

SCHOOL OF PSYCHOLOGY

Anhedonia and other Reward-Related Deficits in Animal Models of Psychiatric Disorder



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Summary

New insights have revealed the complex and heterogeneous nature of reward-related behaviours: not only are different aspects of reward (e.g. reward 'liking' and 'wanting') subserved by dissociable neural mechanisms, but they are differentially expressed across major psychiatric disorders. The aim of this thesis was to investigate discrete reward-related processes, pertaining to the hedonic and cognitive processing of rewards, in relation to schizophrenia and depression preclinical models. The Methylazoxymethanol acetate (MAM) neurodevelopmental model of schizophrenia and the Wistar Kyoto (WKY) inbred depression model were chosen based on their good face and construct validities to the clinical conditions. Microstructural analysis of licking in simple drinking and contrast situations were used to investigate the constructs of consummatory and anticipatory anhedonia in these models. Whilst MAM-treated rats showed no behaviours indicative of consummatory or anticipatory anhedonia, WKY rats showed generally lower consummatory and palatability responses to sweet solutions and failed to suppress their palatability responses to a contrasted solution (when a preferred solution was expected). Therefore, WKY rats demonstrated behaviours analogous to deficits in both consummatory and anticipatory aspects of hedonic processing. To investigate cognitive processing of rewards, outcome devaluation and differential outcome paradigms were adopted, but no impairments on either task were found for the MAM model. In contrast, WKY rats were insensitive to post-conditioning changes in reward value and did not benefit from stimulus-correlated outcomes during the acquisition of a conditional discrimination task. Therefore, WKY rats do not appear to use the nature and /or value of rewards to guide their behaviours in the same manner as controls. In short, MAM-treated animals did not display the hedonic deficits or impaired instrumental behaviours expected for a comprehensive schizophrenia model. In contrast, the WKY inbred rat strain appears to be suitable in investigating manifestations of clinical depression in respect to reward-processing deficits.

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Chapter One

1. General Introduction

1.1 Hedonia and Reward Processing

The term 'hedonia' comes from the ancient Greek word for pleasure ('hedone'), which in turn is derived from the sweet taste of honey ('hedus') (Berridge & Kringelbach, 2015; Rømer Thomsen, Whybrow & Kringelbach, 2015). From an evolutionary perspective, the ability to experience pleasure is essential (Rømer Thomsen et al., 2015). Described by some as 'evolution's boldest trick' (p.230), it ensures that individuals engage in fundamental behaviours, such as food intake and procreation, that are necessary for the survival of the individual and of the species (Kringelbach & Berridge, 2009). Yet, despite its adaptive function, a significant proportion of the general population lack the ability to experience pleasure as a symptom of psychiatric and neurological disorders (Rømer Thomsen et al., 2015).

The term Anhedonia (or anhedonie) was coined by Ribot, a French psychologist, in 1896, stating that "there are, undoubtedly, clinical cases characterised by the isolated lack of pleasure, that render these patients absolutely unable to find gratification from any sexual activity, food, relation or affection" (Ribot, 1896, as cited in Pelizza, Pupo & Ferrari, 2012). Ribot's original definition of anhedonia, as an 'inability to experience pleasure', has remained largely unaltered over the last century (Rømer Thomsen et al., 2015). However, there is now a growing understanding of the complexities of hedonia and how they relate to the processing of rewards (e.g. Der-Avakian & Markou,

2012). As will be discussed in detail below, considerations of reward-related deficits in the clinical context of psychiatric disorders suggests that anhedonia it is not simply a unitary construct related to the isolated loss of subjective experiences of pleasure, as Ribot's definition would imply.

While the term anhedonia does not directly reflect the multifaceted nature of reward-related deficits, it has been retained as a general descriptor of maladjusted reward processing – including problems relating to motivation and learning as well as the hedonic experience. The empirical work reported in this thesis will focus on the assessment of behavioural responses and learning related to rewarding stimuli in selected rat-based models of human psychiatric disorders – in particular schizophrenia and depression. This focus on rodent models in the context of hedonic experience raises the question of whether the subjective states of non-humans are amenable to scientific study. At the risk of dismissing a venerable tradition of philosophical discourse (see for example Nagle's (1974) classic work "What is it like to be a bat"), I will merely note that I will concentrate on objectively observable responses. The fact that that these behavioural measures are lawfully related to the stimulus environment that the rodents are studied in means that these measures provide information about the factors controlling the animals' behaviour (for a more detailed analysis of this issue, see Dwyer, 2012).

1.2 Neurobiological Underpinnings of Hedonia and Reward Processing

Before considering the clinical background to my empirical work, I will first outline some of the neurobiological underpinnings of reward processing and hedonic reactions. Affective neuroscience

has begun to tease apart the underlying brain circuits that serve different aspects of rewarded behaviour. Here I will focus on just two aspects of reward to highlight that different reward-related processes have, at least partially, dissociable neural circuitry. Despite the growing understanding of the complexities of hedonia, a full understanding of the underlying neurobiology is still lacking. Much of the current research has focused on modified orofacial behaviours in rats (see section 1.5.1 for a detailed description of this taste reactivity procedure) in response to neurochemical or neurobiological manipulations. This approach has led to the identification of localised regions in the rodent brain that, in consequence to certain stimulations, causally amplify the number of 'liking' reactions that are elicited by palatable tastes. Such discrete subregions of the brain, or hedonic 'hotspots', have been shown to exist in limbic-related structures - such as the nucleus accumbens (NAc), ventral pallidum (VP) and in the parabrachial nucleus of the brain stem (Berridge & Kringelbach, 2015).

a. Hedonic hotspots in the rodent brain

While orofacial reactions are elicited by sensorimotor circuitry in the brainstem (e.g. Grill & Norgren, 1978a), such hedonic 'liking' responses are not mere brainstem reflexes (as reviewed by Berridge & Kingelbach, 2015). Critically, hedonic responses to a given taste are modified by forebrain structures, thus allowing appropriate modulation by an animal's physiological state (hunger vs. satiation) and prior associative learning (learnt preferences vs. aversions). In particular, enhanced orofacial 'liking' responses are seen after direct stimulation of the NAc hotspot, positioned rostrally in the medial shell, by microinjections of opioid receptor agonists (μ , δ and κ opioids) (Castro

& Berridge, 2014a; Peciña & Berridge, 2005; Smith, Berridge & Aldridge, 2011) and endocannabinoids (such as anandamide, an endogenous ligand for cannabinoid CB1 receptors) (Mahler, Smith & Berridge, 2007). Microinjections in all other sites of the NAc fail to have any effect, whereas stimulation of more caudal areas of the medial shell (in a so-called hedonic 'coldspot') actually suppresses the palatability responses that would normally be elicited by sweet tastes (Castro & Berridge, 2014a). Similarly, microinjections of mu opioids (Smith & Berridge, 2005) or orexin-A (Ho & Berridge, 2013) into a caudal hotspot of the VP result in enhanced hedonic impact of palatable solutions (see also Castro & Berridge, 2013, for an optogenetic confirmation of the location of this effect). Again, stimulation outside this localised region of the VP fails to enhance hedonic reactions (Ho & Berridge, 2013; Smith & Berridge, 2005), while stimulation of the rostral coldspot of the VP can even suppress hedonic responses (Smith & Berridge, 2005). Interestingly, both excitotoxin lesions and temporary inhibition of the VP hotspot not only disrupt hedonic 'liking' reactions to sweet tastes but replace them with aversive 'disgust' reactions (Cromwell & Berridge, 1993; Ho & Berridge, 2014). Indeed, it has now been shown that the negative 'disgust' reactions to food thought to be brought about by lesions of the lateral hypothalamus were actually due to these lesions incorporating the caudal aspects of the VP (as reviewed by Berridge & Kringelbach, 2015).

With regards to the brainstem, there is evidence suggesting a hotspot region in the pontine parabrachial nucleus (PBN), where GABA (γ -aminobutyric acid)-benzodiazepine mechanisms influence hedonic processing (Söderpalm & Berridge, 2000). As reviewed by Castro and Berridge (2014b), systemic injections of the benzodiazepine drug, chlordiazepoxide, which enhances hedonic

'liking' in normal rats, has been shown to enhance 'liking' reactions in decerebrate rats. Microinjection of the benzodiazepine, diazepam, into the fourth ventricle of the brain-stem in 'intact' rats has also been shown to enhance 'liking' reactions to sucrose. Finally, microinjections of midazolam, another benzodiazepine, into the lateral PBN of normal rats enhances the 'liking' reactions elicited when consuming sweet sucrose solutions.

In terms of the circuitry underlying hedonic processing, neural projections exist between NAc, VP and PBN structures but not directly between the specific hotspots located within these structures (reviewed by Castro & Berridge, 2014b). Regardless, it has been shown that NAc and VP at least share a reciprocal functional connection. c-Fos (an indirect marker of neuronal activity) expression studies have shown that stimulation of one of the two hotspots can lead to mutual recruitment of the other (Smith & Berridge, 2007) and blocking one hotspot (e.g. using an opioid receptor antagonist such as naloxone) whilst simultaneously stimulating the other (i.e. with a mu opioid agonist) does not lead to the enhanced 'liking' reactions that would otherwise be expected (see Castro & Berridge, 2014b, for a review).

b. Tentative hedonic hotspots

In addition to the three subcortical limbic structures, there is emerging evidence to suggest further hedonic hotspots in the limbic areas of the prefrontal cortex, including the orbital frontal cortex (OFC) and insula (see Berridge & Kringelbach, 2015). Similar to NAc and VP hotspots, it has been suggested that stimulating opioid or orexin systems in specific subregions of each structure, amplifies

the number of 'liking' reactions elicited by sweet solutions (as reviewed by Berridge & Kringelbach, 2015). This needs to be confirmed but would be consistent with human functional magnetic imaging (fMRI) studies which suggest that the OFC (at a mid-anterior site) codes for the subjective liking of pleasant stimuli on the basis of correlations with changes in subjective hedonic ratings produced by satiety manipulations (Kringelbach, O'Doherty, Rolls & Andrews, 2003). Whilst this study does hinge upon subjective ratings of pleasure rather than direct and objective behavioural responses, it does support a role for the OFC in tracking hedonic changes in a similar fashion to the NAc, VP and PBN, especially when combined with the preliminary analysis performed in rodents.

The striatum (including the NAc hotspot) also receives input from the amygdala, a structure divided into multiple subnuclei including the central (CeA) and basolateral amygdala (BLA) (as reviewed by Cardinal, Parkinson, Hall & Everitt, 2002). Whilst this subcortical structure has long been implicated in emotional processes (Klüver & Bucy, 1939; Weiskrantz, 1956), primarily the processing of fear-related stimuli (e.g. Adolphs, Tranel, Damasio & Damasio, 1995; LeDoux, 1995), its role in reward processing is less clear. Amygdala neurons respond to biologically salient rewards, including the anticipation (O'Doherty, Deichmann, Critchley & Dolan, 2002) and receipt (O'Doherty, Rolls, Francis, Bowtell & McGlone, 2001; Scott, Karadi, Oomura et al., 1993, but see O'Doherty et al., 2002) of pleasant tastes. Amygdala lesions (i.e. of the BLA) cause animals to become insensitive to the devaluation of a reward (e.g. Balleine, Killcross & Dickinson, 2003; Hatfield, Han, Conley, Gallagher & Holland, 1996) and impair pavlovian and instrumental forms of appetitive conditioning (with dissociable effects seen between the BLA and CeA) (See Baxter & Murray, 2002, for a review).

However, studies have shown that opioid stimulations (i.e. by the microinjection of the mu opioid agonist DAMGO), which enhance hedonic 'liking' in hotspot regions, do not increase the 'liking' or palatability of rewards when administered to the BLA or CeA (Mahler & Berridge, 2012). One influential analysis is that the role of the amygdala in reward processing is to underpin the association between neutral environmental cues (Conditioned Stimuli or CSs) and the motivationally significant events they predict (Unconditioned Stimuli or USs). More precisely, it is thought that the BLA supports learning about the specific sensory properties of the US, while the CeA supports learning about the general affective valence of the US (e.g. Balleine & Killcross, 2006; Killcross, 2000).

c. Reward liking vs. reward wanting

Whilst 'liking' is fundamental to reward, another component is reward 'wanting' or the motivation towards a reward. Reward 'wanting' and 'liking' were once thought to be intrinsically linked - you want what you like and vice versa. However, it is now known that this is not necessarily the case. For example, early in drug abuse, 'liking' and 'wanting' are closely correlated, but increased 'wanting' in the absence of increased 'liking' characterises drug addiction (Robinson, Robinson & Berridge, 2013). It has also been shown that motivational aspects of the reward are served by dissociated neuroanatomical structures and different neurochemical mechanisms. For example, while stimulations of the amygdala do not enhance 'liking' (see above), they can increase the incentive salience or motivation to obtain rewards (e.g. DAMGO stimulation of the CeA enhances food consumption) (Mahler & Berridge, 2009).

Returning to the NAc hedonic hotspot, microinjection of mu and delta opioids not only amplify 'liking' reactions towards a reward, but also increase eating behaviour and food intake of that reward (Castro & Berridge, 2014a; Peciña & Berridge, 2005). In contrast, microinjections of other regions outside the hotspots of the structure, which have no effects on 'liking', still amplify core 'wanting' reactions when stimulated by mu opioids (e.g. Castro & Berridge, 2014a). Moreover, mu opioid stimulation of the hedonic coldspot can enhance 'wanting' for a reward, even though it actively suppresses 'liking' (Castro & Berridge, 2014a). This goes at least some way towards demonstrating the differences in brain circuitries underlying these two components of reward, even when focusing on the same brain structures and neurochemical mechanisms (for a comprehensive review of the neurobiology of liking and wanting see Castro & Berridge, 2014b). Intriguingly, co-recruitment of the NAc-VP circuits appears to be necessary for enhancing 'liking' (see above), whereas the simultaneous co-operation of the dual hotspots does not appear necessary for appetitive 'wanting'. While enhanced 'liking' by microinjecting DAMGO into the NAc hotspot was blocked by simultaneous infusion of naxolone into the VP hotspot, 'wanting' stimulation produced by DAMGO in the NAc hotspot was preserved. This dissociation demonstrates that enhanced 'wanting' can occur independently of enhanced 'liking' even from the same anatomical location (as reviewed by Richard, Castro, DiFeliceantonio, Robinson & Berridge, 2013).

Whilst the neuroanatomical location helps dictate whether or not 'wanting' reactions will be enhanced, the neurochemicals by which those structures are stimulated is also critical. As well as opioid stimulation increasing wanting when injected into the NAc hotspot, dopamine agonists and α -

amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R) antagonists have been shown to enhance wanting to a similar degree (Faure, Richard & Berridge, 2010; Smith et al., 2011).

Focusing on the role of dopamine, it must be conceded that this neurotransmitter was once referred to as the pleasure substrate: dopamine junctions represent a "synaptic way station"... where "sensory inputs are translated into the hedonic messages we experience as pleasure, euphoria, or 'yumminess'" (p. 94, Wise, 1980). However, more recent evidence suggests that dopamine is relatively uninvolved in the hedonic impact of rewards, instead being critical for incentive salience or motivation (i.e. core 'wanting') (Berridge & Robinson, 1998). Indeed, studies have shown that 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal and mesolimbic circuits (which greatly reduce dopamine levels in these systems) do not impact on a rodents' palatability responses to sucrose (Berridge & Robinson, 1998; Berridge, Venier & Robinson, 1989). Similarly, patients suffering from Parkinson's disease, who suffer from greatly depleted dopamine levels, give similar hedonic ratings to sweet tastes compared to healthy control subjects (Sienkiewicz-Jarosz, Scinska, Swiecicki et al., 2013). Mutant mice, which over-express striatal dopamine D₂ receptors (D2R-OE), show similar hedonic reactions to appetitive stimuli compared to controls (Ward, Simpson, Richards, Deo, Taylor, Glendinning, Kandel & Balsam, 2012). Furthermore, human studies have shown that an increase in dopamine as a result of L-DOPA administration does not increase a person's subjective hedonic ratings to pleasant stimuli (Liggins, Pihl, Benkelfat & Leyton, 2012). In terms of the incentive salience of rewards, however, dopamine appears to have a critical role. It has been shown that D2R-OE mice are less willing to work for a preferred reward in an effort related choice paradigm, instead opting to

consume a freely available but less preferred reward (Ward et al., 2012). Similar results (i.e. decreased lever pressing but increased chow intake) have also been observed for dopamine receptor antagonists as well as for NAc dopamine depletion (Cousins & Salamone, 1994; Salamone, Correa, Farrar & Mingote, 2007; Salamone, Steinpreis, McCullough, Smith, Grebel & Mahan, 1991). In contrast, increases in dopamine have been demonstrated to enhance the incentive motivation towards rewards. Hyperdopaminergic mutant mice (induced by knocking down dopamine transporter levels to 10%) have been shown to run more 'eagerly' down a runway towards a goal box containing a food reward, suggestive of increased incentive salience for that reward. These mice displayed increased positive orofacial responses to increasing concentrations of sweet tastes, but fewer total positive orofacial reactions to the highest (1.0 M) concentration of sucrose compared to their wild-type controls (Peciña, Cagniard, Berridge, Aldridge & Zhuang, 2003). Such results are reminiscent of other studies, which showed that microinjection of amphetamine into the NAc also increased cue-triggered wanting for a reward (as shown by a study using a Pavlovian-to-instrumental transfer paradigm) even though 'liking' of that reward was slightly decreased (Wyvell & Berridge, 2000). This reiterates the independence between 'wanting' and 'liking' systems and highlights that, whilst dopamine does not have a central role in hedonic liking of a reward, it can induce some changes in 'liking' behaviour, although sometimes in the opposite direction to original proposals.

By highlighting the differences between 'wanting' and 'liking' systems it becomes clear that different aspects of reward processing - hedonic or otherwise - are partially dissociated at behavioural, neuroanatomical and neurochemical levels. As a result, the focus of this thesis is on

parsing out different aspects of reward processing and hedonics in relation to models of psychiatric disorders. How hedonic processing, specifically, and reward processing, more generally, can be further sub-divided will be the focus of upcoming sections.

1.3 Psychiatric Disorders and Anhedonia

Anhedonia has been observed in numerous neurological and psychiatric disorders, including mood disorders (e.g. Schrader, 1997), eating disorders (e.g. Davis & Woodside, 2002), psychosis (e.g. Blanchard & Cohen, 2006) and Parkinson's disease (as shown by a questionnaire assessment method, Isella, Iurlaro, Piolti et al., 2003), to name but a few. Most critically for my current concerns, anhedonia has long been considered as a core symptom of schizophrenia and depression (e.g. Rømer Thomsen et al., 2015). As has been seen in section 1.1 and section 1.2, reward-processing is multifaceted in nature and does not rely on a singular biological circuit. Unfortunately, however, anhedonia has often been used as a blanket term inappropriately incorporating reward-processing deficits beyond pure hedonic capacity (e.g. Der-Avakian & Markou, 2012; Treadway & Zald, 2013). It is only relatively recently that such nuances in reward-related processes have been considered in relation to disease.

The emphasis on anhedonia in the schizophrenia and depression literature, as opposed to other aspects of reward, primarily reflects a historical precedence (Treadway & Zald, 2013). Patient populations have frequently complained about anhedonia, and diminished enjoyment has been captured through self-report and interview-based assessments (Treadway & Zald, 2013; Watson &

Naragon-Gainey, 2009). Furthermore, patients with schizophrenia and depression display decreased goal-directed behaviour (e.g. Barch, Pagliaccio & Luking, 2015, Gard, Kring, Germans Gard, Horan & Green, 2007; Sherdell, Waugh & Gotlib, 2012), which at an intuitive level has been assumed to reflect decreases in hedonic capacity (Treadway & Zald, 2013). However, with increases in our understanding of reward processing, together with affective neuroscience starting to tease apart reward-related processing at the level of the brain (e.g. Berridge & Kringelbach, 2008), efforts have been made to quantify enjoyment in isolation from other aspects of reward (e.g. Cohen & Minor, 2010; Dowd & Barch, 2010; Kring & Moran, 2008). As has been seen above, decreases in the motivation or 'wanting' of a reward does not necessarily mean an accompanying decrease in 'liking' for that reward. Whilst depression may include a reduced ability to experience pleasure together with a reduced ability to pursue pleasurable activities, the picture may be more complicated in schizophrenia (see Rømer Thomsen et al., 2015). As will be discussed in section 1.3.2, there is increasing evidence to suggest that hedonic capacity is unaltered in schizophrenia with patients experiencing as much pleasure from potentially enjoyable stimuli, events and daily experiences as healthy controls. If reduced motivation does not reflect reduced pleasure in this patient population then we need to understand the mechanisms that translate reward into reward-related behaviour and how these might be impaired. My empirical work will concentrate initially on distinguishing between consummatory and anticipatory aspects of hedonic reactions, in relation to schizophrenia and depression, and examine how these relate to the cognitive processing of rewarding events.

1.3.1 Overview of Schizophrenia

Schizophrenia (schizo = split, phrenia = mind) is a highly debilitating neuropsychiatric disorder that affects approximately 1% of the global population (Mueser & McGurk, 2004). As a highly heterogeneous disorder, its clinical presentation is complex, including a range of behavioural traits that are by no means specific to the condition (NICE, 2009). Due to this complexity, efforts have been made to group the symptoms into natural categories or domains, with the latest iteration grouping them into positive, cognitive and negative symptom clusters (Andreasen, 1995). Positive symptoms refer to behaviours that are additional to normal human experience (i.e. 'gain of function'). They consist of hallucinations (often auditory), delusions (usually paranoid in nature) and aggressive or stereotyped behaviours. These symptoms are amenable to the effects of currently available antipsychotic treatments (e.g. Pratt, Winchester, Dawson & Morris, 2012). Cognitive symptoms include inattention, problems with executive control (such as rule learning and selection) and deficits in both working and long-term memory. Negative symptoms refer to the lack of behaviours relative to normal human experience (i.e. 'loss of function'), and encompass blunted affect, anhedonia, avolition, poverty of speech and deficits in general social functioning. These aspects of the disorder often present as prodromal symptoms before cognitive and positive symptoms arise, and can often persist after positive symptoms have subsided (Arango & Carpenter, 2011). Unlike positive symptoms, both negative and cognitive aspects of the disorder are inadequately addressed by currently available medications (e.g. Pratt et al., 2012). Moreover, they have been suggested to contribute more to poor functional outcome and quality of life in schizophrenia patients than do positive symptoms

(Rabinowitz, Levine, Garibaldi et al., 2012). In light of this, the treatment of non-psychotic aspects of schizophrenia represents a vital unmet clinical need.

The aetiology and pathophysiology of schizophrenia is largely unknown. It usually has a post-pubertal onset, with emergence typically between 16 and 30 years old (Mueser & McGurk, 2004). It affects males more than females, with the male to female ratio (median) in the order of 1.4:1 (McGrath, Saha, Welham et al., 2004). Moreover, males tend to have a more chronic form of the disorder (with a greater presentation of negative symptoms) and an earlier age of onset compared to females (Jablensky, 2000; Lewine, 1981). At the broadest level, schizophrenia is thought to result from the complex interplay of environmental factors and biological pre-disposing factors (e.g. van OS & Kapur; van OS, Kenis & Rutten, 2010). At the biological level, genetics, development and neurobiology have been identified as important contributory factors in the risk of developing schizophrenia (e.g. Mueser & McGurk, 2004). At the environmental level, early environment, psychosocial factors and the use of recreational drugs also appear to cause or exacerbate symptoms (van OS et al., 2010; Mueser & McGurk, 2004). Interestingly, both migration and living in urban areas increases the risk of schizophrenia (McGrath et al., 2004; McGrath, Saha, Chant & Welham, 2008). There is also increasing evidence to suggest that early insults to the brain can impact on developmental factors with a subsequent increase in the associated risk of schizophrenia in adulthood due to multiple effects on brain circuitries (Mueser & McGurk, 2004).

The most frequently confirmed neurobiological finding in schizophrenia, as shown by post-mortem studies, is the enlargement of the lateral and third ventricles of the brain (Brown, Colter,

Corsellis et al., 1986; Pakkenberg, 1987). Differences in the total volume of the frontal lobes, hippocampus, amygdala, temporal lobes and thalamus have also been identified (Bogerts, Meertz & Schönfeldt-Bausch, 1985; Brown et al., 1986; Lawrie & Abukmeil, 1998; Pakkenberg, 1992; Wright, Rabe-Kesketh, Woodruff, David, Murray & Bullmore, 2000). As confirmed by meta-analyses, these changes in the volume of brain structures are accompanied by a reduced total brain volume (Wright, et al., 2000), and reduced total brain weight (of approximately 2%: Harrison, Freemantle & Geddes, 2003). Further evidence of neuropathology comes from increased cell packing density in the dorso-lateral prefrontal cortex (Daviss & Lewis, 1995; Selemon, Rajkowska & Goldman-Rakic, 1995; 1998). What is more, many of these neuropathological findings, such as brain volume reductions and increased ventricle size, have been identified in never treated patients and in unaffected 'at-risk' relatives (Fannon, Chitnis, Doku et al., 2000; McDonald, Grech, Touloupoulou et al., 2002, as cited in Mueser & McGurk, 2004). This suggests that such pathologies are not secondary to chronicity of the disorder or to prolonged antipsychotic treatment (Mueser & McGurk, 2004).

a. Dopamine hypothesis

From the discovery that psychostimulant drugs (such as amphetamine) increase neuronal dopamine levels and result in a psychotic state closely resembling schizophrenia, the dopamine hypothesis of the disease was established (as reviewed by Howes, McCutcheon & Stone, 2015). This hypothesis is greatly supported by the fact that all existing therapeutic drugs block dopamine (D₂) receptors at least to some degree (as reviewed by Talbot & Laurelle, 2002). Furthermore,

neuroimaging studies have revealed augmented dopamine synthesis and release, together with higher resting-state concentrations of dopamine in the synapse, during acute psychosis (Howes et al., 2015; van Os & Kapur, 2009). With mesolimbic dopamine involved in assigning motivational salience to events (both internal and external), Kapur (2003) has proposed that aberrant dopamine transmission in the schizophrenic brain causes patients to attribute abnormally high salience to internal representations - essentially generating hallucinations. In turn, the delusions often associated with schizophrenia may be formed as the patient attempts to 'make-sense' of these abnormal experiences (as reviewed by Pratt et al., 2012).

Whilst there is support for dopamine dysfunction underlying the positive symptoms of schizophrenia, hyperactive dopamine in the brain cannot account for negative or cognitive symptoms. The revised dopamine hypothesis proposes a hyperdopaminergic tone (resulting in hyperactivation of D₂ receptors) in mesolimbic circuits (including dopamine dysfunction in the amygdala and overactive dopamine systems in the hippocampus), but hypodopaminergic tone in mesocortical circuits (Brisch, Saniotis, Wolf et al., 2014). Indeed, there is a well-established link between frontal dysfunction and the cognitive impairments exhibited by schizophrenia patients (Barch & Caeser, 2012). That said, there is currently no direct (i.e. in-vivo imaging) evidence for negative and cognitive symptoms attributable to low cortical dopamine (Howes et al., 2015).

b. Glutamate hypothesis

A subset of patients (approximately one-third) does not respond to dopaminergic antipsychotic drugs, suggesting that the pathophysiological basis for their symptoms does not involve a dysregulated dopamine system (Howes & Kapur, 2014). This has lead researchers to investigate other pathways.

The glutamate hypothesis of schizophrenia primarily centres on the observation that non-competitive NMDA (N-methyl-D-aspartate) receptor antagonists (such as phencyclidine, PCP) induce a psychotic state indistinguishable from schizophrenia in healthy human subjects (as reviewed by Howes et al., 2015). Importantly, the administration of NMDA receptor antagonists produces symptoms corresponding to the positive, negative and cognitive symptom domains. It has also been shown that these drugs exacerbate symptoms of people already diagnosed with schizophrenia. At the neuronal level, it is thought that NMDA receptor antagonism reduces the activity of GABA-ergic interneurons. This in turn is thought to disinhibit pyramidal cell firing leading to increased glutamate release in regions including the prefrontal cortex (as reviewed by Pratt et al., 2012).

Further support for the glutamate hypothesis comes from in-vivo imaging studies. Pilowsky and colleagues (2006) in a neuroreceptor occupancy study revealed that patients have reduced NMDA receptor activity in the left hippocampus - but this study has not yet been replicated (Pilowsky, Bressan, Stone et al., 2006). Proton magnetic resonance imaging studies have also revealed that unmedicated patients with first episode psychosis have increased glutamine (a marker of glutamate neurotransmission) in the anterior cingulate cortex (although chronic patients tend to have normal or

reduced levels) and increased glutamate in the NAc. Moreover, such studies have suggested that increased glutamate levels may predict poor treatment response to dopaminergic antipsychotics. Be that as it may, a major limitation of the glutamatergic hypothesis is the fact that there are currently no glutamatergic agents on the market, with clinical trials producing inconsistent results (as reviewed by Howes et al., 2015).

It should be recognised at this stage that dopamine and glutamate hypotheses of schizophrenia are not mutually exclusive. Indeed, there is some suggestion that dopamine dysfunction is secondary to altered glutamate neurotransmission in patients (see Howes et al., 2015). Indeed, as will be highlighted when I discuss preclinical models of schizophrenia (see section 1.4.1), NMDA receptor antagonists such as PCP can alter the dopamine system of the brain and increase sensitivity to subsequent amphetamine challenge (indicating a sensitised dopamine system). Furthermore, in the methylazoxymethanol acetate model of schizophrenia, it has been shown that abnormal hippocampal glutamatergic drive could be the cause of altered dopamine neuronal firing in the midbrain of these animals (Grace, 2012).

c. Neurodevelopmental hypothesis

The neurodevelopmental hypothesis of schizophrenia posits that exposure of genetically predisposed individuals to early life adverse events leads to an altered course of neuronal development, consequently creating a vulnerability to schizophrenia in later life (Lewis & Levitt, 2002).

As mentioned previously, schizophrenic individuals suffering from first-episode psychosis, together with their first degree relatives, exhibit morphological abnormalities in the brain - including ventricular enlargement and brain volume reductions (McDonald et al., 2002; Fannon et al., 2000 - as cited by Mueser & McGurk, 2004). These observations suggest that altered brain morphology is not a pathological consequence of schizophrenia, but constitutes a risk factor for the disease (Mueser & McGurk, 2004). Patients with schizophrenia also have a higher prevalence of physical abnormalities, particularly in the craniofacial area, indicative of a developmental disruption *in utero*, whilst an increased prevalence of cavum septum pellucidum in the brain (a fluid filled space formed from the incomplete closure of the septal leaflets during the first 6 months of life) is consistent with abnormal development during prenatal or early postnatal periods (as reviewed by Brown, 2011). Also consistent with a prenatal developmental disruption, is the lack of gliosis in the schizophrenic brain - a reaction commonly found in adult-onset brain injuries and neurodegenerative disorders such as Alzheimer's disease (Weinberger, 1995). Furthermore, post-mortem analyses of the schizophrenic brain have revealed an inward displacement of cortical neurons which can only be explained in terms of altered early brain development (see Weinberger, 1995, for a review).

Prenatal and perinatal insults including infections, malnutrition, neurotoxin exposure and maternal stress have been shown to increase the risk of developing schizophrenia (see Brown, 2011). In ecologic and birth cohort studies prenatal exposure to rubella (Brown, Cohen, Harkavy-Friedman et al., 2001), maternal respiratory infection (Brown, Schaefer, Wyatt et al., 2000), Herpes simplex virus type 2 (Buka, Cannon, Torrey & Yolken, 2008) and bacterial infection (Sørensen, Mortensen, Reinish

& Mednick, 2009) have each been shown to increase the risk of developing schizophrenia and/or schizophrenia spectrum disorders. Influenza during the first half of pregnancy (Brown, Begg, Gravenstein et al., 2004) as well as increased maternal levels of antibodies against *Toxoplasma gondii* (an intracellular parasite) have also been associated with an increased risk of schizophrenia in offspring (Brown, Schaefer, Quesenberry, Liu, Babulas & Susser, 2005; Mortensen, Nøgaard-Pedersen, Waltoft et al., 2006). Further support has come from preclinical studies which have shown that viral infections during the perinatal period lead to both neuropathological abnormalities and a behavioural phenotype of relevance to schizophrenia (e.g. Piontkewitz, Assaf & Weiner, 2009; Romero, Ali, Molina-Holgado, Castellano, Guaza & Borrell, 2007). What remains unclear is whether the infection per se increases the risk of schizophrenia or whether it is due to the maternal immune response elicited by the infection. Indeed, this latter hypothesis is consistent with the fact that the identity of the pathogen appears to be largely irrelevant. Furthermore, cytokines which are implicated in the differentiation, morphology and survival of developing neural cells, alongside their role in the inflammatory response (see Brown, 2011, for a review), are elevated in the mothers of schizophrenia patients (Brown, Hooton, Schaefer et al., 2004; Buka, Tsuang, Torrey, Klebanoff, Bernstein & Yolken, 2001).

Urban, as opposed to rural, births constitutes another risk factor for developing schizophrenia, perhaps due to higher levels of pollutants or the higher population densities increasing the risk and spread of infection (McGrath & Scott, 2006). The seasonal patterns of infection may also explain why people born in winter and early spring are more likely to develop the disorder (see Brown, 2011).

Alternatively the high percentage of schizophrenia patients born in winter could be due to a reduction in maternal vitamin D levels at this time of year (McGrath, 1999). Whilst the precise mechanisms are not completely understood, preclinical studies have implicated vitamin D in neurogenesis and foetal development (see Brown, 2011).

Prenatal malnutrition also constitutes an important risk factor. A study investigating the Dutch Winter Famine, which occurred during 1944-1945, has revealed that severe famine during conception or pregnancy was associated with an increased susceptibility to schizophrenia in offspring (Susser, Neugebauer, Hoek, et al., 1996). This finding has since been replicated by two ecologic studies investigating famine (between 1956 and 1961) across two different regions of China (St Clair, XU, Wang et al., 2005; Xu, Sun, Liu et al., 2009). Further support for the association between prenatal famine and increased risk of developing schizophrenia has come from preclinical investigations. Pregnant dam mice placed on a protein deficient diet led to morphological and behavioural alterations in the offspring which are of relevance to schizophrenia (See Brown, 2011).

Finally obstetric complications have been shown to increase the risk of schizophrenia (see Cannon, Jones & Murray, 2002, for a meta-analysis). These include complications of pregnancy (e.g. preeclampsia, bleeding, diabetes and rhesus incompatibility), decreased birth weight, and delivery complications (e.g. emergency caesarean section and asphyxia) (Cannon et al., 2002). Hypoxia is linked to many of these obstetric complications but it is unclear whether or not this constitutes a common pathogenic mechanism by which this diverse array of obstetric complications have their effect (see Brown. 2011 for a review).

The reason why prenatal and early postnatal brain alterations lead to the delayed onset of psychosis in adolescence and early adulthood remains unclear. One possibility is that the synaptic pruning that naturally occurs during adolescence causes a threshold level of neuronal loss to be reached, beyond which psychosis occurs. The neuronal loss which occurs with hypoxic birthing complications may further increase the risk of this threshold being reached (see Mueser & McGurk, 2004, for a review).

1.3.2 Anhedonia in Schizophrenia

Anhedonia has been described as one of the core symptoms of schizophrenia since the beginning of the 20th century with classic descriptions from both Kraepelin (1919) and Bleuler (1911). Theorists Rado (1956, as cited in Pelizza & Ferrari, 2009) and Meehl (1962) also assigned anhedonia a prominent role in their aetiological models of schizophrenia, suggesting that it constitutes one of four cardinal symptoms. Meehl (1962) described anhedonia as a "marked, widespread, and refractory defect in pleasure capacity" and "one of the most consistent and dramatic behavioural signs of the disease" (p.829). Rado (see Pelizza & Ferrari, 2009) and Meehl (1962) both considered anhedonia to constitute a genetic vulnerability factor, predisposing individuals to the onset of schizophrenia (see also Horan, et al., 2006; Wolf, 2006). Historical perspectives regarding the importance of anhedonia have been supported by empirical research with the development of clinical assessment scales and self-report questionnaires. For example, the Chapman Anhedonia Scales (Chapman, Chapman & Raulin, 1976), self-report questionnaires which distinguish between physical (e.g. eating) and social

(e.g. friendships) forms of anhedonia, have revealed that schizophrenia patients report less pleasure from both physical and social sources (e.g. Berebaum & Oltmanns, 1992; Blanchard, Horan & Brown, 2001; Blanchard, Mueser & Bellack, 1998; Cohen, Dinzeo, Nienow, Smith, Singer & Docherty, 2005). Similar results have also been found with other self-report methods, such as elevated anhedonia scores on the Snaith-Hamilton Pleasure scale (SHAPS: Snaith, Hamilton, Morley, Humayan, Hargreaves & Trigwell, 1995), which was originally developed to assess anhedonia in depression (Fortunati, Ossola, Camerlengo et al., 2015; Silver & Shlomo, 2002). Self-reported trait anhedonia has been shown to correlate with poor pre-morbid and current community functioning and a reduced quality of life amongst patients (e.g. Horan et al., 2006a; Ritsner, Arbtman & Lisker, 2011). In terms of interview-based assessments, the most frequently utilised is the Scale for the Assessment of Negative Symptoms (SANS) which includes an anhedonia-asociality subscale. As reviewed by Horan and colleagues (2006a), the anhedonia-asociality subscale reveals at least mild anhedonia in the majority of schizophrenia patients, even during the early stages of illness. High anhedonia-asociality scores have also been related to worse pre-morbid adjustment, social incompetency and poor long-term outcome (Horan et al., 2006a).

Findings of reduced pleasure in schizophrenia, however, have not been consistently replicated, and questions have been raised regarding the construct validity of early methods (see Foussias, Siddiqui, Fervaha, Agid & Remington 2015; Strauss & Gold, 2012). Laboratory-based evaluations of hedonic capacity, which are not as noisy or biased as self-report measures, have revealed that schizophrenia patients have an intact capacity to experience pleasant emotions to a

diverse range of emotional stimuli (e.g. Cohen & Minor, 2010; Kring & Moran, 2008; Llerena, et al., 2012). In their seminal work, Berenbaum and Oltmanns (1992) presented schizophrenia patients and healthy controls with emotion-eliciting stimuli which comprised short video clips and different tasting drinks. Whilst schizophrenia patients displayed affective flattening, their subjective experiences did not differ from those of the control group. These results have been replicated for other types of stimuli including food (Horan, Green, Kring & Nuechterlein, 2006), briefly presented pictures (Schlenker, Cohen & Hopmann, 1995; Volz, Hamm & Kirsch, 2003) and simulated social interactions (Aghevli, Blanchard, Horan, 2003). Furthermore, Kring and Colleagues have found normal experience of pleasure (to film clips) in unmedicated patients, suggesting that normal subjective ratings in laboratory settings are not secondary to drug status (Kring, Kerr & Neale, 1993; Kring & Neale, 1996).

Normal hedonic experiences among schizophrenia patients have been shown in daily life with the use of the experience sampling method, a time-sampling self-assessment technique. Gard and colleagues (2007) assessed patients seven times a day (pseudorandomly assigned) across seven consecutive days. Prompted by a pager, patients and controls were required to record what they were doing at the time and rate the enjoyment they were experiencing on a 7-point Likert scale. Compared to healthy control subjects, people suffering from schizophrenia reported similar levels of pleasure in the activities they were engaged in.

Consistent with these objective findings of intact hedonic capacity in schizophrenia, the use of signal detection tasks has revealed normal response biases in patients (Heerey, Bell-Warren & Gold, 2008; Pizzagalli, Jahn & O'Shea, 2005). Furthermore, patients with schizophrenia demonstrate some

intact aspects of memory enhancement for positive stimuli (Hall, Harris, McKirdy, Johnstone & Lawrie, 2007; Horan et al., 2006b, but see Herbener, Rosen, Khine & Sweeney, 2007) and a similar diminished startle response to pleasant stimuli as healthy controls (Curtis, Lebow, Lake, Katsanis & Iacono, 1999; Volz, Hamm, Kirsch & Rey, 2003), both consistent with normal hedonic processing (Barch & Dowd, 2010).

At the level of the brain, the results appear to be more mixed. As reviewed by Kring and Barch (2014), intact striatal responses to the receipt of monetary rewards are often seen in schizophrenia patients, yet some studies have revealed abnormal cortical responses (e.g. reduced reward-related responses in the medial prefrontal cortex (mPFC)). Furthermore, in terms of primary rewards, there is some evidence for reduced activation of the OFC, insula and striatum (Kring & Barch, 2014) - areas that are consistent with Berridge's so-called hedonic hotspots in the rodent brain. The picture is further obscured by reports that patients suffering from schizophrenia show muted neural responses, despite normative self-reports of pleasure (Waltz, Schweitzer, Gold et al., 2009).

The finding that people with schizophrenia score highly on interview-based assessments of anhedonia and self-report lower levels of positive emotions compared to healthy controls, yet display similar amounts of pleasant emotion in response to emotion-eliciting stimuli, has been referred to as the 'emotion paradox' (Strauss & Gold, 2012; Buck & Lysaker, 2014). Efforts to clarify the precise nature of hedonic experience in schizophrenia, and understand this discrepancy in the literature, have drawn attention to the importance of distinguishing between the temporally distinct components of hedonic processing (Buck & Lysaker, 2014). Klein (1984) was the first to distinguish between

consummatory pleasure, which reflects the in-the-moment pleasure experienced while engaged in an enjoyable activity, and anticipatory pleasure, which reflects the pleasure anticipated from future activities. Kring (1999) posited that schizophrenia patients may not experience less pleasure when presented with positive stimuli (consistent with laboratory-based measures), but may be less able to anticipate that events or rewards in the future will elicit pleasure. This is perhaps consistent with self-report measures of anhedonia, which can also reflect retrospective and prospective processing alongside hedonic capacity (e.g. Strauss & Gold, 2012).

Gard and colleagues (2007) were among the first to measure consummatory and anticipatory anhedonia in a schizophrenia sample. Using the temporal experience of pleasure scale (Gard, Germans Gard, Kring & John, 2006), a newer self-report instrument specifically designed to evaluate these distinct aspects of pleasure; they showed deficits in anticipatory but not consummatory pleasure among individuals with schizophrenia. Further, use of an experience sampling method, asking individuals to record how much pleasure they expected from future events throughout the day, also revealed the same pattern of results. That is, patients differed from controls in the pleasure they anticipated they would get from future activities, particularly in relation to goal-directed activities (such as work and school) as opposed to non-goal directed activities (such as watching TV; Gard et al., 2007). Whilst similar results have recently been found in the literature (Chan, Wang, Huang et al., 2010; Fortunati et al., 2015; Wang, Huang, Yang, Lui, Cheung & Chan, 2015), it should be noted that inconsistencies also exist. Strauss and colleagues (Strauss, Wilbur, Warren, August & Gold, 2011) found differences between schizophrenia patients and controls only on the consummatory, and not on

the anticipatory, anhedonia measures. That said, other laboratory-based paradigms might also speak towards an anticipatory hedonic deficit in schizophrenia patients. Delay discounting paradigms measure whether an individual will wait for a better future reward, or opt for a currently available reward of lesser value. This has revealed that patients with schizophrenia more readily discount future rewards, choosing the smaller immediate reward, compared to healthy control subjects, perhaps reflective of anticipatory anhedonia (Heerey, Robinson, McMahon & Gold, 2007).

Reward anticipation is a construct closely related to motivation or reward 'wanting'. As such, both subcomponents of reward are thought to be subserved by overlapping midbrain dopaminergic neurons together with their ventral and dorsal striatum targets (Barbano & Cador, 2006; Kring & Barch, 2014). Anticipatory hedonic processing involves not only an affective component (pleasure expected from future events) but also a prediction component (the ability to predict future events). In the fMRI literature, the most commonly used approach to assessing reward prediction has been through the Monetary Incentive Delay (MID) task. This instrumental task involves the presentation of cues indicating potential monetary gain or loss (vs. no consequence) and has been shown to recruit the dorsal and ventral striatum (including the NAc) in healthy adults (Knutson, Adams, Fong & Hommer, 2001; Knutson, Westdorp, Kaiser & Hommer, 2000). In schizophrenia patients, studies using the MID task have shown reduced ventral striatal activation to reward-predicting cues compared to healthy control subjects. These reduced striatal responses have been demonstrated in unmedicated patients (Juckel, Schlagenhauf, Koslowski et al., 2006b) and in patients taking typical antipsychotics (Juckel, Schlagenhauf, Koslowski et al., 2006a). In contrast, no striatal differences

between patients and controls have been observed when patients are being treated with atypical antipsychotics (Juckel, Schlagenhauf, Koslowski et al., 2006a) or are in a prodromal state (Juckel, Friedel, Koslowski et al., 2012). Dowd and Barch (2012) demonstrated similar reductions in striatal responses to reward-related cues in patients with high levels of self-reported anhedonia.

Interestingly, this was using a passive Pavlovian paradigm, aimed at eliminating confounds associated with the instrumental MID task, such as the execution of motor responses. Grimm, Vollstadt-Klein, Krebs, Zink and Smolka (2012) have also reported similar reductions in striatal activation to appetitive food cues. Importantly, in medicated and unmedicated schizophrenia patients, negative symptom severity has been shown to correlate with the reductions in ventral striatal activity to anticipated monetary reward (Juckel, Schlagenhauf, Koslowski et al., 2006a, 2006b).

The relationship between anhedonia and schizophrenia is clearly very complicated. Overall, it appears that schizophrenia patients display deficits in their anticipatory hedonic capacity, while their in-the-moment (consummatory) pleasure is relatively intact. However, many inconsistencies exist in the empirical literature. For example, while normal hedonic responses have been observed for many emotion-eliciting stimuli (including pictures, films, sounds and drinks), schizophrenia patients display impaired hedonic responses to odours (Kamath, Moberg, Kohler, Gur & Turetsky, 2011). Also, in contrast to the results of Gard et al. (2007) employing the experience sampling method, a similar study showed group differences between schizophrenia patients and controls, with patients reporting less intense positive emotions from their daily experiences (Myin-Germeys, Delespaul & deVries, 2000).

1.3.3 Reward Related Deficits, Beyond Anhedonia, in Schizophrenia

In an attempt to understand the so-called 'emotion paradox', other researchers have considered how cognitive deficits might produce an apparent anhedonic profile in schizophrenia. That is, schizophrenia patients may be unable to appropriately encode the value associated with rewards and/or integrate this representation with knowledge regarding the causal consequences of specific actions. At the anecdotal level, inflexible behaviour is often noted in schizophrenia, with patients unable to adjust their ongoing actions to take into account prior rewards, future goals or current emotional states (Barch & Dowd, 2010). A lost relationship between value representations and action selection is consistent with the reduced goal-directed behaviour characteristic of schizophrenia, despite normative hedonic processing, as well as the dysfunctional decision making commonly observed among patients (see Griffiths, Morris & Balleine, 2014 for a review). What is more, impaired representations of value may also explain the discrepancy between self-report measures of anhedonia, yet normal hedonic responses to evocative stimuli. As discussed by Gold and colleagues (2008), questionnaires such as the Chapmans Anhedonia Scales require participants to generate and maintain representations of the experiences in question, upon which their judgements regarding affective value must be based (Gold, Waltz, Prentice, Morris & Heerey, 2008). A patient who is unable to draw upon value representations may therefore respond in an anhedonia-consistent manner to the true/false question - 'The sound of rustling leaves has never much pleased me' (p. 923) - despite feeling normative pleasure during in-the-moment experiences of 'rustling leaves' (see Winterstein, Silvia, Kwapil et al., 2011, for a list of some of the items on this scale).

A number of studies have reported evidence of impaired value representations and action selection in schizophrenia. Heerey and Gold (2007) used an evoked and representational responding task and demonstrated that, whilst patients rated hedonic experience to emotional stimuli to a similar extent to healthy controls, they were unable to transmit this rating into the effort they were subsequently willing to exert to gain access to the same stimuli in the future. That is, when representations of the stimulus had to be relied upon, schizophrenia patients were unable to couple their in-the-moment pleasure ratings with their behavioural responses.

This inability to maintain value representations may also account for the greater delay discounting seen in schizophrenia, where patients opt for a smaller reward available immediately as opposed to a larger reward available after a delay. As suggested in section 1.3.2, anticipatory anhedonia may account for this deficit. However, an equally plausible explanation is that patients are unable to maintain value representations, preventing them from forming or updating associations between actions and their outcomes. Indeed, patients more readily discount future rewards across longer-term delays compared to shorter delays (Ahn, Rasmussen & Fridberg, 2011; Heerey, Robinson & McMahon, 2007), perhaps reflecting a degradation of internal value representations across time (Heerey, Matveeva & Gold, 2011).

A recent study by Morris, Quail, Griffiths, Green and Balleine (2015) directly investigated whether schizophrenic individuals can integrate causal knowledge of actions and their outcomes with changes in outcome value to flexibly control choice behaviour. In line with outcome devaluation tasks developed in rodents (to be explained in section 1.6.1), subjects were trained to perform two actions

(left and right key presses) to cause the liberation of two different snack foods (e.g. chocolate cookies or barbecue flavoured crackers) from a 'virtual' vending machine. One of these two snack foods (i.e. food A) was then devalued by pairing it with disgust, achieved through a 4 min video in which the snack food was depicted as infested with cockroaches. If an individual is able to flexibly encode reward value and understands the causal consequences of their actions (e.g. left key press leads to snack food A), then they will be less willing to perform actions that lead to the devalued outcome (i.e. they will refrain from pressing the left key). This was found to be the case for healthy control individuals; after pairing one of the snack foods with disgust, healthy subjects reduced the performance of the action associated with the devalued food relative to the alternative action. Furthermore, this was in the absence of the food rewards being delivered and so was in the absence of new learning. In contrast, schizophrenia patients continued to perform the devalued action just as much as the action associated with the valued reward. With the outcome devaluation procedure itself being just as effective in schizophrenia patients as controls (i.e. both groups had reduced subjective ratings to devalued compared to valued foods), this pattern of responding suggests that patients cannot integrate action-outcome learning with changes in outcome value.

The use of outcome devaluation in rodents (and its subsequent use in humans) has uncovered the neural circuits of the brain necessary for promoting flexible goal directed actions. Regions of particular importance include the medial prefrontal cortex (prelimbic cortex in rats) and the dorsal striatum (including the anterior caudate, homologous to dorsomedial striatum in rats) (see Griffiths et al., 2005 and Balleine & O'Doherty, 2010, for a review). Interestingly, the study by Morris

et al. (2015) provided evidence of regional activity differences in the caudate of schizophrenia patients, compared to controls, during the choice phase of the task. What is more, this reduced activity, primarily within the head of the caudate, correlated with the severity of negative symptoms in these patients. This finding is consistent with research in schizophrenia indicating neuropathology in the 'associative striatum' of the brain (de la Fuente-Sandoval, León-Ortiz, Favila, Stephano, Mamo, Ramirez-Bermúdez & Graff-Guerrero, 2011; Howes, Montgomery, Asselin et al., 2009; Kegeles, Abi-Dargham, Frankle et al., 2010), together with evidence for a disconnection between the caudate and its cortical afferents (Fornito, Harrison, Goodby et al., 2013; Quan, Lee, Kubicki et al., 2013; Quidé, Morris, Shepherd, Rowland & Green, 2013). Taken together, this evidence led Morris and colleagues (2015) to propose that the impaired goal-directed behaviour they observed is due to a functional disconnection in the cortico-striatal loops of the schizophrenic brain.

In summary, there is clear evidence that aspects of reward processing in schizophrenia - including flexible goal-directed behaviour - are impaired beyond the narrow conception of anhedonia. Moreover, the neurobiological underpinnings of goal-directed behaviour appear to overlap with at least some of the neurobiological impairments associated with schizophrenia.

