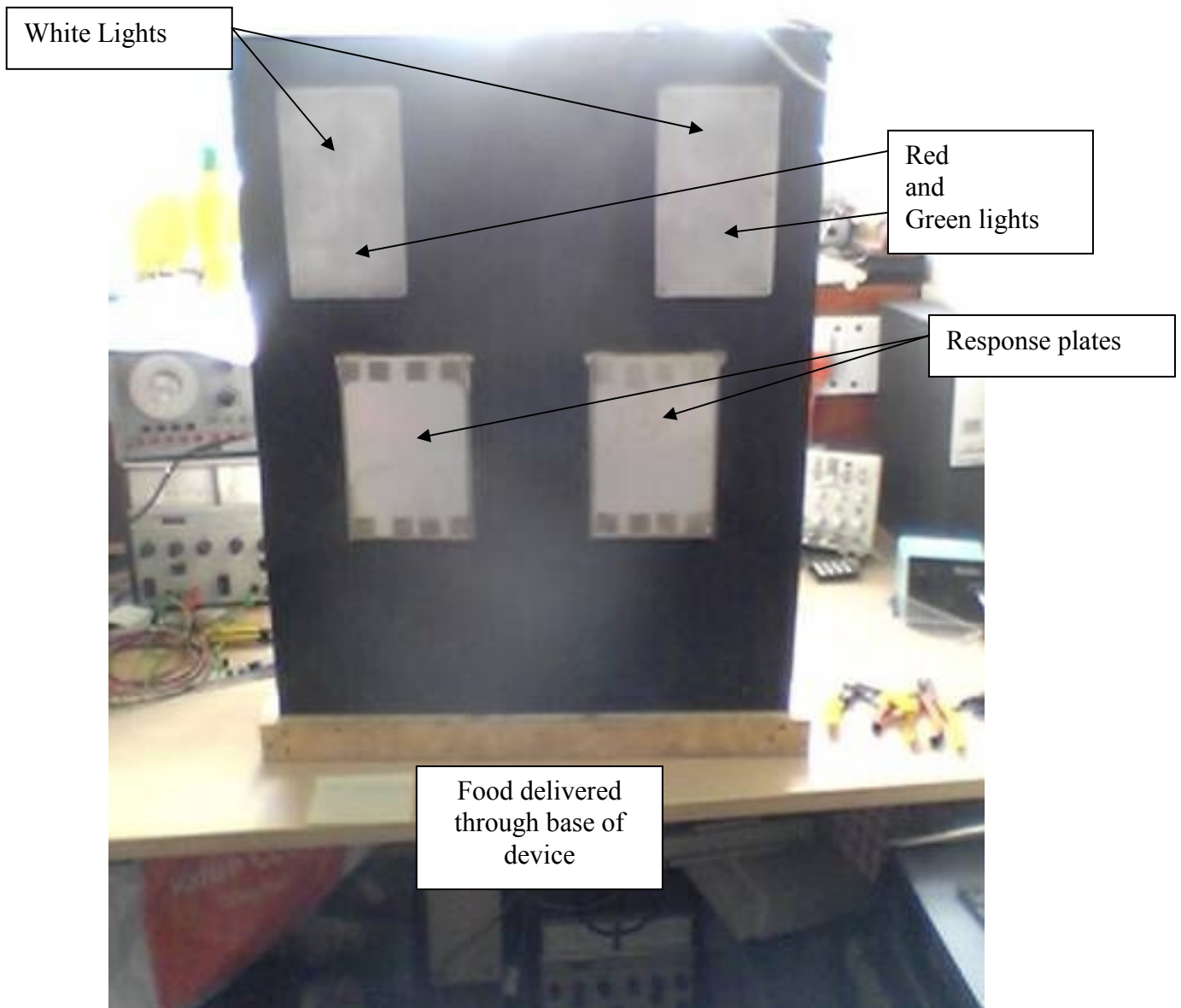
Appendices

Appendix A

Example of route-mapping diagram for Experiment 1. The figure below shows the route taken by one of the subjects in trials 1 and 2.



Appendix B



Photograph of the device used for experiments 3, 4 and 5. On the top of the box were located two steel hooks, which were designed specifically to ‘hang’ the device on the top of a wall or door-frame. Nb., for experiments 4 and 5, the lights and the right response plate were covered, leaving just the left plate visible to the subjects.

Appendix C

Parametric analyses from Experiment 4

A mixed-design, 2×2 ANOVA, with group and trial block as independent measures, was performed on the on-target responses. It revealed no main effect of group, $F(1, 6) = 1.38$, or trial blocks, $F(2, 12) = 1.06$, but a significant interaction between group and trial block, $F(2, 12) = 4.97, p < 0.05$. Simple main effect analyses (Keppell, 1974) indicated that the on-target responses by the groups differed only on trial block 3, $F(1, 18) = 6.79, p < 0.01$, and that there was an effect of trial block for the non-crib-biters, $F(2, 12) = 5.12, p < 0.05$, but not for the crib-biters, $F < 1$.