1.3.4 Overview of Depression

Depression is a highly debilitating disorder with symptoms that manifest at the psychological, behavioural and physiological levels. With higher prevalence than other psychiatric disorders, it has been reported that approximately 16% of people will develop depression at some point over their

lifetime (Kessler, Berglund, Demler et al., 2003). Moreover, depression is predicted to become the second leading cause of disability worldwide by 2030 (second to ischemic heart disease) (Mathers & Loncar, 2006).

According to the diagnostic criteria for Major Depressive Disorder, a diagnosis of depression is contingent on the presence of at least five of the following symptoms: a low or depressed mood, anhedonia, weight disturbances, disturbed sleep, psychomotor abnormalities (i.e. agitation or retardation), fatigue or loss of energy, excessive guilt, difficulty concentrating and suicidal ideation (American Psychiatric Association, 2013). Highlighting the heterogeneity of the disorder, two people could both receive a depression diagnosis but only share a single overlapping symptom (Treadway & Zald, 2011). That said, at least one of the symptoms presented must be either low mood or anhedonia (American Psychiatric Association, 2013).

The aetiology and pathophysiology of depression is not completely understood. It is a recurrent condition, where the likelihood of developing a subsequent depressive episode is positively correlated with the number of previous episodes (Burcusa & Iacono, 2007; Lewinsohn, Zeiss, & Duncan, 1989; Solomon, Keller, Leon et al., 2000). Approximately 60% of patients that have experienced a single depressive episode will succumb to a second, whereas 90% of patients who have experienced three depressive episodes will succumb to a fourth (Winans & Bettinger, 2004). Prevalence is much higher among post-pubertal women than men, with a female:male risk ratio of approximately 2:1 (Kessler, 2003). This gender bias reflects a higher risk of first onset among women, as gender differences do not impact on the persistence of symptoms or the recurrence of

further depressive episodes (Kessler, 2003 – but see Lewinsohn et al., 1989). Whilst adult onset is most prevalent (the average age of onset is in the mid-20s), approximately two percent of children and five percent of adolescents also suffer from depression (Iyer & Khan, 2012; Winans & Bettinger, 2004).

Several hypotheses have been proposed to explain the onset and persistence of depressive symptoms. The monoamine hypothesis, which has dominated the literature since its introduction around fifty years ago, states that depressive symptoms are driven by an absolute or relative deficiency of monoamines in the brain (as reviewed by Willner, Scheel-Krüger & Belzung, 2013). This hypothesis has received support from cerebral spinal fluid (CSF), neuroendocrine and post-mortem analyses which have revealed abnormalities in monoamine precursor/metabolite concentrations and receptor/transporter binding site densities that are consistent with monoamine deficiency in the disease (see Saveanu & Nemeroff, 2012 for a review – see also Belmaker & Agam, 2008). For example, low levels of noradrenaline (NA) and serotonin (5-HT) metabolites have been found in the CSF of depressed patients, while increased density of 5-HT₂ and β -adrenergic receptors has been revealed in post-mortem brain tissue, perhaps reflecting a compensatory mechanism to low synaptic 5-HT and NA concentrations (see Saveanu & Nemeroff, 2012; Belmaker & Agam, 2008). Whilst dopamine has been primarily implicated in the pathophysiology of schizophrenia, there is also evidence to support its role in depression. As reviewed by Pizzagalli (2014), one of the first suggestions of a hypodopaminergic tone in depression came from studies revealing lower levels of homovanillic acid (HVA), a major metabolite of dopamine, in the CSF of depressed patients compared

to healthy controls. Positron emission tomography (PET) studies have also revealed increased postsynaptic striatal dopamine (D₂/D₃) receptor concentrations in depression, possibly reflecting lower dopamine availability in the synapse. Furthermore, post-mortem studies have revealed decreased dopamine transporter (DAT) binding sites in striatal regions (caudate, putamen and NAc) of depressed individuals, again suggestive of blunted DA transmission.

The Role of Stress in Depression

Whilst the monoamine hypothesis is supported by the clinical efficacy of currently available antidepressants, which act to increase monoamine levels, the latency of clinical onset for these drugs (taking several weeks before the full therapeutic effect is achieved), suggest that a cascade of molecular and neural changes occur in the presence of antidepressant therapies. This, together with the fact that up to 30-40% of patients respond poorly to current treatments, has led some researchers to search for alternative, although not mutually exclusive, hypotheses (as reviewed by Willner et al., 2013).

The diathesis-stress hypothesis proposes that an interaction between pre-morbid vulnerability factors (including genetic and neurobiological factors) and stress (external or internal) leads to the development of depressive symptoms (see Willner et al., 2013, for a review).

a. Diathesis.

A variety of factors may contribute to a person's vulnerability (or diathesis) to develop depression. For example, it is thought that approximately 30-40% of depression is inherited, as shown by concordance studies of monozygotic and dizygotic twins (Saveanu & Nemeroff, 2012; Sullivan, Neale & Kendler, 2000). As reviewed by Willner and colleagues (2013) some early life factors also predispose a person to depression, particularly poor parental relationships and childhood abuse. Adverse events in early life, such as criticism, rejection or having a parent with depression, may lead to the development of a negative 'cognitive schema' - causing an individual to have negative world views and heightened attention towards negative information (Beck, 1967 - as cited by Willner et al., 2013). Emotional instability, resulting from poor parental care or loss of a parent, may also mitigate social support in later life. Finally, Willner et al. (2013) recognise that personality factors such as neuroticism, which result from genetic-environmental interactions, also increase the likelihood of developing depression. Not only do these individuals possess a negative-information processing bias (akin to depression), they also act in dysfunctional ways that might increase their exposure to stressful events. As mentioned earlier, female gender is also a predisposing factor, as is the experience of previous depressive episodes. The presence of minor depression also increases risk, with a longitudinal study demonstrating that over 22% of people sampled with minor depression later suffered from a unipolar disorder (Akiskal, Bitar, Puzantian, Rosenthal & Walker, 1978).

b. Stress.

Stress has been strongly implicated in the pathogenesis of depression (e.g. Tennant, 2002; Hammen, 2005). Whilst the stress could be internal (such as a traumatic head injury or hormonal challenge), external stresses are more common precipitants of depression (see Willner et al., 2013). In community samples, stressful life events (e.g. health-related disability and bereavement) have been demonstrated to precede the onset of approximately 80% of depressive episodes (as reviewed in Auerbach, Admon & Pizzagalli, 2014). The accumulation of chronic mild stressors, such as loss of employment, family discord and poverty, has also been implicated in depression onset (see Willner et al., 2013). Moreover, chronic stressors have been strongly linked to poor prognosis, stronger depressive symptoms, and treatment resistance (see Pizzagalli, 2014, for a review).

One of the major physiological responses to stress is the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Briefly, activation of the hypothalamus in response to stress leads to the release of corticotrophin releasing hormone (CRH), which stimulates the pituitary glands to release adrenocorticotrophic hormone (ACTH). This in turn stimulates the adrenal cortex to release glucocorticoids (cortisol in humans, corticosterone in rats) into the blood. In a negative feedback loop, cortisol receptors on the hypothalamus respond by decreasing the production of CRH, thus maintaining a homeostatic state (Smith & Vale, 2006).

In a sub-group of depressed patients, several indicators suggest altered activity of the HPA axis. For example, patients display an altered response to the dexamethasone/CRH challenge (i.e. fail to show the normal suppression of cortisol levels that would suggest a regulated system) (Carroll,

Cassidy, Naftolowitz et al., 2007; Ising, Kunzel, Binder et al., 2005; Saveanu & Nemeroff, 2012); have higher basal levels of CRH (Nemeroff, Widerlov, Bisette et al., 1984); and hypersecrete cortisol (Sachar, Hellman, Fukushima et al., 1970). Whilst activity of the HPA axis is an adaptive response to stress, its prolonged activation can have detrimental consequences including neurotoxic effects (Willner et al., 2013). Reports have shown that depressed patients display the expected neuronal alterations of a hyperactive HPA system (Booij, Wang, Lévesque, Tremblay & Szyf, 2013), demonstrating decreased sensitivity of glucocorticoid receptors (as reviewed by Booij et al., 2013), smaller hippocampal volume (MacQueen, Campbell, McEwen et al., 2003) and a reduced ability to down-regulate the activity of the HPA axis (Maletic et al., 2003; as cited by Booij et al., 2013). Brain-derived neurotrophic factor (BDNF), a neuropeptide involved in neurogenesis that is sensitive to stress (Angelucci, Brenè & Mathè, 2005; Kozlovsky, Matar, Kaplan, Kotler, Zohar & Cohen, 2007), is also reduced in the hippocampus of depressed suicide victims (Karege, Vaudan, Schwald, Proud & La Harpe, 2005), as shown by post-mortem brain tissue. Patients suffering from depression also have elevated levels of pro-inflammatory cytokines (including Interleukin-1, Interleukin-6 and Tumour Necrosis Factor-alpha), with the severity of depressive symptoms correlating with the magnitude of cytokine elevation (as reviewed by Saveanu & Nemeroff, 2012). Consistent with high circulating levels of cortisol, this relationship is particularly interesting given the role of proinflammatory cytokines in modulating the CRH and HPA axis, as well as their role in neurotransmitter metabolism (e.g. 5-HT) (as reviewed by Saveanu & Nemeroff, 2012). Furthermore, neuroimaging studies have shown that

depressed patients exhibit higher activity levels of monoamine-oxidase-A (MAO-A), again consistent with high stress/cortisol levels (Willner et al., 2013).

Whilst stress has been strongly implicated in the development of depression, it should be noted that it does not necessarily predispose an individual to a depressive episode. That is, whilst one person may develop depression from being exposed to a stressor, another person exposed to the same stressor will not. The diathesis-stress model proposes that individuals with a strong depressive diathesis will succumb to minor or trivial stressors, whereas an individual with a weak depression diathesis requires more intense stressors to precipitate depressive symptoms (see Willner et al., 2013). As mentioned earlier, previous experience of depression increases the diathesis for future episodes. Interestingly, evidence suggests that as the occurrence of depressive episodes increases, the importance of stress decreases (Kendler, Thornton & Gardner, 2000). Thought to be due to a sensitisation or 'kindling' effect (see Pizzagalli, 2014), the more a person succumbs to depressive episodes, the more likely these episodes will be independent of large stressors.

In summary, many depressed patients experience a range of abnormalities at both the neurochemical and structural level that resemble those observed following hyperactivity of the HPA axis in response to prolonged stress exposure.

1.3.5 Anhedonia in Depression

Whereas Rado and Meehl (section 1.3.2) were critical in highlighting the role of anhedonia in schizophrenia, its importance in relation to depression was highlighted by Klein: stating that "a sharp,

unreactive pervasive impairment of the capacity to experience pleasure, or to respond affectively, to the anticipation of pleasure" (p. 449) is a central component of endogenomorphic (i.e. 'classic' or 'melancholic') depression (Klein, 1974 - as cited by Der-Avakian & Markou, 2012). Now defined by the DSM-V as a decrease in interest or pleasure in most activities, it constitutes one of the two main symptoms required for depression diagnosis, the second being a generally low or depressed mood (American Psychiatric Association, 2013). As diagnosis depends on only one of these two main symptoms being present (alongside at least four additional symptoms – see above), it is interesting to note that an individual may be diagnosed with depression without them experiencing a depressed mood, if anhedonia is present (Dichter, 2010). The importance of anhedonia in this disorder is further highlighted by studies revealing that low hedonic capacity predicts poor outcome for depressed patients (see Pizzagalli, 2014).

Similarly to schizophrenia, assessments of anhedonia in depression have relied heavily on self-report instruments. Whilst some of these were originally developed with schizophrenia in mind (e.g. the Chapman Anhedonia Scales), others have been developed specifically for hedonic deficits in depression (e.g. SHAPS). Use of the Chapman Social and Physical Anhedonia Scales (Berlin, Givry-Steiner, Lecrubier, Puech, 1998; Loas, Salinas, Guelfi & Samuel-Lajenunesse, 1992), SHAPS (Franken, Rassin & Muris, 2007; Liu, Chan, Wang, Huang, Cheung, Gong & Gollan, 2011) and the Fawcett-Clark Pleasure Scale (Berlin et al., 1998), another self-report instrument, has indicated that depressed individuals exhibit higher levels of anhedonia compared to healthy controls.

The presence of anhedonia measured through self-report instruments has generally been supported by laboratory-based assessments. In contrast to schizophrenia, patients with depression have been shown to rate emotion-eliciting stimuli as being less positive and less arousing compared to healthy controls. Such reduced pleasure ratings have been observed for a range of stimuli including pictures (Dunn, Dalgleish, Lawrence, Cusack & Ogilvie, 2004; Sloan, Strauss, Quirk & Sajatovic, 1997), film-clips (Rottenberg, Gross & Gotlib, 2005; Rottenberg, Kasch, Gross & Gotlib, 2002), emotional words (Liu, Wang, Zhao, Ning & Chan, 2012) and flavoured drinks (Berenbaum & Oltmanns, 1992), although inconsistencies do exist in the literature (Amsterdam, Settle, Doty, Abelman & Winokur, 1987; Berlin et al., 1998; Clepce, Gossler, Reich, Kornhuber & Thuerauf, 2010; Dichter, Smoski, Kampov-Polevoy, Gallop & Garbutt, 2010; Gehricke & Shapiro, 2000). Impaired hedonic capacity has also been examined through a range of other laboratory methods. For example, depressed patients fail to exhibit the typical attenuation of their startle response during the presentation of positive stimuli (Allen, Trinder & Brennan, 1999; Dichter, Tomarken, Shelton & Sutton, 2004; Kaviani, Gray, Checkley, Raven, Wilson & Kumari, 2004). Furthermore, Henriques and colleagues (1994) demonstrated that people suffering from depression do not display a response bias towards rewarding stimuli (Henriques, Glowacki & Davidson, 1994), a result that has since been replicated (Pizzagalli, Iosifescu, Hallet, Ratner & Fava, 2008; Pizzagalli, Jahn & O'shea, 2005). Overall, the literature supports reduced hedonic capacity in depression.

There are many overlaps between the neurobiological changes associated with depression and the brain regions linked to hedonic function. For example, structural MRI studies have revealed a

reduced grey matter volume in the striatum (see Koolschijn, van Haren, Lensvelt-Mulders, Hulshoff Pol & Kahn, 2009 for a review), including the NAc (Wacker, Dillon & Pizzagalli, 2009), of depressed individuals. Moreover, diminished responses of the ventral striatum to the receipt of rewards have been reported in both currently depressed (Epstein, Pan & Kocsis et al., 2006; Pizzagali, Holmes, Dillon et al., 2009; Wacker, et al., 2009) and previously depressed (McCabe, Cowen & Harmer, 2009) individuals compared to healthy controls, with a negative correlation revealed between activity levels and anhedonia severity (Epstein et al., 2006; Wacker et al., 2009). As reviewed by Rømer-Thomsen et al. (2015), alterations in ventral mPFC activity (including activity in the OFC, an area putatively involved in hedonic responses - see section 1.2) have been shown in depressed subjects in response to positive stimuli, while smaller OFC volume has also been reported in the literature (as reviewed by Treadway & Zald, 2011). Furthermore, there is some evidence suggesting that opioid deficiency features in depression. As reviewed by Treadway and Zald (2011), this suggestion first emerged when two convergent studies demonstrated a temporary alleviation of depressive symptoms after the injection of a non-selective opioid receptor agonist (β -endorphin), which occurs endogenously in the brain. However, since these early studies, results in the literature have been largely equivocal (see Hegadoren, O'Donnell, Lanius, Coupland & Lacaze-Mamonteil, 2009, for a meta-analysis of β -endorphin levels in depressed patients). That said, there is emergent interest in the role of kappa opioids antagonists¹ as a treatment of depression (as reviewed by Barch, Pagliaccio & Luking, 2015),

¹ Whilst this might appear to be inconsistent with the role of opioid systems in hedonic processing, it likely reflects kappa stimulation outwith the small rostral hedonic 'hotspot' of the NAc shell. Indeed, while kappa effects induce positive liking reactions inside the hotspot, stimulation of the entire caudal half of the shell produces negative effects as shown by the induction of conditioned place avoidance (Castro & Berridge, 2014a). Systemic activation of the kappa opioid receptor has also

but further work directly addressing the relationships between opioid systems and anhedonic symptoms in depression are needed.

As highlighted earlier, Klein's original definition of anhedonia as it relates to depression included a temporal distinction between consummatory and anticipatory anhedonia. Whilst the importance of this distinction has been clearly demonstrated for schizophrenia patients, studies examining reward anticipation in individuals with depression have produced inconsistent results.

Abnormalities in the anticipation or prediction of rewards in depression is intuitively plausible given that depressed individuals self-report lower levels of pleasure, and exhibit abnormal behavioural and neural responses to positive stimuli. Preliminary work by Sherdell, Waugh and Gotlib (2012) has provided some support for this hypothesis, demonstrating that people suffering from depression self-report reduced levels of anticipatory pleasure. However, this study investigated the construct of anticipatory hedonics with one item from the Hamilton Rating Scale of Depression (HAM-D; Hamilton, 1967). The questions included in this item (e.g. "Have you felt interested in doing...") better reflect motivational capacity of the individual rather than pure anticipatory hedonics. In terms of rating scales, however, promise has come from a Chinese sample demonstrating that clinically depressed subjects report higher anticipatory anhedonia on TEPS (Liu et al., 2011). Whilst the scale used differed from the original designed by Gard and colleagues (2006) (with four factors instead of two), high correlation has been demonstrated across the two versions (Chan, Shi, Lai, Wang, Wang & Kring, 2011). Additionally, work by McFarland and Kein (2009) found that depressed patients

been observed to decrease social play and increase the stimulation threshold of intracranial self-stimulation in rodents (see Lalanne et al., 2014).

reported significantly less pleasure when anticipating monetary rewards compared to healthy individuals, and marginally less pleasure compared to remitted individuals, again consistent with an inability to predict pleasure from future events.

There are some examples of abnormal neurobiological responses in depressed individuals during reward anticipation tasks (e.g. reduced activation in striatal regions; Smoksi, Felder, Bizzell, Green, Ernst, Lynch & Dichter, 2009; Forbes, Hariri, Martin, Silk, Moyles, Fisher, Brown, Ryan, Birmaher, Axelson & Dahl, 2009), but also reports of the absence of such differences (Knutson, Bhanji, Cooney, Atlas & Gotlib, 2008; Pizzagalli, Holmes, Dillon et al., 2009). Given the paucity of work, and potential confounds due to differences in the precise anticipation tasks used, further work using more focused and common methods, such as the TEPS scale, is needed before the neurobiology of anticipatory reward processing in depression is understood.

1.3.6 Reward Related Deficits, Beyond Anhedonia, in Depression

In addition to the disorders of mood that are central to depression, it has long been recognised that depression also involves cognitive and motivational disturbances as well. Indeed, early theoretical analyses of depression placed these cognitive/motivational aspects at the centre of their accounts. For example, both learned helplessness/attributional theory (see Abramson, Seligman & Teasdale, 1978; Alloy, Abramson, Peterson & Seligman, 1984) and Beck's cognitive theory (Beck, 1967; 2008), place a large emphasis on cognitive inflexibility. More recently, it has been suggested (Griffiths et al., 2014) that these cognitive disturbances may be centred on reward processing, with

the result that depressed patients might also fail to flexibly control their actions based on the consequences those actions engender, instead relying on antecedent stimuli to form stimulus-response associations or habits (to be explained in section 1.6.1). This idea is consistent with impaired decision-making abilities in depressed patients (DSM-V; WHO, 1992), together with the high comorbidity seen between depression, impulse control disorder and substance abuse (other habit-based disorders) (Winans & Bettinger, 2004).

Although goal-directed behaviour has not been explicitly investigated in a depressed sample, and thus there has been no direct test of the idea that impairments of goal-directed action are central to depression, the examination of neurobiological factors does provide at least some suggestive evidence. For example, Friedel and colleagues (2009) reported that depressive symptom severity is negatively correlated with connectivity between the medial OFC and the BLA (Friedel, Schlagenhauf, Sterzer et al., 2009). Critically, in both rats (Zeeb & Winstanley, 2013) and monkeys (Baxter, Parker, Lindner, Izquierdo & Murray, 2000), contralateral lesions of the OFC-BLA connections have been shown to prevent subjects from flexibly controlling their behaviour based on reward value. Moreover, investigation of the formation of goal-directed actions in a healthy population have found that goal-directed behaviour correlates significantly with the activity of the OFC (Valentin, Dickinson & O'Doherty, 2007), while abnormal recruitment of the mOFC has been seen when depressed patients perform behavioural tasks (involving planning and behavioural choice) that recruit the frontal system (see Griffiths et al., 2014, for a full review).

Whilst the literature regarding instrumental control in depression per se is extremely underdeveloped, there is converging evidence for the idea that impaired reward processing might contribute to depression from the examination of the effects of stress. As discussed in section 1.3.4, stress is an important risk factor for the development of depression. With this in mind, studies directly investigating the balance between goal-directed and habitual systems in response to stress may provide us with insights into whether similar abnormalities might also exist in depressed patients.

In a seminal study by Schwabe and Wolf (2009), participants were exposed to an acute stress protocol, which combined physical and psychosocial stressors, before being trained to perform different instrumental responses for the delivery of distinct food rewards. After training, participants were invited to consume one of the two rewards to satiety (essentially devaluing this reward relative to the alternative reward). When subsequently given the choice between the two instrumental responses in the absence of any reward delivery, stressed individuals responded in a way consistent with habitual control of behaviour. That is, unlike non-stressed control subjects, stressed individuals were insensitive to the current motivational value of the outcome. Furthermore, this insensitivity was paralleled by a reduction in the participant's causal knowledge of action-outcome contingencies, also consistent with habitual as opposed to cognitive instrumental control.

A similar effect has since been reported in rodents after a chronic mild stress procedure. Results showed that rats chronically stressed across a 21-day period were unable to adjust their behaviour to match changes in outcome value or action-outcome contingency (Dias-Ferreira, Sousa, Melo et al., 2009). Thus, stress applied acutely or chronically appears to bias instrumental control

away from goal-directed systems. Furthermore, studies in humans (Schwabe & Wolf, 2010) and rodents (Braun & Hauber, 2013) have demonstrated that the application of an acute stressor *after* instrumental training of the task also renders performance insensitive to changes in outcome value. Whilst this does not speak to the effects of stress in the acquisition of goal-directed behaviours (which may or may not be intact), it does show that stress can affect the expression of goal-directed actions independent of learning effects. Moreover, in the human study (Schwabe & Wolf, 2010) it was shown that cortisol levels and the participant's behavioural insensitivity to the outcomes value were significantly correlated. Whilst this is not necessarily causative in nature, the fact that elevations in cortisol are also seen in depression (see section 1.3.4) highlights the possibility that impaired goal-directed behaviour and an imbalance towards habitual control could be an important feature in this disorder. In addition, the fact that stress in rodents impacts on corticostriatal circuits implicated in both goal-directed behaviours (Dias-Ferreira et al., 2009) and in depression (Griffiths et al., 2014 – see the start of this section), is also suggestive of potentially impaired goal-directed behaviour in depressed individuals.

In summary, the large overlap between stress responses and depression in terms of endocrine and neurotransmitter systems reinforces the idea that goal-directed behaviours might be impaired in depressed individuals. Like the medial prefrontal cortex in rats, the ventromedial prefrontal cortex (vmPFC, including the OFC) in humans has been implicated in the formation of goal-directed actions (see section 1.6.1 for full discussion). The abnormal recruitment/activity of this area in depression (whilst a contentious issue) might also suggest a dependence on habit (see Griffiths et

al., 2014). Structural abnormalities have also been reported for the frontal cortical regions (e.g. Rajkowka, Miguel-Hidalgo, Wei et al., 1999) (as well as for the caudate nucleus of the striatum (see Griffiths et al., 2014) - another structure implicated in goal-directed behaviour, see section 1.6.1) of depressed individuals. Together with the observation that patients perform poorly on tasks that depend on frontal systems (e.g. Braw, Aviram, Bloch & Levkovitz, 2011; Moritz, Birkner, Kloss et al., 2002), this evidence, strongly suggests impairment of prefrontal-cortex dependent goal-directed behaviours in depressed patients.

1.4 Animal Models of Psychiatric Disorders

Animal models are instrumental in better understanding the mechanisms underlying the symptoms of disease and for the development of novel treatments. This, first and foremost, is because they provide us with a unique opportunity to test hypotheses which are not amenable to human investigation. In many cases, it is animal models that have allowed us to determine what changes occur in the schizophrenic or depressed brain. For example, understanding changes in neurotransmitter levels in humans has had to rely, in large part, on post-mortem analysis (potentially confounded by comorbid disorders and treatment effects) as well as imaging studies that indirectly measure the brains activity levels. Animal models on the other hand allow specific hypotheses regarding the disorder to be tested at the basic science level. They can also side-step the numerous problems associated with human studies such as heterogeneity in symptom course and outcome and the patients' drug medication status.

As well as being able to appropriately measure the discreet constructs of reward processing (and other symptom-like behaviours) (to be discussed in sections 1.5 and 1.6), it is essential that animal models of disease possess good translational value. Models of disorders must be rigorously evaluated for their ability to satisfy requirements of 'face', 'construct' and 'predictive' validities (see Jones, Watson & Fone, 2011). For a model to have good 'face' validity, there must be a large overlap between the behavioural phenotype exhibited by the model and the profile of clinical symptoms in the disorder. Challenges to face validity arise due to the uniquely human characteristics of schizophrenia and depression. After all, measuring hallucinations, or a generally low mood, that require the individual to self-report their experiences, are impossible in non-verbal animals. However, several symptoms can be replicated in rodents and, most importantly for the current thesis, this includes reward-related behavioural deficits.

Construct validity requires the model to replicate the theoretical biological rationale and underlying pathophysiology of the disease. Since the aetiology and pathophysiology of schizophrenia and depression are not clearly established, most animal models have been developed to exhibit some aspect of the neurobiological features that have been detected in schizophrenia (e.g. neurotransmitter deficits, enlarged ventricles and reduced hippocampal volume) and depression (e.g. neurotransmitter deficits, dysregulation of the HPA axis and hypercortisolaemia).

For a model to have predictive validity, the depression- or schizophrenia-like symptoms must be attenuated by clinically effective therapeutic treatments, whilst not being effected by drugs known to be ineffective in the clinic. Typically, in drug discovery research, assessing novel compounds

against current gold-standards provides this level of validation. However, in the case of schizophrenia, gold standard drugs have not yet been developed to target the negative and cognitive symptoms, whereas in depression, all current drugs have the same underlying mechanism on monoamine systems - which makes it hard to identify any drugs with a novel mechanism.

1.4.1 Animal Models of Schizophrenia

Schizophrenia is a disorder in which similar pathology can arise from multiple different aetiologies. As a result, a range of pharmacological (i.e. amphetamine, PCP, ketamine and MK-801), genetic (i.e. mutant DISC-1, knockout of neuregulin-1 and mutant dysbindin), and neurodevelopmental models (i.e. MAM, neonatal ventral hippocampal lesion, post-weaning social isolation and maternal infection) have been introduced to represent schizophrenia in animals (see Jones et al., 2011).

a. Pharmacological models

Pharmacological models have focused in large part on the dopaminergic hypothesis of schizophrenia due to the majority of current antipsychotics having dopamine D₂ receptor antagonist activity. Repeated administration of drugs that increase dopamine levels, such as amphetamine, increase spontaneous locomotor activity as well as activity to subsequent psychostimulant challenge after withdrawal (Miyamoto & Nitta, 2014). Depending on the dosing regimen, amphetamine has also been shown to produce persistent deficits in the prepulse inhibition (PPI) of acoustic startle responses

(Jones et al., 2011), a deficit identified in schizophrenia patients (Braff, Geyer & Swerdlow, 2001), thought to involve impaired pre-attention processing (Young, Powell, Risbrough, Marston & Geyer, 2009). The face validity of such models, however, is limited by the fact that they do not incorporate the negative symptoms associated with the disease (Sams-Dodd, 1995, 1998).

Glutamate (i.e. NMDA receptor hypofunction) has also been implicated in the pathophysiology of schizophrenia and has led to glutamate-based pharmacological models. Chronic PCP administration (a NMDA receptor antagonist) has been shown to be particularly relevant in modelling the disease (see Neill, Barnes, Cook et al., 2010). Not only does subchronic PCP induce a range of neurobiological processes akin to schizophrenia (i.e. mesolimbic DA hyperfunction and mesocortical DA hypofunction) (Jentsch, Taylor & Roth, 1998; Jentsch, Tran, Le, Youngren & Roth, 1997; Jentsch & Roth, 1999), it also induces behaviours reminiscent of the positive, negative (Neil, Harte, Haddad, Lydall & Dwyer, 2014; Sams-Dodd, 1995; 1998) and cognitive symptom clusters (Neill et al., 2010). That said, sub-chronic PCP does not induce behaviours analogous to consummatory (Lydall, Gilmour & Dwyer, 2010) or anticipatory anhedonia (Wright, Dwyer & Gilmour, 2013), nor does it induce impairments in reward valuation (own observation) or motivational competencies (Lydall, 2011). Moreover, this model has resulted in false-positives with respect to currently available antipsychotics, questioning its predictive validity (see Jones et al., 2011).

b. Genetic models

Genetic models of schizophrenia rely on the high heritability of the disorder and as such have good construct validity (McGuffin, Tandon & Corsico, 2003). Example models include DISC-1, neuregulin-1 and dysbindin where susceptible genes that may predispose an individual to develop schizophrenia have been manipulated. Whilst these models show promise, schizophrenia is considered to be both polygenic in nature and involve a complex interplay between genetic and environmental factors. With this in mind, the relevance of a single genetic alteration is hard to determine (see Miyamoto & Nitta, 2014, for a review), although these models may well support the examination of converging 'downstream' effects of the different genetic manipulations investigated (e.g. relating to neural signalling).

c. Neurodevelopmental models

As discussed earlier, the risk of developing schizophrenia is greatly enhanced if the neonate has been exposed to adverse environmental conditions during the critical gestational or perinatal periods of development (Jones et al., 2011). A wide range of perturbations have been considered of relevance, including maternal stress and malnutrition, infections and complications such as hypoxia during birth (Jones et al., 2011). Animal models that utilise perinatal and postnatal insults have been shown to induce behavioural phenotypes of relevance to schizophrenia (Meyer & Feldon, 2010). For example, post-weaning social isolation produces spontaneous locomotor hyperactivity, hyperdopaminergic tone of the mesolimbic system and causes a consistent decrease in PPI (Jones et

al., 2011), whereas maternal malnutrition has similar effects on PPI and increases amphetamine induced hyperlocomotion (e.g. Palmer, Printz, Butler et al., 2004). Neonatal excitotoxic lesions of the ventral hippocampus have also been a popular modelling approach, producing a phenotype emerging during adolescence (see Miyamoto & Nitta, 2014). Behavioural characteristics of relevance to schizophrenia include: increased sensitivity to amphetamine (Beninger, Tuerke, Forsyth et al., 2009), PCP and MK-801; deficits in PPI; impaired latent inhibition; working-memory problems and decreased sociability (a symptom independent of sexual maturity, as reviewed by Lipska & Weinberger, 2000). However, the predictive validity of this model has been questioned and lesioned rats also show increased sucrose preference (potentially questioning the face validity of the model - see Miyamoto & Nitta, 2014, for a review).

Another popular approach is to disrupt neurogenesis using immune activation (via polyriboinosinic-polyribocytidilic acid - PolyI:C) or Methylazoxymethanol acetate. As Methylazoxymethanol acetate is the chosen animal model for this thesis, it will be reviewed in some detail.

Methylazoxymethanol acetate (MAM) is a naturally occurring substance derived from the seeds of cycad plants which, when administered to a pregnant dam rat, disrupts embryonic brain development (Matsumoto & Higa, 1966). As an antimitotic and antiproliferative agent, MAM acts by methylating DNA, specifically targeting developing neuronal cells, without affecting glial cells or peripheral organs (Cattabeni & Di Luca, 1997).

In common with neurodevelopmental models in general, the timing of MAM administration is of utmost importance as neuronal development follows a rigid timetable (see Bayer & Altman, 2004). With MAM preventing cell mitosis for a short time after injection (Balduini, Elsner, Lombardelli, Peruzzi & Cattabeni, 1991; Cattabeni & Di Luca, 1997), it allows for very targeted brain disruption.

Administration of MAM on gestational day fifteen (GD15), when cortical neurogenesis of the developing rat pup is at its peak, produces a disruption which is too widespread to be applicable to schizophrenia (see Jones et al., 2011). For example, at this time, MAM produces gross changes in total cortical mass and brain weight (Moore, Jentsch, Ghajarnia, Geyer & Grace, 2006), which are far removed from the subtle changes in cortical and temporal lobe morphology seen in patients (Shenton, Dickey, Frumin & McCarley, 2001). However, administration of MAM on GD17 is considered to be optimal: Neuronal proliferation is reduced, but not entirely blocked, in most cortical regions by this time point (Jones et al., 2011). As a result, MAM treatment at GD17 causes subtle effects across cortical areas, the hippocampus and the limbic system - all of which are relevant to schizophrenia pathophysiology (Liddle et al., 2006; Lodge & Grace, 2009; Moore et al., 2006).

Structurally, GD17 MAM has been shown to increase ventricle size (including lateral and third ventricles) (Jones et al., 2011, but see Matricon, Bellon, Friedling et al., 2010), reduce hippocampal volume and cause reductions in the thickness of the cortex and mediodorsal thalamus (see Table 1; Chin, Curzon, Schwartz et al., 2011; Flagstad, Mork, Glenthøj, van Beek, Michael-Titus & Didriksen, 2004; Le Pen, Gourevitch, Hazane, Hoareau, Jay & Krebs, 2006; Matricon et al., 2010; Moore et al., 2006). Neuronal packing density is increased in the mPFC (Moore et al., 2006), while disorganisation

of pyramidal neurons features in the hippocampus (Le Pen et al., 2006; Moore et al., 2006), as does a decrease in hippocampal parvalbumin (PV) containing GABA-ergic interneurons (Lodge, Behrens & Grace, 2009; Penschuck, Flagstad, Didriksen, Leist & Michael-Titus, 2006). Decreases in total brain weight have also been seen after GD17 MAM treatment (of approximately 11%) which is also somewhat consistent with schizophrenia (Flagstad et al., 2004).

Table 1 Morphological and neurochemical deficits induced by the administration of MAM on GD17. Due to timing of the intraperitoneal injection, disruptions are restricted to the paralimbic and temporal cortices reflecting the histopathology observed in schizophrenia patients.

Human Schizophrenia	Refs	MAM-Exposed Rat (GD17)	Refs
↓ Tissue volume/thickness in prefrontal cortical and temporal lobe regions	1,2,3,4	↓ Cortical area/thickness <ul style="list-style-type: none"> • <i>mPFC</i> • <i>Hippocampus</i> • <i>Parahippocampal cortices</i> 	5,6,7,8
No change in neocortical neuron number	9	No change in neocortical neuron number	8
Hippocampal pyramidal neurons are disorganised <ul style="list-style-type: none"> • Variation in neuronal orientation 	10	Heterotopias, disorganisation and sporadic density of hippocampal pyramidal neurons	5,6,8
↓ PV-positive GABA-ergic interneurons in cortical and limbic areas	11	↓ PFC/ hippocampal PV-positive GABA-ergic interneurons	12, 13
↓ Size and/or cell number in anterior/medial dorsal thalamus	4, 9,14	↓ size of medial dorsal thalamus	8
↑ Neuron packing density in the dorsal cortex and occipital cortex	15,16	↑ Neuron packing density in mPFC and occipital cortex	8
Hyper-active sub-cortical DA system	17, 18	Increased DA release (i.e. from Nac) in response to amphetamine challenge	5

1) Honea, Crow, Passingham & Mackay (2005), **2)** Nelson, Saykin, Flashman & Riordan (1998), **3)** Shenton et al. (2001), **4)** Wright, Rabe-Hesketh, Woodruff, David, Murray & Bullmore (2000), **5)** Flagstad et al. (2004), **6)** Le Pen et al. (2006), **7)** Matricon et al. (2010), **8)** Moore et al. (2006), **9)** Harrison (1999) as cited by Lodge & Grace (2009), **10)** Kovelman & Scheibel (1984), **11)** Lewis, Hashimoto & Volk (2005), **12)** Lodge, Brehens & Grace (2009), **13)** Penschuck et al. (2006), **14)** Thune & Pakkenberg (2000), **15)** Selemon, Mrzljak, Kleinman, Herman & Goldman-Rakic (2003), **16)** Selemon, Rajkowska & Goldman-Rakic (1995), **17)** Laruelle, Abi-Dargham, van Dyck et al. (1996), **18)** Abi-Dargham, Rodenhiser, Printz et al. (2000).

At the neurochemical level, MAM administration at GD17 produces abnormalities in dopaminergic function of relevance to schizophrenia. Primarily, elevated dopamine release has been reported in the NAc (but not the frontal cortex) of these animals after acute amphetamine challenge – analogous to the mesolimbic hyperdopaminergic tone seen in schizophrenia (Flagstad et al., 2004; Jones et al., 2011; Moore et al., 2006). This is consistent with an increased rate of spontaneous firing of dopamine neurons in the ventral tegmental area (VTA) of these offspring, an area of the brain from which dopamine neurons project to the striatum (as reviewed by Miamoto & Nitta, 2014). With both elevated amphetamine-induced locomotion (see below) and spontaneous increases in VTA neurons being reversed by inactivation of the ventral hippocampus (Lodge & Grace, 2007), it suggests that hippocampal dysfunction may underlie this hyperdopaminergic tone. Indeed, Lodge and Grace (2008) posit that hyperactivity of the ventral subiculum of the hippocampus, which itself may be caused by the loss of inhibitory (parvalbumin-containing) GABA-ergic interneurons (Penschuck et al., 2006), is causative of the hyperactivity displayed by dopamine neurons in the VTA (as reviewed by Jones et al., 2011).

Behaviourally, MAM treatment at GD17 increases spontaneous and PCP-induced orofacial dyskinesias which are thought to reflect fronto-cortical lesions in these animals (Moore et al., 2006). Correlating with increased dopamine release from the NAc, GD17 MAM rats display enhanced hyperlocomotion in response to amphetamine (Flagstad et al., 2004; Le Pen et al., 2006; Lodge & Grace, 2007; Moore et al., 2006). Enhanced hyperactivity to the NMDA receptor antagonist MK-801 is also a robust finding of this model (Le Pen et al., 2006). Further increasing the model's validity,

these altered locomotor responses to psychostimulant drugs (together with basal differences in locomotion) are only found in rats once they have reached puberty (Hazane, Krebs, Jay and Le Pen, 2009; Le Pen et al., 2006; Moore et al., 2006). This time course is seen with other behavioural deficits, such as impaired PPI, and is analogous to the post-pubertal onset profile seen in schizophrenia patients (Hazane et al., 2009; Le Pen et al., 2006). Furthermore, PPI deficits are only observed after GD17 treatment, not GD15 treatment, reinforcing the importance of selecting this treatment window (Moore et al., 2006).

In terms of modelling the non-psychotic symptoms of schizophrenia, MAM treated rats show a range of cognitive deficits such as hippocampal-dependent spatial working memory impairments (Flagstad, Glenthøj & Didriksen, 2005; Gourevitch, Rocher, Le, Krebs & Jay, 2004; Moore et al., 2006). MAM treated rats have also been shown to require significantly more trials to reach criterion during both the reversal and extradimensional shift phases of the bowl-digging attentional set shifting task (Featherstone, Rizos, Nobrega, Kapur & Fletcher, 2007; Gastambide, Cotel, Gilmour, O'Niell, Robbins & Trickleback, 2012; Moore et al., 2006) - the rodent equivalent of the wisconsin card sorting task where schizophrenia patients show an impairment (Tyson, Laws, Roberts & Mortimer, 2004; Jazbec, Pantelis, Robbins, Weickert & Weinberger, 2007). With regards to the negative symptoms, no attempts have been made to assess hedonic ability or reward encoding in this model. However, promise is given by pre-pubertal reduced social interaction (as mentioned negative symptoms are often prodromal) exercised by MAM treated rats, reminiscent of asociality in schizophrenia (Flagstad

et al., 2004). The lack of characterisation of reward processing is a neglect that this thesis will address.

It should be noted that whilst MAM administration at GD17 shows both good face and construct validities, its predictive validity is relatively unknown. To date, studies have investigated the ability of current (Le Pen, Jay & Krebs, 2011) and novel (Gill, Lodge, Cook, Aras & Grace, 2011) antipsychotics to attenuate the locomotor enhancing effects of MK-801 and amphetamine. Clearly, further investigation is required to determine whether or not GD17 MAM administration can be considered as a comprehensive rodent model of schizophrenia, with predictive validity for negative and cognitive symptoms.

1.4.2 Animal Models of Depression

A wide variety of approaches have been taken in attempting to model depression in rodents. These include approaches based on genetic manipulations (e.g. glucocorticoid receptor antisense transgenic mice, CRH receptor subtype knock-outs - see Willner & Mitchell, 2002), lesions (e.g. olfactory bulbectomy - e.g. Slattery, Markou & Cryan, 2007) and pharmacological manipulations (e.g. reserpine - see O'Neil & Moore, 2003) and psychostimulant withdrawal (e.g. Barr & Markou, 2005). While all such approaches have potential advantages – e.g. potentially capturing aspects of the precise genetic basis for the heritability of depression – they all have potential problems with both face and construct validity – e.g. relatively few examples of human depression are reported to stem directly from abuse of stimulants and this approach produces only short-lived effects. Therefore, I will focus

on stress-based models and inbred rat strains – which reflect one of the commonly cited causal factors and produce the long-lasting symptomatology of the disorder. Particular attention will be given to the Wistar Kyoto inbred rat strain, as this model was the focus of the experimental efforts reported in this thesis.

a. Animal models based on stress exposure

Social stresses such as losing social status or rank are considered to be important risk factors in depression (Brown, 1993, as cited in Czéh, Fuchs, Wiborg & Simon, 2015). As a result this has led to the development of paradigms based on social stressors in rodents such as the resident-intruder paradigm (see Czéh for a review, 2015). This model utilises conflict between members of the same species by introducing a novel male 'intruder' rat into the home cage of another 'resident' male. This paradigm has been shown to induce a pessimistic response bias to ambiguous tones after 3 weeks of daily social defeat (Papciak, Popik, Fuchs & Rygula, 2013) as well as a reduction in sucrose preference after 5 weeks of social defeat (Rygula, Abumaria, Flügge et al., 2005). Moreover, it has been shown that the reward related deficits are attenuated by the chronic administration of an antidepressant, suggesting that the model holds predictive validity (Rygula, Abumaria, Flügge et al., 2006).

Feelings of helplessness are a common symptom of depression with patients displaying deficient behavioural control of an aversive stimulus if they have previously experienced aversive stimuli which are uncontrollable (Pryce, Azzinnari, Spinelli, Seifritz, Tegethoff & Meisenschmidt, 2011).

The learned helplessness paradigm was one of the first attempts to replicate depressive like behaviours in rodents (Czéh et al., 2015). Involving the exposure of animals to uncontrollable foot-shocks, it induces helpless behaviour in subsequent escape trials as well as additional behaviours of relevance to depression (as reviewed by Wiborg, 2013). For example, helpless mice have been shown to reduce responding for rewarding brain stimulation (in intracranial self-stimulation, or ICSS, e.g. Zacharko, Bowers, Kokkinidis & Anisman, 1983) while helpless rats fail to suppress corticosterone after a dexamethasone challenge (Greenberg, Edwards & Hen, 1989), suggesting hyperactivity of the HPA axis. Whilst this model holds promise, paradigm differences across the literature as well as strain effects question the generalisability of the results (see Willner & Mitchell, 2002). The spontaneous recovery of the animals also means that this model is less amenable to drug discovery efforts (as reviewed by Wiborg, 2013). What is more, the 'shock duration order effect' suggests that the rats are not really 'helpless' so much as directly conditioned to be unresponsive (Balleine & Job, 1991; Prabhakar & Job, 1996).

Chronic mild stress (CMS) is one of the most validated preclinical models of depression. It involves exposing the animals to sequential chronic mild stressors (i.e. social isolation, pair-housing, dampening bedding, disruption of the light-dark cycle, and food and water deprivation) in an unpredictable manner for at least two weeks (as reviewed by Czéh et al., 2015). This induces a long-lasting behavioural phenotype in susceptible rodents as well as a range of neurochemical, neuroendocrinological and neuroimmune abnormalities reminiscent of those observed in depressed patients (see Czéh et al., 2015, and Willner, 1997, for reviews). For example, rats susceptible to

stress have lower consumption of sucrose in both single bottle consumption tests and two-bottle preference (sucrose vs. water) tests (reviewed by Willner, 1997). Also in line with reward-related deficits in depression, they demonstrate impaired food-induced place-preference conditioning (Papp, Willner & Muscat, 1991) and an increased stimulation threshold in the ICSS paradigm (Moreau, Jenck, Martin, Mortas & Haefely, 1992). Stress-susceptible rats also show hypercortisolaemia and a dysregulation of the HPA axis (although these have been shown to recover after eight weeks of chronic stress exposure, see Wiborg for a review, 2013), while all rats subjected to the CMS procedure have working memory impairments (Henningesen, Andreasen, Bouzinova et al., 2009), a pessimistic response bias (Harding, Paul & Mendl, 2004) and altered sleep architecture (i.e. REM sleep occurs earlier in the cycle - Cheeta, Ruigt, van Proosdij & Willner, 1997; Grønli, Murison, Bjorvatn, Sørensen, Portas & Ursin, 2004).

Originally, the chronic stress paradigm was developed by Katz and colleagues and utilised harsher physical stressors (i.e. electric shock, shaker stress and cold swim) (Katz & Hersh, 1981). The modified paradigm used today (developed by Willner, e.g. Willner, Towell, Sampson, Sophokleous & Muscat, 1987) has been argued to better reflect the inducing factors of depression in humans (see Wiborg, 2013, for a review). The unpredictable nature of the stressors used is also of importance, as it prevents the animals from habituating over time. As a result, deficits in reward-related behaviours have been shown to persist as long as stressors are repeatedly applied. After cessation of stress, the animals recover after a period of four to five weeks allowing the possibility of subsequent stress-induced behaviours in the future. This gives the chronic mild stress model a

unique ability to replicate the repetition of episodes seen in depression (as reviewed by Czéh et al., 2015). The use of outbred rat strains in this model means that some animals are highly susceptible to stress whereas others show stress resilience (as is also the case for other stress-based paradigms including social defeat and learned helplessness, see Czéh et al., 2015 and Wiborg, 2013 for reviews). Furthermore, of the susceptible animals, only half respond to chronic antidepressants (Christensen, Bisgaard & Wiborg, 2011). Both these latter features of the model make it highly consistent with features of the general (i.e. heterogeneity in stress susceptibility) and depressed populations (i.e. therapeutic treatment refraction). Unfortunately, however, the CMS procedure is labour-intensive, and has not produced consistent results in some laboratories (as reviewed by Willner, 1997). Furthermore, not all the reward-related deficits thought to feature in depression are found after CMS exposure. For example, stressed rats do not differ in their lever press responses for sucrose under a progressive ratio schedule of reinforcement (Barr & Phillips, 1998).

b. Animal models based on selective breeding

Rats displaying helpless behaviour after exposure to inescapable shocks have been selectively bred to produce the congenital learned helplessness rat strain (cLH). Conversely, their controls (cNLH) were developed from inbreeding rats which showed stress resilience on the learned helplessness paradigm. In terms of reward-related behaviours, cLH and cNLH rats are identical prior to precipitating stressors. After either social isolation (Sanchis-Segura, Spanagel, Henn & Vollmayr, 2005) or foot-shock stress (Enkel, Spanagel, Vollmayr & Schneider, 2010), however, cLH rats

consume significantly less sweet solution compared to their controls. Furthermore, stress challenge has been shown to induce a significantly reduced pleasure-attenuated startle response for sweet solutions in cLH rats (Enkel et al., 2010). As such, the cLH rat strain perhaps provides a model of predisposition to depression. Consistently, neuroimaging studies have revealed that metabolism is significantly lower in various cortical regions of the cLH rat (i.e. dorsal frontal, medial orbital and anterior cingulate cortex), but significantly higher in the subgenual cingulate cortex - similar to changes which have been previously demonstrated in depression (as reviewed by Willner & Mitchell, 2002). That said, unlike the rats from which this strain was developed, cLH rats do not show adrenal responsiveness to stress (King, Abend & Edwards, 2001). Indeed, some authors, based on hyporesponsiveness of the HPA axis, together with the cognitive impairments and stress-induced analgesia displayed by the strain, have posited that cLH rats better reflect post-traumatic stress disorder (King et al., 2001).

Similarly to the cLH rat, the Roman Low Avoidance (RLA) rat strain was selectively bred for impaired performance in the shuttle box paradigm, whereas their controls (RHA) were developed for good performance on this task. Compared to the RHA strain, RLA inbred rats exhibit behaviours in the open field test and elevated plus maze which are consistent with high emotionality/anxiety or an inability to cope with stress (as reviewed by Willner & Mitchell, 2002). Consistently, when RLA rats were given daily access to a 22% sucrose solution they exhibited a larger suppression in their consummatory behaviour after a downward shift to a less valued 4% sucrose solution, as compared to their control strain. This may reflect an increased level of disappointment or frustration in this rat

strain, or a greater susceptibility to the stress-inducing effects of reward loss (Gómez, Escarabajal, de la Torre, Tobeña, Fernández-Teruel & Torres, 2009). Inconsistent with depression, RLA and RHA rats show similar behaviour in response to social defeat (as reviewed by Willner & Mitchell, 2002). Moreover, RLA rats are (potentially) unimpaired in their ability to predict future rewards, and adjust their behaviour in light of this prediction (as shown by the development of a between-subjects anticipatory contrast effect in consumption), suggesting that anticipatory hedonics are normal in this rat strain (Gómez et al., 2009).

The Flinders sensitive line (FSL) is another inbred rat strain where rats were selected based on their sensitivity to the hypothermic effects of cholinergic agonists, thus mimicking the increased cholinergic sensitivity reported in depression (reviewed by Willner & Mitchell, 2002). Alongside cholinergic hypersensitivity, FSL rats display altered serotonergic and dopaminergic systems compared to their controls (the Flinders Resistant Line, FRL) (Willner & Mitchell, 2002), as well as decreased BDNF in the hippocampus (but not in the frontal cortex) (see Neumann, Wegener & Homberg et al., 2011 for a review) and smaller hippocampal volume (Chen, Madsen, Wegnwe & Nyengaard, 2010). Phenotypically, the FSL strain exhibits behaviours which resemble symptoms of depression. For example, FSL rats exhibit weight disturbances (Overstreet, 1993), sleep disturbances (i.e. decreased latency to the onset of REM sleep and increased REM sleep episodes) (Shiromani, Overstreet, Levy, Goodrich, Campell & Gillin, 1988) and increased immobility in the forced-swim test (which may or may not resemble behavioural despair) (as reviewed by Willner & Mitchell, 2002). Similarly to the cLH rat strain, FSL rats do not display deficits in reward sensitivity

under basal conditions, as shown by normal preference for sweet solutions (Pucilowski, Overstreet, Rezvani & Janowsky, 1993) and similar response rates to the rewarding effects of ICSS (Matthews, Baldo, Markou, Lown, Overstreet & Koob, 1996). However, after being subjected to a CMS procedure, FSL rats displayed lower intake of sweet solutions in one-bottle and two-bottle preference tests compared to their controls (Pucilowski et al., 1993). Whilst the face validity of this model is generally good, FSL rats display reduced anxiety behaviours on the elevated plus maze (although anxiogenic behaviour has been demonstrated in social interaction tasks, see Abildgaard, Solskov, Volke, Harvey, Lund & Wegener, 2011; Braw, Malkesman, Dagan et al., 2006 and Overstreet, Keeney & Hogg, 2014), and exhibit a reduced, as opposed to elevated, HPA axis activity with lower basal ACTH levels (as reviewed by Czéh et al., 2015).

The Wistar-Kyoto (WKY) rat line was originally developed as the normotensive control strain for the spontaneously hypertensive rat derived from a Wistar outbred stock (Okamoto & Aoki, 1963). However, in the late 1980s and early 1990s, it was discovered that WKY rats display behaviours which are perhaps relevant to depression symptomatology. For example, WKY rats, compared to an outbred Wistar control strain, display an increased susceptibility to develop stress-induced ulcers (Paré, 1989a; Paré, 1989b; Paré & Redei, 1993), increased immobility on the forced swim test (Paré, 1989a; Paré, 1989b; Paré & Redei, 1993) and deficient behaviour after being exposed to uncontrollable stressors which is consistent with learned helplessness (Paré, 1989a; Paré & Redei, 1993). WKY rats also demonstrate abnormal behaviour in the defensive burying task, with higher levels of freezing behaviour and less defensive burying (perhaps linked to passive coping) (Paré,

1992; Paré & Redei, 1993). Also consistent with depression (Nutt, Wilson & Paterson, 2008), WKY rats demonstrate weight abnormalities (e.g. Nam, Clinton, Jackson & Kerman, 2014) and sleep disturbances with increased REM sleep and sleep fragmentation (Dugovic, Solberg, Redei, Van Reeth & Turek, 2000).

WKY rats exhibit neurochemical and neuroendocrine abnormalities reminiscent of those observed in depressed patients. For example, WKY rats display altered serotonergic, noradrenergic and dopaminergic systems (as reviewed by Jiao, Beck, Pang & Servatius, 2011), abnormalities of the thyroid stimulating hormone system (Solberg, Olson, Turek & Redei, 2001) and hyperactivity of the HPA axis (Pare & Redei, 1993; Redei, Pare, Aird & Klucynski, 1994). Compared to Sprague-Dawley rats, WKY animals exhibit lower tissue content of 5-HT (Scholl, Renner, Forster & Tejani-Butt, 2010), decreased expression of tryptophan hydroxylase 2 mRNA (encoding an enzyme involved in 5-HT synthesis) (Lemos, Zhang, Walsh et al., 2011), and decreased neuronal excitability of 5-HT neurons in the dorsal raphe nucleus (DRN) (a serotonergic nucleus) of the brain stem (Lemos et al., 2011). As reviewed by Jiao, Beck, Pang and Servatius (2011), WKY rats also exhibit abnormalities of their dopaminergic function in multiple brain regions. Briefly, reports have shown that dopamine turnover is higher in the NAc shell compared to Wistar control animals. WKY animals also exhibit altered levels of dopamine transporters (DAT) and altered dopamine D₁ and D₂ receptor densities across multiple brain regions, compared to controls. Noradrenergic transmission is also altered in the WKY rat with attenuated noradrenaline activity in response to acute stress (Pardon et al., 2002), lower noradrenaline concentrations in the locus coeruleus and reduced noradrenaline reuptake in cortical

areas (as reviewed by Bruzos-Cidón, Llamosas, Ugedo & Torrecilla, 2015). By contrast, chronic stress is thought to lead to greater sensitisation of the noradrenergic system in WKY rats compared to Sprague-Dawleys controls (see Morilak, Barrera, Echevarria, Garcia, Hernandez & Petre, 2005). Hypercortisolism has also been observed in this rat strain: At baseline, WKY rats exhibit higher levels of ACTH and corticosterone during the light phase compared to their Wistar counterparts (Solberg, Loose Olson, Turek & Redei, 2001). Furthermore, in response to both acute and chronic stressors, the increase in plasma ACTH levels is exaggerated in WKY rats (Pare & Redei, 1993; Redei, Pare, Aird & Kluczynski, 1994). For a summary of neurochemical and neuroendocrine abnormalities seen in WKY animals and how these might relate to human depression, please refer to Table 2.

As anhedonia is one of the cardinal symptoms of depression it is of paramount importance that rodent models of the disorder incorporate a reduced hedonic capacity. However, results in the WKY rat strain have been somewhat equivocal. For example, Mileva and Bielajew (2015) investigated the WKY rats' response in a two-bottle (water vs. 1% sucrose) preference test and found no strain differences in sucrose preference between female WKY and Wistar rats. Similarly, male WKY rats were shown to have increased preference for 1% sucrose over water, comparable to the Wistar control strain (but greater than a Sprague-Dawley control strain). Moreover, WKY rats in this experiment were shown to consume more sucrose solution overall than the other strains relative to their body weight (Nam et al., 2014). A study by Malkesman and colleagues investigated reward-related behaviour in pre-pubertal WKY rats. Whilst signs of hedonic impairment were seen in a saccharin preference test, WKY animals showed normal acquisition of a conditioned place preference

induced by social interaction with a con-specific (Malkesman, Braw, Zagoory-Sharon et al., 2005). In contrast, WKY male rats were shown to display reduced investigatory behaviour of a novel female stimulus compared to Wistar animals, consistent with the female interaction being less rewarding (Paré, 2000). In an instrumental learning paradigm, WKY animals were also found to display less lever-press behaviour for sucrose pellet rewards when they were delivered on both a fixed-ratio (FR-1) and progressive ratio (e.g. 3, 6, 10, 16, 23, 32, 44 etc.) schedule of reinforcement (De La Garza, 2005). This may be consistent with reduced hedonic capacity of the WKY rat, but equally plausible explanations would be motivational incompetency or psychomotor retardation displayed by these animals.

Whilst the discrepancies across the literature may result from paradigm, age or gender differences, it is interesting to note that testing conditions and prior experience of the animals may also have a role. Where reductions in hedonic capacity have been found, animals were often pre-exposed to a physical stressor. For example, reduced saccharin preference in WKY animals occurred after they had been exposed to a five minute forced-swim test (Malkesman et al., 2005). In contrast, where normal sucrose preference was exhibited by the WKY strain, testing was performed in the animals home-cage environment and no prior stress manipulation was performed (Mileva & Bielajew, 2015; Nam et al., 2014). Furthermore, reduced investigatory behaviour of a novel female was amplified for WKY rats after being exposed to either a tail-shock or water-restraint stressor - a trend not seen in the control strains (Paré, 2000). Clearly, further investigations regarding the hedonic capacity of WKY rats are warranted. Combining stress with the genetic vulnerability model

may result in a more comprehensive depression-like phenotype, and could offer a more valid way to model the disease since depression in the clinic often involves an innate predisposition to depression, which is precipitated by stressful life events.

Table 2 Morphological, neurochemical and behavioural abnormalities in the WKY inbred rat strain. WIS and SD refer to Wistar and Sprague-Dawley controls, respectively.

Human depression	Ref	Wistar Kyoto rat strain	Ref
↓ in hippocampal volume	1	↓ hippocampal volume in female WKY rats compared to WIS control; ↓ in hippocampal volume in male WKY rats compared to SD control	2, 3
↓ in BDNF in the hippocampus	4	↓ in BDNF in the CA3 of the hippocampus compared to WIS rats (pre-pubertal rats)	5
HPA axis hyperactivity	e.g. 6	↑ in ATCH and corticosterone after the diurnal peak ↑ in ACTH after acute and chronic stressors	7,8,9
↑ basal levels of CRH	10	↓ in CRH binding/ CRH Receptor mRNA expression	11
Abnormalities of the monoamine system	e.g. 6	Abnormal levels of monoamines in the limbic system	e.g. 12
Abnormalities in sleep architecture	e.g. 13	↑ REM Sleep ↑ Sleep Fragmentation	14
↑ Prevalence in females	15	Some evidence for greater depression-like behaviours in female WKY rats	16

1) Czéh & Lucassen (2007), **2)** Tizabi, Hauser, Tyler et al. (2010), **3)** Cominski, Jiao, Catuzzi, Stewart & Pang (2014), **4)** Shimizu, Hashimoto et al. (2003), **5)** Malkesman & Weller (2009), **6)** Saveanu & Nemeroff (2012), **7)** Solberg et al. (2001), **8)** Pare & Redei (1993), **9)** Redei et al. (1994), **10)** Nemeroff et al. (1984), **11)** Hauger, Shelat & Redei (2002), **12)** Jiao et al. (2011), **13)** Nutt et al. (2008), **14)** Dugovic et al. (2000), **15)** Kessler (2003), **16)** Pare & Redei (1993b).

Comorbidity of psychiatric conditions is common. In one study, 67% of those with depression had a current comorbid anxiety disorder, while 75% had a lifetime comorbid anxiety disorder (Lamers, van Oppen, Comijs et al., 2011). Moreover, anxiety and depression may exert potentiating effects as

individuals suffering from both experience longer duration of symptoms and higher symptom severity (Lamers et al., 2011). Anxiety-like behaviours have been investigated in the WKY strain. WKY rats display hypoactivity in response to novel environments (Malkesman et al., 2005), the open-field test (Malkesman et al., 2005; Paré, 1989a; Paré & Redei, 1993) and the elevated plus maze (Paré, 1992) - each of which may be consistent with anxiety related behaviours in this rat strain (although see Nam et al., 2014, where WKY animals spent more time in the centre square of the elevated plus maze which is perhaps more consistent with reduced activity or ambivalent behaviour of the strain). WKY rats also rapidly acquire a passive avoidance response, perhaps indicative of increased anxiety or reduced locomotor behaviour (Paré, 1993; Paré & Redei, 1993). Furthermore, WKY rats display impaired gastric tone, most likely due to their exaggerated stress response (Gunter, Shepard, Foreman, Myers, & Greenwood-Van Meerveld, 2000; Nielsen, Bayati, & Mattsson, 2006). This is a primary characteristic of irritable bowel syndrome, a condition that has long been associated with both depression and anxiety (O'Mahony, Bulmer, Coelho et al., 2010; O'Malley, Julio-Pieper, Gibney, Dinan, & Cryan, 2010; Spiller, 2004). Combined, this evidence suggests that WKY rats perhaps best constitute a model of comorbid depression and anxiety disorders.

As mentioned previously, predictive validity constitutes another important criterion against which a putative model is assessed. Several studies have shown that the increased immobility in the forced swim test displayed by WKY rats is attenuated by chronic (but not acute) administration of antidepressants, consistent with chronic antidepressant efficacy in the clinic (Lahmame, del Arco, Pazos, Yritia & Armario, 1997). However, antidepressant effects only occur with certain drug types:

desipramine (an NA reuptake blocker tricyclic antidepressant) was found to increase swimming time in the forced swim test whereas fluoxetine (a 5-HT reuptake inhibitor, SSRI) had limited effect (López-Rubalcava & Lucki, 2000; Tejani-Butt, Kluczynski & Paré, 2003; Will, Aird & Redei, 2003).

Desipramine has also been shown to have an effect in the open field test. While ACTH responses to the open field test were increased by prior restraint stress in both WKY and Wistar strains, desipramine attenuated this effect in WKY rats only (Durand, Aguerre, Fernandez et al., 2000). That said, desipramine has been found to have no effect on the amount of REM sleep observed in WKY rats (Ivarson, Paterson & Hutson, 2005). Kappa opioid receptor antagonists have also been found to reduce forced swim test immobility in the WKY strain (Carr, Bangasser, Bethea, Young, Valentino & Lucki, 2010). This is interesting in light of recent evidence suggesting the antidepressant properties of kappa opioid receptor antagonists in the clinic (Berton & Nestler, 2006). Considered together, these results suggest that WKY rats might constitute a model of SSRI-resistant depression. Whilst effective antidepressants are only efficacious against some of the depressive-like behaviours observed in the WKY rat model, it suggests that the model has predictive validity (Will et al., 2003).

In summary, while there are many possible approaches to modelling depression in rodents, in the work reported in this thesis I have focused on the WKY rat as it potentially captures aspects of the comorbidity between anxiety and depression and may model the SSRI-resistance seen in some patients.

1.5 Measuring Reward Processing in Animal Models

Alongside choosing valid animal models, it is essential that appropriate and reliable measuring techniques are employed to assess disorder-like characteristics of the model. The importance of adopting suitable behavioural measures becomes most apparent when we consider the reward-processing deficits which feature in both schizophrenia and depression. Whilst certain reward processes (such as reward 'wanting' and reward 'liking') were once thought to be inextricably linked, it is now known that they are served by dissociable neural mechanisms and as such should be considered as distinct, but related, entities. The final section of the general introduction will introduce the behavioural paradigms which I adopted to investigate the potential reward processing deficits of the neurodevelopmental MAM model of schizophrenia and the inbred WKY model of depression. The techniques were chosen for their ability to tease apart precise aspects of reward-related behaviours, in terms of both hedonic and cognitive processing of rewarding events.

1.5.1 Consumption Measures and Orofacial Reactivity

As briefly mentioned in section 1.2, one approach to investigate 'liking' or palatability responses, unconfounded by other aspects of reward, is to study the affective orofacial reactions elicited by the hedonic impact of sweet tastes. Such facial 'liking' reactions were initially described in newborn babies - with each displaying characteristic lip licking and tongue protrusions to the sweet taste of sucrose (Steiner, 1973). Since then, the technique has been extended to rodents (Pfaffmann et al., 1977; Grill & Norgren, 1978a, b): rodents also elicit positive facial reactions to sweet tastes (e.g.

tongue protrusion) whereas bitter tastes elicit disliking facial reactions (e.g. gaping). With taste reactivity profiles being highly conserved across species, studies in rodents can provide us with useful information regarding human pleasure and how this might be affected in disease states. Furthermore, studies using taste reactivity in rodents have demonstrated the importance of not using consumption measures in isolation (e.g. sucrose consumption/preference tests) as the early studies using this technique demonstrated that consumption and hedonic reactions can dissociate (e.g. Pelchat, Grill, Rozin, & Jacobs, 1983).

Whilst the benefits of using taste reactivity are clear, the technique has several limitations that prevent it from being effective for examining the presence of reduced hedonic capacity in rodent models of disease. The most crucial of these is that taste reactivity tests typically provide categorical information as to whether something is palatable or aversive to an animal. In short, gaping reactions are only elicited by nausea and never by sweet tastes. That said, it can be argued that taste reactivity responses lie on a continuum from appetitive to aversive and so do, in fact, allow some quantitative distinctions to be made (e.g. Beslin, Grill & Spector, 1992). Indeed, this approach of considering taste reactivity responses as a continuum, from appetitive to aversive, is central to the work reviewed in section 1.2. Regardless of this issue, taste reactivity is a subjective (although inter-rater reliability is very high) and highly time-consuming method, which does not lend itself to the high-throughput environment of the drug discovery process.

1.5.2 Microstructural Analysis of Licking

Another method by which the value of a reward can be measured in animals, which is both more objective and less labour intensive, is to examine the licking microstructure of rats as they voluntarily consume a solution. First utilised by Davis and his co-workers in the 1970s, microstructural analysis of licking is grounded on the observation that rats ingest fluids in sustained runs of rapidly occurring rhythmic licks (herein referred to as *clusters*) separated by pauses of varying length (Davis, 1973). Critically, the number of licks in each licking cluster (lick cluster size) is lawfully related to the nature or concentration of the solution being consumed. That is, it bears a positive monotonic relationship with the concentration of sweet pleasant tasting solutions, such as sucrose and saccharin (Davis & Smith, 1992; Spector, Klumpp, & Kaplan, 1998). Conversely, it bears a negative monotonic relationship with the concentration of bitter, unpleasant solutions such as quinine (Hsiao & Fan, 1993; Spector & St John, 1998).

Of critical importance in understanding the emotional competence of an animal, certain manipulations can induce lick cluster size (LCS) changes when the solution itself is physically unaltered. For example, pairing saccharin with lithium chloride (LiCl)-induced nausea has been shown to reduce the size of licking clusters when an animal is given subsequent access to that solution in extinction (Dwyer, Boakes, & Hayward, 2008). That is, the lick cluster sizes elicited by the rat become similar to those that would be elicited if the rats were drinking quinine. With the solution being physically unchanged, the change in LCS licenses the inference that the change must lie with the animal and more specifically, with the primary driving force behind LCS being the nature of the

solution, inferences can be made that the change lies with the perception or evaluation of the solution made by the animal (See Dwyer, 2012, for a review). Importantly, the use of LCS as a measure of hedonic evaluation is supported by the fact that a variety of manipulations influence LCS in ways analogous to their effects on taste reactivity. Taking the above example, pairing saccharin with LiCl causes rats to display a taste reactivity profile (consisting of behaviours such as mouth gapes) similar to one typically elicited by bitter solutions (e.g. Baird, St John, & Nguyen, 2005).

Also of importance to the use of LCS as a measure of hedonic value is the fact that LCS is not a proxy measure of consumption, the latter often reflecting factors such as motivation that are unrelated to pure hedonic processing. Whilst pairing saccharin with LiCl results in both reduced LCS and reduced consumption, evidence of a dissociation between the two parameters can be seen when pairing saccharin with amphetamine, as here only consumption measures and not LCS measures are reduced (Dwyer et al., 2008). Furthermore, consumption displays an inverted U-shaped function with the concentration of palatable solutions (e.g. Richter & Campbell, 1940) which means that at higher solution concentrations (at the peak and descending limb of the function) increases in LCS should be met with unchanged or decreasing consumption levels – which is exactly the relationship observed in the foundational studies of licking microstructure (e.g. Davis & Smith, 1992; Spector, Klumpp, & Kaplan, 1998). Moreover, studies of conditioned flavour preference demonstrate that LCS can be raised through learning in testing situations that prevent conditioned changes in the amount consumed (Dwyer, 2008).

Given the fact that taste reactivity and microstructural analysis of licking both show comparable responses to different manipulations, but microstructural analysis is a less labour-intensive technique, and perhaps more sensitive to small changes in hedonic value, this method was used in the current series of experiments. An additional benefit of this technique is that it allows the experimenter to determine the length of time between one lick made by the rat and the next. This parameter of interlick interval (ILI) usually shows very little variability across animals, except in cases where motoric disturbances are present (e.g. altered posture). The importance of measuring this parameter is high, not only when we consider that motor abnormalities could confound lick cluster measurements, but also when we consider the psychomotor retardation that is thought to be present in depression and its WKY rat model. An observation of reduced LCS without corresponding changes in ILI would provide strong evidence for reduced hedonic capacity in animal models of psychiatric and mood disorders.

1.6 Measuring Reward Processing Deficits, Beyond Anhedonia, in Animal Models

Alongside pure hedonic reactions, be it in-the-moment or expected in the future, appropriate processing of rewards also depends on intact cognitive and motivational competencies. Within the reward processing construct are subdomains such as incentive salience, motivation, cost-benefit decision making, and the formation of cognitive representations of reward value and their integration with ongoing actions (Der-Avakian & Markou, 2012). Of the reward-related deficits associated with both schizophrenia and depression, beyond impaired hedonic processing, patients may be unable to

form, maintain or update value representations and use this information to motivate behaviour and inform decision making. Indeed, as has been discussed earlier, a recent experiment revealed that patients suffering from schizophrenia were unable to adjust their behaviour to take into account reduced reward value when the representation of the reward had to be relied upon (Morris et al., 2015). Whilst no direct experiments testing reward valuation have been performed in a depressed sample, stress-reactivity in these patients may also render their behaviours as inflexible and independent of reward (Schwabe & Wolf, 2011). Regardless of stress reactivity, at the intuitive level, it is plausible that people suffering from depression would no longer perform actions based on the consequences those actions engender if they were not pleasurable upon their receipt (Griffiths et al., 2014).

1.6.1 Procedures that Assess the Use of Reward Representations

To determine whether MAM-exposed or WKY inbred rats are able to form flexible representations of reward value, two behavioural paradigms were adopted: the first an outcome devaluation procedure (investigating whether ongoing behaviour is sensitive to current reward value) and the second a differential outcomes procedure (investigating whether reward identity can guide and direct behaviour). As results for the outcome devaluation procedure have been reported across various species, (including monkeys, West et al., 2011, and humans, Klossek et al., 2008; Valentin et al., 2007), it suggests that this paradigm has translational relevance for assessing the capacity to update the value of future rewards (as reviewed by Markou, Salamone, Bussey et al., 2013).

1.6.1a Action and Habits: Behaviour

Instrumental performance, such as pressing a lever for a food reward, is thought to be mediated by two dissociable processes: a goal-directed process in which action selection is governed by an association between the response and the outcome engendered by that response (action-outcome, or A-O, associations), and a stimulus-driven habitual process in which action selection is governed by a direct association between the stimulus and response (stimulus-response, or S-R, associations) without any link to outcome value (e.g. Balleine & O'Doherty, 2010). Crucially, because the associations which underlie habitual behaviours do not include outcome representation, responding by an individual is independent of any explicit motivation to obtain a reward. By contrast, responding controlled by goal-directed processes depends explicitly on the anticipated outcome and its motivational value (Adams & Dickinson, 1981; Adams, 1982; Balleine & Dickinson, 1998; Dickinson, 1985). That is, the assessment of the degree to which instrumental behaviour is goal-directed or habitual is a direct test of the nature of reward processing in a given circumstance. Given this, the most common probe of the difference between actions and habits has been to examine the effects of devaluing the reward between training and test (Balleine & O'Doherty, 2010). This can be achieved either by specific satiety (during which the rat is allowed free access to the instrumental outcome) (e.g. Balleine & Dickinson, 1998) or by pairing the outcome with LiCl-induced nausea (i.e. taste aversion conditioning) (e.g. Adams & Dickinson, 1981; Adams, 1982). If the animals' performance is goal-directed (i.e. sensitive to changes in the current value of a reward) then it should be reflected by a lowered willingness to perform the response that produces the devalued outcome –

even when the test is conducted in the absence of any reward delivery (Balleine & O'Doherty, 2010).

It is also possible to assess goal-directed behaviour by pre-test degradation of the response-outcome contingency, although this method has been less utilised in practice (Balleine & Dickinson, 1998).

In behavioural terms, the balance between goal-directed and habitual performance has been shown to reflect several aspects of the training situation. One of the first mediators of this difference to be identified was the length of training. During initial instrumental learning in rats, actions are largely goal-directed and performed on the basis of their consequences (i.e. sensitive to outcome devaluation or contingency degradation). However, as training continues, action-outcome associations are inhibited and reflexive habit behaviour predominates (e.g. Adams, 1982; Dickinson, Balleine, Watt, Gonzalez, & Boakes, 1995). Similarly, if human subjects are trained to key press for different food types, they will decrease their responding for a devalued food after moderate training, but not after overtraining (Tricomi, Balleine & O'Doherty, 2009).

1.6.1b Action and Habits: Neuroanatomy

The neuroanatomy of goal-directed and habitual control of instrumental action has been well characterised - with the main focus on cortico-striatal circuits (reviewed by Balleine & O'Doherty, 2010). In brief, for rodents areas implicated in flexible goal directed behaviour include the prelimbic PFC and the connecting dorsomedial striatum. Lesions of either of these two regions prevents goal-directed behaviour, rendering performance insensitive to both outcome devaluation and contingency degradation, even after only minimal levels of training (i.e. behaviour is stimulus bound and habitual)

(Balleine & Dickinson, 1998; Corbit & Balleine, 2003; Yin, Knowlton & Balleine, 2005). While the examination of temporary inactivation and lesion timing suggests that the dorsomedial striatum is necessary for both the acquisition and expression of goal-directed behaviours, the prelimbic PFC appears to be crucial for acquisition only (Ostlund & Balleine, 2005; Yin et al., 2005).

The formation of stimulus-response habits appears to be driven by separate (but interacting) frontal and striatal systems. Lesions of either the dorsolateral striatum (Yin, Knowlton & Balleine, 2004), or the infralimbic PFC (Killcross & Coutureau, 2003), maintain goal directed responding in rodents (i.e. maintain performance sensitivity to changes in reward value) even after extended training. Intriguingly, even once habitual behaviour has become established with overtraining of an instrumental response, disruption of the infralimbic cortex will reinstate goal-directed responding (Coutureau & Killcross, 2003). Importantly, this finding suggests that the transition from goal-directed to habitual responding is not an absolute process: A-O associations are not forgotten or removed over the course of training. Together with results described above, showing that disrupting goal-directed control results in reflexive responding, these studies suggest that S-R and A-O associations develop in parallel, with factors such as training length (as well as reinforcement schedule, drug exposure and, as we have seen earlier, stress) determining the relative influence that these two processes have over instrumental behaviour (Dickinson et al., 1995). Indeed, in terms of the infralimbic cortex, some argue that this structure is not involved in the formation of S-R associations per se, but instead inhibits goal-directed processes, therefore allowing habitual behaviour to predominate (Killcross & Coutureau, 2003).

These cortico-striatal circuits first identified in rodents also appear to underpin goal-directed and habitual behaviour in humans (see Balleine & O'Doherty, 2010, for a full review). For example, fMRI analysis reveals that activity of the (medial and lateral) OFC was significantly correlated with goal-directed behaviour (i.e. the decreased performance of an action related to a devalued outcome relative to an action related to a valued outcome) (Valentin, Dickinson & O'Doherty, 2007). In addition, activity of the vmPFC (including the mOFC and mPFC) was elevated during the performance of high contingency schedules compared to low contingency schedules. Furthermore, the activity of the anterior caudate nucleus (a target area of these structures and analogous to the rat dorsomedial striatum) was also found to be modulated as a function of A-O contingency (Tanaka, Balleine & O'Doherty, 2008: for converging evidence from an overtraining procedure see Tricomi et al., 2009). Finally, these functional studies are supported by more recent diffusion tensor imaging research (de Wit, Watson, Harsay, Cohen, van de Vijver & Ridderinkhof, 2012), which revealed that the integrity of white matter tracts connecting the posterior putamen (analogous to the dorsolateral striatum in rats) with pre-motor cortical regions was predictive of habitual behaviour, autonomous of outcome value, in healthy humans. Conversely, the integrity of white matter tracts between the vmPFC and the caudate was shown to be positively correlated with goal-directed control of choice behaviours.

1.6.1c Action and Habits: Neurochemistry

Much of the analysis of the neurochemistry of goal-directed and habitual behaviour has focused on the actions of psychostimulant drugs such as amphetamine and cocaine, which sensitise

dopamine systems in the brain (as reviewed by LeBlanc, Maidment & Ostlund, 2013). Chronic exposure to either amphetamine (Nelson & Killcross, 2006), or cocaine (Schoenbaum & Setlow 2005; LeBlanc, Maidment & Ostlund, 2013), reduced or completely blocked the sensitivity of animals to pre-test reward devaluation. The actions of these treatments may be mediated by long-term neural adaptations in the regions that are implicated in the transition from goal-directed to habitual responding, including the striatum (dorsal and ventral) and mPFC (Wickens, Horvitz, Costa & Killcross, 2007).

The involvement of dopaminergic mechanisms is suggested by infusion studies. For example, infusion of dopamine directly into the mPFC of over-trained rats, produced a bidirectional effect with rats demonstrating decreased responding for a devalued outcome, but increased responding for a non-devalued outcome (Hitchcott, Quinn & Taylor, 2007). This is further supported by 6-hydroxy dopamine (6-OHDA) lesions of the dorsomedial striatum and prelimbic cortex which impaired goal-directed responses (Naneix, Marchand, Di Scala, Pape & Coutureau, 2009; Lex & Hauber, 2010), whereas 6-OHDA lesions to the nigrostriatal pathway blocked habitual behaviour (Faure, Haberland, Conde & Massioui, 2005).

In summary, there is good cross-species consistency regarding the brain structures which subserve goal-directed and habitual instrumental responding. Given reports of hypofrontality in schizophrenia, together with the observation that both patients suffering from schizophrenia and depression are impaired in their performance on pre-frontal dependent tasks, this might suggest that psychiatric and mood disorders are associated with greater habitual control of behaviour (as

discussed in earlier sections, this has been explored in schizophrenic but not depressed subjects).

Dopamine dysregulation in both of these disorders may also be involved in the shift between goal-directed and habitual systems, although this as yet has not been directly assessed. But to the extent that altered dopamine signalling is important in both schizophrenia and depression pathophysiology (and their animal model counterparts), an impaired balance between goal-directed and habitual systems may feature in both of these disorders. Thus, the outcome devaluation procedure will form a valuable assessment of reward processing deficits in the context of models of these disorders.

1.6.2 Differential Outcomes Procedure (DOE)

The outcome devaluation procedure provides useful information regarding the transition of association from outcome expectancy to S-R mechanisms. Whilst an accelerated transition to habitual systems is clearly maladaptive for behaviour, the devaluation procedure only speaks to whether animals are sensitive to changes in outcome value, and not whether reward identity can guide and direct the acquisition of a set of responses. Therefore, in order to further examine the precise nature of reward representations in the MAM-exposed and WKY inbred rat strains, the differential outcomes procedure will be utilised. This is a unique paradigm that was originally developed by Trapold (1970) to assess whether specific anticipatory reward information can be used by an organism to guide instrumental choice behaviour (as reviewed by Savage & Ramos, 2009).

Trapold posited that animals encode many properties of a reward, including sensory and magnitude properties, each of which can enter into association with both the environmental stimuli

and the response requirement. This procedure uses a conditional discrimination, where an animal is trained to choose from two available responses (e.g. left and right lever press) in the presence of different discriminative stimuli (e.g. tone and clicker). In one group of animals (the experimental or differential outcomes group), outcomes are stimulus correlated. That is, a correct response (e.g. left lever press) in the presence of stimulus one (e.g. a tone) is always reinforced by outcome one (e.g. a food pellet), whereas a correct response (e.g. right lever press) in the presence of stimulus two (e.g. a clicker) is always rewarded by outcome two (e.g. sucrose solution). In contrast, for the control group (the non-differential outcome group), the reward identity is not contingent on the correct stimulus-response pairing. That is, subjects are exposed to either a single common reinforcer or multiple reinforcers that are uncorrelated with the stimuli or response (e.g. left lever press response in the presence of a tone leads to both pellet and sucrose rewards at equal probability). In both groups, incorrect responses made in the presence of each discriminative stimulus are without consequence (i.e. non-reinforced). Trapold found that rats trained with stimulus-correlated outcomes (i.e. in the differential group) master the discrimination more rapidly and reach a higher asymptote than rats trained with uncorrelated outcomes. Termed the Differential Outcomes Effect (DOE), this finding has since been observed across different tasks, parameters and, importantly, species - giving it good translational validity (e.g. Carlson & Wielkiewicz, 1976; Miller, Waugh & Chambers, 2002; Urcuioli & Zentall, 1992).

There have been numerous explanations as to why faster acquisition occurs when differential as opposed to non-differential outcomes are used, including improved cue salience and acquired

distinctiveness of the discriminative cues (see Urcuioli, 2005, for a review). When the differential outcomes effect was first discovered, Trapold and Overmier (1972) posited that the animals are forming associations between the stimuli and the specific sensory properties of the rewards (through Pavlovian S-O conditioning), with the resultant specific reward expectations evoked by each stimulus also acting as a cue to guide instrumental behaviour (above and beyond the reward expectations general motivational properties). In other words, better task performance in a differential outcomes procedure is thought to be due to stimulus-outcome, outcome-response (S-O, O-R) chaining (reviewed by Delamater, Kranjec & Fein, 2010). Rats under the differential condition learn differential associations between each stimulus and the distinct sensory properties of its outcome (S1-O1 vs S2-O2). Since an appropriate response made by the animal is reinforced during the presence of an outcome representation or expectancy (brought about by the stimulus presentation), differential associations also form between the outcome representation and the response (O1-R1 vs. O2-R2) (Blundell, Hall, & Killcross, 2001; Urcuioli, 2005). As a result, alongside instrumental S-R associations, each stimulus and the expectation/representation of the reward evoked by the presentation of the stimulus signal the appropriate response. By contrast, rats trained with non-differential outcomes can only rely on stimulus-response associations to direct their responding. Expectation of uncertain outcomes does not provide any additional discriminative cue to the animals as the same expectation would be elicited in the presence of *both* discriminative stimuli (Urcuioli, 2005).

As a correlation between each choice alternative and a particular reward is also embedded in the differential outcomes procedure, differential R-O associations can also mediate the enhanced performance observed as part of the differential outcomes effect. For example, in one study, the differential outcomes paradigm was set up in such a way that the differential outcomes group were able to form differential R-O associations uncorrelated with the stimuli. Despite eliminating differential S-O associations, the differential outcomes group more rapidly acquired the task, compared to their non-differential controls (e.g. DeMarse & Urcuioli, 1993).

Regardless of the relative contribution of differential S-O and R-O associations in controlling performance, both associations require that the outcome is part of what is learned in discrimination learning. That is, better performance in the differential outcomes group cannot be explained by the outcome merely acting as a catalyst (i.e. not explicitly encoded) to enhance S-R relations. Furthermore, the reinforcer expectancies that develop in the differential outcome task are unique to each of the two rewarding outcomes - non-specific (motivational) effects of S-O/R-O relations would not aid choosing between two response alternatives (see Urcuioli, 2005).

In summary, the differential outcomes effect requires that the reinforcer becomes part of the learning matrix. In contrast to the outcome devaluation procedure, it is the specific association between the stimuli and the unique sensory properties of the outcomes that are required to confer any advantage on the task. With a higher cognitive load than in a typical outcome devaluation procedure, the organism must also be capable of monitoring and using multiple unique S-R-O associations

(Savage & Ramos, 2009). Thus, impairments that do not emerge in an outcome devaluation procedure may emerge in a differential outcomes task.

Whilst relatively little research has been performed to determine the precise neuroanatomical underpinnings of the differential outcomes effect, lesion studies have suggested a role for the BLA and interconnected OFC (Ramirez & Savage, 2007 - see also Ghashghaei & Barbas, 2002). Lesioning of the BLA prior to training abolishes the differential outcomes effect, as well as preventing specific pavlovian-to-instrumental transfer and making an animals' performance insensitive to post-conditioning changes in outcome value (see Blundell et al., 2001). Pre-training lesions of the OFC also prevents differential outcome expectancies from guiding instrumental choice behaviour across training (McDannald, Saddoris, Gallagher & Holland, 2005): although this effect may only emerge later in training (Ramirez & Savage, 2007).

How these neurobiological underpinnings relate to schizophrenia and depression is uncertain with no studies explicitly investigating the differential outcomes effect in either of these patient populations. As has been previously described, both disorders may involve aberrant activity of the OFC. In terms of the amygdala, however, results in the literature have been somewhat equivocal. Some meta-analyses of structural imaging data have revealed volume reductions of the amygdala in both schizophrenic (e.g. Honea, Crow, Passingham & Mackay, 2005) and depressed patient (e.g. Sacher, Neumann, Fünfstück et al., 2012) populations. However, other, more recent analyses have not supported these findings (e.g. Vita, De Peri, Silenzi & Dieci, 2006) in the context of schizophrenia. Furthermore, there is some suggestion of greater amygdala activation in schizophrenia patients

compared to their healthy counterparts during the processing of fearful and neutral stimuli (Holt, Kunkel, Weiss et al., 2006). Similarly, some studies have revealed that patients with depression exhibit increased cerebral blood flow and metabolism in the amygdala (as reviewed by Gold & Chrousos, 2002). Consistently, stress and stress hormones have also been revealed to increase the amygdala activity (reviewed by Schwabe & Wolf, 2009). In recognising that outcome value insensitivity is at odds with increased BLA activity in response to stress, Schwabe and Wolf (2009) suggest that the amygdala may be exerting its effects through modulating other brain systems. However, it has been shown that depressed patients exhibit decreased resting-state functional connectivity between the amygdala and other brain regions, including the ventrolateral PFC and caudate region of the striatum (Ramasubbu, Konduru, Cortese, Bray, Gaxiola-Valdez & Goodyear, 2014, see also Friedel et al., 2009, section 1.3.6). Resting state functional connectivity has also been shown to be reduced between the amygdala and ventral PFC in schizophrenia patients (Hoptman, D'Angelo, Catalano et al., 2010)

In summary, the DOE procedure offers a sensitive test of whether animals are able to use specific information regarding the identity of rewards to acquire and direct their behaviour. Although the presence of a disrupted DOE has not been explicitly identified in either schizophrenia or depression, the procedure itself will help characterise the nature of any reward processing deficit in models of these disorders.

1.7 Summary and Guide to Thesis

Historically, anhedonia, defined as a loss of interest or pleasure, was proposed to be a core symptom of both schizophrenia and depression. Today, however, it is becoming clear that a whole host of dissociable reward-related deficits are being inappropriately labelled under the anhedonia umbrella. When we start to parse out the precise aspects of reward, we can see how subtle differences can exist across the different disorders. Indeed, consummatory hedonic processing appears to be unimpaired in schizophrenia, with anticipatory anhedonia perhaps better reflecting the disorder.

There is still a long way to go in the human literature but as advances are constantly being made it is essential that the animal models of the disorders keep pace. Furthermore, we must develop reliable measuring techniques which precisely assess the intended reward-related construct, without being confounded by other aspects of reward processing. Comprehensive, robust animal models together with reliable measuring techniques will provide a platform from which to better understand the biological underpinnings of reward-related behaviours and will inform future investigations in the clinic.

Many animal models of both schizophrenia (e.g. the MAM neurodevelopment model) and depression (i.e. the WKY inbred rat strain) have been developed matching the necessary face, construct and predictive validities - however, rigorous behavioural characterisation of these models, particularly in terms of the hedonic and cognitive processing of reward are lacking.

The experiments reported in this thesis aimed to address two general and related issues with respect to the processing of rewarding stimuli and its relationship with modelling schizophrenia and depression in animals: examining first, the hedonic responses to rewarding stimuli and second, the ability of rewarding stimuli to motivate and control ongoing behaviour.

With respect to the first of these issues, Chapter two (EXP 1) develops a method of assessing anticipatory hedonics in rodents - a within-subject negative anticipatory contrast procedure. Chapter three assesses the consummatory (EXP 2) and anticipatory (EXP 3) hedonic responses of rats prenatally exposed to MAM through the detailed analysis of their licking behaviour during ingestion of non-contrasted and contrasted sweet solutions. Regarding the second issue, Chapter four utilises the outcome devaluation procedure (EXP 4 and 6) and the differential outcomes procedure (EXP 5) to determine whether MAM-exposed rats can learn that their actions are reliably connected to the delivery of pleasurable rewards, and use this information to guide responding in instrumental tasks.

The experiments reported in Chapters five and six address the hedonic and cognitive aspects of reward-processing in the WKY model of depression. EXP 7 and EXP 8 specifically address affective responses of WKY rats, looking at consummatory and anticipatory aspects of hedonic processing in turn. EXP 9 and 10 employed behavioural assessments (outcome devaluation and the differential outcomes procedure) to address the more cognitive aspects of reward-processing in this model.

Chapter Two

2. Methods Development

2.1 Introduction

2.1.1 The Assessment of Anticipatory Anhedonia

Dating back to Klein's original definition of anhedonia in the context of depression, a distinction has been made between the temporal components of pleasure. That is, not only can an organism experience pleasure in-the-moment when engaged in an enjoyable activity, but pleasure can also be anticipated from future events. The importance of making this distinction has been realised in the schizophrenia literature. Using methods such as the Temporal Experience of Pleasure Scale, reports suggest that schizophrenia patients cannot anticipate pleasure, particularly from future goal-directed activities, whereas their consummatory pleasure may be relatively intact (Gard et al., 2007). Some attempts have been made to assess reward anticipation and expectancy in rodent models of disease. Briefly, these have included successive contrast effects, where the animals behaviour is evaluated after an unexpected downshift in the value of reward (i.e. successive negative contrast), and anticipatory locomotor responses in the context of sexual behaviour or a preferred food type (see Barnes, Der-Avakain & Markou, 2014; Foussias et al., 2015 for a review). However, whilst these methods assess the ability of an animal to anticipate or predict future rewards, they do not necessarily look at the animals hedonic responses in light of those predictions, with potential confounds such as motor abnormalities and motivational incompetency also obscuring the results.

This chapter focuses on the use of Negative Anticipatory Contrast as a means of directly assessing hedonic responses in anticipation of a future event.

2.1.2 Negative Anticipatory Contrast

Negative anticipatory contrast specifically looks at the adjustment of an animal's behaviour toward currently available stimuli (food) in light of a future rewarding event. For example, a rat given brief daily sequential access to two solutions will learn to expect the second upcoming solution and will adjust its consumption of the currently available solution accordingly. If the second solution is preferred over the first, intake of the first solution will be suppressed (e.g., Flaherty, Coppotelli, Grigson, Mitchell, & Flaherty, 1995). The suppression of the first solution's intake has been ascribed to a contrast effect based on the comparison between the levels of reward available at the time and the level of reward expected in the near future. This suppression appears to be genuinely anticipatory because, in within-subjects designs, intake from the first bottle available in a day is low when the upcoming solution is valuable, while the value of the solution consumed the previous day has little effect (Flaherty et al., 1995; Flaherty & Rowan, 1985). More generally, the existence of within-subjects anticipatory contrast demonstrates that the effect cannot simply be due to a comparison between the currently available solution and the animal's previous overall experience. Furthermore, increasing the interval between solutions within a day reduces contrast, which would not be the case if the reduction in consumption was based simply on comparison with previous experience in that context (e.g., Flaherty & Checke, 1982; Lucas, Gawley, & Timberlake, 1988).

2.1.3 Mechanisms underpinning Negative Anticipatory Contrast

While the behavioural phenomenon of anticipatory contrast is well established, few mechanisms have been put forward to explain how current behaviour can be suppressed by the expectation of a more rewarding event. Flaherty and Rowan (1985) proposed that exposure to the first solution, together with the context of its presentation, allows a comparison between the different solution values by invoking an internal representation of the impending preferred solution. Flaherty (1996) considered three general mechanisms by which this might lead to a reduction in consumption of the first solution: a relative devaluation of the first solution; spatial competition from goal tracking (i.e. the animal repeatedly approaches the location of the not-yet accessible second solution); or response inhibition, where the animal learns to inhibit intake of the first solution because the second solution is more rewarding (see also Flaherty et al., 1995; Onishi & Xavier, 2011). Of these, the devaluation account is perhaps the most plausible. This account dictates that as an animal learns to expect a valuable stimulus in the near future, there is a functional devaluation of the currently available reinforcer of lesser value - hence, a lower consummatory response.

While intuitively plausible, the devaluation account appears to be inconsistent with some previous results. Indeed, Weatherly, Nurnberger, and Sturdevant (2006) found that a 1% sucrose solution subject to anticipatory contrast did not suffer a reduction in its ability to act as a reinforcer for operant behaviour, as compared with a non-contrasted 1% solution of sucrose. Furthermore, when different spout cues were paired with contrasted and non-contrasted solutions, Flaherty et al. (1995) found that cues paired with the contrasted substance were not avoided in preference tests, as

compared with cues paired with the control (non-contrasted) substance. The fact that solutions subject to anticipatory contrast do not suffer a reduction in their ability to act as reinforcers in either instrumental (Weatherly et al., 2006) or Pavlovian (Flaherty et al., 1995) situations would seem to suggest that their rewarding value has not been diminished by being reliably presented in advance of a preferred solution. That said, neither Weatherly et al. (2006) nor Flaherty et al. (1995) actually assessed the value of the solution subject to contrast, so they do not directly demonstrate that the value of the contrasted solution is maintained. More importantly, the nature of the anticipatory contrast procedure means that the contrasted substance is set up as a perfectly reliable cue for a highly rewarding event. It has long been known that otherwise neutral cues paired with rewarding events can themselves support instrumental or Pavlovian conditioning as secondary reinforcers (see Mackintosh, 1974). Thus, a solution subject to contrast might have supported subsequent responses as a secondary, rather than a primary, reinforcer even if anticipatory contrast had reduced the intrinsic value of the initial solution itself. Direct measurement of the hedonic response to the solution subject to contrast would address these issues.

2.1.4 Combining the Negative Anticipatory Contrast procedure with Lick Analysis

The negative anticipatory contrast paradigm can be used in combination with microstructural analysis of licking. The benefits from this are two-fold. Primarily, it allows the assessment of a rodent's hedonic reactions as they learn to anticipate a more rewarding solution being made available

in the future. This has important implications as it allows the first analysis of anticipatory hedonics in non-verbal rodents.

The second benefit is that it allows for a more direct assessment of the devaluation hypothesis of negative anticipatory contrast effects. There has been one study to my knowledge that combined lick microstructure measures with an anticipatory contrast paradigm: Arthurs, Lin, Amodeo, and Reilly (2012) found a difference in lick cluster size for a saccharin solution as a factor of whether it was followed by higher valued sucrose or more of the same solution. Such suppressed lick cluster sizes appear to be wholly consistent with a reduction in the first solution's rewarding value, relative to appropriate controls. However, the analysis of the results offered by Arthurs et al. led them to conclude that this difference was not, in fact, a product of devaluation (I will address these differences of interpretation more fully in the Discussion section of this chapter).

Regardless of the interpretation of the results, it should be noted that the Arthurs et al. used a between-subjects design in their study, which meant that animals in the contrast and control conditions differed in their exposure to concentrated sucrose. Repeated exposure to concentrated sucrose in the contrast group could have resulted in a shift in their general adaptation levels to sweet and, thus, lowered their sensitivity to the relatively weak sweet taste of dilute saccharin (Albertella, Harris, & Boakes, 2008; Boakes, Albertella, & Harris, 2007). Although general differences in experience with different concentrations of sucrose cannot explain all previously observed anticipatory contrast effects (see the comments above regarding within subjects and intersolution time effects), it

remains the case that the suppressed lick cluster sizes observed by Arthurs et al. may reflect differences in overall experience, rather than being the product of anticipatory contrast.

2.1.5 Design of the Current Paradigm

The present study used a within-subjects design to address the reliability and source of lick cluster size changes in anticipatory contrast. Importantly, in this design, all animals received exposure to all test solutions, eliminating any differences in the level or type of solution exposure. This has further importance when the paradigm is used to assess animal models of disease, as adaptation level effects may be more prevalent in experimental animals compared to their controls. Different contextual cues (chosen on the basis of the work of Flaherty et al., 1995) were used to signal which of the two solution pairings (either a low-reward solution followed by more of the same solution or a low-reward solution followed by a high-reward solution) was in operation each day. We reasoned that if the reduction in consumption (i.e. negative anticipatory contrast) of the initial solution when it precedes a preferred solution occurs because the first solution is devalued, it will be mirrored by a similar reduction in lick cluster size.

2.2 Experiment 1 - Materials and Methods

2.2.1 Subjects

Male Lister-hooded rats (n=8, Harlan, UK), weighing 300-340g on ad libitum food (approximately 12 weeks of age), were used in the experiment. They were paired-housed, under a 12 hour light/dark cycle. Experimental sessions were performed during the light phase, beginning approximately 3 hours after 'lights-on', and were conducted 6 to 7 days per week. Prior to the start of the experiment, all animals were placed on a food-restricted diet, which maintained them between 85 to 95% of their free feeding weights. Their food ration was given in their home cage 30 min after the end of each daily session. The experiment (together with the other experiments reported in this thesis) was conducted in accordance with the United Kingdom Animals Scientific Procedures Act, 1986, and was subject to Home Office approval (Project License PPL 30/2703). In the interest of meeting the three 'R's (Reduce, Refine and Replace), the guiding principles underpinning the humane use of animals in scientific research, power analyses were performed to determine the sample size appropriate for each experiment.

2.2.2 Apparatus

Testing was conducted in six automated drinking chambers² (Med Associates Inc., St Albans, VT, USA) arranged in a 3 × 2 array. Each chamber, measuring 30 × 24 × 29 cm, was comprised of

²This experiment was conducted alongside a secondary experiment not reported in this thesis. Only 4 of the 6 available chambers were used in the current experiment.

two aluminium side walls, a clear Perspex back wall and a clear Perspex ceiling. The front wall was also made of clear Perspex and served as the door to the chamber. The chamber floor consisted of 19 steel rods, 4.8 mm in diameter, spaced 16 mm apart. Approximately 5 cm above the grid floor, two holes, each 1 cm in diameter, were positioned on each side of the right wall to allow the rat access to the solutions. Solutions were delivered through the left (referring to the back of the chamber) and right (referring to the front of the chamber) access holes by 50 ml cylinders with ball-bearing metal drinking-spouts. These were mounted to the cage via motorized holders that held the spout flush with the outside of the chamber and retracted it as required. This allowed sequential access to the two solutions. During access to the solution, contact sensitive lickometers registered the timing of each lick made by the animal to the nearest 0.01 s, and a computer running MED-PC software controlled the equipment and recorded the data. The solutions used were 4% and 32% (w/w) sucrose formulated daily using commercial-grade cane sugar and deionised water.

Distinct lighting conditions were created in the room that housed the chambers. Room lighting provided a bright light condition for the animals, while an angle-poise lamp (positioned underneath the chambers) provided a dim light condition with the room lighting switched off. A stainless steel mesh insert could be slotted over the top of the grid floor of the chamber to provide an alternative tactile floor cue. White-noise could also be provided by a de-tuned radio that was on constantly throughout the session.

2.2.3 Procedure

On the first day of the experiment, the animals (which had been water restricted for 22 hours) were habituated by leaving them in the drinking chambers with 10 min access to water from both bottles. During this initial training, drinking spouts were positioned inside the chamber to allow for easy detection by the rats. After pre-training the animals were returned to an ad libitum water supply for the remainder of the experiment. On each of the following training days, rats were given access to solution pairings that were manipulated within-subject. Rats were presented with either a 4% sucrose solution followed by more 4% sucrose (the 4-4 condition or control condition) or a 4% sucrose solution followed by a 32% sucrose solution (the 4-32 condition or contrast condition). These daily solution pairings were presented in double alternation (e.g. ABBAABBA) and different contextual cues were used to signal which of the two solution pairings was in operation each day. For half the animals, the 4-4 condition was presented in context 1 (consisting of bright light, white noise and normal grid floor), and the 4-32 condition was presented in context 2 (consisting of dim light, no background noise and wire mesh floor insert). The remaining subjects had the opposite assignment. The first solution in the pair was made available for 3 min on the right-hand side of the chamber. Following a 4 sec inter-solution interval, the second solution was then made available for 6 min on the left-hand side of the chamber. Across training, the position of the spout started inside the chamber and was progressively moved back, until it was flush with the outside of the chamber (taking around 3 days). Training continued across 32 days until a contrast effect had developed.

2.2.4 Data analysis

Consumption was assessed by weighing the bottle before and after each experimental session. Lick cluster size (defined as the mean number of licks per cluster) was extracted from the MED-PC data. As in previous experiments using these general methods and equipment in Cardiff (e.g. Dwyer, Lydall, & Hayward, 2011; Lydall, Gilmour, & Dwyer, 2010), a cluster was defined as a series of licks, with each lick separated by no more than a 0.5 s interval. The same criterion had been adopted by Davis and his colleagues (Davis, 1989; Davis & Perez, 1993; Davis & Smith, 1992). Although other criteria have been used (e.g. 1 s by Spector, Klumpp, & Kaplan, 1998), there is little practical difference between them as most pauses greater than 0.5 s are also greater than 1 s (Davis & Smith, 1992). Mean interlick-interval (ILI: the timing between one lick and the next) was also extracted from the MED-PC data. Measuring ILI is essential in determining whether or not other licking parameters (namely LCS) are confounded by motoric/ motivational abnormalities displayed by the animal. ILI is usually a very constant measure; with variability (particularly long ILIs) potentially reflecting motor impairments, posture changes, or other abnormal drinking patterns. Across the thesis, this parameter will only be reported when inclusion of ILI might reflect important differences across strains, groups or conditions.

In the current experiment, drinking data were collated into two-session blocks. Data were analysed using repeated-measures ANOVA with factors of block (1-8) and contrast condition (4-4 or control condition vs. 4-32 or contrast condition). All statistical tests reported in this thesis were performed using SPSS 20.0 (SPSS Inc., Chicago). Where the assumption of Sphericity was not met,

Greenhouse-Geisser corrections have been reported, as is the case across the thesis. An alpha level of .05 was adopted as the level of significance throughout.

2.3 Results

Figure 1 depicts the consumption (Panel A) and lick cluster size measures (Panel B), across the eight 2-session blocks, of the initial 4% solution as a factor of whether it was followed by 4% sucrose (the 4-4 condition) or 32% sucrose (the 4-32 condition). Inspection of Figure 1A suggests that intake of the initial 4% solution increased across blocks to a greater extent for the 4-4 condition than the 4-32 condition, representing an anticipatory contrast effect. A repeated measured ANOVA with factors of block (1 to 8) and contrast condition (4-4 vs. 4-32) revealed a non-significant main effect of contrast condition ($F(1, 7) = 2.785, p = .139, MSE = 1.125$), a significant main effect of block ($F(7, 49) = 17.876, p < .001, MSE = 6.271$) and a significant contrast condition by block interaction ($F(7, 49) = 2.310, p = .041, MSE = 0.279$). Simple-effects analysis of the interaction suggest no difference between the 4-4 and 4-32 conditions for blocks 1, 2, 3, and 5 (largest $F(1, 7) = 1.175, p = .314, MSE = 0.051$, for block 2), while there were significant differences for blocks 4, 6, 7 and 8 (smallest $F(1, 7) = 5.755, p = .048, MSE = 0.014$, for block 7).

Inspection of Figure 1B indicates that the anticipatory contrast effect on consumption was associated with lower lick cluster sizes in the contrasted (4-32) than non-contrasted (4-4) condition during intake of the initial solution. ANOVA revealed a significant main effect of contrast condition ($F(1, 7) = 24.568, p = .002, MSE = 1203.074$), a significant main effect of block ($F(1.947, 13.626) =$

7.112, $p = .008$, $MSE = 2750.778$) and a significant contrast condition by block interaction ($F(7, 49) = 3.367$, $p = .003$, $MSE = 189.502$). Follow-up analysis revealed no significant differences between contrast conditions during blocks 1, 2, 3, 4 and 7 (largest $F(1, 7) = 5.448$, $p = .052$, $MSE = 11.102$, for block 4), and that there were significant differences on blocks 5, 6 and 8 (smallest $F(1, 7) = 7.218$, $p = .031$, $MSE = 15.272$, for block 6). This result indicates that following 4% sucrose with 32% sucrose (in the 4-32 condition) suppresses the increase in lick cluster size for 4% sucrose across sessions that would have otherwise occurred if it had been followed by more of the same solution (the 4-4 condition).

Figure 1, panels C and D, shows the consumption and lick cluster size measures for the second sucrose solution in conditions 4-4 and 4-32 over the eight 2-day blocks of the experiment. As can be seen in Panel C, the consumption of the second solution (4% sucrose) remained consistently low across blocks for the 4-4 condition. In contrast, the consumption of the second solution (32% sucrose) in the 4-32 condition increased over the blocks. ANOVA revealed significant main effects of contrast condition ($F(1, 7) = 39.169$, $p < .001$, $MSE = 198.254$), of block ($F(2.155, 15.082) = 38.367$, $p < .001$, $MSE = 96.863$), and an interaction between them ($F(2.667, 18.666) = 6.457$, $p = .004$, $MSE = 11.374$). Post-hoc tests showed that there was no significant difference between conditions at block 1 ($F < 1$), while there were significant differences at blocks 2 - 8 (smallest $F(1, 7) = 12.738$, $p = .009$, $MSE = 0.701$, for block 8). Inspection of Panel D reveals a similar pattern of results for lick cluster size in that the lick clusters were consistently higher, at least numerically so, for the 4-32 than the 4-4 condition across blocks. ANOVA revealed significant main effects of contrast condition ($F(1, 7) =$

31.393, $p = .001$, $MSE = 4664.573$) and block ($F(7, 49) = 14.961$, $p < .001$, $MSE = 813.666$) plus a significant contrast condition by block interaction ($F(2.288, 16.018) = 6.735$, $p = .006$, $MSE = 1439.224$). Simple-effects analysis revealed no significant difference between conditions at block 1 ($F(1, 7) = 4.177$, $p = .080$, $MSE = 20.142$), significant differences on blocks 2 to 7 (smallest $F(1, 7) = 6.545$, $p = .038$, $MSE = 9.229$, for block 7), but no difference in block 8 ($F(1, 7) = 2.345$, $p = .170$, $MSE = 15.085$). This pattern of effects appears to be largely driven by the gradual reduction in lick cluster sizes during consumption of 32% sucrose from blocks 4 to 8. The reason for this downward trend is not clear, however, I have also observed similar reductions across exposure sessions when animals were repeatedly presented with sucrose in the absence of an anticipatory contrast procedure. It is possible that it might reflect within-session adaptation to the concentrated sucrose (Dwyer, 2012) that is exacerbated as consumption levels increase³.

³ It is also possible that the reduced lick cluster size seen for the 32% solution across training is due to sucrose-induced insulin resistance. High sucrose diets have previously been shown to impair insulin action in rats (e.g. Storlien, Kraegen, Jenkins, and Chisholm, 1988). Furthermore, Ribeiro, Lutt, Legare, and Macedo (2005) gave Sprague-Dawley rats free access to a 35% sucrose solution (along with food and water ad libitum) and found that insulin resistance was expressed as early as 2 weeks in this strain. Since exposure to concentrated sucrose is restricted to 6 min a day in our paradigm, this possibility may be unlikely but cannot be ruled out on the basis of the current data alone.

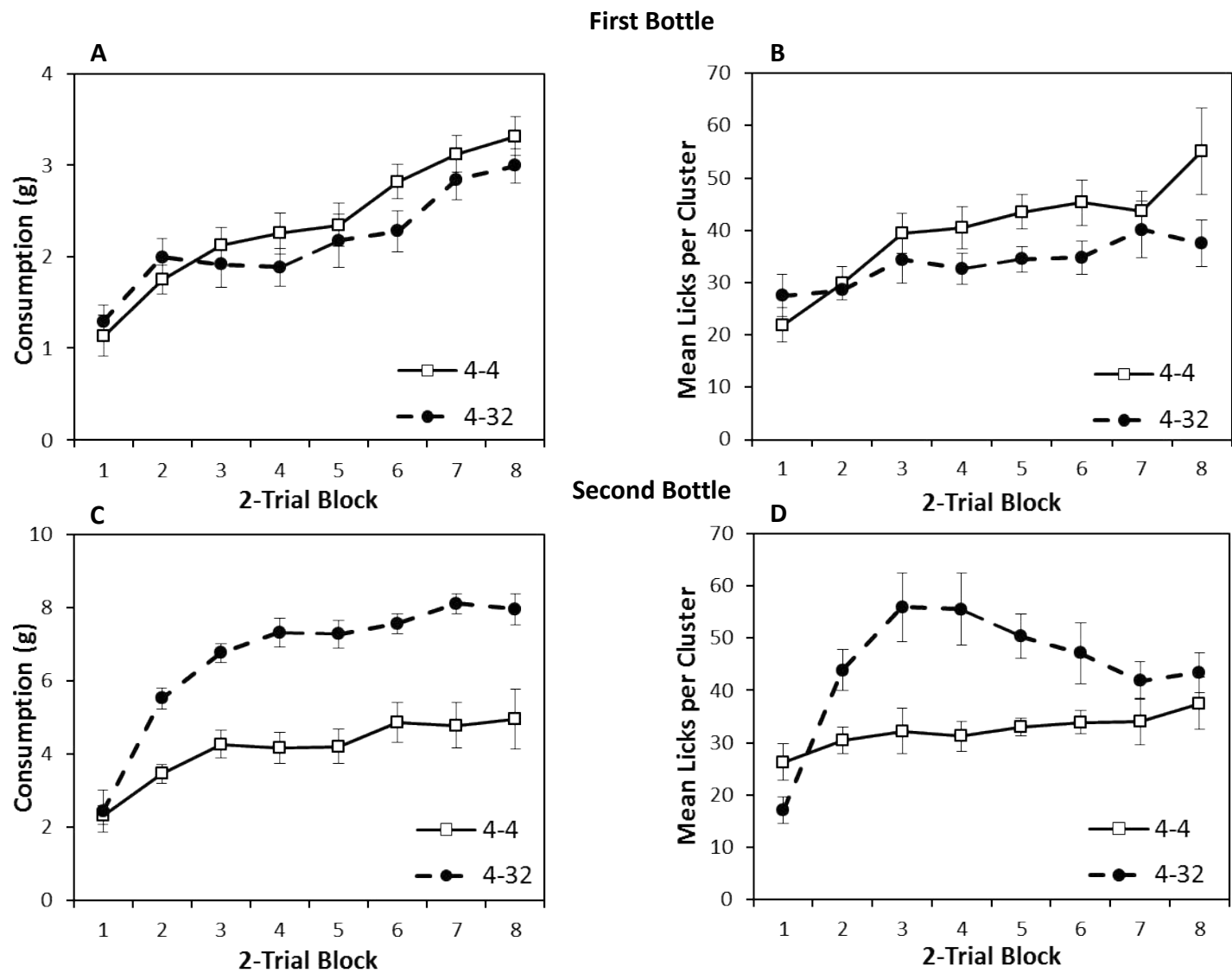


Figure 1 Panel A shows the mean (\pm SEM) consumption data from the first bottle available (containing 4% sucrose) each day during negative anticipatory contrast for the 4-4 (control) and 4-32 (contrast) conditions. Panel B shows the mean lick cluster size (\pm SEM) for the first bottle available each day as a factor of contrast condition. The first bottle was available for 3 min. Panel C shows the mean (\pm SEM) consumption data from the second bottle available each day during negative anticipatory contrast for the 4-4 (control) and 4-32 (contrast) conditions. Panel D shows the mean lick cluster size (\pm SEM) for the second bottle available each day as a factor of contrast condition (4-4 vs. 4-32). The second bottle was available for 6 min (beginning 4 sec after the first bottle had been retracted). The data is averaged over two trial blocks.

2.4 Discussion

In one context, rats received access to 4% sucrose from one bottle followed by access to 4% sucrose from a second bottle, while in a different context, they received access to 4% sucrose from one bottle followed by access to 32% sucrose from a second bottle. The rats' consumption of 4% sucrose was lower on days when 4% sucrose preceded access to 32% sucrose than when it preceded access to more 4% sucrose. This reflects a within-subjects anticipatory contrast effect on consumption. Moreover, an analysis of licking microstructure revealed that this contrast effect was also reflected in the size of licking clusters. That is, the same 4% sucrose elicited lower lick cluster sizes on days when it was followed by 32% sucrose than on days when it was followed by 4% sucrose. Since lick cluster size is directly related to the perceived value or concentration of sucrose and the first solution was physically unchanged, this effect is consistent with anticipatory contrast producing a devaluation of 4% sucrose relative to an appropriate control. That is to say, the differences in the mean number of licks per cluster between the 4-4 and 4-32 conditions result from a change in the perceived value of the initial solution by the anticipation of future rewards. Moreover, because a within-subjects procedure was used, the effects observed here cannot be attributed to a general reduction in the sensitivity to sweet tastes as a result of adapting to high sucrose concentrations. Contrary to the majority of previous analyses, this suggests that negative anticipatory contrast does indeed include a devaluation of the initial solution.

While the use of a within-subjects design means that the suppressed lick cluster sizes observed cannot be attributed to a general reduction in the rat's sensitivity to sweetness as a result of shifts in their overall adaptation level (Boakes et al., 2007), there is evidence for context-specific adaptation level effects (Albertella et al., 2008). In this light, it is thus possible that the reduced lick cluster sizes are due to a comparison between the concentration of sucrose previously experienced in a particular context and the currently available solution, rather than being the product of an anticipatory comparison process. That said, it should be remembered that the interval between two solutions within a day influences consumption effects in anticipatory contrast (e.g., Flaherty & Checke, 1982; Lucas et al., 1988). This timing effect would not be expected if anticipatory contrast were actually due to a comparison between the currently available solution and the stored value of previous solutions experienced in the same context. Since inter-solution intervals have not been manipulated here, the possibility that context-dependent adaptation effects are contributing to the lick microstructure results cannot be ruled out. Thus, the suppression seen in both lick-microstructure and consumption might reflect different causal mechanisms. That said, it might be suggested that it is more parsimonious to assume that contrast effects on consumption and on lick cluster size share a common cause. This is especially so given that the effects of contrast on consumption and lick cluster size emerged at roughly the same point in the experiment.

The idea that the lower lick cluster sizes for 4% sucrose in the 4-32 than in the 4-4 condition reflects devaluation in the former condition might seem to be a relatively direct corollary of the generally observed relationship between lick cluster size and solution concentration or value.

However, Arthurs et al. (2012) previously reported similar results from a between-subjects design, while concluding that devaluation was not involved. This conclusion was based on the fact that, in animals for which saccharin preceded sucrose, the cluster size for saccharin remained relatively consistent across training, while in animals for which saccharin preceded further saccharin access, the cluster size for saccharin increased across sessions. That is, there was no evidence from the lick cluster size measure that the value of saccharin reduced from its initial level as a result of anticipatory contrast (essentially the same pattern of results was observed here with 4% sucrose). However, it should be remembered that rodents typically show a neophobic response to novel tastes that dissipates with experience. Indeed, Lin, Amodeo, Arthurs, and Reilly (2012) reported that lick cluster sizes increase over exposure for a variety of solutions and similar results were seen by Dwyer (2009). Lin et al. neatly summarised that the clear implication of these results is that “the pleasure of drinking increases as the novel, potentially dangerous tastant becomes accepted as safe” (p. 515). In this light, the failure to see an increase in the lick cluster size for saccharin (by Arthurs et al., 2012) or 4% sucrose (here) as a result of anticipatory contrast does represent a devaluation, relative to the state that would have occurred had the solution simply been exposed on its own. To be sure, pairing saccharin or sucrose with illness can produce devaluations relative to the initial state (e.g. Arthurs et al., 2012; Dwyer, 2009), but the mere fact that other treatments produce larger effects does not mean that contrast is not producing a devaluation at all.

A devaluation account of anticipatory contrast seems intuitively plausible: The decrease in responding for a low valued solution when a high-valued solution will be available in the near future

occurs because the initial solution has become one of functionally lower hedonic value. However, this devaluation interpretation has generally been rejected; largely because solutions that have been subject to anticipatory contrast appear to operate as positive rewards in both instrumental (e.g., Weatherly et al., 2006) and Pavlovian (e.g., Flaherty et al., 1995) situations. But, as was noted in section 2.1.3, these are not direct tests of the functional value of the solution subject to contrast, and more critically, the reinforcing value of the contrasted solutions could be attributed to a process of secondary reinforcement. Since the present study directly addressed the value of the contrasted solution via the analysis of licking microstructure and did see a functional devaluation, it would appear that previous theorists might have been premature in rejecting the devaluation account.

What should be stressed at this point in time, however, is that an equally plausible explanation is that, rather than the devaluation of the first solution driving the contrast effect in consumption, the changes in solution value and amount consumed could be independent aspects of experiencing contrast. This issue will be returned to later on in the thesis (see section 5.9).

To summarise, the present study is the first to combine microstructural lick analysis with a within-subjects negative anticipatory contrast procedure and, thus, avoids the problems either of using indirect assessments of reward value or of confounds relating to differences in adaptation level to sweet tastes between groups. The results obtained suggest, contrary to prevailing assumptions, that anticipatory contrast does produce a functional devaluation of the solution subject to contrast, but whether this is the mechanism behind the reduced consumption remains unclear. Regardless of the mechanism, combining negative anticipatory contrast with lick analysis provides us with a unique

opportunity to assess how an animal responds when a predictable rewarding event will be made available in the near future. Specifically, measuring contrast effects in consumption allows us to determine whether the animal is able to anticipate or predict the future event, whereas measuring contrast effects in lick cluster size allows us to determine how this anticipation impacts upon their hedonic systems. An animal that fails to expect the second solution in the pairing, or cannot adequately predict or 'care' about that solution's value, would show a much diminished or even absent contrast effect. This technique will be returned to in the coming chapters to assess the presence of anticipatory anhedonia in the MAM-exposed and WKY rodent models.

Chapter Three

3. Hedonic Deficits in the MAM Model

3.1 Introduction

Early definitions of schizophrenia routinely included anhedonia as a core symptom of the disease (e.g. Kraepelin, 1919; Bleuler, 1911), falling within the negative symptom cluster (e.g. Andreasen, 1995). As has been discussed in the general introduction (section 1.3.2), the empirical evidence for anhedonia was largely driven by interview based assessments and self-report questionnaires (Horan et al., 2006). These findings of reduced hedonic capacity have not been supported by laboratory-based assessments, which evaluate an individual's in-the-moment pleasure when exposed to a range of emotion-eliciting stimuli (e.g. pictures, film-clips and flavoured drinks)

(Cohen & Minor, 2010; Kring & Moran, 2008; Llerena et al., 2012). In an attempt to reconcile this apparent 'emotion paradox' it has been suggested that anticipatory anhedonia may better reflect the hedonic deficits reported in this patient group (e.g. Gard et al., 2007).

This chapter investigates the hedonic capacity of the MAM-exposed neurodevelopmental model of schizophrenia. As injection of MAM on GD17 has been shown to be optimal in producing neuroanatomical and behavioural alterations of relevance to schizophrenia, this time-point was used for all experiments reported in the upcoming chapters (Chapters three and four). Whilst this model shows promise, its behavioural phenotype has not been subjected to robust characterisation, particularly with regard to the negative symptoms of the disease. For example, the hedonic impact of sweet tastes has not been investigated in this model, nor has there been an assessment of behaviours analogous to anticipatory anhedonia. Microstructural analysis of licking of non-contrasted and contrasted solutions (as part of the negative anticipatory contrast procedure developed in Chapter two) provides a unique opportunity to assess consummatory and anticipatory anhedonia in this model, whilst factoring in potential motor confounds (by the inclusion of the ILI parameters - see sections 1.5.2 and 2.2.4) that have been seen to potentially influence other putative schizophrenia modelling approaches (e.g. Lydall et al., 2011). Experiment two investigates the consummatory hedonic capacity of MAM-exposed rats while Experiment three uses the negative anticipatory contrast procedure to investigate anticipatory hedonics in the model. In light of the clinical literature, animal models that exhibit reduced palatability responses to sucrose solutions may not necessarily be comparable to the hedonic deficits, or lack thereof, in schizophrenia. Investigating consummatory

hedonics in this model might be seen as a negative control. Based on current understanding, the presence of anticipatory hedonic deficits in the absence of consummatory hedonic deficits, would suggest that the MAM neurodevelopmental model has good translational validity to the clinic.

3.2 Experiment 2 - Materials and Methods

3.2.1 Subjects

Chapters three and four pertain to a series of studies done on several cohorts of MAM treated rats and their controls, with multiple experiments performed on each cohort of animals (please refer to Appendix A for experimental order). The data from two separate cohorts of rats were combined for Experiment two. Both cohorts were comprised of male Sprague-Dawley rats supplied by Charles River UK. For cohort one, twenty-four rats were prenatally exposed to Methylazoxymethanol acetate (MAM: Midwest Research Institute, Kansas City, Missouri) and eighteen acted as control animals having been prenatally exposed to saline. For cohort two, twenty-four rats were MAM exposed and twenty-four were saline exposed, again at the embryonic stage. All animals were bred and given MAM/ saline treatment at Charles River as previously described by Moore et al. (2006). Briefly, a single dose of MAM (diluted in 0.9% saline) was delivered at a 28 mg/kg dose (expressed as salt weight) by intraperitoneal (i.p) injection in a volume of 1ml/kg to pregnant dams on gestational day 17. MAM is a light sensitive compound and was maintained in light-restricting bottles. Control females received injections of 0.9% saline (1ml/kg, i.p.) at the equivalent time point. MAM- and saline-treated rats from multiple litters were delivered to Cardiff at 9 weeks of age (both cohorts). Rats were housed

in groups of two or three for cohort one and pair housed for cohort two in a climate-controlled vivarium. Animals were tested approximately 1 hour after 'lights-on', as was the case for all subsequent experiments reported in this thesis. At the start of the experiment, rats were approximately 11 weeks old (both cohorts). Characteristics of both cohorts and can be found in Table 3. For dates of when the experiments were conducted, please refer to Appendix A.

Table 3 Cohort characteristics for first and second cohort used in Experiment two. Data is taken prior to food deprivation.

	Cohort 1		Cohort 2	
	MAM-Exposed (n = 24)	Saline-Exposed (n = 18)	MAM-Exposed (n = 24)	Saline-Exposed (n = 24)
Age	~ 11 weeks		~ 11 weeks	
Weight range (g)	329 - 397	335 - 483	306 - 389	288 - 392
Mean ad-lib weight (g)	357	403	336	351
MAM-Sham comparison (Independent (2-tailed) T-test)	MAM rats significantly lighter than Shams ($t(20.663) = -3.664, p = .001$)*		MAM rats significantly lighter than Shams ($t(46) = -2.161, p = .036$)**	

* Equal variances not assumed

** No assumptions violated

Rats were placed on a food restricted diet to reduce them to ~ 85% of their free feeding weights. Moderate weight gain was allowed over the course of the experiment to match expected increases of free-feeding animals, as determined by growth charts. Rat weights were carefully monitored throughout to ensure that weights, as percentages of free-feeding weight, did not differ significantly between MAM- and saline-treated control rats. Food rations were given in the rats' home cage one hour after the end of each daily session.

3.2.2 Apparatus

Cohort One: Testing was conducted in a room containing 16 drinking chambers. Chambers were white plastic boxes measuring 32 × 15 × 12 cm arranged in an 8 × 2 array. Both the floor and lid of the chamber consisted of metal grids. Solutions were given in 50 ml cylinders with stainless-steel ball-bearing drinking spouts. These were positioned on the left hand side of the chamber at the start of each experimental session and removed by hand at the end of the session. As described in Experiment one, a contact sensitive lickometer recorded the timing of each lick made by the animal to the nearest 0.01s. This was recorded by a computer with MED-PC software. The solutions used for this experiment were 4%, 8% and 16% sucrose formulated daily (w/w) with commercial-grade sugar and deionised water. For pre-training, rats were given 8% maltodextrin, formulated in the same manner as the sucrose solution. Maltodextrin was used as rats seem to process this hydrolysed starch as entirely separate from sucrose, even though both types of solution appear to be 'liked' (see Sclafani, 1987; 2004 for a review).

Cohort Two: Testing for cohort two was conducted in the same six automated drinking chambers described in Experiment one (Section 2.2.2). Solutions were delivered through the left access hole by 50 ml cylinders with ball-bearing metal drinking spouts. The spouts were brought flush with the outside of the chamber at the start of the session and were retracted automatically at the end of the session. Solutions were again 4%, 8% and 16% sucrose made daily (w/w) with deionised water.

3.2.3 Procedure

Pre-training. Pre-training was conducted differently across the two cohorts: Cohort one were water restricted for 22 h prior to pre-training day one only. Eight pre-training days were given in total, each comprised of 20 min exposure to an 8% maltodextrin solution; for cohort two, animals were water restricted for 22 h prior to each of the three daily pre-training sessions. During each 20 min session, water was made available in the operant chamber. Rats were given free access to water in their home cages 1 h after the session for 1 h duration. Rats were then returned to ad lib water for the remainder of the experiment.

Test. All rats (cohorts one and two) were given access to one of three (4%, 8% and 16%) sucrose concentrations in the drinking chambers for 20 min each day for five days (Monday-Friday, where possible). The order of sucrose presentations was counterbalanced with half of the rats receiving the sucrose in order of increasing concentration (4-8-16) and the other half receiving them in order of decreasing concentration (16-8-4). Two-days rest was given before the next concentration in the sequence was presented.

3.2.4 Data analysis

Consumption was assessed by weighing the bottle before and after each experimental session. Lick cluster size (LCS) was extracted from the MED-PC data. The same parameters were used as previously described (section 2.2.4). Inter-lick interval (ILI) was also extracted from the data.

As this normally shows very little variability, any differences in ILI between exposure conditions (MAM vs. saline) may be indicative of motor abnormalities in the experimental rats.

To overcome potential neophobic effects, consumption, LCS and ILI data were analysed for the last three days of test only for each of the three different concentrations of sucrose (i.e. after two days of solution exposure). Data were analysed by a repeated-measures ANOVA with a within-subject factor of solution concentration (4, 8 and 16% sucrose) and between-subject factors of replication (cohort one vs. cohort two), treatment (MAM vs. Sham) and sequence order (4-8-16 or ascending vs. 16-8-4 or descending). All graphs show the data collapsed across the two cohorts with an n of 42 for saline-treated animals and 48 for MAM-treated animals.

3.3 Results

The mean amount of sucrose consumed in grams, the mean number of licks per cluster (LCS) and the mean inter-lick interval (ILI) at each sucrose concentration are represented in panels A, B and C, respectively, of Figure 2. Inspection of Figure 2A indicates that increasing sucrose concentration did not produce an overall increase in the amount consumed, with the moderate (8%) concentration instead eliciting the highest intake. Whether the rats had been prenatally exposed to MAM or saline did not appear to influence the amount consumed at any of the three concentrations.

A mixed ANOVA revealed a significant main effect of concentration ($F(1.565, 128.361) = 55.099, p < .001, MSE = 564.992$). There was also a main effect of sequence order with rats generally consuming more under the descending sequence order condition (16-8-4: 23.13 g)

compared to the ascending condition (4-8-16: 21.67 g; $F(1, 82) = 4.476, p = .037, MSE = 47.384$).

The ANOVA also revealed a concentration \times sequence order interaction ($F(2, 164) = 76.681, p < .001, MSE = 615.347$) with consumption of the 4% and 16% solutions differing significantly across the two conditions (4%: $F(1, 82) = 51.260, p < .001, MSE = 1098.796$; 8%: $F < 1$; 16%: $F(1, 82) = 24.878, p < .001, MSE = 260.729$). This likely occurred due to lower consumption levels being exhibited by rats for the first solution experienced in the sequence, regardless of that solution's intrinsic value. According to the description of the data, prenatal MAM treatment had no effect on the amount consumed by the rats ($F < 1$). There was also no interaction between drug treatment and sucrose concentration ($F(2, 164) = 1.610, p = .203, MSE = 12.924$), nor was there a treatment \times sequence order interaction ($F(1, 82) = 1.383, p = .243, MSE = 14.646$).

In terms of replication, rats in cohort two generally consumed more sucrose (25.75 g) than rats in cohort one (19.05 g; $F(1, 82) = 93.813, p < .001, MSE = 993.181$). The ANOVA also revealed a replication \times treatment interaction ($F(1, 82) = 5.314, p = .024, MSE = 56.255$) and a replication \times sequence order interaction ($F(1, 82) = 9.775, p = .002, MSE = 103.488$). Further analysis of these interactions revealed that MAM treated rats consumed non-significantly more sucrose than their saline counterparts during the initial replication ($F(1, 82) = 3.701, p = .058, MSE = 39.176$) whereas saline-treated rats consumed non-significantly more than MAM animals during the second replication ($F(1, 82) = 1.730, p = .192, MSE = 18.315$). Inspection also revealed that higher consumption for descending than ascending conditions occurred for replication one only (Replication one: $F(1, 82) = 12.759, p = .001, MSE = 135.072$; Replication two: $F < 1$). A concentration \times replication \times sequence

order interaction was also revealed by the analysis ($F(2, 164) = 7.593, p = .001, MSE = 60.928$) with significantly different consumption levels between ascending and descending sequence orders for 4% (ascending < descending: $F(1, 82) = 25.540, p < .001, MSE = 547.472$) and 8% (ascending < descending: $F(1, 82) = 9.115, p = .003, MSE = 144.872$) concentrations for replication one and for 4% (ascending < descending: $F(1, 82) = 25.806, p < .001, MSE = 553.181$) and 16% (ascending > descending: $F(1, 82) = 51.154, p < .001, MSE = 536.115$) for replication two (16% Replication one: $F < 1$; 8% Replication two: ascending > descending, $F(1, 82) = 3.661, p = .059, MSE = 58.190$). There was no concentration \times replication interaction ($F < 1$), no three-way interaction between replication, treatment and concentration ($F(2, 164) = 2.770, p = .066, MSE = 22.229$), nor between replication, treatment and sequence order ($F < 1$). Finally there was no four-way interaction between replication, treatment, concentration and sequence order ($F < 1$). Whilst it is important to recognise the replication differences, it should be stressed that the differences do not detract from the general interpretation of the results – MAM rats do not exhibit any changes in consumption indicative of an affective impairment when compared with the saline-treated control animals.

Inspection of Figure 2B suggest that the number of licks per cluster increased with increasing concentration of sucrose for both MAM-treated and saline-treated rats. ANOVA performed with the same factors revealed a significant main effect of concentration ($F(2, 164) = 28.225, p < .001, MSE = 23754.707$), no main effect of sequence order ($F < 1$) and no interaction between these two factors ($F(2, 164) = 2.151, p = .120, MSE = 1799.575$). There was no main effect of prenatal treatment ($F(1, 82) = 1.641, p = .204, MSE = 2594.872$) and no concentration \times treatment interaction ($F(2, 164) =$

1.729, $p = .181$, $MSE = 1445.972$). Further, there was no treatment \times sequence order interaction ($F < 1$) and no treatment \times concentration \times sequence order interaction ($F(2, 164) = 1.955$, $p = .145$, $MSE = 1635.454$).

Briefly, in terms of replication, there was a significant main effect with higher LCSs exhibited by rats in cohort two (mean of 86.05 licks per cluster) than cohort one (mean of 61.99 licks per cluster) ($F(1, 82) = 8.111$, $p = .006$, $MSE = 12824.598$). There was no replication \times concentration interaction ($F(2, 164) = 2.452$, $p = .089$, $MSE = 2051.179$) no replication \times treatment interaction ($F < 1$) and no replication \times sequence order interaction ($F < 1$). Furthermore, no three-way interaction was revealed between replication, treatment and sequence order ($F < 1$) or between replication, concentration and sequence order ($F(2, 164) = 2.279$, $p = .106$, $MSE = 1906.045$), nor was a four way interaction revealed between these factors ($F < 1$). There was, however, a replication \times treatment \times concentration interaction ($F(2, 164) = 6.407$, $p = .002$, $MSE = 5358.710$). Further inspection of pairwise comparisons revealed no significant differences in LCS between MAM and saline-treated rats for any solution concentration across either replication (largest $F(1, 82) = 3.224$, $p = .076$, $MSE = 9033.679$ - Replication one (16% sucrose) with MAM rats exhibiting higher licks/cluster than saline-treated animals). Again, replication effects do not question the original interpretation of the results. That is, MAM rats did not display licking patterns suggestive of an affective deficit when compared against the saline-treated control strain.

With respect to ILI, inspection of Figure 2C suggests that this measure was stable across MAM and saline-treated animals and across the three different concentrations of sucrose. ANOVA

analysis revealed no significant main effect of concentration, treatment or sequence order (All $F_s < 1$).

There was also no treatment \times concentration interaction ($F(2, 164) = 2.613, p = .076, MSE = 36.530$),

treatment \times sequence order interaction ($F < 1$) or treatment \times concentration \times sequence order

interaction ($F(2, 164) = 1.083, p = .341, MSE = 15.137$). There was, however, a concentration \times

sequence order interaction ($F(2, 164) = 3.319, p = .039, MSE = 46.386$), further analysis of which

revealed that rats experiencing the solutions in ascending order of concentration displayed higher ILIs

when consuming 16% sucrose compared to 8% sucrose ($F(1, 82) = 6.591, p = .012, MSE = .446$).

No other significant differences were seen across any other solution concentration within each

sequence order condition, nor were significant differences seen in ILI for any of the three

concentrations of sucrose between sequence order conditions (all $F_s < 1$).

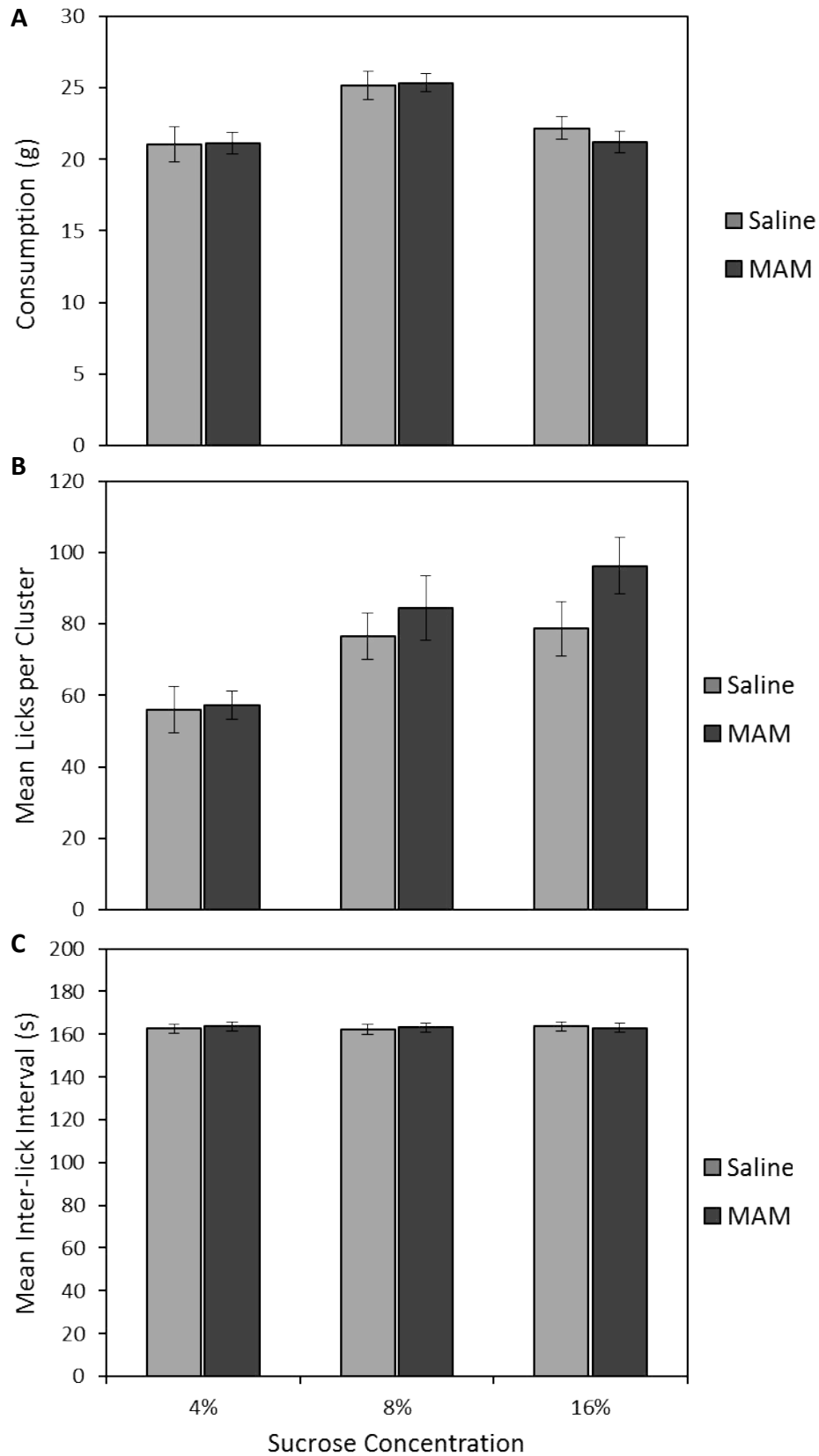


Figure 2 Data from the consumption of three sucrose concentrations following prenatal treatment with either MAM or Saline. A – C: mean amount of sucrose, mean number of licks per cluster and mean inter-lick interval, respectively (each displayed with \pm SEM). Saline-treated animals $n = 42$; MAM-treated animals $n = 48$. Data has been collapsed across two cohorts.

ANOVA analysis of ILI revealed a significant main effect of replication ($F(1, 82) = 105.577, p < .001, MSE = 8158.522$) with rats in cohort two exhibiting higher ILIs when consuming the sucrose solutions. There was also a replication \times concentration interaction ($F(2, 164) = 4.699, p = .010, MSE = 65.678$) as well as a replication \times concentration \times sequence order interaction ($F(2, 164) = 4.460, p = .013, MSE = 62.345$). Critically, ILI did not vary significantly across MAM and saline-treated rats (all $F_s < 1$; with the exception of the replication \times treatment \times concentration interaction where $F(2, 164) = 1.157, p = .317, MSE = 16.175$). As such, the pattern of licks/cluster previously reported was not confounded by motoric differences induced by prenatal drug treatment.

3.4 Summary

Using microstructural analysis of licking together with consumption measures, Experiment two investigated the hedonic capacity of MAM-treated rats. Rats prenatally exposed to MAM on GD17 demonstrated consumption and palatability responses to sweet solutions which were comparable to their saline-treated controls. Given that normal consummatory hedonic capacity is a relatively consistent finding in the schizophrenia clinical literature, the results of Experiment two may further indicate the validity of prenatal MAM-exposure as a schizophrenia model. Experiment three investigates whether or not MAM-exposed rats are impaired in their ability to anticipate the value associated with future rewards, a deficit perhaps more relevant to schizophrenia symptomatology. To investigate anticipatory anhedonia in this model the negative anticipatory contrast procedure described in Chapter two was utilised. A failure of MAM exposed animals to anticipate the second

solution in the pairing, or accurately predict its value, would lead to the loss or attenuation of the negative anticipatory contrast effect, with an increase in the consummatory and palatability responses towards the first solution across training regardless of second solutions intrinsic value.

3.5 Experiment 3 - Materials and Methods

3.5.1 Subjects

A third cohort was used in Experiment three (see Table 4 for cohort characteristics). This cohort was comprised of forty-eight male Sprague-Dawley rats supplied by Charles River UK. Of these, sixteen rats had been prenatally exposed to MAM, sixteen had been prenatally exposed to saline and sixteen had not been exposed to any solution (non-exposed control group). For full details of the MAM treatment please refer to Experiment two (Section 3.2.1). Pups were delivered to Cardiff at 8 weeks of age and pair housed. All aspects of animal husbandry were as previously described. At the start of the experiment, rats were approximately 10 weeks old. One week prior to the start of the experiment, all rats were placed on a food restricted diet to reduce them to ~85% of their free feeding weights. Weight gain was allowed over the course of the experiment and particular attention was given to ensure that weights did not differ significantly across the three treatment groups (in terms of percentage free-feeding weight).

Table 4 Characteristics of cohort three: data is from the day of food deprivation (ad lib weights) prior to the experiment start.

	MAM-Exposed (n= 16)	Saline-Exposed (n = 16)	Non-Exposed (n = 16)
Age	~ 10 weeks		
Weight range (g)	222 - 355	292 - 369	258 - 389
Mean ad-lib weight (g)	312	329	334
Weight comparison (Independent 2-tailed T-test)	MAM vs. Saline $t(30) = 1.625, p = .115$ MAM vs. Non-Exposed $t(30) = 1.783, p = .085$ Saline vs. Non-Exposed $t(30) = -.512, p = .613$		

3.5.2 Apparatus

Testing was conducted in the same six automated drinking chambers as described in Experiment one (Section 2.2.2). The solutions used in the experiment were 4% and 32% sucrose made daily w/w with deionised water. Distinct light conditions were created by room lighting (bright light condition) and an angle-poise lamp fitted with a red bulb (dim light condition). A metal grid floor insert could also be inserted into the bottom of the drinking chamber to create a distinct tactile cue. Unlike for Experiment one, white noise was not included to create the contexts. This was to allow consistency between MAM and WKY experiments. For subsequent experiments with WKY rats, strain differences in reactions to white-noise needed to be considered due to the stress sensitivity of this strain.

3.5.3 Procedure

Pre-training. Pre-training was conducted as described for Experiment one (Section 2.2).

Rats were water restricted (22 h max) during this initial training and drinking spouts were positioned inside the chamber to allow them to be easily detected. After pre-training, rats were returned to *ad libitum* water and remained so for the duration of the experiment.

Acquisition Training. Acquisition training of anticipatory contrast was conducted as described in Experiment one. It consisted of daily drinking sessions 9 min in duration. During sessions, rats received 3 min access to an initial 4% sucrose solution made available from right-hand side of the chamber, followed by access to a second solution for 6 min made available from the left-hand side of the chamber. There was a 4 s inter-solution interval. The identity of the second solution in the pairing - more of the same 4% sucrose solution (the 4-4 or control condition) or a more palatable 32% sucrose solution (the 4-32 or contrast condition) - was signalled by the context in which the rats were placed. Accordingly, the second solution identity was manipulated within subject with all rats experiencing both 4-4 and 4-32 conditions during training. In this experiment, context one consisted of bright light and the mesh floor insert put in the base of the chamber while context two consisted of dim light and the normal grid floor of the chamber. The position of the chamber, whether it was one of the top or bottom chambers of the 3 × 2 array, was also factored in to the context assignment. Solution pairing and context assignments were fully counterbalanced across rat strains. That is, for half the animals in each group (MAM-treated, saline-treated and non-exposed rats), context one was

paired with the 4-4 condition and context two was paired with 4-32 condition, while for the other half the opposite assignment was given. Acquisition training lasted twenty-four days and was carried out on consecutive days where possible. Initially spouts for both the left and right bottles were positioned within the chamber, but were gradually moved back across sessions (taking approximately three days but done on a rat by rat basis) until they were flush with the outside of the chamber.

3.5.4 Data analysis

Data collection was performed as described for Experiment one (Section 2.2.4). Drinking data were collated into two-session blocks and analysed using a repeated measures ANOVA with between-subject factor of prenatal treatment (saline-treated, non-exposed and MAM-treated) and within-subject factors of block (1-6) and contrast condition (4-4 or control condition vs. 4-32 or contrast condition).

3.6 Results

Figure 3 shows the consumption data for the anticipatory contrast drinking sessions, across six 2-Trial blocks. Panel A summarises the data for saline-treated control rats, panel B the data for non-exposed control rats and Panel C the data for MAM-treated experimental rats. For all panels, the data represents consumption of the first 4% sucrose solution, presented for the first 3 min of each drinking session, as a factor of whether the second solution was 4% sucrose (the control condition) or 32% sucrose (the contrast condition). An anticipatory contrast effect emerged across training with

suppressed consumption of the initial 4% sucrose solution on days in which animals received 32% sucrose in the second bottle (the 4-32/contrast condition) relative to days in which more 4% sucrose was received (the 4-4/control condition). Whilst prenatal treatment appeared to have impacted on the total amount consumed, there was no suggestion that treatment affected the development of anticipatory contrast. ANOVA results were consistent with these impressions: there were significant effects of test block ($F(3.172, 142.719) = 49.274, p < .001, MSE = 36.760$), and contrast condition ($F(1, 45) = 49.304, p < .001, MSE = 42.058$), as well as a significant interaction between them ($F(2.896, 130.326) = 22.212, p < .001, MSE = 10.746$). There was a significant main effect of treatment ($F(2, 45) = 8.038, p = .001, MSE = 59.029$) but no block \times treatment interaction ($F(10, 225) = 1.683, p = .086, MSE = .796$) and, critically, no contrast condition \times treatment interaction ($F(2, 45) = 2.035, p = .143, MSE = 1.736$) or block \times contrast condition \times treatment interaction ($F(10, 225) = 1.681, p = .086, MSE = .471$). Simple effect analyses revealed that the difference in consumption between 4-4 and 4-32 conditions was significant on trial blocks 2-6 (smallest $F(1, 45) = 5.273, p = .026, MSE = .009$ - block 2) but not on trial 1 ($F(1, 45) = 3.810, p = .057, MSE = .031$).

Figure 4 shows the lick cluster size data for the initial bottle across six 2-trial blocks as a factor of whether the second solution in the pairing was 4% or 32% sucrose. Panels A – C depict the data for saline-treated, non-exposed and MAM-treated rats, respectively. Inspection of this figure indicates that the anticipatory contrast effect on consumption was accompanied by lower lick cluster sizes for the initial 4% sucrose solution in the 4-32 than the 4-4 conditions, and that this was consistent across treatment groups. ANOVA revealed a significant effect of block ($F(2.665, 119.928)$

= 35.668, $p < .001$, $MSE = 35785.341$), a significant effect of contrast condition ($F(1, 45) = 32.368$, $p < .001$, $MSE = 14445.455$) and a significant block \times contrast condition interaction ($F(2.333, 105.003) = 9.284$, $p < .001$, $MSE = 6264.475$). Further analysis of the interaction revealed significantly higher LCSs for the contrast compared to control condition for trial block 1 ($F(1, 45) = 4.419$, $p = .041$, $MSE = 3.508$), no significant difference for trial block 2 ($F < 1$), and significantly smaller LCSs for the contrast compared to control condition for trials 3 - 6 (smallest $F(1, 45) = 12.706$, $p < .001$, $MSE = 48.025$ - block 6). There was a main effect of treatment ($F(2, 45) = 5.267$, $p = .009$, $MSE = 16666.788$) with saline-treated rats performing significantly more licks per cluster than MAM-treated rats ($F(1, 46) = 10.523$, $p = .002$, $MSE = 32.959$) and non-significantly more than non-exposed rats ($F(1, 46) = 2.952$, $p = .093$, $MSE = 32.959$). There was no significant difference between the number of licks per cluster exhibited by MAM-treated and non-exposed rats ($F(1, 46) = 32.959$, $p = .134$, $MSE = 32.959$). There was also a block \times treatment interaction ($F(10, 225) = 2.277$, $p = .015$, $MSE = 1217.468$), further analysis of which revealed higher LCSs for saline-treated rats relative to MAM-treated rats for blocks 3 - 6 (smallest $F(1, 46) = 6.814$, $p = .012$, $MSE = 157.578$ for block 6). Critically, there was no contrast condition \times treatment interaction ($F(2, 45) = 1.430$, $p = .250$, $MSE = 638.403$) or block \times contrast condition \times treatment interaction ($F < 1$), indicating that the contrast effect in LCS developed equally across all treatment groups.

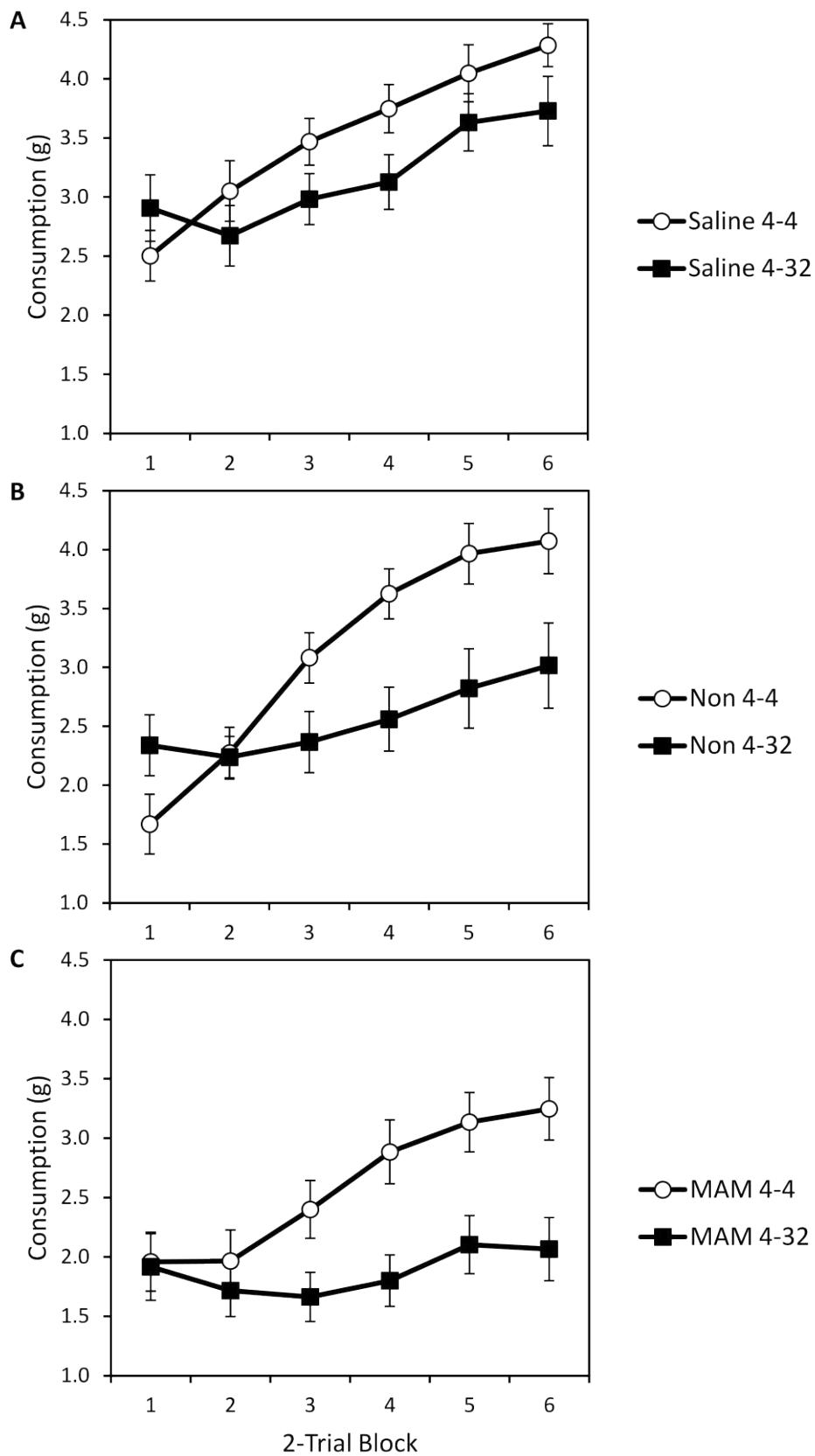


Figure 3 Mean (\pm SEM) consumption data from the first bottle available each day as a factor of contrast condition (4-4 vs 4-32 - numbers refer to the concentration of sucrose presented first and second in each daily drinking session) for saline-treated control (Panel A), non-exposed control (Panel B) and MAM-treated experimental rats (Panel C). The data is presented averaged over two-trial blocks. N = 16 in each condition.

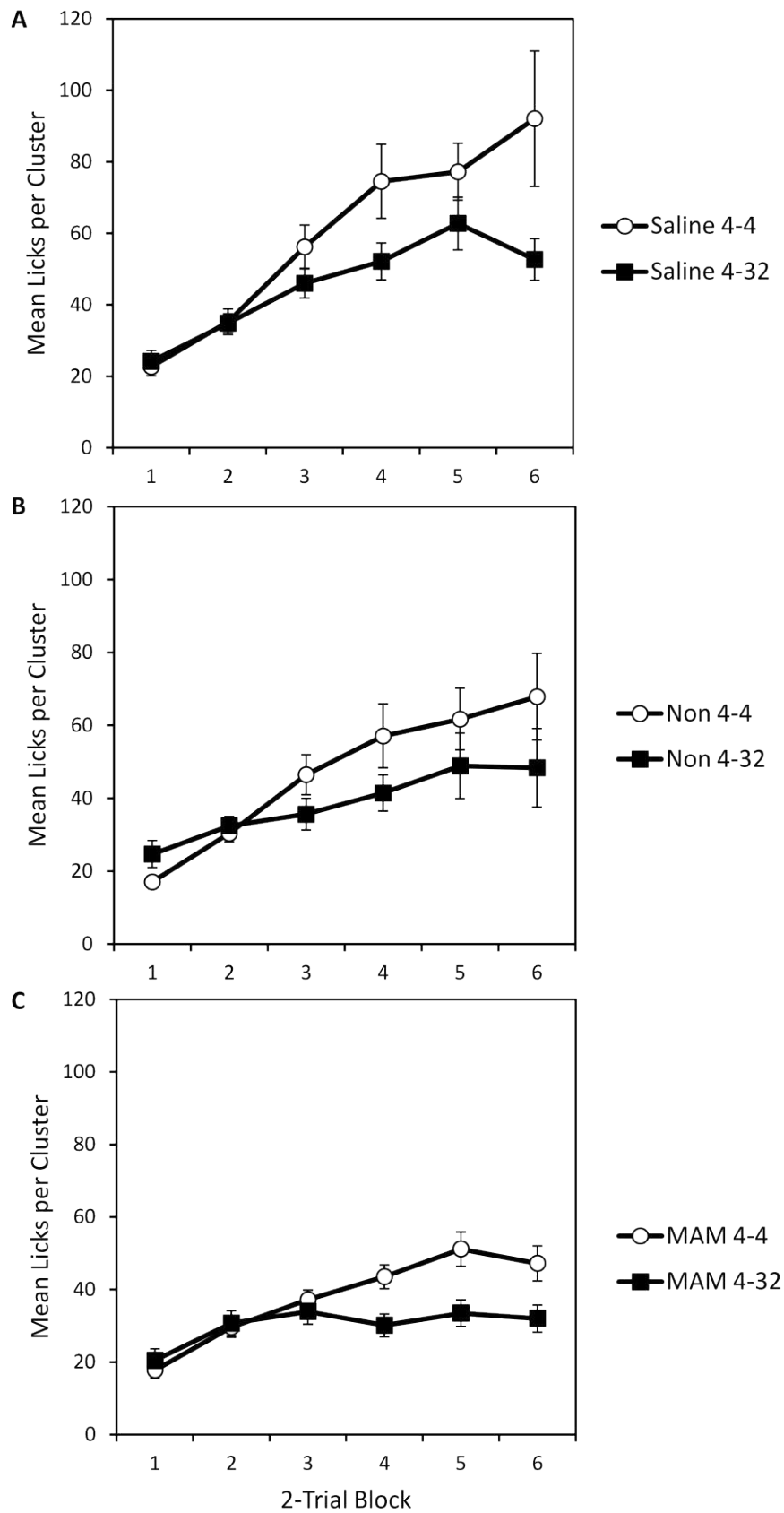


Figure 4 Mean (\pm SEM) Lick Cluster Size data from the first bottle available each day as a factor of contrast condition (4-4 vs 4-32 - numbers refer to the concentration of sucrose presented first and second in each daily drinking session) for saline-treated control (Panel A), non-exposed control (Panel B) and MAM-treated experimental rats (Panel C). The data is presented averaged over two-trial blocks. N = 16 for all groups.

LLIs were extracted from the record of licks and are summarised in Figure 5. Data are displayed in one figure panel as similar LLIs were displayed by all treatment groups. ANOVA analysis with factors of block, contrast condition and treatment revealed a main effect of block ($F(2, 188, 98.447) = 49.682, p < .001, MSE = 8104.750$) and a main effect of contrast condition ($F(1, 45) = 10.817, p = .002, MSE = 1256.218$). The main effect of block is likely driven by the lower LLIs found at block one, which results from the bottle spout being positioned within the chamber. The spout was progressively moved backwards with normal spout position achieved by block two. There was no block \times contrast condition interaction ($F(1.553, 69.879) = 2.968, p = .071, MSE = 657.985$), no main effect of treatment ($F(2, 45) = 2.303, p = .112, MSE = 1299.285$) and no interaction between treatment and any other factor: block \times treatment ($F < 1$); contrast condition \times treatment ($F(2, 45) = 2.123, p = .131, MSE = 246.556$; block \times contrast condition \times treatment ($F(10, 225) = 1.028, p = .421, MSE = 70.759$).

Figure 6 (Panels A - C) depicts the mean consumption levels for the second solution (4% or 32% sucrose) which was presented in the final six min of each daily drinking session. Again, data are collated into 2-trial blocks. As expected, consumption of 32% sucrose was higher than that of 4% sucrose. Whilst this pattern of consumption was consistent across the three treatment groups, saline-treated rats appeared to consume more overall. ANOVA was consistent with these impressions revealing a significant main effect of block ($F(2.680, 120.582) = 156.400, p < .001, MSE = 301.000$), a main effect of solution concentration ($F(1, 45) = 206.771, p < .001, MSE = 1499.787$) and a main effect of treatment ($F(2, 45) = 5.715, p = .006, MSE = 104.249$) with saline-treated rats consuming

significantly more than MAM rats ($F(1, 46) = 11.414, p = .002, MSE = .190$) and non-significantly more than non-exposed rats ($F(1, 46) = 3.192, p = .081, MSE = .190$). Consumption levels did not differ significantly between MAM-treated and non-exposed animals ($F(1, 46) = 2.534, p = .118, MSE = .190$). There was no block \times solution concentration interaction ($F(2.951, 132.812) = 1.987, p = .120, MSE = 3.086$), no block \times treatment interaction ($F < 1$), no solution concentration \times treatment interaction ($F(2, 45) = 1.822, p = .173, MSE = 13.215$) and no block \times solution concentration \times treatment interaction ($F(10, 225) = 1.486, p = .145, MSE = 1.363$).

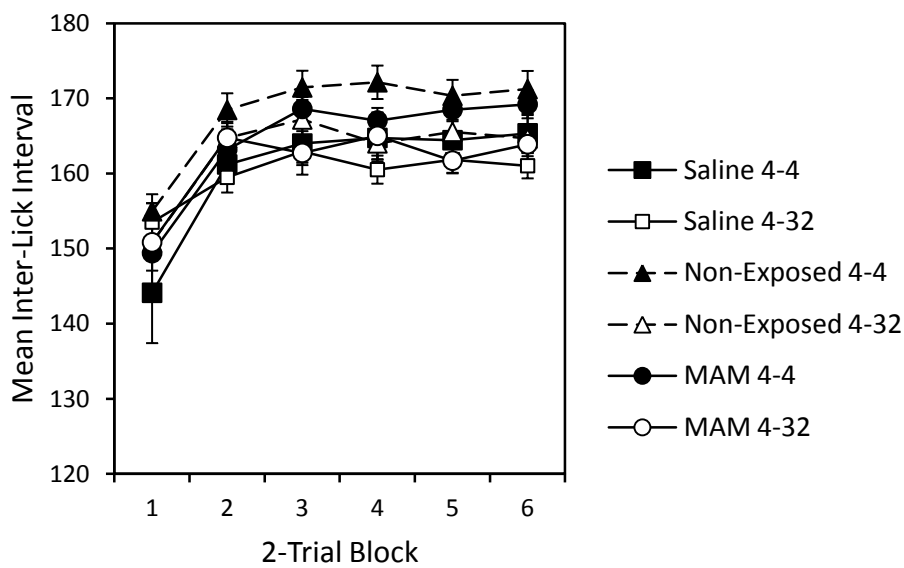


Figure 5 Mean inter-lick intervals from the first bottle available each day as a factor of contrast condition (4-4 vs 4-32 (numbers refer to the concentration of sucrose presented first and second in each daily drinking session) and treatment. Filled symbols represent the inter-lick interval to 4% sucrose in the 4-4 condition; open symbols represent the inter-lick interval to 4% sucrose in the 4-32 condition. Squares represent the responses of saline-treated rats, Triangles represent the responses of non-exposed rats and Circles represent the responses of MAM-treated experimental rats. Data is collated into 2-trial blocks. N = 16 for all groups.

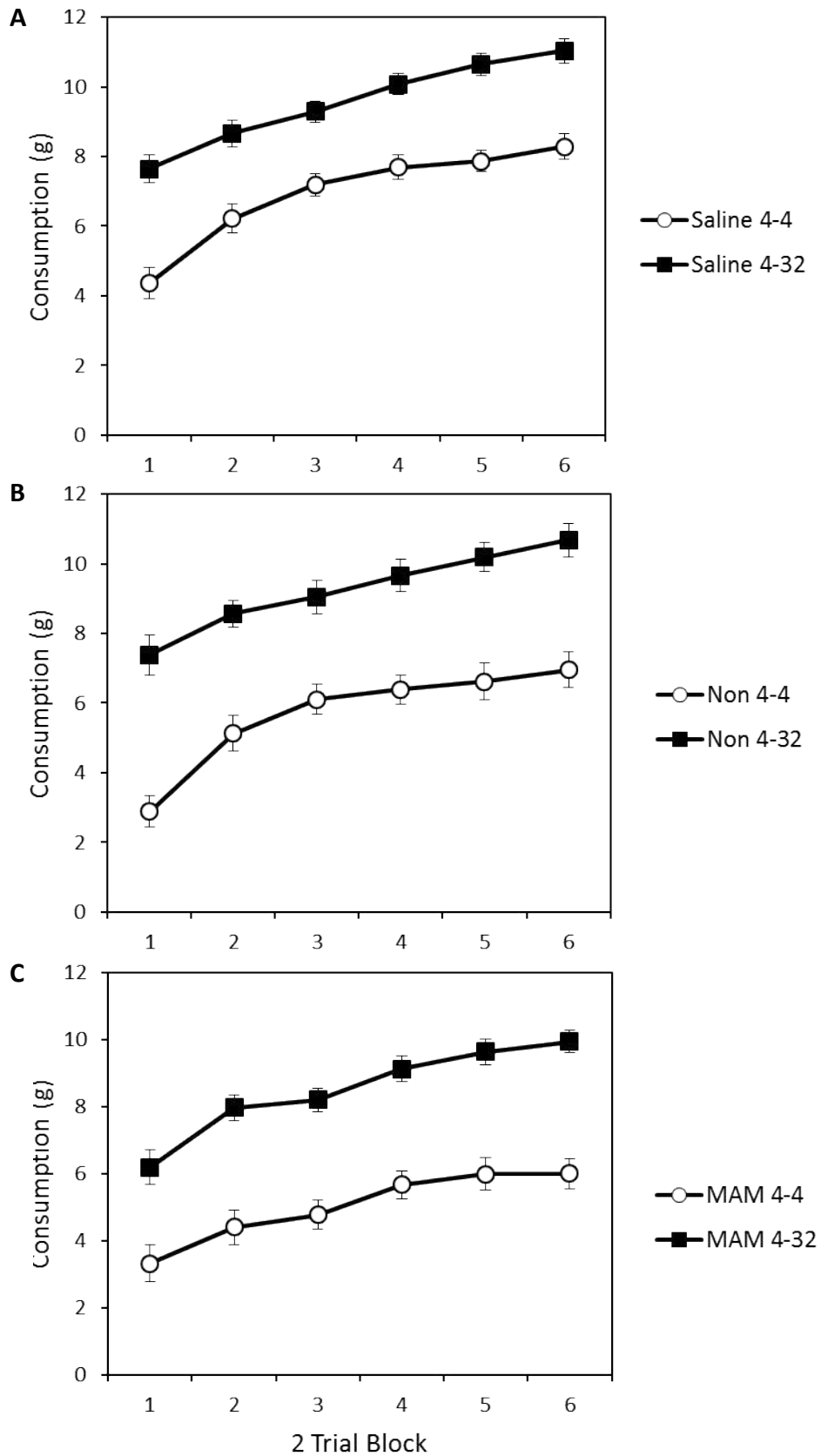


Figure 6 Mean consumption (\pm SEM) for the second bottle made available each day for saline treated (Panel A), non-exposed (Panel B) and MAM-treated (Panel C) rats. Solutions in the second bottle were made available for 6 min. In all cases, open symbols represent the rat's intake of the 4% sucrose solution, whereas the filled symbols represent the rat's intake of the 32% sucrose solution made available. Data is collated into 2-trial blocks. N = 16 for all groups.

Inspection of Figure 7 (Panel A - C) suggests that 32% sucrose was associated with higher lick cluster sizes. Again, it appears that LCS was generally higher for saline-treated rats compared to the other treatment groups. ANOVA results showed a significant main effect of block ($F(2, 163, 97.328) = 28.714, p < .001, MSE = 74856.913$), solution concentration ($F(1, 45) = 32.919, p < .001, MSE = 155385.671$) and a significant interaction between these two factors ($F(2, 198, 98.904) = 10.822, p < .001, MSE = 24903.632$). There was also a main effect of treatment ($F(2, 45) = 3.439, p = .041, MSE = 25922.827$) with saline-treated rats displaying significantly more licks per cluster than both non-exposed ($F(1, 46) = 5.476, p = .024, MSE = 78.517$) and MAM rats ($F(1, 46) = 4.818, p = .033, MSE = 78.517$). No significant difference was seen for mean licks per cluster between MAM-treated and non-exposed animals ($F < 1$).

Inter-lick intervals were extracted from the record of licks (Figure 8). ANOVA revealed a main effect of block ($F(1, 600, 72.022) = 108.213, p < .001, MSE = 16919.254$), a main effect of solution concentration ($F(1, 45) = 66.026, p < .001, MSE = 6277.663$) and a block \times solution concentration interaction ($F(1, 698, 76.405) = 4.750, p = .015, MSE = 480.213$). There was no significant main effect of treatment ($F(2, 45) = 1.508, p = .232, MSE = 837.055$) and no solution concentration \times treatment interaction ($F < 1$). There was, however, a significant block \times treatment interaction ($F(10, 225) = 2.922, p = .002, MSE = 146.243$), further analysis of which revealed that ILI was higher for non-exposed rats than MAM rats for block 1 only ($F(1, 46) = 5.764, p = .021, MSE = 12.048$).

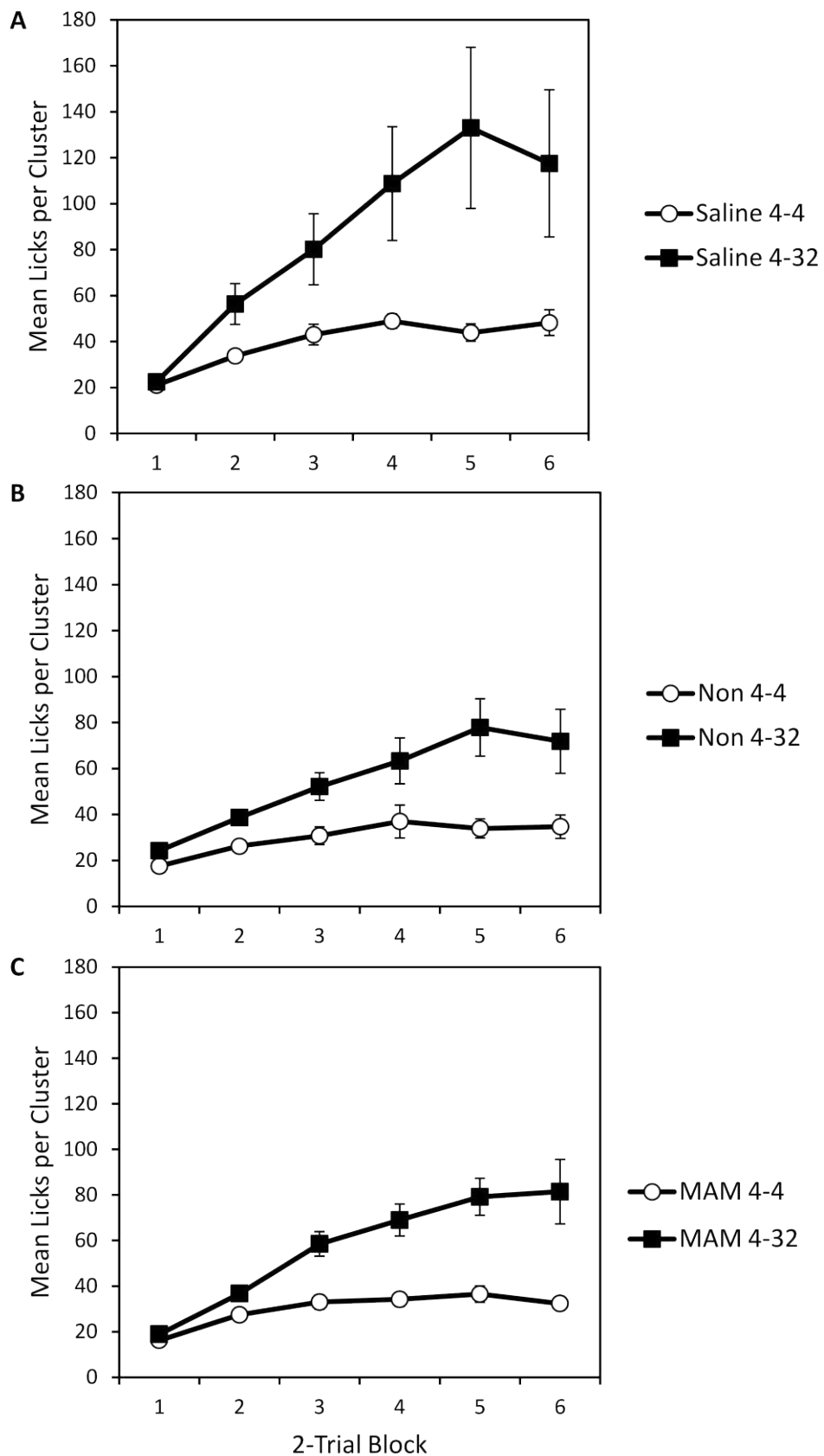


Figure 7 Mean Lick Cluster Size (\pm SEM) for the second solution available (4% vs. 32%) for saline-treated (Panel A), non-exposed (Panel B) and MAM-treated rats (Panel C). The second solution was made available for 6 min each day. In all cases, open symbols represent the rat's response to the 4% sucrose solution made available in the second bottle, whereas the filled symbols represent the rat's response to the 32% sucrose solution made available in the second bottle. Data is collated into 2-trial blocks. N = 16 for all groups.

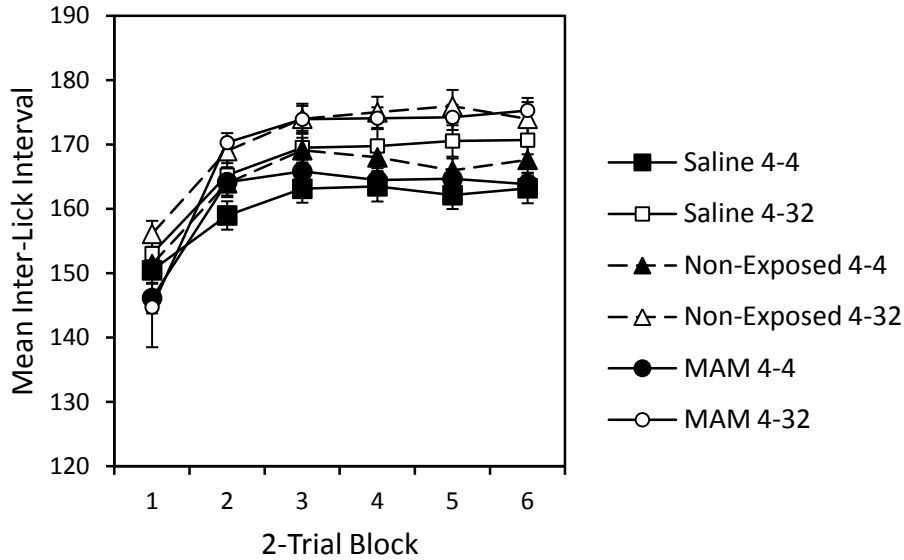


Figure 8 Mean Inter-lick Intervals (\pm SEM) as a factor of treatment and solution concentration (4% vs. 32%) for the second solution made available each day. Filled symbols represent the inter-lick interval to 4% sucrose; open symbols represent the inter-lick interval to 32% sucrose. Squares represent the responses of saline-treated rats, Triangles represent the responses of non-exposed rats and Circles represent the responses of MAM-treated experimental rats. Data is collated into 2-trial blocks. N = 16 for all groups.

3.6.1 Additional analysis

While the analysis of anticipatory contrast did not suggest any detrimental effect of MAM treatment, the overall trend of higher lick cluster sizes in the saline control compared to the MAM group suggests a potential inconsistency with the results of Experiment two. However, the difference in lick cluster size between the saline and non-exposed controls makes this result hard to interpret. Therefore, this cohort of animals was re-tested in the absence of contrast. The experiment was conducted as described for cohort two (Experiment two, Section 3.2.3), with three exceptions: Firstly, the solution concentrations used were 2, 6 and 18% sucrose (because rats had already been exposed to 4% sucrose during negative anticipatory contrast); secondly, drinking sessions were 10 min in duration (as very high consumption levels had been previously seen) and thirdly, each

concentration was run for three consecutive days only (deemed appropriate due to the rats previous drinking experience with sucrose). The animals were approximately 28 weeks old at the start of the experiment and remained on a food-restricted diet as previously described (see Table 5 for cohort characteristics at this age). A single pre-training session was given prior to the experiment start to re-familiarise the rats with the drinking equipment. This was deemed necessary due to the intervening period between the contrast and non-contrast drinking experiments (see Appendix A for details). During pre-training, water restricted rats (22 h/day maximum) were exposed to water for 10 min in the drinking chambers. Rats were then returned to an ad lib water supply for the remainder of the experiment.

Table 5 Characteristics of cohort 3. Data is taken from the first day of the experiment (after pre-training). Rats are on a food restricted diet to maintain them at 85% of their free feeding weights.

	MAM-Exposed	Saline-Exposed	Non-Exposed
Age	~ 28 weeks		
Weight range (g)	391 - 528	403 - 540	411 - 582
Mean ad-lib weight (g)	468	466	488
Weight comparison (Independent (2-tailed) T-test)	MAM vs. Saline $t(30) = -.096, p = .924$ MAM vs. Non Exposed $t(30) = 1.313, p = .199$ Saline vs. Non-Exposed $t(30) = -1.451, p = .157$		

Similarly to Experiment two (Section 3.2.4) data were analysed by a repeated measures ANOVA with a within subject factor of concentration (2, 6, 18%) and between subject factors of treatment (saline, non-exposed and MAM) and sequence order (2-6-18 or ascending vs. 18-6-2 or descending). To overcome potential neophobic effects, data were analysed averaged across the

second and third days of exposure for each solution concentration. Three animals (rats 9, 13 and 28 (two saline-treated and one non-exposed rat)) were excluded from all data analysis due to their LCSs being outside of 3 standard deviations of the mean. Rats 9 and 13 also displayed low ILIs relative to the group mean. Further inspection of the anomalous data points revealed very low numbers of bouts for these animals. The reason behind such infrequent but long bouts is unknown and has not been seen in previous or subsequent experiments.

3.6.2 Results

Figure 9A displays the mean consumption levels for saline-treated, non-exposed and MAM-treated rats in cohort three at each of the three different concentrations of sucrose: 2%, 6% and 18%. An ANOVA with factors of treatment, solution concentration and sequence-order revealed a main effect of concentration ($F(1.578, 61.539) = 155.711, p < .001, MSE = 895.305$). There was no main effect of sequence-order ($F < 1$) but a sequence order \times concentration interaction ($F(2, 78) = 6.187, p = .003, MSE = 28.066$). There was also a main effect of treatment ($F(2, 39) = 4.244, p = .021, MSE = 85.508$) with saline-treated rats consuming significantly more solution than MAM rats ($F(1, 39) = 8.400, p = .006, MSE = .910$) and non-significantly more than non-exposed rats ($F(1, 39) = 3.176, p = .083, MSE = .939$). No significant difference in intake was seen between MAM and non-exposed rats ($F(1, 39) = 1.240, p = .273, MSE = .869$). There was no treatment \times concentration interaction ($F < 1$), no treatment \times sequence-order interaction ($F < 1$) and no treatment \times concentration \times sequence order interaction ($F < 1$). Further analysis of the concentration \times sequence order interaction revealed

varying patterns of consumption depending on sequence order. That is, rats given the solutions in ascending order of concentration demonstrated an inverted U-shaped pattern of consumption with marginally higher consumption at the intermediate concentration of sucrose (2% < 6%, $F(1, 39) = 190.868$, $p < .001$, $MSE = .368$; 18% < 6%, $F < 1$; 2% < 18%, $F(1, 39) = 111.696$, $p < .001$, $MSE = .585$). In contrast, rats given the sucrose in descending order of concentration demonstrated increased consumption with increasing solution concentration (2% < 6%, $F(1, 39) = 72.590$, $p < .001$, $MSE = .391$; 6 < 18%, $F < 1$; 2% < 18%, $F(1, 39) = 54.456$, $p < .001$, $MSE = .621$). At no concentration were there any significant differences between intake levels due to sequence order effects (largest $F(1, 39) = 2.691$, $p = .109$, $MSE = 31.786$).

Figure 9B depicts the LCSs elicited by the three treatment groups in cohort three (saline, non-exposed and MAM) when consuming each of the three sucrose concentrations. Regardless of treatment, rats increased the size of their licking clusters as the solution consumed increased in concentration. An ANOVA with factors of treatment, concentration and sequence-order revealed a significant main effect of concentration ($F(1.697, 66.201) = 54.263$, $p < .001$, $MSE = 57411.452$), no main effect of treatment ($F < 1$) and no treatment \times concentration interaction ($F(4, 78) = 1.156$, $p = .337$, $MSE = 1037.740$). There was also no main effect of sequence order ($F(1, 39) = 2.908$, $p = .096$, $MSE = 11832.124$), no treatment \times sequence order interaction ($F < 1$), no concentration \times sequence order interaction ($F(2, 78) = 1.449$, $p = .241$, $MSE = 1301.607$) and no three-way interaction between treatment, concentration and sequence order ($F(4, 78) = 1.081$, $p = .372$, $MSE = 970.789$).

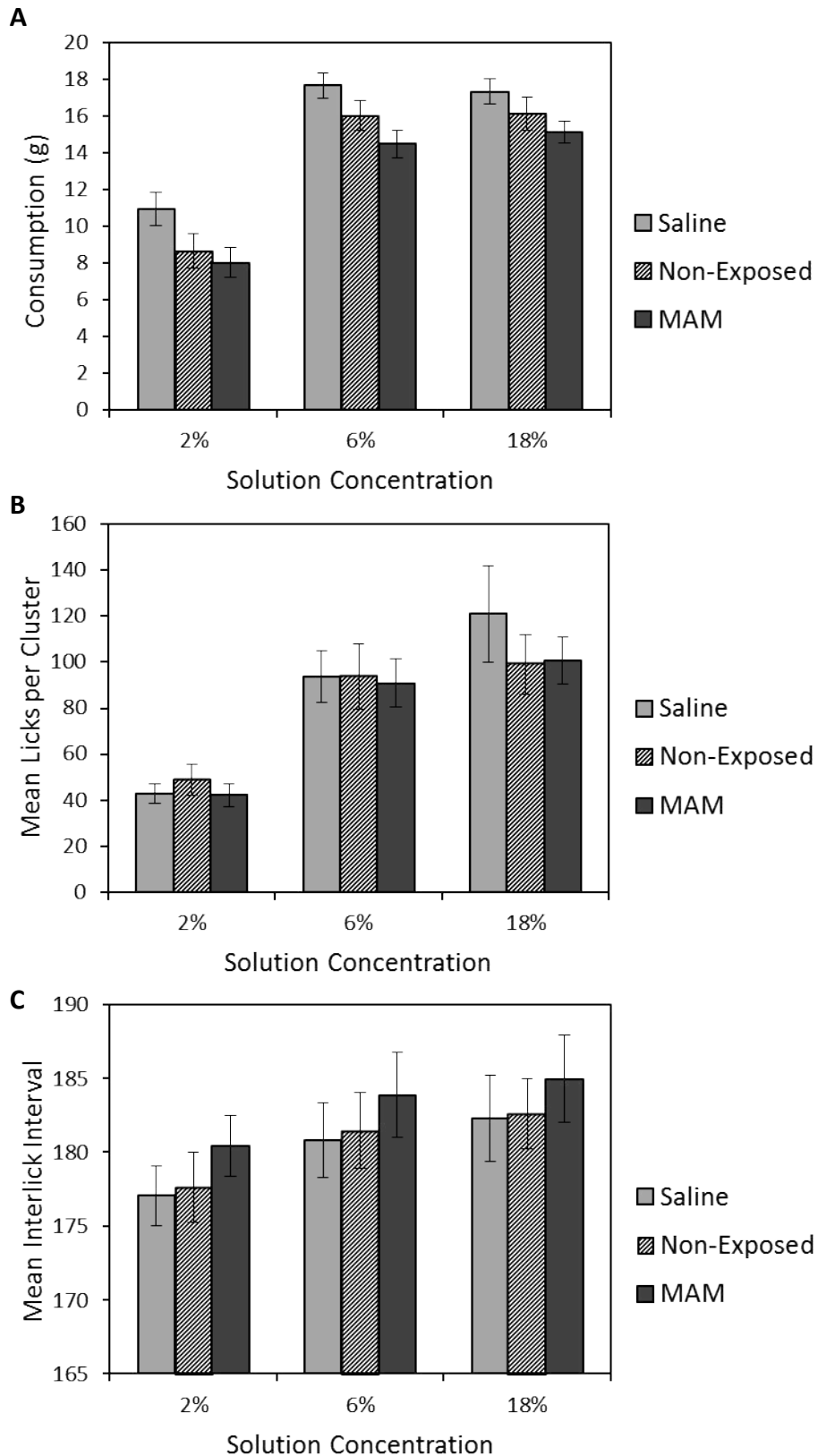


Figure 9 Mean Consumption (Panel A), mean Lick Cluster Size (Panel B) and mean Inter-lick intervals (Panel C) for three different concentrations of sucrose solution (2, 6 and 18%) for saline-treated (light grey bars), non-exposed (striped bars) and MAM-treated (dark grey bars) animals. Error bars represent \pm SEM. *N*: saline = 14, non-exposed = 15 and MAM = 16.

Figure 9C displays the mean inter-lick intervals extracted from the record of licks. An ANOVA on this data revealed a significant main effect of concentration ($F(2, 78) = 19.280, p < .001, MSE = 270.057$), no significant main effect of treatment ($F < 1$) and no main effect of sequence order ($F(1, 39) = 3.503, p = .069, MSE = 841.295$). There was also no treatment \times concentration interaction ($F < 1$). There was, however, a significant concentration \times sequence order interaction ($F(2, 78) = 16.435, p < .001, MSE = 230.210$) and a treatment \times concentration \times sequence order interaction that approached significance ($F(4, 78) = 2.478, p = .051, MSE = 34.709$). Further analysis of the concentration \times sequence order interaction revealed that ILI was lower for both 6 and 18% sucrose solutions for rats experiencing the solutions in order of decreasing concentration (18-6-2 sequence order) compared to increasing concentration (2-6-18 sequence order): 6% $F(1, 39) = 7.275, p = .010, MSE = 671.867$; 18% $F(1, 39) = 6.250, p = .017, MSE = 629.259$; 2% $F < 1$. There were no significant differences in ILI between the three solution concentrations in the descending order group (largest $F(1, 39) = 1.490, p = .230, MSE = .949$). In contrast, significant differences in ILI were seen between solutions of different concentrations for animals in the ascending order group, with ILI for 2% being significantly lower than for 6% and 18% (6%: $F(1, 39) = 45.207, p < .001, MSE = 1.290$; 18%: $F(1, 39) = 49.658, p < .001, MSE = 1.481$). No significant differences were seen between 6% and 18% concentrations ($F < 1$).

3.7 Summary

Experiment three investigated whether evidence of anticipatory anhedonia could be found in rodents prenatally exposed to MAM on GD17. Based on our current understanding of the hedonic deficits in schizophrenia, it is thought that people suffering from the disorder may be unable to anticipate or predict that future events will be pleasurable and adjust their ongoing behaviour in light of these predictions. The results of Experiment three provide further evidence that anticipatory contrast effects develop for both consumption and palatability measures. Lower consumption and lick cluster sizes were exhibited by the rats during the presentation of the initial 4% sucrose solution when it was followed by a more palatable 32% sucrose solution. Critically, the development of this contrast effect was not affected by prenatal MAM treatment. Thus, despite the biological and supposed phenotypic validity of the MAM model, there is no evidence that this model includes behaviours analogous to anticipatory anhedonia.

Unlike for Experiment two, MAM-exposed rats in the current experiment demonstrated reduced overall consumption and palatability responses for the (contrasted) solutions, but only when compared to saline-treated controls and not the non-exposed control. However, further analysis of the cohort in non-contrasted consumption tests did not reveal any differences between the MAM-treated rats and either of the control groups. This provides further evidence that the MAM model of schizophrenia does not produce consummatory hedonic deficits.

The general implications of these findings, combined with those from the analysis of other reward-processing studies, will be discussed at the end of Chapter four. It should be recognised that

the evaluation of anticipatory hedonic deficits in schizophrenia patients has produced some inconsistent findings (see Strauss et al., 2011). Therefore, the presence of an anticipatory deficit needs to be clarified in the clinical literature. A lack of anticipatory anhedonia after prenatal MAM treatment may highlight a short-coming of the model in recapitulating negative symptoms, or could reflect an absence of anticipatory anhedonia as a primary symptom of the disease. The critical deficit may lie in using reward values to guide action selection (see section 1.3.3 of the general introduction). Therefore, the possibility of aberrant encoding of rewards by MAM-exposed rats will be investigated in Chapter four.

Chapter Four

4. Value Representations in the MAM Model

4.1 Introduction

As was seen in Chapter three, MAM-treated rats showed no evidence of impaired hedonic reactions, consistent with the absence of consummatory and anticipatory anhedonia. Therefore, Chapter four examines the possible reward processing deficits beyond anhedonia in the MAM GD17 neurodevelopmental model.

As has been discussed in section 1.6.1 of the general introduction, both manipulations of the frontal cortex (section 1.6.1b) and repeated exposure to psychostimulants (section 1.6.1c) influence the transition from goal-directed to habitual behaviour. This suggests that this transition is under the

control of the type of 'frontal' and neurochemical mechanisms thought to be disrupted in schizophrenia patients. Furthermore, direct assessment of an outcome devaluation test in people with schizophrenia has demonstrated that integration of reward values with action selection may be impaired in these patients (Morris et al., 2015). Impaired performance in this task was accompanied by regional activity differences in the caudate between schizophrenia patients and healthy controls. Taken together, these impairments suggest that schizophrenia patients may over rely on habitual strategies, perhaps due to impaired cortico-striatal circuitry.

Experiment four used an outcome devaluation procedure (described in section 1.6.1a) to determine whether rats prenatally exposed to MAM are able to form and update value representations and use these representations to motivate behaviour. MAM-exposed animals and their saline-treated counterparts were trained on a random interval schedule of reinforcement before their sensitivity to outcome devaluation was tested in extinction. Outcome devaluation was achieved by LiCl-induced gastric malaise as this manipulation has been demonstrated to produce a larger and more robust devaluation effect compared to satiety manipulations (see Nelson & Killcross, 2006). If the animal's performance is goal-directed (i.e. sensitive to changes in the current value of the reward), then it should be reflected in a lowered willingness to perform the response associated with the devalued outcome. Sensitivity to the outcome devaluation manipulation in the absence of consummatory feedback provides evidence that the outcome is encoded as part of the associative framework driving behaviour and that this representation has been updated to reflect the change in reward value. Alternatively, if the animal's performance is habit-driven (i.e. insensitive to the change in value of the

reward) then the subject will perform the devalued action at an equivalent rate to animals for which the action has not been devalued. Under these circumstances, the outcome is not included as part of the associative framework controlling behaviour, with the animals performance instead being controlled by antecedent stimuli (Balleine & O'Doherty, 2010).

Whilst interval schedules are usually used to promote habitual responding, limited training can maintain goal-directed behaviour (as indexed by sensitivity to outcome devaluation e.g. Dickinson et al., 1995). Importantly, the use of three days training for a total of 120 rewards (adopted in the current experiment) has been shown to maintain sensitivity to outcome value in normal, untreated animals whereas amphetamine-treated animals display habitual behaviours (Nelson & Killcross, 2006). The use of a 30 s random interval schedule (RI30s) was employed in the current experiment as this reinforcement schedule maintains a high level of lever pressing during extinction (Nelson & Killcross, 2006), thus allowing good sensitivity to outcome devaluation manipulations in goal-directed animals.

Given the likelihood that MAM disrupted prefrontal cortex function and cortical-striatal dopamine systems (Flagstad et al., 2004; Lavin, Moore, & Grace, 2005) it might be expected that MAM-treated animals would undergo a faster transition to habitual behaviour, exhibiting insensitivity to the outcome devaluation manipulation after three days of training.

4.2 Experiment 4 - Materials and Methods

4.2.1 Subjects

The two cohorts combined in Experiment two were also using in the current experiment (see Appendix A for experiment order for each cohort). At the start of the experiment rats in cohort one (n: Sham, 18; MAM, 24) were approximately 17 weeks old with saline-treated animals weighing between 393 – 543 g and MAM-treated animals weighing between 376 – 455 g (ad libitum weights). Rats in cohort two were approximately 16 weeks old at the start of the experiment. Saline-treated animals weighed between 326 – 486 g and MAM-treated animals weighed between 374 – 470 g (ad libitum weights). For all aspects of animal husbandry, please refer to Experiment two. All animals were food restricted to ~85% of their free-feeding weights prior to the experiment start. Moderate weight gain was allowed over the course of the experiment.

4.2.2 Apparatus

The training apparatus comprised eight chambers (Med Associates Inc., St Albans, VT), each measuring 30 × 24 × 21 cm, arranged in a 2 × 4 array. The door, back wall and ceiling were made of clear Perspex while the left and right-hand walls were made of aluminium. Chocolate flavoured sugar pellets (45 mg; Test Diet, Richmond, IN) were delivered into a recessed magazine located at the centre of the right-hand wall. Access to the magazine could be determined by the means of infrared detectors mounted across the mouth of the recess. Flat-panel retractable levers could be made

available to both the left and right of the magazine, although only the left lever was used in this experiment. Each chamber was housed within a sound-attenuating cabinet ventilated by low-noise fans. A computer equipped with MED-PC (Med Associates) software controlled the equipment and recorded the data.

4.2.3 Procedure

Training consisted of two stages: magazine training and lever-press training. This was followed by an extinction test after devaluation of the instrumental reward by LiCl-induced nausea. Each rat was assigned to one of the eight experimental chambers, and thereafter always trained in the same chamber (see Figure 10).

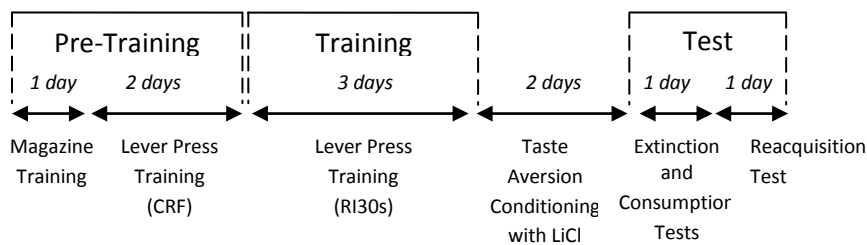


Figure 10 Schematic depicting the experimental protocol for the Outcome Devaluation Task with limited levels of training.

Magazine training. All rats were trained to collect food rewards during a single magazine training session. Chocolate flavoured sugar pellets were delivered on a random time (RT) 60s schedule whereby a single pellet was delivered, on average, every 60 s. The session ended once 20 pellets had been delivered. Rats that did not collect the food were given a repeat of the session later the same day.

Lever-press training. Two initial sessions of lever press training were given during which chocolate flavoured sugar pellets were delivered on a continuous (CRF) schedule of reinforcement (i.e. each lever press led to reward delivery). The lever was inserted into the chamber at the start of the session and retracted at the end of the session. The session ended once the rat had earned 20 reinforcers. In the subsequent three sessions of training, chocolate pellets were now delivered according to a random interval (RI) 30 s schedule whereby the reward was made available, on average, every 30 s and delivered on the next lever press. During this RI30s training the session ended once rats had earned 40 reinforcers.

Devaluation by LiCl. Animals received two days of devaluation with LiCl. On each day, the rats were placed in individual cages measuring 32 × 15 × 12 cm in a separate test room and given free access to the chocolate pellets for 30 min. Once 30 minutes had past, the devalued group (Cohort one: 12 MAM-treated, 9 Saline-treated; Cohort two: 12 MAM-treated, 12 saline-treated) received intraperitoneal injections of 0.15 M, 15ml/kg LiCl solution (dissolved in deionised water, LiCl from Sigma-Aldrich). The non-devalued group (Cohort one: 12 MAM-treated, 9 saline-treated; Cohort one: 12 MAM-treated, 12 saline-treated) were given intraperitoneal injections of the equivalent volume of 0.9% saline. Animals were matched according to lever press levels during the final day of acquisition to determine their devaluation group. Twenty-four hours after the second taste aversion session, the animals' sensitivity to outcome devaluation was assessed via a 10 min extinction test. Rats were given the opportunity to lever press during this session but no rewards were available.

Consumption test. Immediately after the extinction session, all rats underwent a consumption test to ensure that the devalued group had acquired an aversion to the instrumental outcome. During this, animals were placed in test cages and given 30 min free access to the reward.

Reacquisition test. Twenty-four hours after the extinction and consumption tests, rats were given a further session in the instrumental chambers. Sessions lasted 25 min and rats were allowed to lever press on a RI30s schedule of reinforcement with chocolate flavoured pellets delivered.

4.2.4 Data analysis

Statistical analysis was performed using ANOVA with between-subject factors of replication (cohort one vs. cohort two), treatment (saline treated vs. MAM treated), and devaluation condition (devalued vs. valued). Lever presses per minute and magazine approaches per minute during the extinction test were calculated as a proportion of baseline. Baseline was taken as the data from the final day (day three) of acquisition.

4.3 Results

Instrumental training. Across the three days training, MAM-treated and saline-treated rats acquired the lever press response at the same overall rate. Importantly, there were no differences in baseline lever press responses (day three) as a function of devaluation condition (see Table 6).

Consistent with this impression, ANOVA analysis on lever press responses (day three only) revealed

no effect of prenatal treatment ($F < 1$) or of devaluation condition ($F < 1$) and no interaction between these two factors ($F < 1$). There was no significant main effect of replication ($F(1, 82) = 2.582, p = .112, MSE = 101.283$) and no interactions between replication and any other factor (All $F_s < 1$, except for the replication \times condition interaction where $F(1, 82) = 2.528, p = .116, MSE = 99.178$).

Table 6 Lever press responses per minute for day three of acquisition (used as 'baseline').

Prenatal Treatment	Devaluation Condition	Mean Lever press/min (\pm SEM)
Saline	Valued	20.069 (\pm 1.500)
	Devalued	18.562 (\pm 1.147)
MAM	Valued	19.004 (\pm 1.418)
	Devalued	18.939 (\pm 1.232)

In terms of magazine approach behaviour (see Table 7), analysis suggested no effect of prenatal treatment. Importantly, there also appeared to be no differences in baseline magazine entries between the valued and to-be-devalued conditions. In line with this impression, ANOVA revealed no main effect of treatment ($F < 1$), devaluation condition ($F < 1$), and no interaction between these two factors, $F(1, 82) = 1.883, p = .174, MSE = 39.814$. There was a main effect of replication ($F(1, 82) = 6.608, p = .012, MSE = 139.692$) with higher magazine entries elicited by rats in cohort two (Mean of 16.220 entries per min) compared to cohort one (Mean of 13.708 entries per min). Replication did not interact with any factor or combination of factors (All $F_s < 1$).

Table 7 Magazine approach behaviour as responses per minute for day three of acquisition (used as 'baseline').

Prenatal Treatment	Devaluation Condition	Mean Magazine Entries/min (\pm SEM)
Saline	Valued	15.132 (\pm 1.033)
	Devalued	14.472 (\pm 0.913)
MAM	Valued	14.324 (\pm 0.737)
	Devalued	16.284 (\pm 1.188)

Extinction – Lever press performance. The mean lever press response rates as a proportion of baseline for the 10 min extinction test are displayed in Figure 11A. Both MAM- and saline treated animals' performance was sensitive to post-conditioning changes in the value of the instrumental outcome. That is, regardless of prenatal treatment, animals performed fewer lever presses as a proportion of baseline when the outcome was previously paired with LiCl-induced nausea (devalued condition – grey bars) compared with those animals where the outcome was not previously paired with LiCl-induced nausea (valued condition – white bars).

The description of the data was confirmed by statistical analysis. Between-subjects ANOVA with factors of replication (cohort 1 vs. cohort 2), treatment (saline-treated vs. MAM-treated) and devaluation condition (valued vs. devalued) yielded a main effect of devaluation condition ($F(1, 82) = 7.417, p = .008, MSE = 2.503$), no main effect of treatment ($F(1, 82) = 1.375, p = .244, MSE = .464$) and no devaluation \times treatment interaction ($F(1, 82) = 1.653, p = .202, MSE = .558$). The ANOVA also yielded no main effect of replication ($F(1, 82) = 2.538, p = .115, MSE = .856$) and replication did not interact with any other factor: briefly, replication \times treatment ($F(1, 82) = 2.042, p = .157, MSE = .689$); replication \times condition ($F < 1$); replication \times treatment \times condition ($F(1, 82) = .707, p = .403, MSE = .239$).

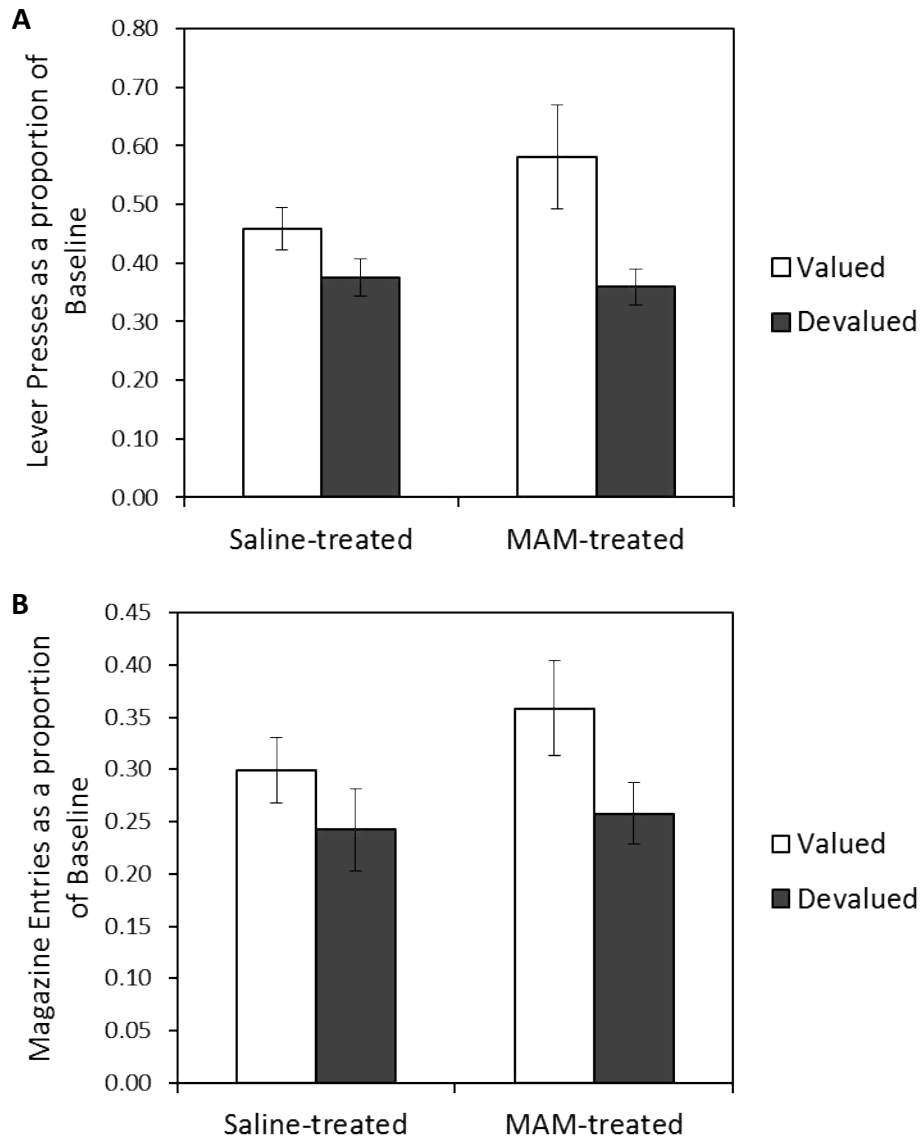


Figure 11 Effect of prenatal treatment on sensitivity of lever pressing (Panel A) and magazine entries (Panel B) to reward devaluation by LiCl-induced nausea. Mean lever presses/ magazine entries per minute as a proportion of baseline (\pm SEM) in the extinction test are shown, where grey bars represent responses in the devalued condition and white bars represent responses in the valued condition. N = 21, saline-treated rats and 24, MAM-treated rats.

Extinction – Magazine approach performance. Figure 11B shows magazine approach

behaviour as a proportion of baseline during the 10 min extinction test. As indicated in the figure,

animals with an aversion to the reinforcer (devalued condition – grey bars), regardless of prenatal

treatment, performed fewer magazine entries compared to animals not averted to the reinforcer

(valued group – white bars). ANOVA was consistent with this impression: whilst there was a main effect of devaluation condition ($F(1, 82) = 4.795, p = .031, MSE = .711$), there was no main effect of prenatal treatment ($F(1, 82) = 1.511, p = .222, MSE = .224$) and no interaction between these two factors ($F < 1$). The ANOVA also yielded a main effect of replication ($F(1, 82) = 7.785, p = .007, MSE = 1.154$) with rats in the cohort two undergoing more magazine entries as a proportion of baseline compared to rats in cohort one (.337 and .235, respectively). The factor of replication did not interact with any other factor ($F < 1$).

Outcome devaluation. The data from the devaluation phase are presented in Figure 12.

Taste aversion learning was not affected by prenatal MAM treatment as LiCl injection produced a strong aversion to the instrumental outcome in both treatment groups. In contrast, all animals in the valued group (where the outcome had been paired with a saline injection) continued to consume the outcome across sessions. An ANOVA with factors of session (day one, day two and test), replication (cohort one vs. cohort two) prenatal treatment (MAM-treated vs. saline treated) and devaluation condition (valued vs. devalued) was performed. This revealed a significant main effect of devaluation ($F(1, 82) = 160.618, p < .001, MSE = 1147.445$), session ($F(1.621, 132.884) = 152.280, p < .001, MSE = 776.115$) and a significant interaction between these two factors ($F(2, 164) = 54.115, p < .001, MSE = 223.475$), reflecting the development of the aversion across sessions. There was no main effect of prenatal treatment ($F < 1$) and no significant interaction between treatment and any other factor ($F < 1$). The ANOVA did yield a main effect of replication ($F(1, 82) = 64.331, p < .001,$

$MSE = 459.578$) with rats generally consuming more across the sessions in cohort two (mean of 6.335 g) compared to cohort one (mean of 3.706 g). Importantly, there was no replication \times treatment interaction ($F < 1$). Significant interactions (e.g. the replication \times condition interaction ($F(1, 82) = 9.704, p = .003, MSE = 69.322$)), were all driven by lower overall consumption for cohort one. Importantly, inspection of the replication \times condition interaction revealed that rats in the devalued condition consumed significantly less than their non-devalued counterparts in both cohort one ($F(1, 82) = 42.419, p < .001, MSE = 101.013$) and cohort two ($F(1, 82) = 125.026, p < .001, MSE = 321.540$).

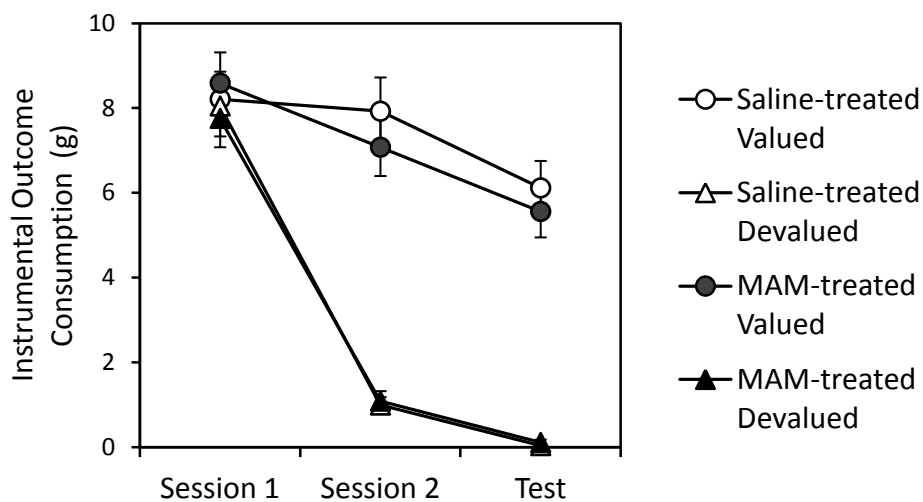


Figure 12 Mean chocolate pellet consumption (\pm SEM) over 2 days of taste aversion training and 1 post-extinction consumption test for MAM- and saline-treated rats. Rats received LiCl injections (Devalued) or saline injections (Valued) after 30 min free access to the instrumental outcome. The test phase also took place in a 30 min period immediately after the 10-min extinction test.

Reacquisition Test – Lever Press Performance. The mean lever press responses per minute for the 25 min rewarded reacquisition test are shown in Figure 13. Inspection of the figure shows that rats in the devalued group, regardless of prenatal treatment, performed fewer responses compared to

rats in the valued group. Statistical analysis by a between-subject ANOVA with factors of replication, prenatal treatment and devaluation condition yielded a significant main effect of devaluation condition ($F(1, 82) = 86.413, p < .001, MSE = 1411.552$). The effect of aversion conditioning on lever press performance during this test was comparable across prenatal treatment groups as there was no main effect of treatment ($F < 1$) and no treatment \times devaluation condition interaction ($F < 1$). The ANOVA also revealed that cohorts one and two responded similarly on the lever during the reacquisition test, as there was no main effect of replication and no interaction between replication ($F < 1$) and any other factor ($F_s < 1$, except for the replication \times condition interaction where $F(1, 82) = 1.062, p = .306, MSE = 17.351$).

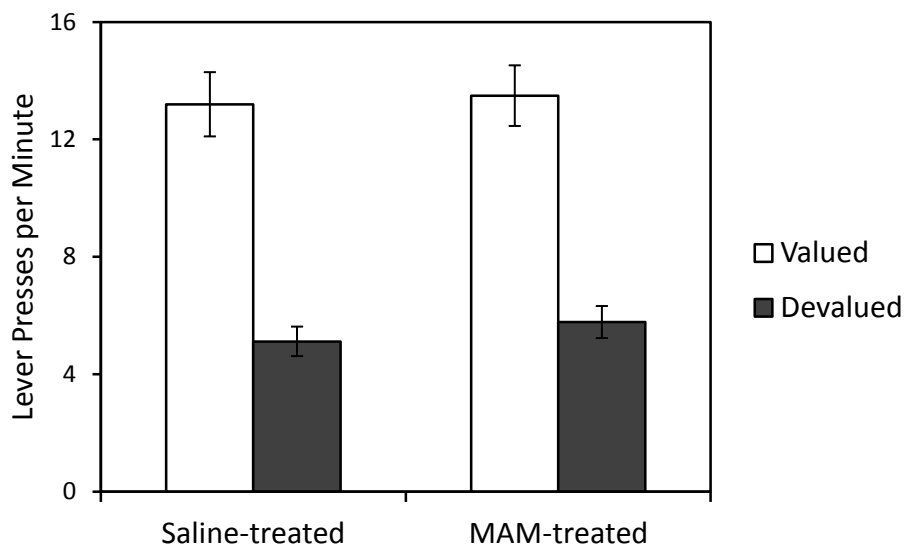


Figure 13 Effect of prenatal treatment (saline vs. MAM) on lever press reacquisition after reward devaluation by LiCl-induced nausea. Mean lever presses per minute (+ SEM) in the rewarded reacquisition test after devaluation with LiCl (grey bars) or no devaluation (white bars) are shown. The reacquisition test was given 24 hours after the extinction and consumption tests and lasted for 25 min.

Reacquisition Test – Magazine Approach Behaviour. The mean number of magazine entries per minute (\pm SEM) for saline-treated and MAM-treated rats was as follows: Saline-treated Valued =

10.844 (\pm .934); Saline-treated Devalued = 5.037 (\pm .562); MAM-treated Valued = 9.370 (\pm .446); MAM-treated Devalued 6.121 (\pm .682). Rats in both devalued groups showed a marked suppression in their magazine approach behaviour compared with rats in the equivalent valued group. In support of this, ANOVA yielded a main effect of devaluation condition ($F(1, 82) = 43.190, p < .001, MSE = 451.417$), no main effect of treatment ($F < 1$) and no treatment \times condition interaction ($F(1, 82) = 3.394, p = .069, MSE = 35.474$). There was also no main effect of replication ($F < 1$) and replication did not interact with any other factor (All $F_s < 1$).

4.4 Summary

Experiment four assessed the control of instrumental actions in MAM-treated rats after limited levels of training. The rats' sensitivity to reward value was assessed by a post-training devaluation procedure before testing in extinction. Both MAM-treated rats and saline-treated controls in the devalued group displayed a reduction in their lever press behaviour compared to rats in the non-devalued group, suggesting that their behaviour was still contingent on outcome value (i.e. goal-directed). If anything, the difference in response rates between valued and devalued groups was more pronounced for rats prenatally exposed to MAM. Contrary to the original prediction, rats prenatally exposed to MAM on GD17 are unimpaired in their ability to use and update internal representations of reward value to modify ongoing behaviour.

As intended (see section 1.6.2 of the general introduction), this result was followed with a differential outcomes procedure in Experiment five. As this procedure affords a reversal of the

conditional discrimination, and reversal deficits have previously been reported for MAM-treated animals (e.g. Featherstone et al., 2007; Flagstad, Glenthøj & Didriksen, 2005; Gastambide, et al., 2012; Moore et al., 2006), Experiment five also incorporated a reversal phase.

4.5 Experiment 5 - Materials and Methods

4.5.1 Subjects

Cohort three (16 saline-treated, 16 non-exposed and 16 MAM-treated rats) was used for Experiment six (please refer to Appendix A to see the timing of this experiment). At the start of the experiment, rats were approximately 19 weeks old. Saline treated animals weighed between 351 – 430 g, non-exposed animals weighed between 345 – 475 g and MAM-treated animals weighed between 300 – 430g. Animals remained on food deprivation from the previous experiment (reported as Experiment three in this thesis). Moderate weight gain was allowed throughout to match the expected increase of free-feeding animals. For all aspects of animal husbandry, please see Experiment two.

4.5.2 Apparatus

The same eight experimental chambers used in Experiment Four were also used in the current experiment. Now, however, the chambers were illuminated by a 3-W light bulb positioned at the top centre of the left wall. Two flat-panel retractable levers could be inserted to the left and right

of the central magazine positioned on the right wall. Distinct rewards, a single plain food pellet (45 mg) and a 20% (w/w) sucrose solution (made daily) could be delivered into the same magazine. The sucrose solution was delivered at a volume of 0.05 ml by a motorized dipper. Auditory stimuli consisted of a 3 kHz tone (delivered at 80 dB) and a 10 Hz train of clicks delivered from speakers located at the top left and right of the left-hand wall, respectively.

4.5.3 Procedure

Each rat was assigned to one of the eight experimental chambers, and thereafter always trained in the same chamber. Training consisted of three stages: magazine training, lever press training and acquisition training on a continuous performance conditional discrimination task (see Figure 14). Rats then underwent a test session performed in extinction and a reinforcer-only test. The two test sessions were separated by one day of reacquisition training. Following a further day of reacquisition training, all rats entered the reversal phase of the experiment.

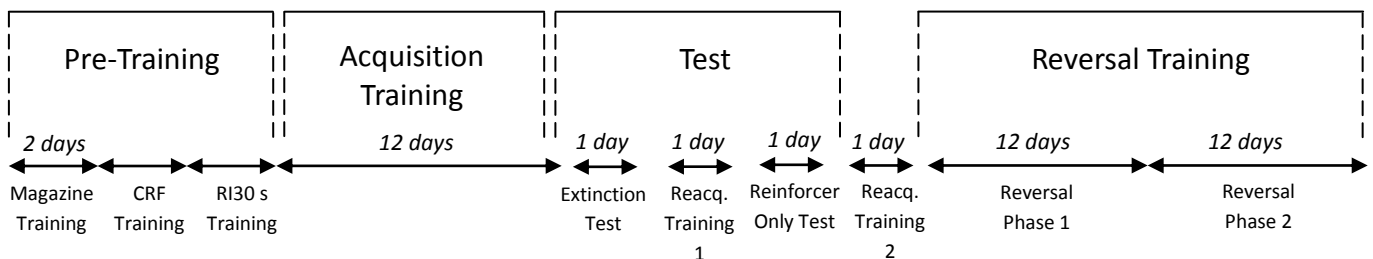


Figure 14 Schematic depicting the experimental protocol for the differential outcomes procedure.

Magazine training. Rats were trained to collect the two different food rewards across consecutive days. Half of the rats (equal numbers of MAM-exposed, saline-exposed and non-

exposed rats) were trained to collect the food pellet reward on day one and the sucrose solution reward on day two, while the other half were trained in the reverse order. Rewards were delivered on a random time (RT) 60s schedule and sessions finished once 20 rewards had been delivered.

Lever-press training. Rats were initially trained to lever press on a continuous reinforcement (CRF) schedule, where each lever press led to reward delivery. Half the rats were trained on the left lever on day one and the right lever on day two. The other half was trained on the right lever on day one and the left lever on day two. For each day, the appropriate lever was inserted into the chamber at the start of the session, and retracted at the end of the session. Lever presses were reinforced with pellet and sucrose rewards at equal probability. The session ended once the rats had received 40 rewards.

Across the next two days, lever presses were reinforced on a RI30 s schedule. Rats were trained in the same order as CRF training. That is, rats trained on the left first and right lever second on the CRF schedule also received training on the left lever first and right lever second during the RI30s stage. Again, food pellets and sucrose solution were rewarded with equal probability and sessions finished once 40 rewards had been earned. Additional training, where necessary, was given to overcome any lever biases displayed by the rats.

Discrimination Training. Instrumental training of the conditional discrimination occurred across twelve days; until the rats performed the task with discrimination ratios consistently above .70

(see section 4.4.4 for the calculation of discrimination ratios). Each daily session lasted 48 min and consisted of sixteen 3 min presentations of the auditory stimuli. Therefore, rats experienced eight presentations of the 3 kHz tone and eight presentations of the 10 Hz train of clicks during each session. The identity of the first stimulus was randomly allocated, and stimulus identity was then strictly alternated thereafter. Illumination of the house light signaled the active session. Throughout the session, both levers were inserted into the instrumental chamber but only one lever was reinforced during the presentation of each stimulus. That is, during the presentation of the tone, pressing the left lever *may* have been reinforced while the right lever would be non-reinforced. Similarly, during the presentation of the train of clicks, pressing the right lever *may* have been reinforced while the left lever would be non-reinforced. Appropriate lever press responses were reinforced on a RI30s schedule. Before acquisition training, rats were divided into two groups, differential vs. non-differential, with equal numbers of MAM-exposed, saline-exposed and non-exposed rats in each. Training was identical across the two groups except the stimulus-outcome contingencies (See Table 8). For rats trained under the differential condition, an appropriate lever press was reliably followed by a reward of specific identity. For example, an appropriate lever press (e.g. left) in the presence of the tone stimulus would be consistently followed by the pellet reward, whereas an appropriate lever press (e.g. right) in the presence of the click stimulus would be consistently followed by the liquid sucrose reward. In contrast, rats trained under the non-differential conditions would receive each reward identity (pellet and sucrose rewards) at equal probability after each appropriate lever press. The conditions were fully counterbalanced: For example, during the

presentation of the tone half of the rats would need to press the left lever and half the right lever as the appropriate response; similarly, for the differential group, an appropriate response during the tone stimulus was reinforced by a food pellet for half of the rats and by the delivery of sucrose solution for the other half.

Table 8 Stimulus-outcome contingencies for the Differential and Non-differential groups across acquisition training of the continuous performance conditional discrimination.

Group	Stimulus 1	Stimulus 2
Differential	R1 → O1	R1 → ∅
	R2 → ∅	R2 → O2
Non-Differential	R1 → O1/O2	R1 → ∅
	R2 → ∅	R2 → O1/O2

Stimulus 1 and 2 represent the tone and click stimuli; 'R' represents lever press responses (left vs. right); 'O' represents rewards (pellet vs. sucrose); ∅ represents 'no reward'.

Extinction test. The extinction test was performed twenty-four hours after the last day of training. Rats underwent a 48 min session similar to a training day, except that reinforcers were omitted for duration of the session. The purpose of this test was to ensure that the rats had learnt to solve the discrimination based on the auditory stimuli and were not relying on the presentation of the rewards alone to direct their responding. If discriminative performance was based on the auditory cues, differential responding should be maintained in this extinction test.

Reinforcer only test. The reinforcer only test was carried out twenty-four hours after reacquisition training (which occurred the day after the extinction test). Parameters were again the same as during the training phase, with the exception that the auditory stimuli were omitted for the

duration of the session. The purpose of this test was to determine whether the animals could use the rewards themselves as discriminative stimuli. Indeed, it is possible that rats trained under differential conditions, but not under non-differential conditions, could use reward identity to indicate which lever press response to make through the formation of back-associations (O-R). If animals were using this cue to solve the original discrimination, performance would be maintained in this reinforcer-only test but decline to chance levels in the extinction test.

Reversal Training. During reversal training the opposite stimulus-response contingencies were given to all rats. For example, rats which had received a sucrose reward after pressing the *left* lever in the presence of a tone and a pellet reward after pressing the *right* lever in the presence of a clicker would now receive a sucrose reward after pressing the *right* lever in the presence of a tone and a pellet reward after pressing the *left* lever in the presence of a clicker. After twelve days of reversal training (first reversal), stimulus-response pairings were reversed back to the original contingencies (second reversal). Training then continued for an additional twelve days.

4.5.4 Data analysis

For all analysis, correct and incorrect lever press responses were converted into discrimination ratios, calculated as mean number of correct responses in a session/ mean number of correct plus mean number of incorrect responses. Discrimination ratios were used to counteract the higher overall response rates displayed by MAM-treated animals compared to the other treatment

groups. Data were analysed via a three-way ANOVA with factors of session (days 1 – 12), treatment (saline-treated, non-exposed and MAM-treated) and group (differential vs. non-differential).

4.6 Results

Acquisition of the conditional discrimination. Discrimination ratios for saline-treated, non-exposed and MAM-treated rats during acquisition of the conditional discrimination are displayed in Figure 15, panels A, B and C, respectively. Generally, the discrimination ratio was higher for the differential than non-differential groups across all treatment conditions, indicative of a differential outcomes effect. A three-way ANOVA with factors of session, treatment and group revealed a main effect of session ($F(4.978, 209.056) = 171.740, p < .001, MSE = .461$) a main effect of group ($F(1, 42) = 5.982, p = .019, MSE = .078$) but no session \times group interaction ($F < 1$). There was no main effect of treatment ($F < 1$), no treatment \times group interaction ($F < 1$) and no session \times treatment \times group interaction ($F(22, 462) = 1.201, p = .241, MSE = .001$). A main effect of group without interactions with treatment indicates that a differential outcomes effect was established for saline-treated, non-exposed and, importantly, MAM-treated animals.

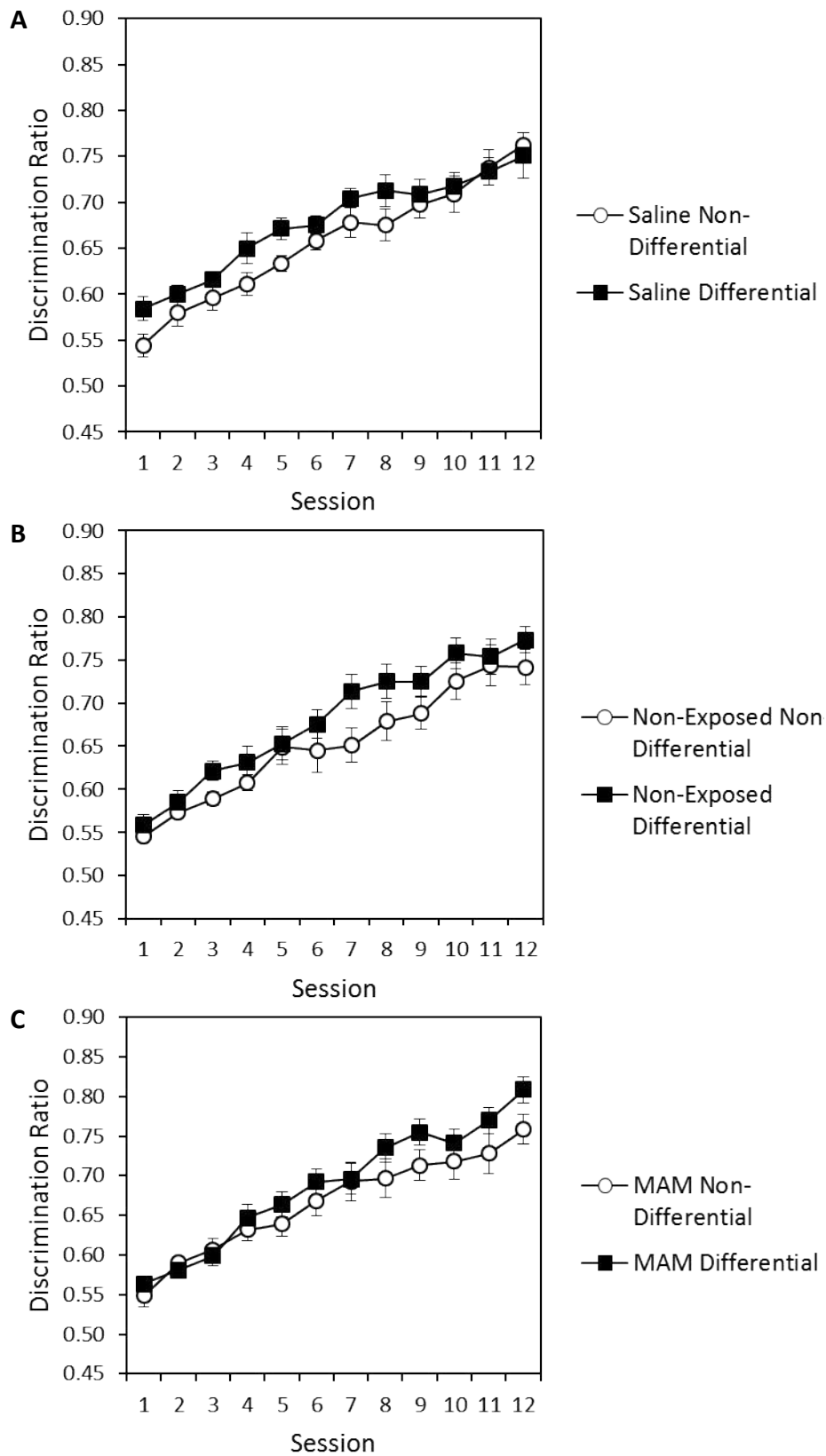


Figure 15 Discrimination ratios (Mean number of correct responses/Total number of responses) across acquisition training of the conditional discrimination: saline-treated rats (Panel A), non-exposed (Panel B) and MAM-treated rats (Panel C). Open symbols indicate the Non-Differential group; filled symbols indicate the Differential group. Error bars represent \pm SEM.

Extinction test. Figure 16 shows the mean discrimination ratios for the 48 min extinction test.

All groups maintained the discrimination: 95% CI, Sham Non-differential (.63 - .72); Sham Differential (.63 - .72); Non-Exposed Non-differential (.65 - .74); Non-Exposed Differential (.67 - .76); MAM Non-differential (.59 - .68); MAM Differential (.67 - .76). This suggests that the rats had learnt the discrimination based on environmental cues (tones and clicks) and not merely by the cues supplied on the delivery of the rewards. A between-subject ANOVA of the data summarised in Figure 16 with factors of treatment and group revealed no main effect of treatment ($F(2, 42) = 1.392, p = .260, MSE = .006$), no main effect of group ($F(1, 42) = 3.505, p = .068, MSE = .014$) and no significant interaction between these two factors ($F(2, 42) = 1.898, p = .163, MSE = .008$).

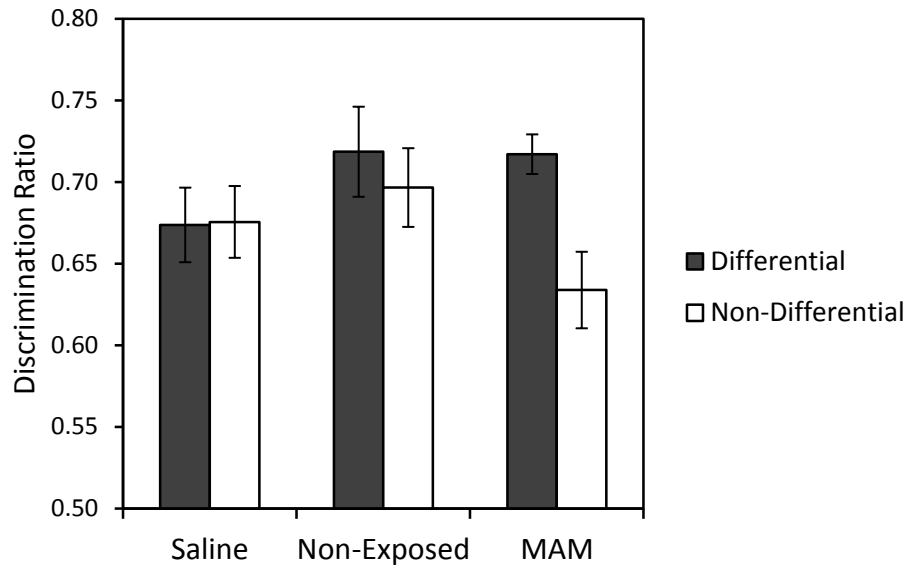


Figure 16 Discrimination ratios during the extinction test for saline-treated, non-exposed and MAM-treated rats. White bars represent the Non-Differential group; Grey bars represent the Differential group. Error bars represent \pm SEM.

Reinforcer Only Test. Figure 17 shows the discrimination ratios for the 48 min reinforcer only test. All rats showed discrimination ratios above 0.50 (95% CI, Sham Non-differential (.58 - .63); Sham Differential (.57 - .61); Non-Exposed Non-differential (.55 - .60); Non-Exposed Differential (.60 - .65); MAM Non-differential (.56 - .60); MAM Differential (.61 - .66)). This suggests that presentation of the reinforcer alone could serve as a cue to control performance, presumably with the rats adopting a win-stay strategy whereby they would continue to respond on the same lever once they had received a reward, combined with a contribution of O-R associations in the differential conditions. An ANOVA performed on the data summarised in Figure 17 revealed a main effect of group ($F(1, 42) = 9.546, p = .004, MSE .011$), no main effect of treatment ($F < 1$) and a treatment \times group interaction ($F(2, 42) = 4.663, p = .015, MSE = .005$). Further analysis of this interaction revealed significantly better performance for rats in the differential compared to non-differential group for both non-exposed ($F(1,$

42) = 8.338, $p = .006$, $MSE = .010$) and MAM-treated animals ($F(1, 42) = 9.989$, $p = .003$, $MSE = .012$). No difference in performance was seen for differential and non-differential groups for the saline-treated control animals ($F < 1$).

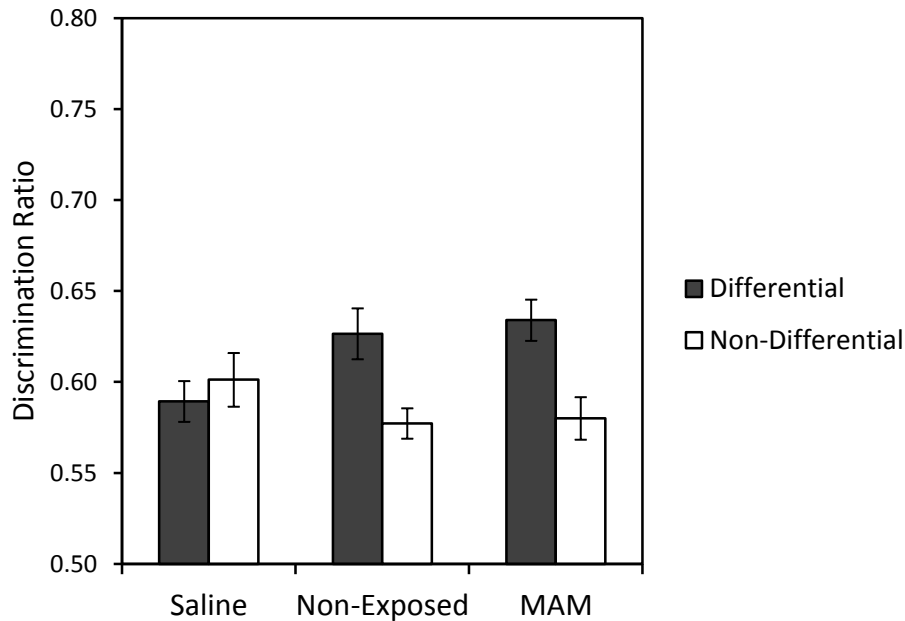


Figure 17 Discrimination ratios for the reinforcer-only test for saline-treated, non-exposed and MAM-treated rats. White bars represent the Non-Differential group; Grey bars represent the Differential group. Error bars represent + SEM.

Reversal Phase. After extinction and reinforcer only tests, all rats underwent additional training (one day of reacquisition training with inclusion of both auditory stimuli and reinforcers). Subsequently the response outcome contingencies were reversed for each rat. Figure 18 shows the discrimination ratios during the acquisition of the first reversal phase of the experiment. Early in training, rats demonstrated more responding on the previously correct/now incorrect lever. Higher discrimination ratios for rats in the differential compared to non-differential group is suggestive of a differential outcomes effect. ANOVA analysis with factors of session, treatment and group was

consistent with this impression revealing a significant main effect of session ($F(3.426, 143.874) = 212.835, p < .001, MSE = .901$), and a main effect of group ($F(1, 42) = 11.111, p = .002, MSE = .144$).

There was also a session \times group interaction ($F(11, 462) = 12.956, p < .001, MSE = .017$), further analysis of which revealed that rats in the non-differential group outperformed rats in the differential group for session one ($F(1, 42) = 12.088, p = .001, MSE = .025$) with the reverse pattern seen (with rats in the differential group outperforming rats in the non-differential group) for sessions 5 through to 12 (smallest $F(1, 42) = 6.774, p = .013, MSE = .024$ – session 12). The ANOVA also revealed a main effect of treatment ($F(2, 42) = 6.183, p = .004, MSE = .080$) with MAM-treated rats displaying significantly higher discrimination ratios than their saline-treated ($F(1, 42) = 7.610, p = .008, MSE = .000$) and non-exposed counterparts ($F(1, 42) = 10.731, p = .002, MSE = .000$). There was no treatment \times group interaction ($F(2, 42) = 2.012, p = .146, MSE = .026$), treatment \times session interaction ($F(22, 462) = 1.371, p = .122, MSE = .002$), nor session \times treatment \times group interaction ($F(22, 462) = 1.426, p = .096, MSE = .002$). Higher overall discrimination ratios for MAM-treated rats, together with the presence of a differential outcomes effect during the reversal phase, provides no evidence of impairment in these animals.

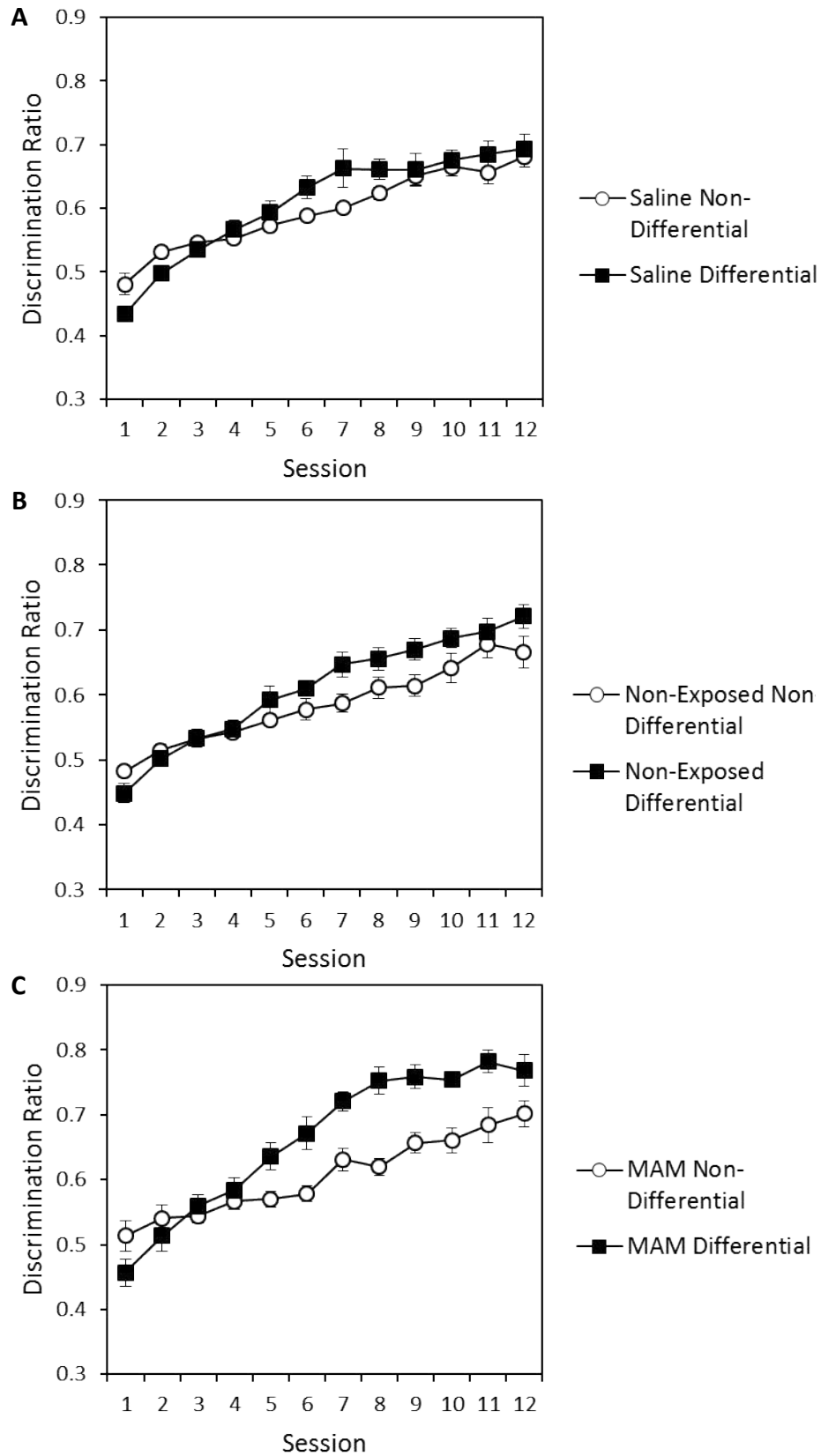


Figure 18 Discrimination ratios for saline-treated (Panel A), non-exposed (Panel B) and MAM-treated (Panel C) rats for the first reversal phase. Open symbols indicate the Non-Differential group; filled symbols indicate the Differential group. Error bars represent \pm SEM.

Figure 19 shows the discrimination ratios for saline-treated, non-exposed and MAM-treated rats for the second reversal phase of the experiment, where response-reward contingencies were the same as original acquisition. An ANOVA was performed with variables of session, treatment and group. This revealed a main effect of session ($F(4.372, 183.603) = 138.534, p < .001, MSE = .434$), a main effect of treatment ($F(2, 42) = 4.293, p = .020, MSE = .095$) but no session \times treatment interaction ($F < 1$). Simple effects analysis revealed that MAM-treated animals had significantly higher discrimination ratios compared to their non-exposed controls ($F(1, 42) = 8.218, p = .007, MSE = .0002$), but non-significantly higher ratios compared to their saline-treated controls ($F(1, 42) = 4.000, p = .056, MSE = .0002$). No significant difference in performance was seen between the two control groups ($F < 1$). The ANOVA analysis also revealed a main effect of group ($F(1, 42) = 26.303, p < .001, MSE = .580$), with rats in the differential group outperforming those in the non-differential group, as well as a session \times group interaction ($F(11, 462) = 16.656, p < .001; MSE = .021$). No treatment \times group interaction ($F < 1$) nor session \times treatment \times group interaction ($F < 1$) was yielded by the analysis.

Further analysis of the session \times group interaction revealed higher discrimination ratios for non-differential compared to differential groups for session one ($F(1, 42) = 14.184, p = .001; MSE = .025$). This pattern quickly reversed and stayed reversed (with the differential group outperforming the non-differential group) from session 3 onwards (smallest $F(1, 42) = 9.415, p = .004, MSE = .022$ – session 3).

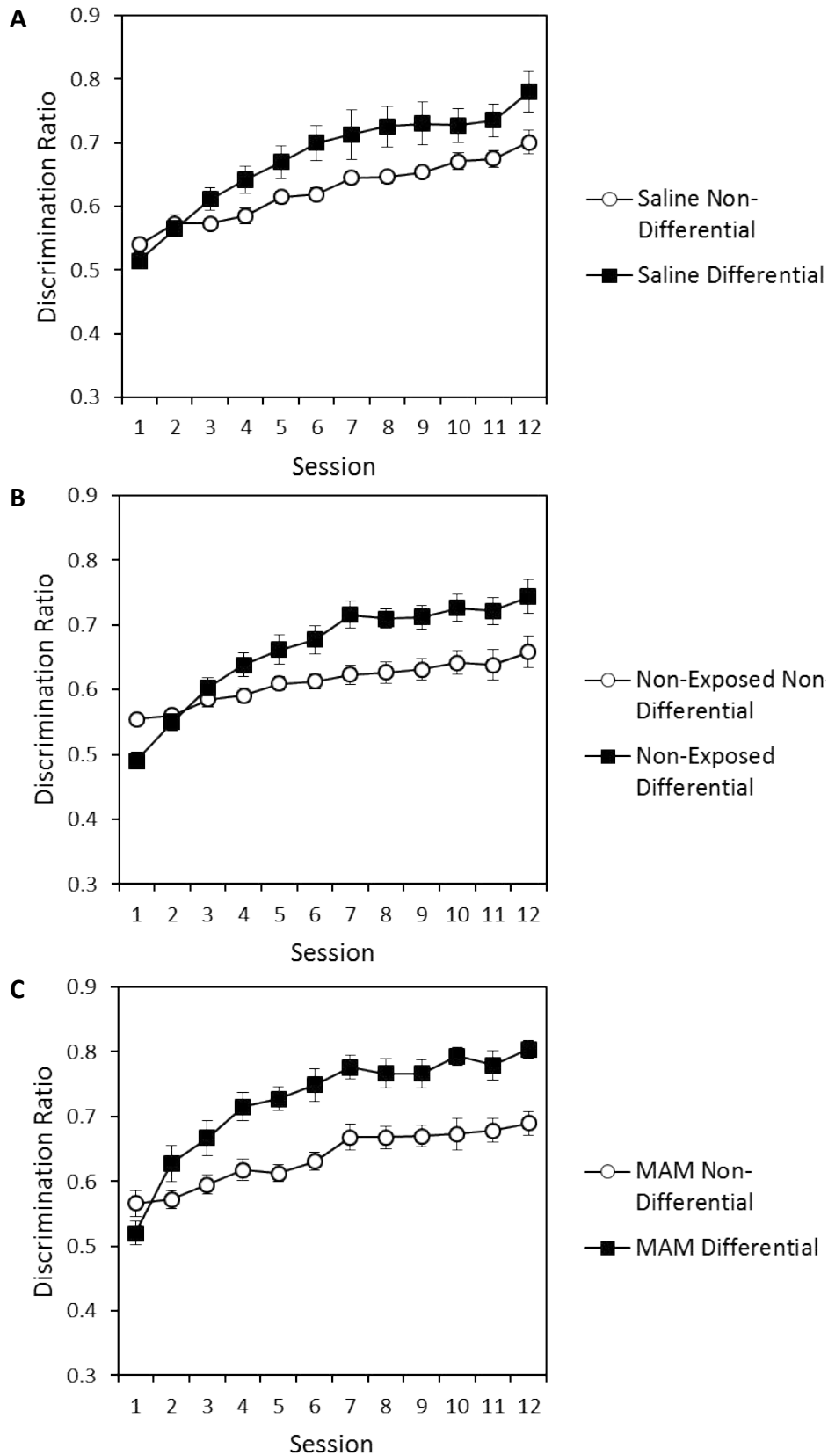


Figure 19 Discrimination Ratios for saline-treated, non-exposed and MAM-treated rats during reversal phase two. Open symbols indicate the Non-Differential group; filled symbols indicate the Differential group. Error bars represent \pm SEM.

4.7 Summary

Rats trained on a conditional discrimination showed better performance when given stimulus-correlated outcomes (the differential outcomes group) compared to rats for which the outcomes were not correlated with the stimuli (the non-differential outcomes group), regardless of prenatal treatment. This differential outcomes effect, which could not be explained purely on the basis of backward associations formed between the outcome and the response (O-R), demonstrates that rats prenatally exposed to MAM are able to use specific anticipatory reward information to guide their instrumental choice behaviour. No deficits were seen during either reversal phase of the task. Consistent with results from Experiment four, this demonstrates that these animals are sensitive to changes in action-outcome (A-O) contingency.

Based on the observations that MAM rats are marginally more sensitive to outcome devaluation (Experiment four) and demonstrate higher discrimination ratios during the reversal of a conditional discrimination, Experiment six aimed to determine whether or not the behaviour of these animals is less sensitive to the effects of overtraining. Whilst this would be inconsistent with the schizophrenia phenotype, lack of specificity of prenatal MAM treatment could mean that the structural abnormalities present may actually block habit formation - as is the case for rats with lesions of the infralimbic PFC (Killcross & Coutureau, 2003) and dorsolateral striatum (Yin et al., 2004). Previous studies have shown that typically nine to ten sessions (with 360-400 reinforced responses) promotes habitual performance in untreated animals, indexed by a lack of sensitivity of the instrumental

response to reward devaluation (e.g. Powell, 2013; Wickens et al., 2007). Nine days training was used in the current experiment.

4.8 Experiment 6 - Materials and Methods

4.8.1 Subjects and Apparatus

Cohort three (16 Saline-treated, 16 non-exposed and 16 MAM-treated rats) was used for Experiment five (please refer to Appendix A for the timing of this experiment). Rats were approximately 16 weeks old at the start of the experiment. Ad libitum weights were as follows: saline treated, 345 – 437 g; non-exposed, 329 – 483 g; MAM-treated, 289 – 430 g. Rats were placed on a food restricted diet prior to the start of the experiment to reduce them to 85% of their free-feeding weights. Moderate weight gain was allowed over the course of the experiment. For all aspects of animal husbandry, please see Experiment two (section 3.2.1). The training apparatus was as described in Experiment four (section 4.1.2).

4.8.2 Procedure

As in Experiment four, training consisted of two stages: magazine training and lever-press training. This was followed by an extinction test after devaluation of the instrumental reward by lithium-chloride (LiCl)-induced nausea (see Figure 20). Each rat was assigned to one of the eight experimental chambers, and thereafter always trained in the same chamber.



Figure 20 Schematic depicting the Outcome Devaluation Task with extended training. EXT = Extinction test; CT = Consumption Test and RT = Reacquisition Test.

Magazine training. All rats were trained to collect food rewards during a single magazine training session. 45 mg sucrose pellets (Test Diet, Richmond, IN) were delivered on a RT60s schedule. The session ended once 20 pellets had been delivered. Rats that did not collect the food were given a repeat of the session later the same day.

Lever-press training. The next day, a single session of lever press training was given during which sucrose pellets were delivered on a CRF schedule of reinforcement. The left lever was inserted into the chamber at the start of the session and retracted at the end of the session. The session ended once the rat had earned 20 reinforcers. Subsequently, animals underwent nine days training on a RI30s schedule of reinforcement. Sessions ended once rats had earned 40 reinforcers.

Devaluation by LiCl. After the last day of training, all rats were given free access to sucrose pellets for 30 min in individual test cages (as described for Experiment four). Once 30 minutes had past, the devalued group (8 MAM-Exposed, 8 Sham and 8 Non-exposed) received intraperitoneal injections of 0.15 M, 15ml/kg LiCl solution (Sigma-Aldrich). The non-devalued group (8 MAM-

Exposed, 8 Sham and 8 Non-exposed) were given intraperitoneal injections of the equivalent volume of 0.9% saline. Animals were matched according to lever press levels during the final day (day nine) of acquisition to determine their devaluation group. Outcome-LiCl/Saline pairings were repeated across two conditioning days. Twenty-four hours after the second conditioning day, the animals' sensitivity to outcome devaluation was assessed by a 10 min extinction test. Here, rats were given the opportunity to lever press but no rewards were available.

Consumption test and Reacquisition test. Immediately after the extinction session, all rats underwent a consumption test to ensure that the devalued group had acquired an aversion to the instrumental outcome. This was followed twenty-four hours later by a reacquisition test. Both tests were conducted as previously described in Experiment four.

4.8.3 Data analysis

Statistical analysis was performed with a repeated measures ANOVA with within-subject factor of time bin (five 2 min bins) and between-subject factors of treatment (saline-treated, non-exposed and MAM treated) and devaluation condition (devalued vs. valued). Lever presses and magazine entries per minute were calculated as a proportion of baseline. Baseline was taken as the data from the final day (day nine) of acquisition. Using the data averaged across the final three days of acquisition (days seven – nine) did not affect the statistical outcome. It should be noted that bin was included in the analysis here, unlike for previous and subsequent outcome devaluation

experiments, due to the suggestion of a devaluation effect, seemingly during the middle of the extinction test. To not include 'bin' in the analysis may have excluded potential significant results. It should be highlighted that the factor of bin did not interact with any other factor for the other outcome devaluation experiments reported in this thesis.

4.9 Results

Instrumental training. At the end of training, rats prenatally exposed to MAM performed more lever press responses than both saline-treated and non-exposed control animals (See Table 9). Importantly, there appeared to be no difference in baseline lever press responses as a function of devaluation condition for any treatment group.

Consistent with the overall impression, ANOVA revealed a main effect of treatment ($F(2, 42) = 3.763, p = .031, MSE = 978.897$). Importantly, valued and to-be-devalued groups responded at a similar level as there was no main effect of devaluation condition ($F < 1$) and no treatment \times devaluation condition interaction ($F < 1$).

Table 9 Lever press response per minute for day 9 of acquisition training (used as 'baseline').

Treatment	Devaluation Condition	Mean lever press/min (\pm SEM)
Saline-treated	Valued	30.516(\pm 3.268)
	Devalued	30.920(\pm 3.633)
Non-exposed	Valued	37.123(\pm 3.733)
	Devalued	33.923(\pm 4.276)
MAM-treated	Valued	48.566(\pm 4.706)
	Devalued	43.461(\pm 3.991)

Magazine approach behaviour appeared to be similar across all treatment groups and devaluation conditions at the end acquisition training (See Table 10). ANOVA showed no main effect of treatment ($F < 1$), no main effect of devaluation condition ($F(1, 42) = 1.083, p = .304, MSE = 48.758$) and no treatment \times devaluation condition interaction ($F(2, 42) = 1.332, p = .275, MSE = 266.524$).

Table 10 Magazine approach performance per minute for day 9 of acquisition training (used as 'baseline').

Treatment	Devaluation Condition	Mean lever press/min (\pm SEM)
Saline-treated	Valued	14.693(\pm 2.536)
	Devalued	17.803(\pm 2.773)
Non-exposed	Valued	17.816(\pm 2.581)
	Devalued	16.092(\pm 2.822)
MAM-treated	Valued	12.097(\pm 2.343)
	Devalued	16.759(\pm 2.372)

Extinction – Lever Press Performance. The mean lever press response rates as a proportion of baseline across five 2 min bins for the extinction test are displayed in Figure 21 panels A – C. Inspection of this figure shows that performance of saline-treated rats (Panel A) and MAM-treated rats (Panel C) appeared, to some extent, to be sensitive to the current value of the goal. That is, saline-treated and MAM-treated animals performed fewer lever presses as a proportion of their baseline rates after the outcome had been paired with LiCl-induced nausea (devalued – closed symbols) compared to those animals that had not received this pairing (valued – open symbols). In contrast, non-exposed animals (Panel B) responded on the lever at equivalent rates regardless of whether the outcome had been devalued.

Statistical analysis was performed by repeated-measures ANOVA with a within-subject factor of bin (1-5) and between-subject factors of prenatal treatment (saline-treated, non-exposed and MAM-treated) and devaluation condition (valued vs. devalued). This yielded a main effect of bin ($F(2.932, 123.148) = 14.633, p < .001, MSE = .838$), no main effect of devaluation condition ($F < 1$) but a bin \times devaluation condition interaction ($F(4, 168) = 4.900, p = .001, MSE = .206$). There was no main effect of prenatal treatment ($F < 1$) and no significant interactions between treatment and any other factor: critically, bin \times treatment, $F(8, 168) = 1.392, p = .203, MSE = .058$; treatment \times devaluation condition, $F < 1$; bin \times treatment \times devaluation condition, $F < 1$.

Pairwise comparisons of the bin \times devaluation condition interaction revealed significantly higher lever press response rates for rats in the valued group compared to rats in the devalued group for bins 3 and 4 (smallest $F(1, 42) = 5.568, p = .023, MSE = .363$). After this level of training, it would be expected that rats would be insensitive to post-conditioning changes in outcome value. That is, their behaviour would be habitual and lever press responses would be equivalent across valued and devalued groups. The suppressed responding after devaluation treatment is instead indicative of goal-directed behaviour.

Although there was no significant interaction between prenatal treatment and the effect of devaluation (nor between the factors of bin, treatment and devaluation), inspection of Figure 21 hints that goal-directed behaviour was not necessarily present in all treatment groups. In fact, when examined separately, neither the main effect of devaluation, nor the bin \times devaluation condition interaction was significant in any individual group: Saline-treated animals, condition, $F < 1$, bin \times

condition, $F(4, 56) = 2.373$, $p = .063$, $MSE = .145$; Non-exposed animals, condition, $F < 1$, bin \times condition, $F(4, 56) = 1.064$, $p = .383$, $MSE = .028$; MAM-treated animals, condition, $F(1, 14) = 1.233$, $p = .286$, $MSE = .525$, bin \times condition, $F(4, 56) = 1.793$, $p = .143$, $MSE = .070$. Thus, although there was a hint of goal-directed behaviour in some time-bins when the data was collapsed across all treatment groups, there was no support for this being selective to any individual group (or indeed, no support for it being present in any individual group).

Extinction – Magazine Approach Behaviour. Magazine approach behaviour for the extinction test is displayed in Figure 22. As ANOVA revealed no main effect of bin ($F(1.855, 77.906) = 1.713$, $p = .189$, $MSE = .567$) or interaction between bin and any other factor (All $F_s < 1$), the data in Figure 22 is summarised as mean responses per minute (as a proportion of baseline responding) collapsed across the 10 min session. ANOVA results revealed no main effect of treatment ($F < 1$), a significant main effect of devaluation condition ($F(1, 42) = 5.150$, $p = .028$, $MSE = 1.358$), with rats nose-poking more in the valued than devalued group, and no treatment \times devaluation condition interaction ($F(2, 42) = 2.704$, $p = .079$, $MSE = .713$).

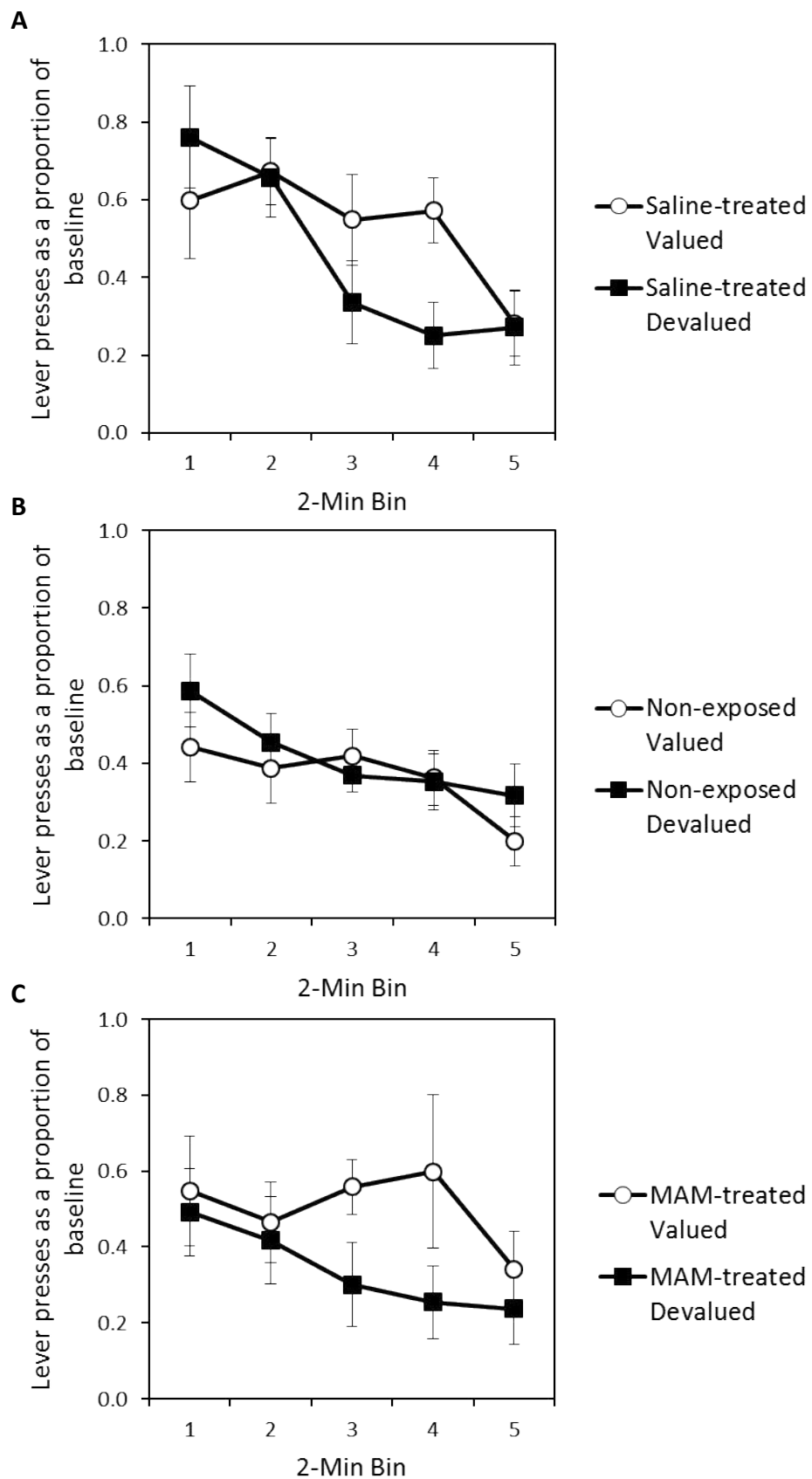


Figure 21 Mean Lever Press responses as a proportion of baseline responding across the 10 min extinction test, separated into 2 min bins, for saline-treated control (Panel A), non-exposed control (Panel B) and MAM-treated experimental rats (Panel C). Open symbols represent responding for the valued group, filled symbols represent responding for the devalued group, which had received taste-aversion conditioning. Error bars represent \pm SEM.

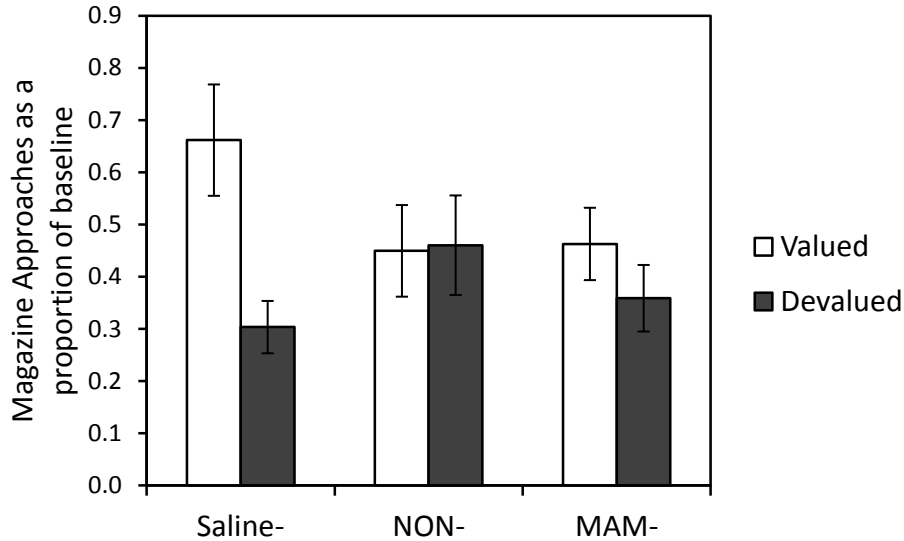


Figure 22 Magazine approach behaviour as a proportion of baseline responding across the 10 min extinction test for saline-treated, non-exposed and MAM-treated rats. Error bars represent \pm SEM.

Outcome Devaluation. Figure 23 shows the consumption of the instrumental outcome across the taste-aversion conditioning sessions and test (which immediately followed the extinction test). Inspection of the figure suggests that effective taste aversion was acquired by all three treatment groups. Rats in the devalued group exhibited a strong aversion to the instrumental outcome while rats in the valued group (where the outcome had been paired with saline) continued to consume the outcome across sessions. An ANOVA was performed with factors of session (conditioning day one, two and test), treatment and devaluation condition (valued vs. devalued). The ANOVA revealed a significant main effect of session ($F(2, 84) = 186.429, p < .001, MSE = 710.167$), a significant effect of devaluation condition ($F(1, 42) = 84.864, p < .001, MSE = 637.142$) and a significant session \times condition interaction ($F(2, 84) = 50.919, p < .001, MSE = 193.968$). There was no main effect of treatment ($F < 1$), nor was there an interaction between treatment and any other factor: treatment \times

devaluation condition, $F < 1$; session \times treatment, $F(4, 84) = 1.913$, $p = .116$, $MSE = 7.287$; session \times treatment \times devaluation condition ($F(4, 84) = 1.84$, $p = .134$, $MSE = 6.909$).

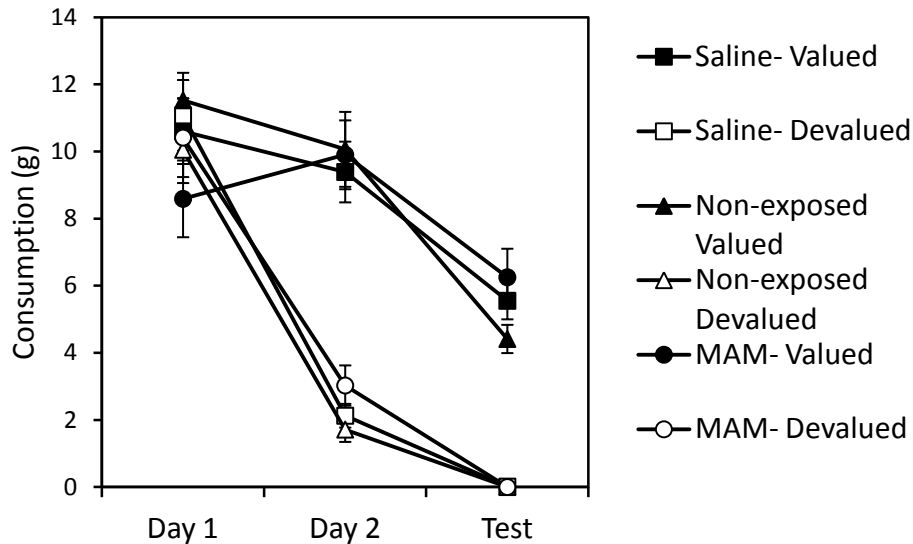


Figure 23 Mean sucrose pellet consumption (\pm SEM) over two days of taste aversion training and one post-extinction consumption test for saline-treated, non-exposed and MAM-treated rats. Rats received LiCl injections (devalued – open symbols) or saline injections (valued – filled symbols) after 30 min free access to the instrumental outcome. The test phase also took place in a 30 min period immediately after the 10 min extinction test.

Reacquisition test – Lever Press Performance. The reacquisition test was performed twenty-four hours after the extinction test. The data for lever press performance and magazine entries are displayed as responses per min in Figure 24 panels A and B, respectively. An ANOVA was performed on the data summarised in Figure 24A and included factors of treatment and devaluation condition. This revealed a significant main effect of prenatal treatment ($F(2, 42) = 5.443$, $p = .008$, $MSE = 665.712$) with MAM-treated rats performing significantly more lever presses than both saline treated ($F(1, 42) = 9.631$, $p = .003$, $MSE = 15.288$) and non-exposed rats ($F(1, 42) = 6.360$, $p = .016$, $MSE = 15.288$). There was also a main effect of devaluation condition ($F(1, 42) = 30.938$, $p < .001$, $MSE = 3783.640$) but no treatment \times condition interaction ($F(2, 42) = 1.100$, $p = .342$, $MSE =$

134.584). ANOVA analysis on magazine approach behaviour did not reveal a significant main effect of treatment ($F < 1$). There was a main effect of devaluation condition ($F(1, 42) = 10.540, p = .002, MSE = 193.181$) but no treatment \times condition interaction ($F(2, 42) = 2.703, p = .079, MSE = 49.541$).

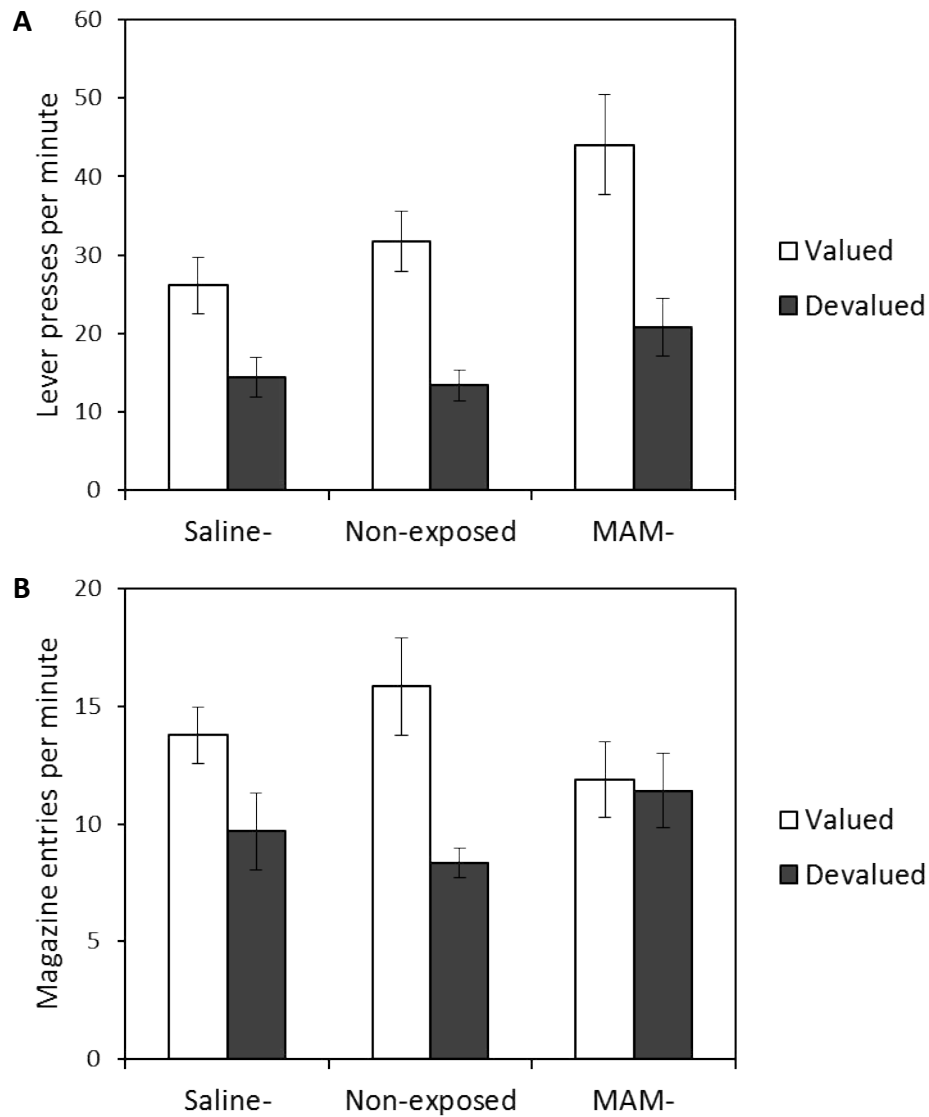


Figure 24 Effect of prenatal treatment on lever press performance (Panel A) and magazine approach behaviour (Panel B) during reacquisition after reward devaluation by LiCl-induced nausea. Mean lever presses and magazine entries per minute (\pm SEM) in the rewarded reacquisition test after devaluation with LiCl (grey bars) or no devaluation (white bars) are shown. The reacquisition test was given 24 hrs after the extinction and consumption tests.

4.10 Summary

After extended training of an instrumental response, the control of behaviour usually becomes dominated by reflexive stimulus-response associations, as indexed by performance insensitivity to manipulations of outcome value. The current results are somewhat equivocal with some hint that MAM-exposed rats may maintain goal sensitivity after extended training, but with the interpretation obscured by a similar finding in saline-treated rats. The results of the current experiment would need to be repeated before any firm conclusions can be drawn regarding the reward encoding deficit, or lack thereof, in the MAM neurodevelopmental model of schizophrenia. All that can be said at this stage is that MAM rats are capable to forming mental representations of outcome value and using these representations to both motivate and direct behaviour. Whether a deficit lies with the transition to habitual behaviour remains unclear. That said, cognitive, as opposed to reflexive, behavioural control in this model may go some way towards explaining the superior performance of MAM-treated rats during reversal learning.

4.11 Validation of Cohorts one, two and three

The three cohorts presented across Chapters three and four underwent additional analyses to validate the effectiveness of MAM treatment on GD17. Cohort one was challenged with the psychostimulant MK-801 (an NMDA-R antagonist) and the subsequent enhancement of hyperlocomotion caused by prenatal MAM treatment was recorded. Hypersensitivity to the locomotor enhancing effects of this drug has been found to be a robust finding for the MAM model (e.g. Le Pen

et al., 2006; 2011). The full details of the apparatus, experimental protocol and results can be found in Appendix B of this thesis. MK-801 produced higher locomotor activity in MAM-treated rats compared to their saline-treated controls.

For each of the three cohorts, brains were extracted and their wet weights recorded.

Evidence suggests that rats prenatally treated with MAM display an approximate 11% decrease in total brain weight (Flagstad et al., 2004). Reductions of 12.39%, 11.66% and 12.21% were found for absolute brain weights for cohorts one, two and three, respectively. Please refer to Appendix B further details.

4.12 Discussion of Chapters 3 and 4

Disruption of neurogenesis by MAM on gestational day 17 has been suggested to be a comprehensive rodent model of schizophrenia, producing a schizophrenia-like pattern of pathophysiological abnormalities and a range of behavioural alterations reminiscent of the positive, cognitive and negative symptoms of the disease (e.g. Jones et al., 2011). However, despite these claims, the MAM GD17 model has not undergone robust behavioural characterisation, particularly with regard to the negative symptom cluster. This is a neglect that the current thesis aimed to address.

Chapter three investigated the presence of hedonic deficits in the MAM GD17 model. When exposed to three different concentrations of sucrose solution, prenatal MAM treatment did not affect reward value, as evidence by similar consumption and palatability responses by MAM and saline-

treated animals. This demonstrates that during reward consumption, MAM-exposed rats do not display a hedonic deficit. This result is consistent with the normal hedonic capacity to emotional stimuli that is often reported in the schizophrenia literature. Of direct relevance to the current results, Berlin et al (1998) found equivalent hedonic responses to a sucrose solution between schizophrenia patients and healthy volunteers, as assessed by a sweetness rating scale. Similar results to sweet solutions have also been demonstrated by Berenbaum and Oltmanns (1992), despite schizophrenia patients displaying affective flattening to positively-valenced stimuli, while Horan et al (2006b) found in-the-moment pleasure for pleasant foods to be intact in schizophrenia.

If the actual consummatory pleasure experienced from a reward is intact, then a critical deficit may lie with anticipating hedonic consequences from future rewarding events. Therefore, Experiment three investigated the occurrence of anticipatory anhedonia in the MAM model by examining the development of negative anticipatory contrast. Regardless of prenatal treatment, all animals acquired a strong contrast effect across training. This was indicated by the lower consumption and lick cluster size measures associated with the initial 4% sucrose solution when it was consistently followed by a more palatable 32% sucrose solution. Whilst these results are consistent with Experiment one (i.e. the first solutions' perceived palatability is reduced when it is followed by a solution of higher hedonic value), no evidence was provided for an anticipatory hedonic deficit in the MAM model of schizophrenia. Whilst some studies have found increased anticipatory anhedonia in schizophrenia patients (Gard et al., 2007), others have found normal (or even increased) anticipatory hedonic behaviour (e.g. Gard, Sanchez, Cooper, Flsher, Garrett, Coleman & Vinogradov, 2014; Strauss et al.,

2011). As such, the results reported here may highlight either a shortcoming of prenatal MAM treatment as a model of negative symptomatology, or an absence of anhedonia as a primary feature of schizophrenia.

Chapter four investigated whether the MAM-exposed rats can use reward values to guide action selection. In Experiment four, rats were trained to lever press for a reward for three days on a random interval schedule of reinforcement. This level of training was selected because it maintains goal-directed behaviour in healthy, untreated animals but is sufficient to push instrumental responding towards habitual systems in amphetamine-treated rats (Nelson & Killcross, 2006). Similarly, rats with prefrontal lesions have shown habitual responding after minimal levels of training (e.g. Killcross & Couturea, 2003). However, in the current experiment it was found that rats prenatally exposed to MAM in the devalued group (i.e. where the outcome had been paired with LiCl-induced nausea) reduced their lever press behaviour relative to rats in the valued group (i.e. where the rats had not been averted to the outcome), consistent with goal-directed responding. This demonstrates that MAM-treated rats can alter their behaviour based on changes in outcome value even when that outcome is not present at the time of responding. Moreover, there was even a suggestion that MAM-treated animals showed a trend towards greater sensitivity to outcome devaluation than the control group.

Experiment five further investigated reward value representations for the MAM-model using the differential outcomes procedure. MAM rats demonstrated superior performance when trained on stimulus-correlated outcomes (e.g. the differential group), compared to uncorrelated outcomes (e.g.

the non-differential group), consistent with the differential outcomes effect. This demonstrates that MAM-treated animals can include a representation of the outcome as part of the learning matrix, but more precisely that they can use the specific sensory information regarding the identity of different rewards to direct their choice behaviour. The results of Experiments four and five combined highlight that the MAM model may not incorporate the problems seen with value representation in schizophrenia patients. The inability of this prenatal MAM treatment to comprehensively model the negative symptoms of schizophrenia will be considered in the general discussion.

Many studies have shown that when contingencies are reversed, people suffering from schizophrenia show significant impairments (Murray, Cheng, Clark et al., 2008; Waltz & Gold, 2007). Similar results have also been seen for MAM-treated animals (Featherstone et al., 2007; Flagstad, Glenthøj & Didriksen, 2005; Gastambide, et al., 2012; Moore et al., 2006). However, when the instrumental contingencies were reversed in Experiment five, MAM rats outperformed control animals with higher overall discrimination ratios. This result, together with the findings from Experiment four, raises the possibility that the MAM rats in the current investigation may rely more on A-O associations than their controls.

Investigating this possibility in Experiment six did not provide conclusive evidence for impaired habit transition in MAM treated animals. Indeed, whilst there was a hint towards continued goal-directed behaviour after extended training in MAM-treated rats, a similar pattern of results was seen for saline-treated rats. This may suggest that saline-treated animals are perhaps not the most

appropriate control for the MAM model and highlights the importance of including an additional non-exposed group in future experiments.

In summary, the experiments reported in Chapters three and four provide no evidence for behavioural impairments in MAM-treated rats reminiscent of either the negative or cognitive symptoms of the disease. Importantly, it is highly unlikely that the lack of behavioural impairments reported can be attributed to ineffective MAM treatment. Not only were multiple litters included in each cohort, but multiple cohorts were often run through the same experiment. As reported in the results sections of Chapters three and four, there were no cases where 'cohort' significantly altered the results to question their interpretation. The lack of behavioural impairments reported in my experiments also occurred despite reduced brain weights for each MAM-cohort compared to their control animals (Please refer to Appendix B). Furthermore, I found a lack of deficits for cohort one (included in Experiments two and four of this thesis), despite the same animals showing enhancement of MK-801 induced hyperlocomotion.

While the current results certainly suggest that MAM-treated animals do not show either hedonic or reward-processing deficits, such a conclusion relies on the adequacy of the behavioural methods. While the outcome devaluation and DOE methods are relatively well characterised and validated in terms of neurobiological manipulations, it is certainly possible that the microstructural analysis of licking assay is simply insensitive to changes in hedonic state. Thus, it would be useful to assess the methods against other models of disease characterised by reward-processing impairments. Reward processing deficits feature in depression, and deficits in hedonic capacity in

particular are less controversial in this patient group (e.g. it comprises one of two main symptoms required for a depression diagnosis). As Chapters five and six will detail the investigation of the WKY inbred depression model, I will postpone further consideration of how the behaviour of MAM-treated animals in the hedonic tests used here relates to the clinical presentation of schizophrenia to the general discussion.

Chapter Five

5. Hedonic Deficits in WKY Rats

5.1 Introduction

Unlike for schizophrenia, where anhedonia is not directly part of the diagnostic criteria, the DSM-V criteria for major depression includes a reduction or loss of interest or pleasure in usually enjoyable activities in relation to hedonic deficits (American Psychiatric Association, 2013). As has been seen in section 1.2 of this thesis, these criteria present problems in that it assumes equivalence between hedonic and motivational capacity, despite these reward processes being subserved by distinct neural mechanisms. Regardless of this issue, the importance of reduced hedonic capacity per se in this patient population has been highlighted by both increased self-reports of anhedonia (e.g. Berlin et al., 1998; Franken et al., 2007) and a reduction in the pleasure and arousal ratings elicited by positive stimuli (e.g. Dunn et al., 2004; Rottenberg et al., 2002; 2004; Sloan et al., 1997).

The WKY inbred rat strain is a putative model of depression and comorbid anxiety replicating important pathophysiological and behavioural deficits associated with these disorders (e.g. Overstreet, 2012). Some attempts have been made to characterise hedonic deficits in this model, but the results have been somewhat ambiguous (see section 1.4.2 of the general introduction). There is some suggestion that, in line with depression in the clinic, the application of an external stressor may be necessary for the symptom-like behaviours to manifest. Therefore, the experiments reported in Chapters five and six use a factorial design (i.e. with both stressed and non-stressed conditions for

the WKY rats and their controls) to determine stress effects in WKY rats on reward-related behaviours. It should be noted that because WKY rats were originally derived from a Wistar outbred strain, Wistar rats are used as the comparison strain for the battery of tests reported here.

A subset of stressors used by Willner and colleagues (e.g. Willner, Muscat & Papp, 1992; Willner, Towell, Sampson, Sophokleous & Muscat, 1987) were used in the experiments reported here, chosen primarily based on their ability to be applied in a semi-random fashion within the constraints of the Cardiff University animal unit. Unlike Willner's work, a forced swim test was also used in the current experiments to act both as a stressor and to allow me to assess whether previous reports of forced swim test behavioural deficits in the WKY strain are replicable. Furthermore, unlike for Willner's protocol, where stressors are applied daily, a maximum of three stressors were applied per week. In light of this, the current stress protocol may be viewed as marginally less stressful overall compared to Willner's design.

The first question addressed in relation to the WKY model was whether microstructural analysis of licking can detect hedonic impairments (whether inherent or stress-induced) during the consumption of palatable solutions. Referring back to the MAM results in Chapter three, it is possible that lick cluster size is somehow insensitive to anhedonia. In this light, the presence of a hedonic deficit in WKY rats would serve as a useful positive control for the MAM results.

5. 2 General Methods and Materials

5.2.1 Subjects

Twenty-four male Wistar rats and twenty-four male Wistar Kyoto (WKY) rats were used in the experiments reported in Chapters five and six. Both experimental and control strains were from Charles River (UK) breeding stocks and were delivered to Cardiff University at approximately 11 weeks of age. On arrival both the Wistars and WKY rats were split in to two weight-matched groups of twelve. Rats in one group were assigned to the 'No-stress' condition while rats in the second group were assigned to the 'Stress' condition (Mean Weights (\pm SEM): Wistar No-Stress 177.8 g (\pm 3.9); Wistar Stress 182 g (\pm 6.8); WKY No-stress 182.3 g (\pm 4.2); WKY Stress 178.4 g (\pm 7.9)). No-stress rats were housed in pairs and their home cages included standard environmental enrichment (tubes and gnawing sticks). Stress rats were singly housed in a separate room and no environmental enrichment was provided. All other aspects of animal husbandry were kept the same across the two groups and were as previously described in Experiment one. Rats were placed on a food-restricted diet to reduce them to 85% of their free feeding weights prior to testing. Careful monitoring was employed throughout to ensure that rat weights, as a percentage of free-feeding weights, did not differ significantly between the two strain and stress conditions.

5.2.2 Stress manipulation

Rats in the Stress condition underwent a series of mild social and environmental stressors which commenced a week prior to testing. This continued throughout the course of these experiments. Each week, rats in the stress group were exposed to three of five possible stressors: wet bedding, overnight illumination, cage swap with an unfamiliar rat, pair-up with an unfamiliar rat and the forced-swim test. Details of the stress procedures, including their duration, are shown in Table 11. The identity of the stressor was randomly allocated, as was the day on which it was given. When stress manipulations were to occur on the same day as an experimental session, the stressor was applied after the training or test session had been carried out. Rats in the No-stress condition were gently handled on a daily basis.

Forced Swim Test. During the forced swim tests, the rats' behaviour was recorded via a camcorder mounted above the water cylinders. Data was scored using a time sampling technique, whereby the rats' predominant behaviour was noted every 2 s across the 120 s test, giving a total of 60 scores. Recording commenced as soon as the rats had entered the water. Their behaviour was scored as either 'Active', 'Escape' or 'Immobile'. Active behaviour was recorded when the rat was swimming, climbing or diving. Thus, rats would be considered 'active' if they made upward-directed movements of the forepaws, horizontal movements across the cylinder (including rapid changes in the rat's direction) or dived to the bottom of the cylinder before resurfacing. Immobile behaviour was recorded if rats were floating in the water without any signs of struggling. Tiny movements of the back

limbs were permitted in this category if they served only to keep the animals head out of the water.

Escape behaviour was recorded if the rat was able to leave the cylinder. This would be considered as one escape. For every subsequent 2 s period where the rat was out of the water, an 'X' would be recorded so that it was not included in subsequent analysis. Percentage time spent active, immobile or escaping was then calculated for each animal.

As it was impossible for the primary observer to be blind to the rat strain being scored, a single session ($n = 24$), chosen at random, was re-scored by a secondary observer (who was blind to the strain) using the criterion outlined above. Inter-rater reliability was then assessed for this single session via a correlation. Analysis revealed a strong positive correlation between the two observers immobility scores, $r(22) = .975$, $p < .001$. Thus, analysis of the forced swim test could commence with the confidence that the observer was not biasing the results. Immobility data (the main parameter of interest) was analysed with a repeated-measures ANOVA with factors of session (1 – 7) and strain (Wistar vs. WKY).

Table 11 Social and environmental stressors for the stress condition. Three of the five stressors were randomly applied each week in addition to social isolation and unenriched home cages. Stressor identity and the day that the stressor was given were randomly allocated to minimise habituation to the stress procedure over time.

<i>Stressor</i>	<i>Duration</i>	<i>Description</i>
Wet Bedding	< 4 Hours	Rats were transferred to a different cage where the sawdust had been dampened with approximately 300 ml of cold water.
Overnight Illumination		Light-dark cycle was temporarily reversed. This manipulation was never done on consecutive days.
Cage Swap		The cages of two rats were randomly swapped including water bottles. Rats remained in the cage of the unfamiliar rat until their cages were next cleaned (maximum of 7 days).
Pair-Up	< 12 Hours	Rats were paired at random, within their strain, and left housed together overnight. All pair-ups included a defending male and an intruder male, as one rat was placed into the home cage of another instead of into clean 'neutral' home cage. Which rat was to be the intruder/defending male was randomly allocated. During food restriction, both of the rat's food rations were placed both in the food hopper and inside the cage. *
Forced-Swim Test	< 2 Minutes	Rats were carefully lowered into a black container of water, measuring 33.5 cm by 23 cm (H x D). The temperature of the water was maintained at 20-22 ° C. To minimize escape, the water surface was kept at 16-17 cm from the lip of the container. Any rat which escaped was placed back in the water for a maximum of four times after which the trial was terminated. As two rats (1 Wistar and 1 WKY) were run simultaneously in separate containers, trials were terminated for both rats. Upon trial termination or once 2-min had elapsed, rats were removed from the water and carefully dried off before being replaced in their home cage. Water was replaced after four rats had been tested.

*Rats were carefully monitored for signs of fighting. One rat received bite marks after being paired up and so no longer underwent this manipulation.

5.3 Experiment 7 - Materials and Methods

5.3.1 Apparatus

The apparatus used in the consumption experiment was as described for Experiment one, Section 2.2.2. The solutions used were 2%, 8% and 24% sucrose made daily (w/w) with deionised water.

5.3.2 Procedure

Pre-training. The order of the experiments presented here is not the order in which they were conducted (please refer to Appendix A for the order of each experiment). Since rats had already undergone anticipatory contrast training involving multiple drinking sessions (reported as Experiment eight, Section 5.7), no pre-training or habituation was deemed necessary for these rats for the non-contrast consumption tests.

Test. Rats were given access to one of the three sucrose concentrations which were always made available from the left hand side of the drinking chamber. Each concentration was given for three consecutive days and the order of sucrose presentations was counterbalanced so that half of the rats received the sucrose in order of increasing concentration (2-8-24) and the other half received them in order of decreasing (24-8-2) concentration. A two-day rest was given before the next concentration in the sequence was presented. All solutions were made available for 10 min each day.

5.3.3 Data analysis

Data analysis was conducted using the same parameters described for Experiment one (Section 2.2.4). To overcome potential neophobic effects, consumption, LCS and ILI data were analysed across the last two days of exposure only for each solution concentration. One animal (#39, a WKY No-stress rat) was excluded from all descriptive and inferential statistics reported. This was due to abnormally high LCSs displayed by this animal, more than 3 standard deviations of the group mean. All other parameters, however, (i.e. ILI and volume/1000 licks) appeared to be within the normal range.

5.4 General Results

5.4.1 Body Weight and Food Intake

Body weight and food rations (i.e. the amount given to rats to maintain them around 85% of their free feeding weights) are displayed in Figure 25 panels A and B, respectively. Body weight was recorded at regular intervals (every 2 - 3 days) across the experimental timescale (Experiments seven to ten). The data here represents the averages for the cohort across the four experiments. Average body weight was lower for WKY rats compared to their Wistar counterparts (strain: $F(1, 44) = 11904.686$, $p < .001$, $MSE = 4301725.490$). As the food rations were adjusted to maintain a steady bodyweight, the application of a chronic mild stress regime did not affect the rats body weight for either strain (stress: $F(1, 44) = 2.233$, $p = .142$, $MSE = 807.014$; stress \times strain: $F < 1$). To maintain

these consistent weights across stress conditions, larger food rations were given to rats in the stress group (stress: $F(1, 44) = 88.386, p < .001, MSE = 62.431$). This was true regardless of strain (stress \times strain: $F < 1$). Overall, however, larger rations were required to maintain Wistar rats at 85% of their free-feeding weights compared to WKY rats (strain: $F(1, 44) = 65.333, p < .001, MSE = 46.147$).

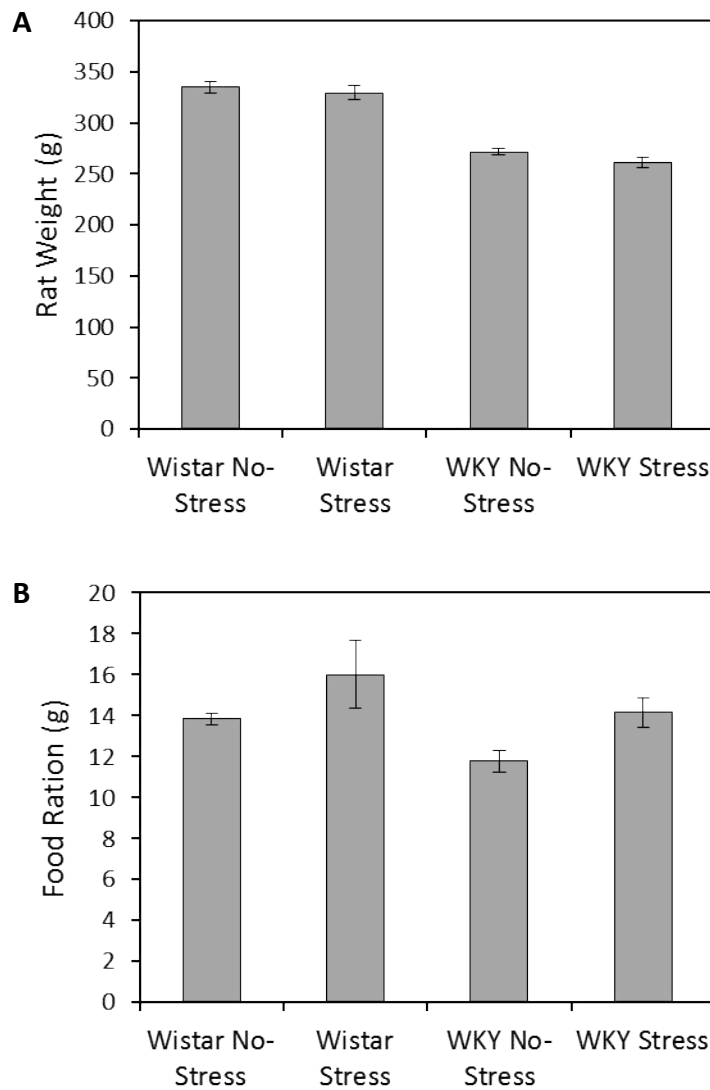


Figure 25 Body weight (Panel A) and food rations (Panel B) for Wistar and WKY rats averaged across Experiments 7-10. Food rations were calculated to maintain rats at 85% of their free feeding weights. Moderate weight gain was allowed over the course of each experiment. Error bars represent \pm SEM.

5.4.2 Forced Swim Test

The percentage time spent immobile for Wistar and WKY strains is displayed in Figure 26.

As can be observed from this figure, WKY rats spent more time immobile compared to their Wistar counterparts across all seven sessions (to see when each forced swim test was performed in relation to other experiments, please refer to Appendix C). Time spent immobile also generally increased across sessions, unlike for the Wistar strain. The ANOVA analysis yielded a main effect of session ($F(3.764, 82.811) = 7.809, p < .001, MSE = 1531.553$) and a main effect of strain ($F(1, 22) = 161.090, p < .001, MSE = 96439.796$). There was also a session \times strain interaction ($F(6, 132) = 14.532, p < .001, MSE = 1788.013$), further inspection of which revealed that percentage time spent immobile increased across sessions for WKY rats only ($F(6, 17) = 21.144, p < .001$; Wistar: $F < 1$). Inspection of this interaction also revealed that immobility increased significantly for session two compared to session one for WKY rats ($p < .001$).

The finding of increased immobility for WKY rats during the forced swim test is consistent with previous results (e.g. López-Rubalcava & Lucki, 2000; Paré, 1989a; Paré & Redei, 1993; Rittenhouse, Lopez-Rubalcava, Stanwood & Lucki, 2002; Tejani-Butt et al., 2003;). The finding of increased immobility on day one of test has also been previously reported, as has the increased immobility between test days one and two (Nam et al., 2014). As most forced swim test paradigms reported in the literature involve a 15 min pre-test and a 5 min test period, the current results are novel in showing significant differences between Wistar and WKY rats despite a significantly reduced swim time. Whilst Nam et al. (2014) suggest that increased immobility on day one of test may reflect

psychomotor retardation in the WKY strain, the subsequent experiments reported in Chapters five and six demonstrate that this alone cannot explain the WKY rats' behavioural phenotype.

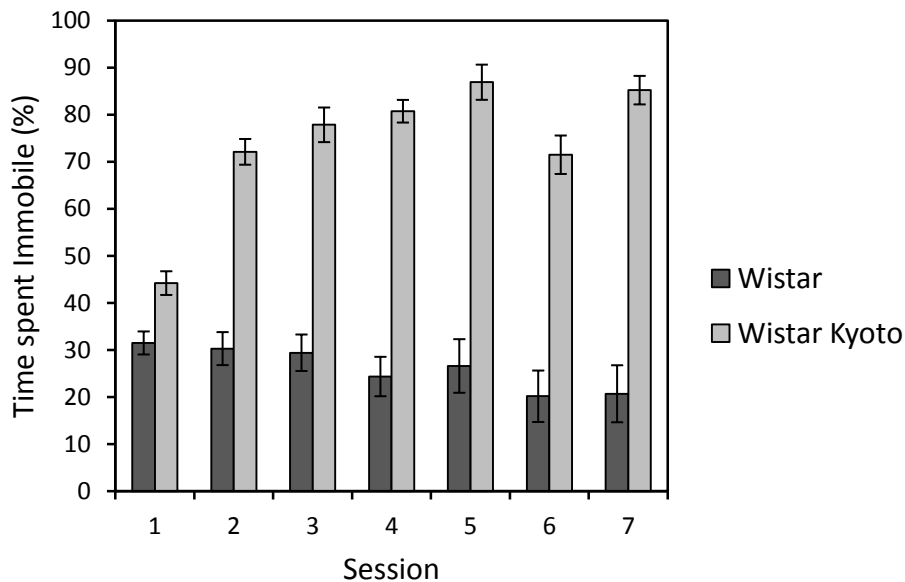


Figure 26 Percentage Time Spent Immobile for Wistar (Dark grey bars) and WKY rats (Light grey bars) during the 2 min Forced Swim Test. Data is displayed per session. Error bars represent \pm SEM.

5.5 Experiment 7 Results

Figure 27 (Panels A and B) depicts the mean consumption at each of the three sucrose concentrations (2, 8 and 24%) for Wistar and WKY rats, separated into Stress and No-stress groups.

Inspection of this figure suggests that increasing sucrose concentration did not produce an overall increase in the amount consumed for either strain or stress group, with the moderate (8%) solution instead eliciting the highest intake. Regardless of solution concentration, WKY rats appeared to consume less than their Wistar counterparts. Rats in the Stress groups, regardless of strain, also appeared to reduce their intake of the solution at each concentration. The data summarised in Figure

27 was analysed with a mixed ANOVA with factors of sucrose concentration (2, 8 and 24%), sequence order (2-8-24 vs. 24-8-2), strain (WKY vs. Wistar) and stress (Stress vs. No-stress). This was subsequently collapsed across sequence order due to a lack of over-arching effects for this factor: There was no main effect of sequence order ($F(1, 40) = 1.925, p = .173, MSE = 12.990$) and no interactions between sequence order and any other factor (All $F_s < 1$, except sequence order \times strain ($F(1, 40) = 1.786, p = .189, MSE = 12.047$) and sequence order \times stress ($F(1, 40) = 1.777, p = .190, MSE = 11.989$).

Consistent with the description of the data, ANOVA analysis revealed a main effect of concentration ($F(1.573, 67.628) = 194.244, p < .001, MSE = 346.670$), a main effect of strain ($F(1, 43) = 15.219, p < .001, MSE = 108.595$) and a main effect of stress ($F(1, 43) = 7.093, p = .011, MSE = 50.613$). There was no significant strain \times stress interaction ($F < 1$), indicating that stress did not differentially affect intake levels across the Wistar and WKY strains. There was also no strain \times concentration interaction ($F < 1$), nor strain \times stress \times concentration interaction ($F < 1$).

Pairwise comparisons for the concentration effect revealed that rats consumed significantly more 8% sucrose than both 2% ($F(1, 43) = 367.747, p < .001, MSE = .054$) and 24% ($F(1, 43) = 10.614, p = .002, MSE = .036$) sucrose solutions. Consumption of 24% sucrose was also significantly higher than of 2% sucrose ($F(1, 43) = 164.080, p < .001, MSE = .089$). Importantly, further investigation of the strain effect revealed that WKY rats consumed less sucrose than their Wistar counterparts at all three solution concentrations: 2% ($F(1, 43) = 9.081, p = .004, MSE = 46.924$), 8% ($F(1, 43) = 7.288, p = .010, MSE = 22.086$) and 24% ($F(1, 43) = 24.214, p < .001, MSE = 42.248$).

Likewise, the ANOVA revealed that rats in the Stress conditions consumed less sucrose at each of the three concentrations, with non-significantly lower intake of 2% sucrose ($F(1, 43) = 1.335, p = .254, MSE = 6.900$) and significantly lower intake of 8% ($F(1, 43) = 7.712, p = .008, MSE = 23.372$) and 24% sucrose ($F(1, 43) = 13.544, p = .001, MSE = 23.630$).

Figure 28 (Panels A and B) depicts the mean LCS elicited by Wistar and WKY rats, separated into the two stress conditions, when consuming each of the three sucrose concentrations. Wistar rats in both the Stress and No-stress groups increased the size of their licking clusters as the solution consumed increased in concentration. LCS also appeared to increase slightly with concentration for both WKY Stress and No-stress rats. However, their overall affective response to each solution appeared to be extremely blunted.

An ANOVA was performed with factors of concentration, sequence order, strain and stress. As sequence order did not impact on the interpretation of the results, the results presented here are collapsed across this factor (sequence order: $F < 1$; all interactions $F < 1$ except sequence order \times strain \times stress ($F(1, 40) = 2.130, p = .152, MSE = 4074.382$); sequence order \times concentration \times strain ($F(2, 80) = 1.022, p = .365, MSE = 1379.732$); and sequence \times concentration \times strain \times stress ($F(2, 80) = 1.306, p = .277, MSE = 1762.672$). The ANOVA revealed a main effect of solution concentration ($F(1.497, 64.378) = 49.942, p < .001, MSE = 17458.341$), a main effect of strain ($F(1, 43) = 30.713, p < .001, MSE = 26117.736$) and a strain \times solution concentration interaction ($F(2, 86) = 17.943, p < .001, MSE = 4695.319$). In contrast to the consumption data, ANOVA revealed no main

effect of stress ($F < 1$). There was also no strain \times stress interaction ($F < 1$) or concentration \times strain \times stress interaction ($F < 1$)⁴.

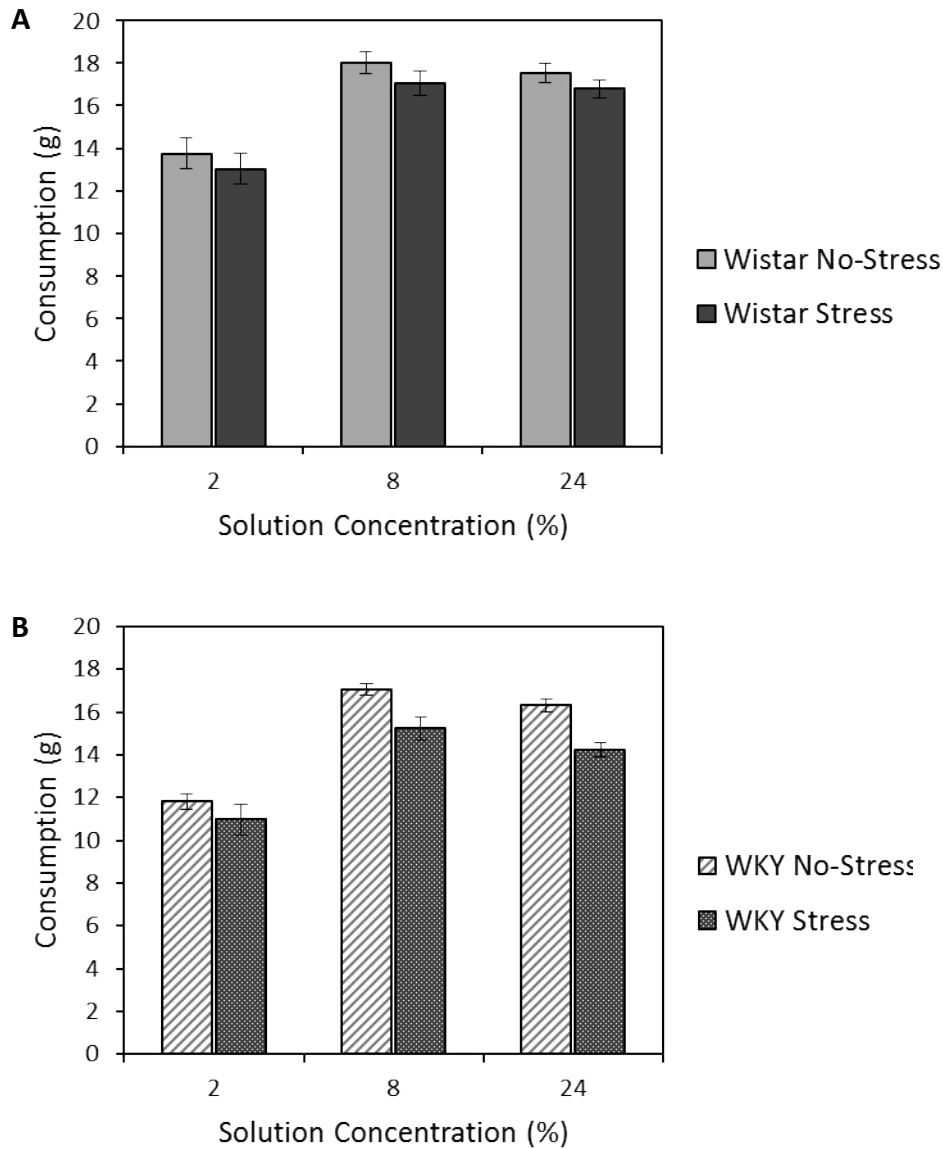


Figure 27 Mean consumption of three different concentrations of sucrose (2, 8 and 24%) for Wistar rats (Panel A) and WKY rats (Panel B). Light grey bars represent the No-Stress condition; dark grey bars represent the Stress condition. N = 12, except for the WKY No-stress group where N = 11. Error bars represent \pm SEM.

⁴ Depression is associated with a two - three times higher prevalence of diabetes (Adriaanse & Bosmans, 2010; Golden, 2007). Similarly, chronically activated glucocorticoids, as a result of stress, also leads to insulin resistance (Sominsky & Spencer, 2014). With studies demonstrating a link between insulin resistance and opioid dysfunction (Berent-Spillson, Love, Pop-Busui et al., 2011), it is at least plausible that dysregulated insulin activity may account for the current results in WKY rats.

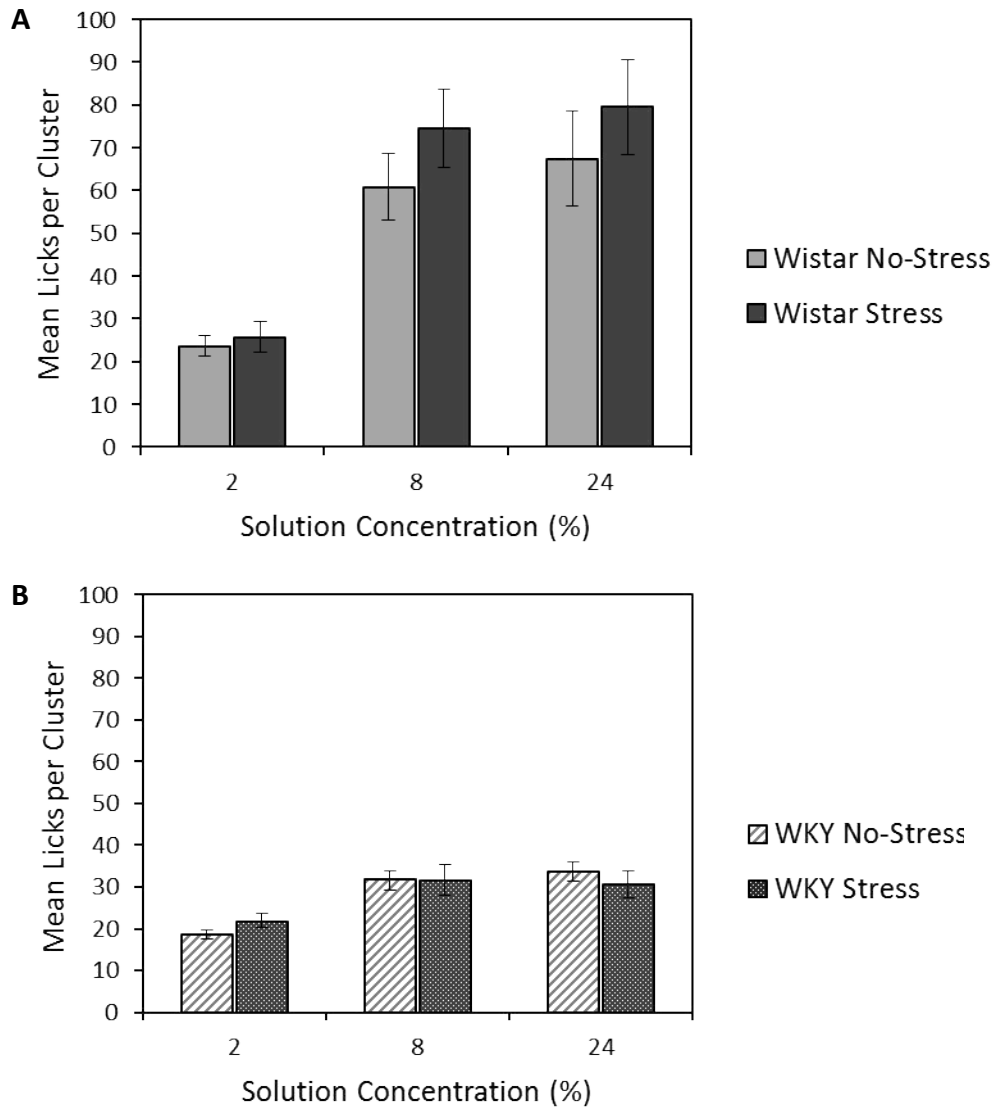


Figure 28 Mean Lick Cluster Size for each of the three concentrations of sucrose for Wistar (Panel A) and WKY (Panel B) rats. N = 12, except for the WKY No-stress group where N = 11. Error bars represent ± SEM.

Pairwise comparisons of the strain × solution concentration interaction revealed that LCS was significantly higher for Wistar rats consuming the 8% solution compared with the 2% solution ($F(1, 43) = 108.334, p < .001, MSE = 17.065$) and during consumption of the 24% solution compared with the 2% solution ($F(1, 43) = 69.595, p < .001, MSE = 34.328$). There was no significant difference in LCS between 24% sucrose and 8% sucrose ($F(1, 43) = 2.465, p = .124, MSE = 14.033$). For WKY

rats LCS was higher during the consumption of 8% sucrose than 2% sucrose ($F(1, 43) = 7.227, p = .010, MSE = 17.834$). There was also a trend toward higher LCSs for 24% sucrose compared with 2% sucrose but this did not reach significance ($F(1, 43) = 3.916, p = .054, MSE = 35.880$). There was no difference in the LCSs exhibited when the rats were consuming 8% and 24% sucrose solutions ($F < 1$). Comparing across the two strains revealed significantly lower LCSs exhibited by WKY rats for 8% sucrose ($F(1, 43) = 30.374, p < .001, MSE = 15200.990$) and 24% sucrose ($F(1, 43) = 25.005, p < .001, MSE = 20085.333$). WKY rats also exhibited lower LCSs when consuming the 2% sucrose solution, but non-significantly so ($F(1, 43) = 3.172, p = .082, MSE = 222.052$).

Figure 29 displays the mean inter-lick intervals (ILI) extracted from the record of licks. There was a tendency for marginally higher ILIs for WKY rats compared to the Wistar strain, primarily at the highest sucrose concentration. ANOVA revealed a main effect of solution concentration ($F(1.739, 74.763) = 15.006, p < .001, MSE = 221.666$) with the lowest ILIs occurring during consumption of the 2% sucrose solution. It also revealed a strain \times concentration interaction ($F(2, 86) = 8.191, p < .001, MSE = 105.181$) but, critically, no main effect of strain ($F(1, 43) = 3.102, p = .085, MSE = 316.681$). There was also no main effect of stress ($F < 1$) and no interaction between stress and any other factor (All $F_s < 1$, except concentration \times stress ($F(2, 86) = 1.136, p = .326, MSE = 14.587$)).

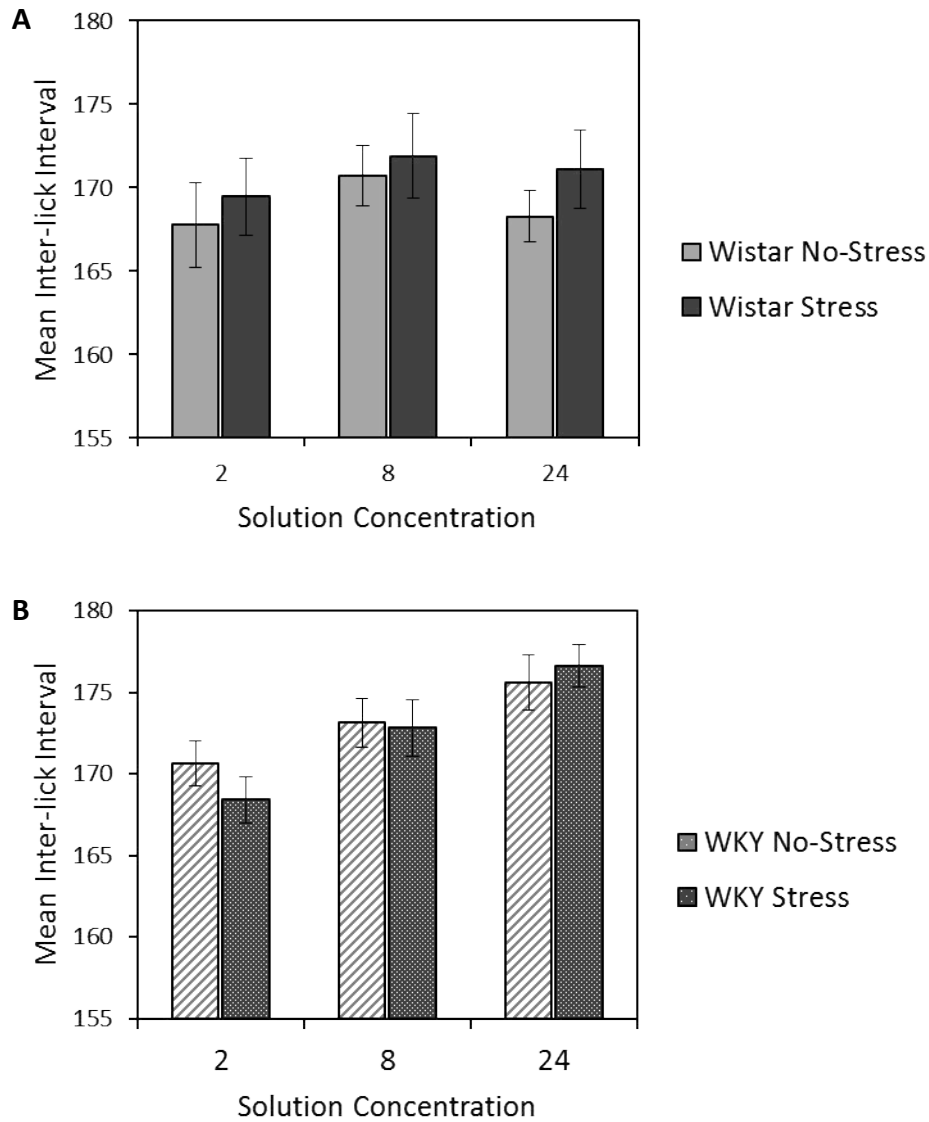


Figure 29 Mean Interlick interval for Wistar and WKY strains when consuming each of the three concentrations of sucrose (2, 8, and 24%), separated into Stress (dark grey bars) and No-stress (light grey bars) groups. N = 12, except for the WKY No-stress group where N = 11. Error bars represent \pm SEM.

It is important to recognise that the longer the ILI the greater the chance that the next lick performed by the rat will be grouped into the subsequent cluster, leading to generally lower LCSs. The lack of a strain effect here implies that ILI differences cannot fully explain the lower LCSs experienced by WKY rats. The absence of a reciprocal relationship between ILI and LCS is further highlighted by analysing the strain \times concentration interaction. ILI was significantly longer for WKY

rats compared to Wistar rats for the 24% solution only ($F(1, 43) = 13.366, p = .001, MSE = 484.869$). However, LCS was significantly smaller for WKY rats compared to Wistar rats for not only the 24% solution but also for the 8% solution. Clearly the shorter LCS at the moderate solution of sucrose cannot be explained by strain differences in ILI.

5.5.1 Additional analysis

Although the test consumption of sucrose represents only a fraction of the rats overall energy intake, the caloric requirements of animals generally scale such that they relate to weight $^{0.75}$ (Kleiber, 1947). If this scaling is applied to the consumption tests described above, the main effect of rat strain is removed ($F < 1$), but the remaining features of the analysis are unaffected. Therefore, differences in bodyweight may have contributed to the lower overall consumption exhibited by WKY rats – this, together with the discrepancy between stress effects on consumption and LCS measures, emphasises that consumption measures alone can be ambiguous indicators of hedonic responses.

5.6 Summary

The aim of Experiment seven was to determine whether WKY rats display behaviours analogous to consummatory anhedonia compared to an outbred Wistar control strain. Reduced reward value was observed for WKY rats as indexed by reduced palatability and consumption across the sucrose concentrations. Importantly, whilst ILI was generally higher for WKY rats, strain differences were not statistically significant. The lack of a reciprocal relationship between ILI and LCS for WKY animals further suggests that increased ILI cannot fully account for the decreased LCSs seen. Based on the current measures, WKY rats appear to demonstrate a reduced hedonic tone that is not dependent on the application of external stressors. Furthermore, as LCS is modulated by solution concentration in the WKY strain, these results suggest that their hedonic responses to rewards are blunted but not entirely absent.

The results from this experiment demonstrate that microstructural analysis of licking is a sensitive measure of hedonic capacity in rodent models of disease, more so than consumption measures. The results both suggest a hedonic deficit in the WKY strain, and provide an important contrast to the lack of such an effect in MAM-treated animals. These issues will be considered in greater depth in the general discussion. To determine whether the WKY rats also include deficits in the anticipatory aspects of hedonic processing, Experiment eight adopted the negative anticipatory contrast procedure that relies on the accurate prediction of future appetitive rewards.

5.7 Experiment 8 - Materials and Methods

5.7.1 Apparatus

Testing was conducted in the same six automated drinking chambers as described in Experiment one (section 2.2.2). The solutions used in the experiment were 4% and 32% sucrose made daily w/w with deionised water. Distinct light conditions (room lighting vs. angle-poise lamp fitted with a red bulb) and a metal grid floor insert were used to create distinct contexts. As in Experiment three (Section 3.5.2), white noise was not included to create the contexts. This was to avoid potential strain \times stress interactions as the anxiety phenotype of WKY rats means that they are potentially hypersensitive to external stressors. Previous findings in our lab have found that particular noises can be aversive to different rat strains (unpublished observation).

5.7.2 Procedure

Pretraining. All rats were habituated to the drinking boxes for 10 minutes each day for three days prior to the pre-training phase of the experiment. This was to overcome stress effects caused by a novel environment which may have differentially affected the stress-sensitive WKY rats. No solutions were made available during this habituation. During pre-training, rats were water restricted for 22 hours and then given access to water for 10 min from both the left and right hand side of the drinking chamber. During this initial training, drinking spouts were positioned inside the chamber to allow for easy detection by the rats. Only one pre-training day was given,

after which rats were returned to *ad libitum* water and remained so for the duration of the experiment.

Acquisition Training. Acquisition training of anticipatory contrast consisted of daily drinking sessions, 9 min in duration, as described in Experiment one and three (section 2.2 and section 3.5). Briefly, rats received 3 min access to an initial 4% sucrose solution that was followed by access to a second solution for 6 min, the identity of which was either more 4% sucrose or preferred 32% sucrose depending on the context. Solution to context assignments, as described in Experiment three, were fully counterbalanced across strain and stress conditions: For half the animals in each strain and stress group, context one was paired with the 4-4 condition and context two was paired with 4-32 condition; while for the other half the opposite assignment was given. Acquisition training lasted thirty-two days and was carried out on consecutive days where possible. Initially spouts for both the left and right bottles were positioned within the chamber, but were gradually moved back across sessions (taking approximately three days but done on a rat by rat basis) until they were flush with the outside of the chamber.

5.7.3 Data analysis

Data analysis was performed as described in Experiment one (Section 2.2.4). Data were analysed with a repeated measures ANOVA with factors of block (1-8), contrast condition (4-4 or

control condition vs. 4-32 or contrast condition), strain (Wistar vs. WKY) and stress (Stress vs. No stress).

5.8 Results

Figure 30 (Panels A – D) depicts consumption of the 4% sucrose solution presented first each day across eight two-session blocks. Data in the top two panels depicts the data for the Wistar control strain, separated into No-stress (Panel A) and Stress conditions (Panel B). Data in the bottom two panels depicts the data for the WKY experimental strain, again separated into the two stress conditions, No-stress (Panel C) and Stress (Panel D). Across training an anticipatory contrast effect emerged such that consumption levels of the initial 4% sucrose solution depended upon the identity of the subsequent solution in the pairing. That is, consumption of the initial 4% solution was higher when it preceded more of the same solution compared to when it preceded the more palatable 32% solution. Critically, an anticipatory contrast effect in consumption appeared to develop for both Wistar and WKY strains and did not seem to be overly influenced by exposure to an unpredictable chronic mild stress procedure. ANOVA results were consistent with this impression: There was a main effect of block ($F(4.552, 198.981) = 59.884, p < .001, MSE = 30.420$), and contrast condition (4-4 vs. 4-32) ($F(1, 44) = 28.357, p < .001, MSE = 10.961$) as well as a significant interaction between these two factors ($F(3.996, 175.843) = 3.968, p = .004, MSE = 1.868$). The ANOVA yielded a main effect of strain ($F(1, 44) = 13.770, p = .001, MSE = 41.557$), with WKY rats generally consuming less than their Wistar counterparts. There was also a main

effect of stress ($F(1, 44) = 6.074, p = .018, MSE = 18.330$), with lower consumption levels for Stress compared to No-stress rats. There was no interaction between contrast condition and strain ($F < 1$), or between contrast condition and stress ($F < 1$). There was also no three-way interaction between either of these pairs of factors and block (block \times contrast condition \times strain ($F < 1$); block \times contrast condition \times stress ($F < 1$)) and no four-way interaction between these factors ($F(7, 308) = 1.996, p = .055, MSE = .537$).

Figure 31 (Panels A - D) depicts the average size of licking clusters elicited by the rats when consuming the first 4% sucrose solution presented each session across the eight 2-session blocks. Contrast dependent changes in lick cluster size were evident in the Wistar No-stress (Panel A) and Stress groups (Panel B). That is, as training progressed, lick cluster sizes were suppressed when the second solution in the pairing was the more palatable 32% solution compared to when the second solution was of equal palatability. In contrast to this, the anticipatory contrast effect was severely attenuated for WKY No-stress (Panel C) and Stress rats (Panel D), such that lick cluster size of the initial solution was comparable across the two contrast conditions.

ANOVA analysis revealed a main effect of block ($F(3.556, 156.449) = 7.455, p < .001, MSE = 4751.408$), a main effect of contrast condition ($F(1, 44) = 9.392, p = .004, MSE = 6191.711$) and a significant interaction between these factors ($F(3.996, 175.822) = 2.987, p = .020, MSE = 727.600$). Overall, WKY rats exerted fewer licks per cluster compared with Wistar animals as the ANOVA also yielded a main effect of strain ($F(1, 44) = 17.221, p < .001, MSE = 47578.086$).

Regardless of strain, LCS was not influenced by the application of an external stressor with no main effect of stress ($F < 1$) or stress \times strain interaction ($F < 1$) yielded by the analysis.

ANOVA analysis revealed a significant contrast condition \times strain interaction ($F(1, 44) = 4.442, p = .041, MSE = 2928.399$) but no contrast condition \times strain \times stress interaction ($F < 1$).

There was no three-way interaction between block, contrast condition and strain ($F < 1$); block, contrast condition and stress ($F < 1$); and no four-way interaction between block, contrast condition, strain and stress ($F(7, 308) = 1.427, p = .194, MSE = 198.370$). Further analysis of the contrast condition \times strain interaction revealed significantly higher LCSs for the control (4-4) than contrast (4-32) conditions for Wistar rats ($F(1, 44) = 13.376, p = .001, MSE = 6.870$). No difference in LCS for the initial solution was seen between contrast and control conditions across the two stress groups for WKY rats ($F < 1$). That is, only Wistar animals showed a negative anticipatory contrast effect in their affective responses to the initial solution.

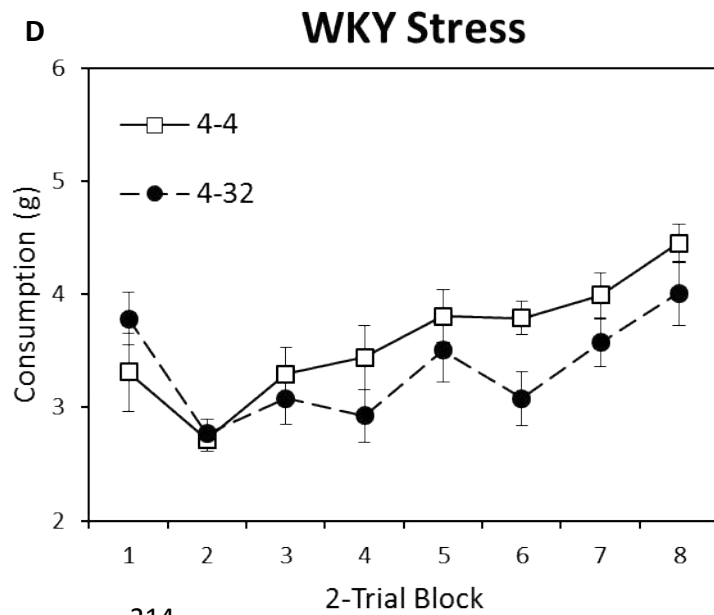
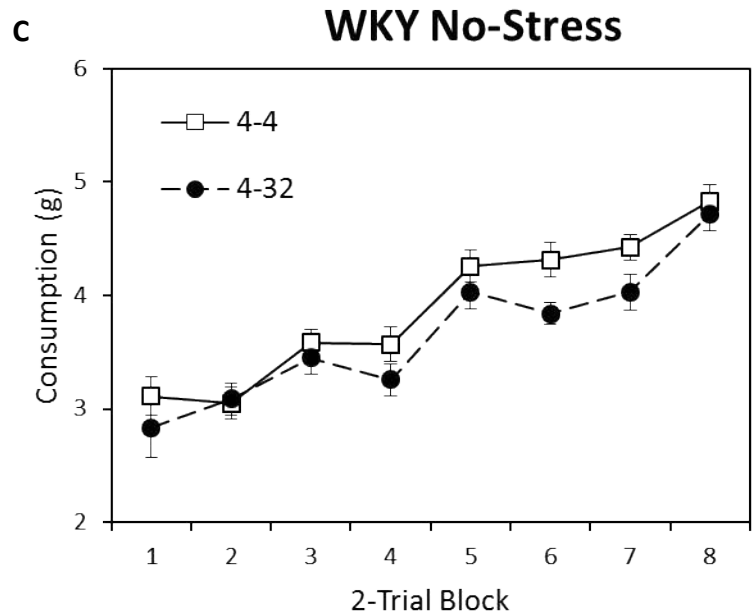
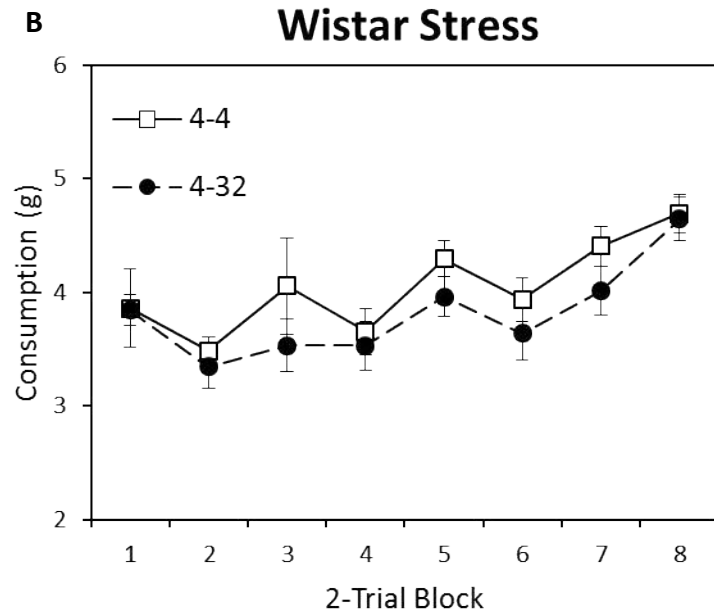
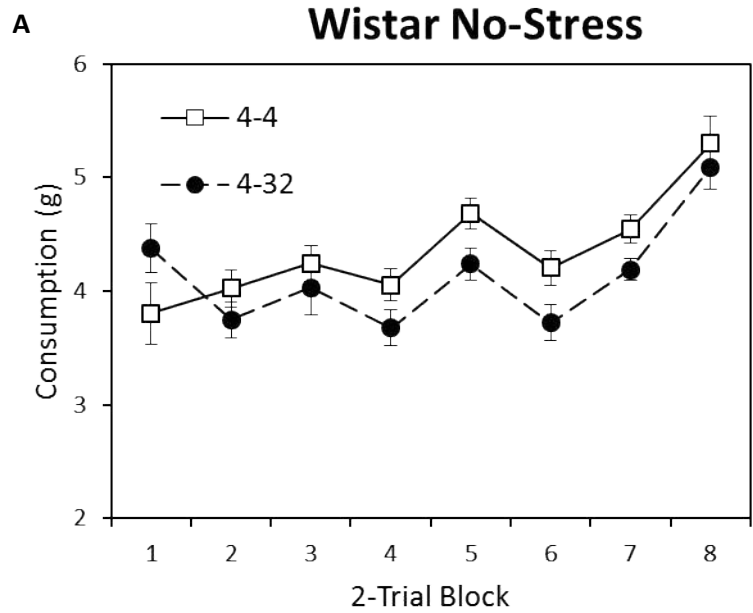


Figure 30 Mean Consumption (\pm SEM) of the initial 4% solution made available each day as a factor of strain (Wistar: Panels A and B; WKY: Panels C and D) and stress groups (No-Stress: Panels A and C; Stress: Panels B and D). Open symbols represent the consumption from the initial bottle in the control condition (when 4% sucrose is available in the second bottle) and filled symbols represent the consumption from the initial bottle in the contrast condition (when the preferred 32% solution is available in the second bottle). The data presented is averaged over two-day blocks. The first bottle was available for 3 min per day.

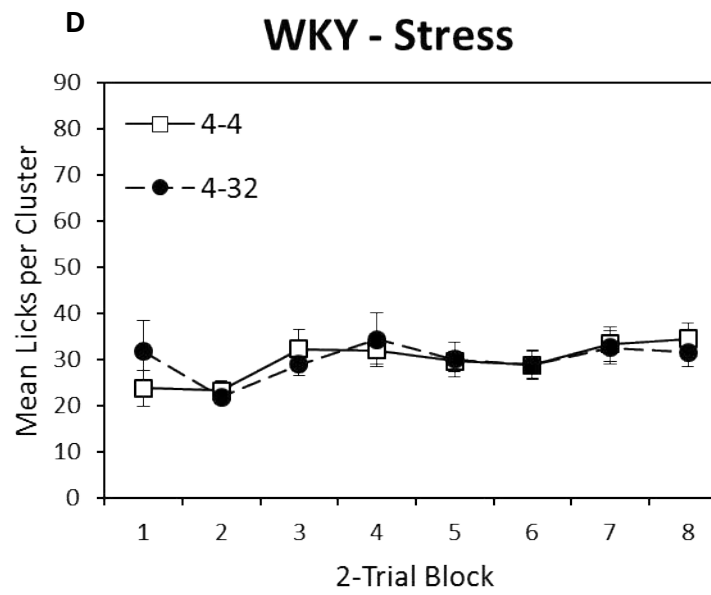
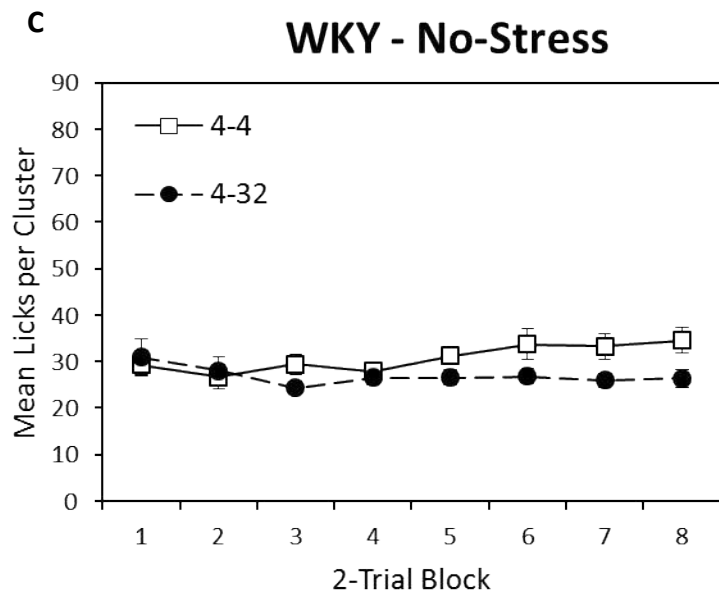
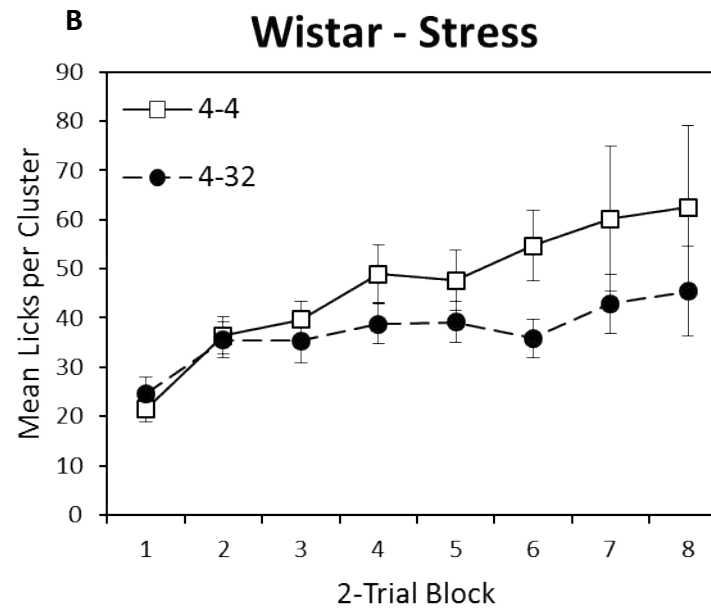
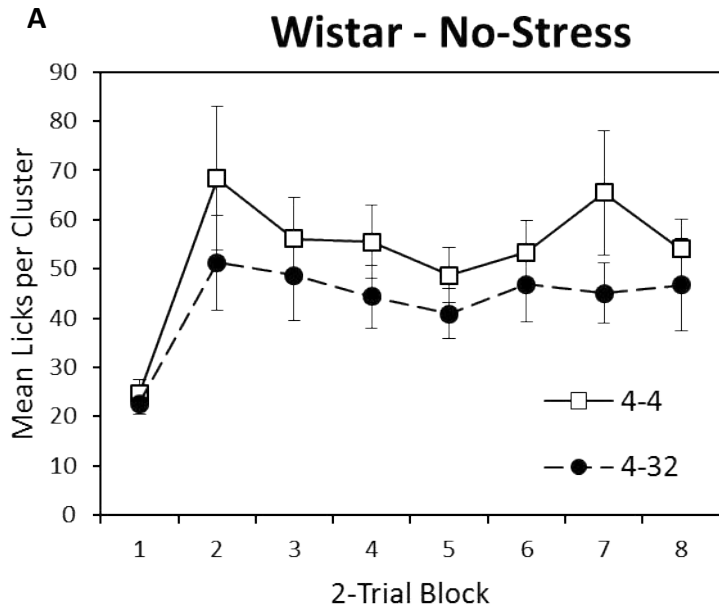


Figure 31 Mean number of licks per cluster (\pm SEM) during consumption of the initial 4% sucrose solution presented each day as a factor of strain (Wistar: Panels A and B; WKY: Panels C and D) and stress (No-Stress: Panels A and C; Stress: Panels B and D). Open symbols represent the mean LCS for the initial solution in the control condition (when the second bottle in the pairing also contains 4% sucrose) and filled symbols represent the mean LCS for the contrast condition (when the second bottle in the pairing contains preferred 32% sucrose). The data presented is averaged across 2-session blocks. The first solution in each condition was made available for 3 min.

ILI was extracted from the record of licks for the initial 4% solution. Similarly to Experiment seven, WKY rats displayed longer ILIs compared with their Wistar counterparts ($F(1, 44) = 9.474, p = .004, MSE = 3663.765$) (see Table 12 for details). There were no significant differences in ILI between Stress and No-stress groups ($F < 1$) and no interaction between strain and stress ($F < 1$). Importantly, there was also no contrast condition \times strain interaction ($F < 1$) and no block \times contrast condition \times strain interaction ($F(7, 308) = 1.224, p = .289, MSE = 39.067$).

Table 12 Mean Interlick Intervals as a factor of strain, stress and pairing (4-4 = control condition; 4-32 = contrast condition) for the first solution presented each day.

Strain	Stress	Pairing	First bottle Mean ILI (\pm SEM)
Wistar	No-stress	4-4	166.154 (\pm 1.735)
		4-32	164.361 (\pm 1.536)
	Stress	4-4	166.658 (\pm 2.299)
		4-32	164.064 (\pm 2.147)
WKY	No-stress	4-4	171.189 (\pm 2.082)
		4-32	170.286 (\pm 2.348)
	Stress	4-4	171.356 (\pm 1.518)
		4-32	165.878 (\pm 3.165)

Figure 32 (Panels A – D) depicts the average consumption levels for the second solution presented to the rats (4 or 32% sucrose) separated into the four strain and stress conditions across the eight 2-session blocks. Consumption levels were larger compared to the initial solution due to the different durations of bottle access: The first bottle was only made available for 3 minutes; the second bottle was made available for 6 minutes. Similarly to the first solution data, consumption levels from the second bottle were larger for the Wistar strain compared to the WKY strain. For both strains, there was higher consumption of the more palatable 32% sucrose solution compared with the moderately palatable 4% sucrose solution. ANOVA results showed a main effect of block ($F(3.308, 145.530) = 91.795, p < .001$,

$MSE = 168.344$) and solution concentration (4 vs. 32%) ($F(1, 44) = 23.846, p < .001, MSE = 159.824$) as well as a main effect of strain ($F(1, 44) = 65.228, p < .001, MSE = 539.434$). There was no solution concentration (4 vs. 32%) \times strain interaction ($F(1, 44) = 1.127, p = .294, MSE = 7.551$). There was a main effect of stress ($F(1, 44) = 8.060, p = .007, MSE = 66.652$), with rats consuming generally less from the second bottle if they were in the stress group, but no strain \times stress interaction ($F < 1$). The ANOVA yielded a block \times stress interaction ($F(7, 308) = 2.473, p = .018, MSE = 2.143$), further analysis of which revealed significant differences in total consumption between stress and no stress conditions for block 2-7 (smallest $F(1, 44) = 5.260, p = .027, MSE = 3.953$). Non-significantly higher consumption was seen for stressed rats compared to No-stress rats during block one ($F < 1$). Thereafter, consumption was always lower for the Stress group.

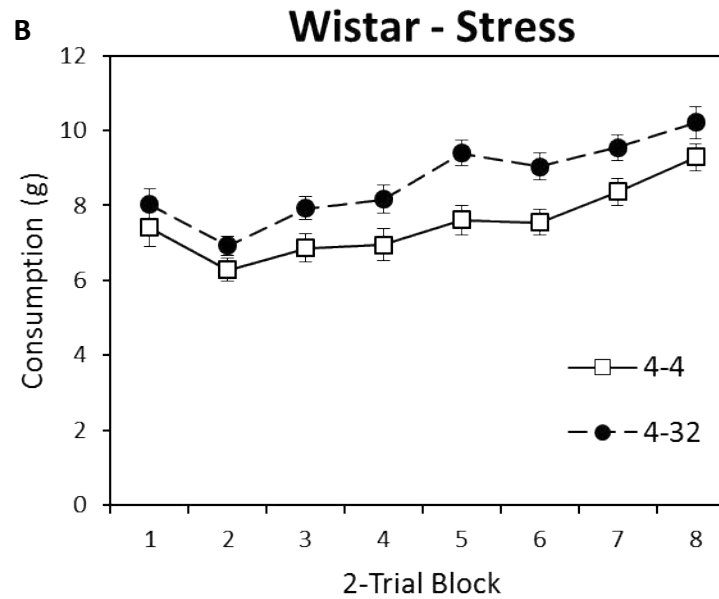
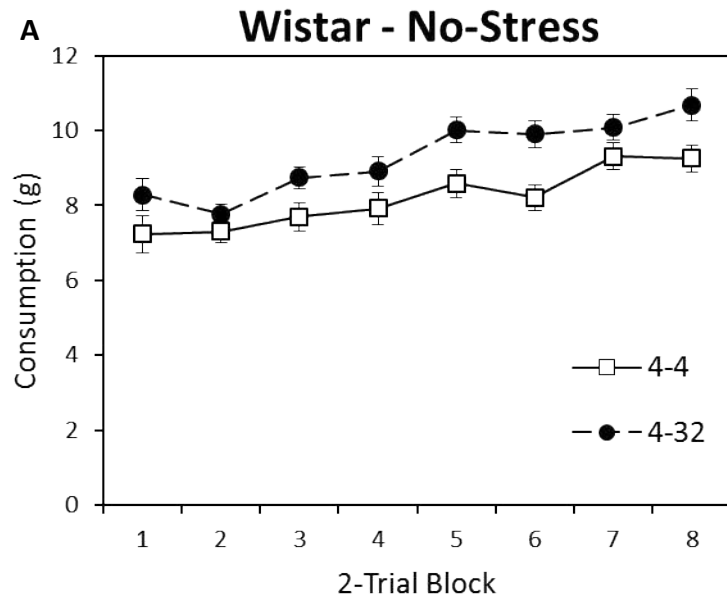
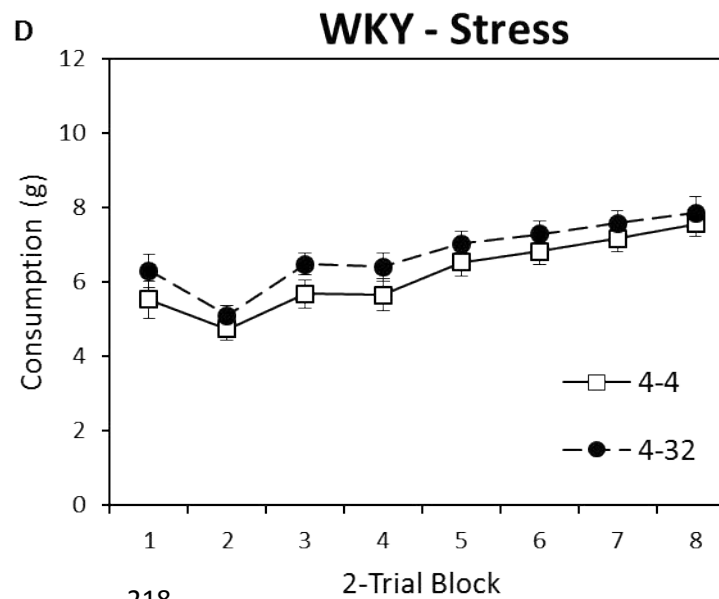
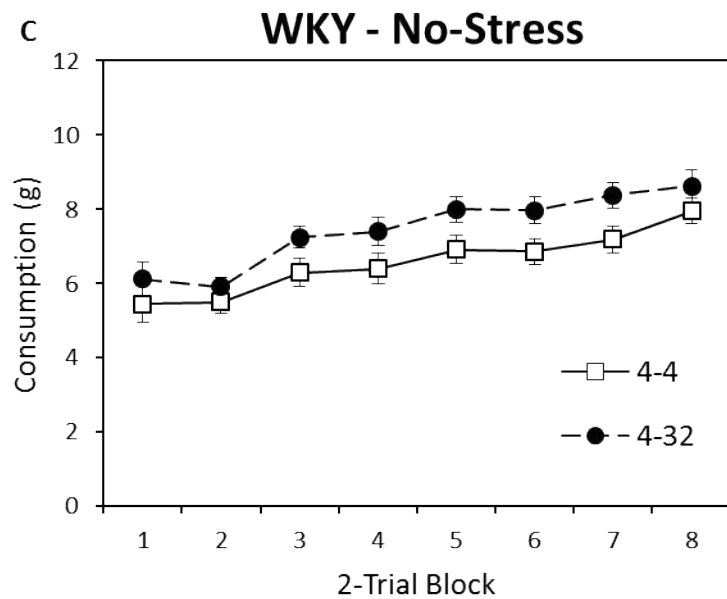


Figure 32 Mean consumption (\pm SEM) for the second bottle made available each day. Open symbols represent the consumption of 4% sucrose in the second bottle and filled symbols represent the consumption of 32% sucrose in the second bottle. Data presented is averaged across 2-session blocks. Solutions in the second bottle were made available for 6 min.



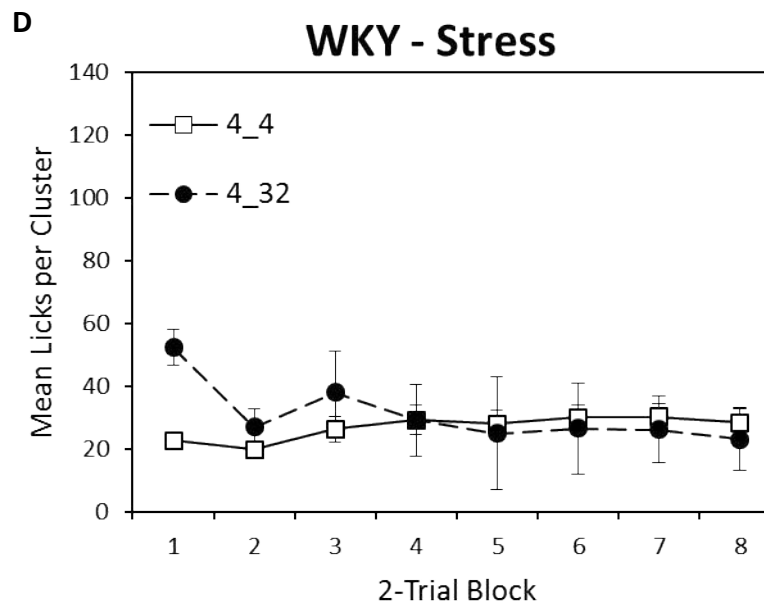
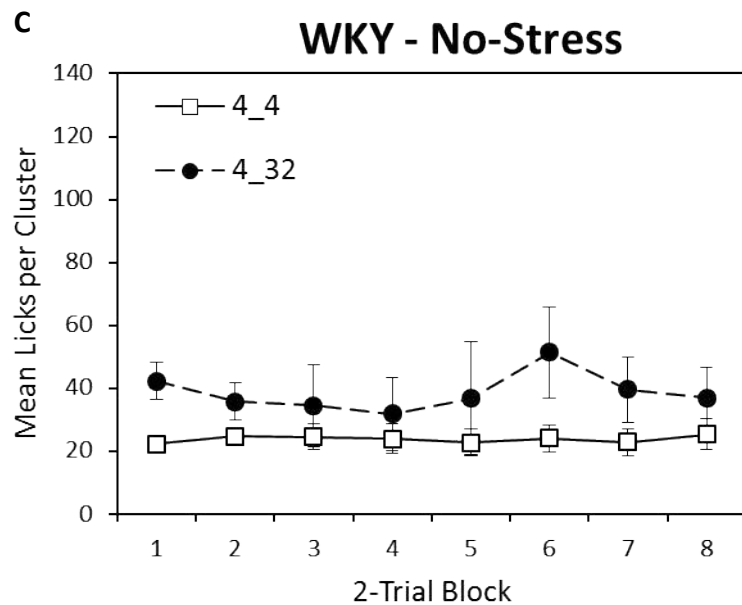
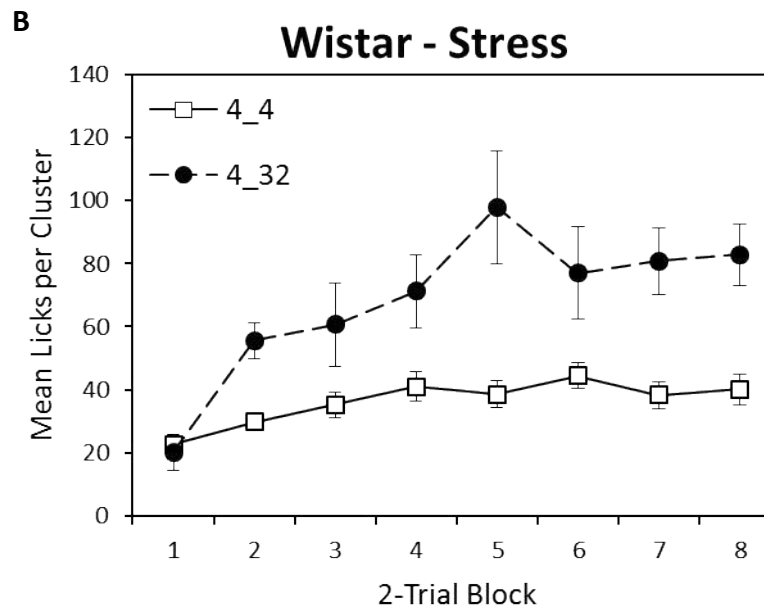
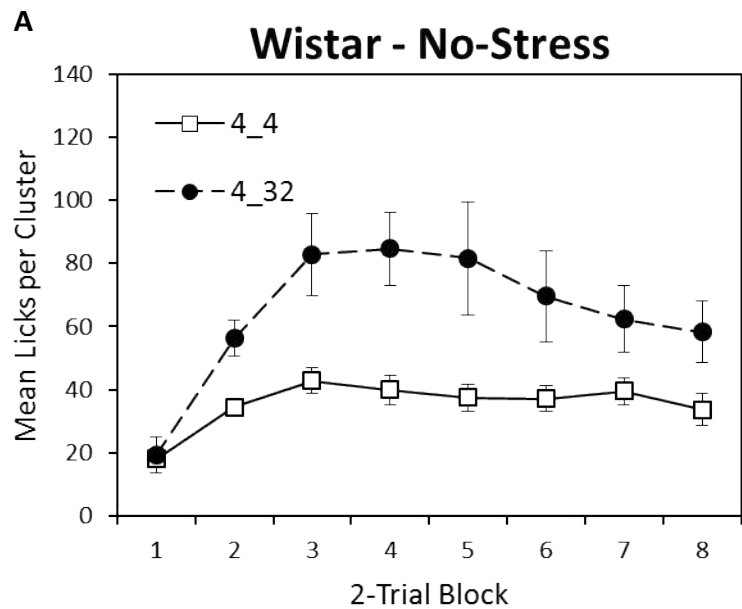


Figure 33 Mean Lick Cluster Size (\pm SEM) for the second solution available each day (4% vs. 32%) for Wistar No-stress (Panel A), Wistar Stress (Panel B), WKY No-stress (Panel C) and WKY Stress (Panel D) rats. In all cases, open symbols represent the rat's response to the 4% sucrose solution made available in the second bottle, whereas the filled symbols represent the rat's response to the 32% sucrose solution made available in the second bottle.

Figure 33 (Panels A – D) depicts the average size of licking clusters performed by the rat when consuming solution from the second bottle (4 vs. 32% sucrose) again collated into eight 2-session blocks. In accordance with data from the initial solution in the pairing, WKY rats exerted fewer licks per cluster compared to their Wistar counterparts. For Wistar rats, in both Stress and No-stress groups, LCSs elicited during consumption of the 32% solution were higher compared with when the 4% solution was consumed, reflecting the greater palatability of this solution. For WKY Stress and No-stress rats, LCS was also marginally higher for the 32% sucrose solution than for the 4% sucrose solution, at least early in training.

ANOVA revealed a main effect of block ($F(3.406, 149.882) = 7.825, p < .001, MSE = 7581.702$) and a main effect of sucrose concentration (4 vs. 32%) ($F(1, 44) = 21.043, p < .001, MSE = 76764.604$). There was also a main effect of strain ($F(1, 44) = 18.616, p < .001, MSE = 85267.132$) but not a main effect of stress ($F < 1$) and no interaction between these two factors ($F < 1$). There was, however, a block \times strain \times stress interaction ($F(7, 308) = 2.201, p = .034, MSE = 1037.675$), further inspection of which revealed that WKY rats exhibited higher LCSs, regardless of stress treatment, compared to their Wistar counterparts for block one (smallest $F(1, 44) = 10.349, p = .002, MSE = 1533.681$). For all subsequent blocks, Wistar rats exerted higher LCSs than WKY animals, which trended towards being more pronounced for animals in the Stress group (Stress: smallest $F(1, 44) = 2.588, p = .115, MSE = 1490.426$; largest $F(1, 44) = 19.842, p < .001, MSE = 7646.762$; No-stress, smallest $F(1, 44) = 2.076, p = .157, MSE = 1494.208$; largest $F(1, 44) = 15.149, p < .001, MSE = 7153.925$). Importantly, the ANOVA yielded a sucrose concentration \times strain ($F(1, 44) =$

5.817, $p = .020$, $MSE = 21222.110$) and block \times sucrose concentration \times strain interaction ($F(7, 308) = 5.628$, $p < .001$, $MSE = 2676.065$). Simple effect analyses revealed that WKY rats exerted lower lick cluster sizes compared to Wistar rats for both the 4% sucrose solution ($F(1, 44) = 11.390$, $p = .002$, $MSE = 1338.229$) and the 32% sucrose solution ($F(1, 44) = 13.142$, $p = .001$, $MSE = 11972.926$). Furthermore, it revealed that LCS was significantly larger for 32% sucrose than 4% for Wistar rats ($F(1, 44) = 24.494$, $p < .001$, $MSE = 37.995$). Whilst there was no overall difference in LCS between the two solution concentrations for WKY animals ($F(1, 44) = 2.366$, $p = .131$, $MSE = 37.995$), further analysis of the block \times sucrose concentration \times strain interaction revealed that WKY rats exerted a significantly higher LCS for 32% sucrose compared to 4% sucrose for blocks one and two (smallest $F(1, 44) = 5.221$, $p = .027$, $MSE = 15.912$).

Separate analysis of the right bottle data for LCSs exhibited by WKY rats was then performed. Critically, this revealed a main effect of solution concentration ($F(1, 22) = 4.843$, $p = .039$, $MSE = 8631.161$) and no effect of stress ($F < 1$). Further analysis again revealed that, whilst LCS was always higher for the 32% solution compared with the 4% solution, significant differences were only found early in training (Blocks 1-3: smallest $F(1, 22) = 4.920$; $p = .037$, Block 3).

Mean inter-lick intervals (ILI) were extracted from the record of licks for the second solution presented each day and are displayed in Table 13. WKY rats, regardless of stress treatment, appeared to display higher ILIs, particularly during the consumption of 32% sucrose. This was supported by ANOVA analysis: There was a main effect of solution concentration ($F(1, 44) = 51.117$, $p < .001$, $MSE = 5302.505$), a main effect of strain ($F(1, 44) = 35.781$, $p < .001$, $MSE = 11658.048$)

and a significant interaction between these two factors ($F(1, 44) = 61.057, p < .001, MSE = 6333.633$). Further investigation of the interaction revealed that WKY rats displayed significantly higher ILIs compared to the Wistar strain when consuming 32% sucrose ($F(1, 44) = 93.218, p < .001, MSE = 2.198.592$). No significant difference was found between the two strains for the 4% sucrose solution ($F(1, 44) = 3.024, p = .089, MSE = 313.703$). In terms of the stress manipulation, ANOVA results yielded no significant main effect ($F < 1$). Further, there were no significant interactions between strain and stress ($F < 1$), solution concentration and stress ($F(1, 44) = 3.024, p = .089, MSE = 313.703$), or solution concentration, strain and stress ($F < 1$). It should be noted that higher ILIs demonstrated for the WKY strain for the higher concentration of sucrose may well be contributing to the lower LCSs seen for this solution concentration. That said, previous non-contrast consumption tests (Experiment seven, Section 5.5) have demonstrated that WKY rats exhibit reduced affective responses to sucrose that are not confounded by differences in ILI.

Table 13 Mean Interlick Intervals as a factor of strain, stress and solution concentration (4% vs. 32%) for the second solution presented each day.

Strain	Stress	Solution Concentration	Mean ILI (\pm SEM)
		(%)	
Wistar	No-stress	4	166.137 (\pm 1.662)
		32	164.174 (\pm 1.507)
	Stress	4	165.273 (\pm 2.088)
		32	166.258 (\pm 1.951)
WKY	No-stress	4	168.381 (\pm 2.169)
		32	178.297 (\pm 1.543)
	Stress	4	167.126 (\pm 2.275)
		32	179.207 (\pm 1.696)

5.9 Summary

Experiment eight aimed to determine whether anticipatory hedonic deficits are present in the WKY model of depression using a negative anticipatory contrast procedure. For the outbred Wistar control strain, the rats' consumption of the initial 4% sucrose solution was lower on days when 4% sucrose preceded access to 32% sucrose than when it preceded access to more 4% sucrose. Consistent with the findings presented in Experiment one and Experiment three, Wistar rats also exhibited contrast dependent changes in their palatability responses, with lower LCSs for the initial solution in the contrast (4-32) condition compared to the control (4-4) condition. For the inbred WKY strain, rats also demonstrated contrast dependent changes in their consumption levels as, across training, they consumed less of the initial solution in the contrast condition compared to the control condition. However, unlike for the outbred strain, WKY rats failed to show any contrast dependent changes in their palatability responses towards the initial solution. That is, the LCSs exhibited by these animals for the initial solution remained equally low, regardless of the second solutions identity. This dissociation suggests that WKY rats are able to form some expectation of the second solution in the pairing, but are not able to modulate their affective responses in light of this expectation. These results are consistent with anticipatory anhedonia in this rat strain but it must be acknowledged that hedonic range is extremely blunted in these animals (as will be discussed fully in the discussion section after Chapter six). Similar to Experiment seven, antecedent stressors were not necessary for this anticipatory hedonic deficit to manifest. Chapter six reports the investigation of whether the deficits present in the WKY model also include impaired reward processing in instrumental conditioning situations.

Before moving to the analysis of reward processing, it is worth briefly noting the implications of the dissociation seen between the consumption and palatability measures in the WKY rats for the analysis of negative anticipatory contrast more generally. Whilst 'normal' animals usually show suppressed intake and palatability responses towards an initial solution that is reliably followed by a preferred solution, the behaviour of the WKY animals suggests that these might be two independent consequences of experiencing contrast. That is, the suppressed intake may not be due to the contrasted solution becoming one of functionally lower hedonic value as would be suggested by a devaluation mechanism. However, given the possibility of floor effects on the LCS measure in the WKY rats, the current results do not conclusively rule out devaluation as a mechanism for producing negative anticipatory contrast – although they do clearly suggest that additional experimental evaluation of this possibility is required.

Chapter 6

6. Value Representations in WKY Rats

6.1 Introduction

Based on the negative anticipatory contrast results presented in the previous chapter, there might not be any expectation of seeing problems in using outcome expectancy in WKY animals to motivate their behaviour per se. After all, reward anticipation requires a mental representation of the reward from previous experience to be projected into the future and the cognitive expectancies of WKY animals appear to be spared based on the consumption results of Experiment eight alone. However, as detailed in section 1.3.6, there is at least some suggestion of inflexible habit-based behaviour in response to stress (which in turn is linked to depression). Thus, it is not immediately obvious whether a general deficit in reward processing would be expected in WKY rats. Furthermore, it is unclear whether current value representations can be integrated with ongoing instrumental behaviour in the WKY rat.

Chapter six (Experiments nine and ten) investigates the cognitive processing of rewards in the WKY model. More specifically, it investigates whether or not WKY rats can use reward representations to motivate and direct their interactions with their environment. In a novel or changing environment, it is necessary to accurately represent both the relationship between an animal's behaviour and its outcomes, and the value of those outcomes. Such representations allow the production of goal-directed behaviour whereby actions are performed to attain currently valuable rewards (or allow unpleasant outcomes to be avoided). Experiment nine utilised an outcome devaluation procedure whereby WKY rats and their Wistar

control strain were trained for three days to lever press for a food reward on a RI30s schedule of reinforcement (a schedule that promotes goal-directed behaviour in healthy rats). After this training, the food reward was paired with LiCl-induced nausea (devaluing the reward) so that the degree of goal-directed responding in these animals could be assessed.

Experiment ten utilised a differential outcomes procedure to further investigate whether or not WKY rats can form explicit knowledge of the reinforcer. Similar to Experiment five reported for MAM rats, animals were trained to solve a conditional discrimination where correct responding during the presence of discriminative stimuli (here distinct light cues) was rewarded. Whilst the conditional discrimination can be solved based on stimulus information alone (with the presence of stimulus-response contingencies), the presence of correlated outcomes in the 'differential' group (e.g. flashing light → pellets; steady light → sucrose solution) enables animals to form and use specific reinforcer representations that provide additional information to aid learning. As a result, better learning of the conditional discrimination occurs for when correlated, as opposed to uncorrelated (i.e. correct responses are rewarded with pellets and sucrose at equal probability), outcomes are provided.

6.2 Experiment 9 - Materials and Methods

6.2.1 Apparatus and Procedure

The experiment was conducted in the same chambers as described for Experiment four (section 4.1.2). Training consisted of two stages: magazine training and lever-press training, followed by an extinction test after devaluation by LiCl-induced nausea (see Figure 34). Each rat was assigned to one of the eight experimental chambers, and thereafter always trained in the same chamber.

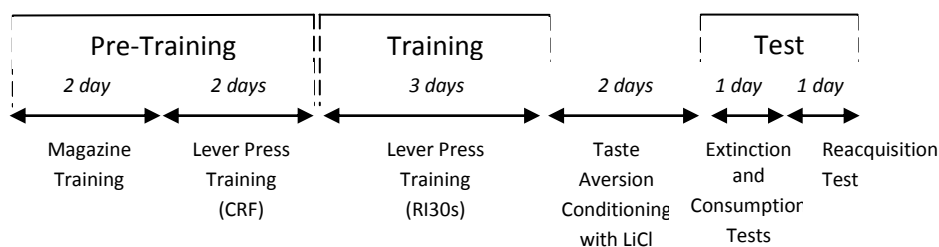


Figure 34 Schematic depicting the experimental protocol for the Outcome Devaluation Task.

Magazine training. All rats were trained to collect food rewards during two magazine training sessions. 45 mg chocolate flavoured sugar pellets (Test Diet, Richmond, IN) were delivered on a RT60s schedule. The session ended once 10 pellets had been delivered. Rats that did not collect the food were given a repeat of the session later that day. Extra magazine training, compared to Experiment Four, was deemed necessary to habituate the stress-sensitive WKY rats to the new environment.

Lever-press training. As described for Experiment four (Section 4.1.3), animals received two days of CRF training, earning 20 rewards in each session, followed by three days training on a RI30s schedule, earning 40 rewards in each session.

Devaluation by LiCl. After the final day of instrumental lever press training, animals received two days of taste aversion conditioning with LiCl. This was conducted in the same manner as described for Experiment four, Section 4.1.3. Valued and devalued groups comprised 12 Wistar control and 12 WKY experimental rats divided equally among the Stress and No-stress conditions. After taste aversion conditioning days, the rats' sensitivity to changes in outcome value was assessed via an extinction test (See Experiment four for details on the experimental procedure).

Consumption and Reacquisition tests. As described for Experiment four, Section 4.1.3, but the reacquisition test was 20 min.

6.2.2 Data analysis

Statistical analysis was performed using ANOVA with between-subject factors of devaluation (devalued vs. valued), strain (Wistar vs. WKY) and stress (Stress vs. No-Stress). Lever presses per minute and magazine approaches per minute during the extinction test were calculated as a proportion of baseline. Baseline was taken as the data from the final day (day three) of acquisition.

6.3 Results

Instrumental training. Across the three days of acquisition training, WKY rats exerted a greater number of lever press responses per minute compared with the Wistar strain. Levels of responding did not

appear to be affected by stress. Importantly, there were no differences in baseline lever press responses (taken as day three) as a function of devaluation condition for either strain (see Table 14).

Table 14 Lever press response per minute for day three of acquisition training (used as 'baseline').

Strain	Stress	Devaluation Condition	Mean lever press/min (\pm SEM)
Wistar	No-stress	Valued	24.517 (\pm 1.367)
		Devalued	23.552 (\pm 3.530)
	Stress	Valued	19.650 (\pm 2.308)
		Devalued	23.086 (\pm 4.083)
WKY	No-stress	Valued	30.651 (\pm 4.535)
		Devalued	26.558 (\pm 3.318)
	Stress	Valued	27.679 (\pm 2.139)
		Devalued	27.296 (\pm 3.220)

Consistent with this impression, ANOVA revealed a main effect of strain ($F(1, 40) = 5.517, p = .024, MSE = 342.815$), and no main effect of stress ($F < 1$) and no strain \times stress interaction ($F < 1$).

Importantly, there was no effect of devaluation condition and no interaction between this and any other factor ($F_s < 1$).

In terms of magazine approach behavior, analysis also suggested an effect of strain but this time with WKY rats exerting fewer nose pokes into the magazine compared to Wistar rats (Table 15).

Importantly, there were no differences in baseline magazine entries between the valued and to-be-devalued conditions.

Table 15 Magazine entries per minute for day three of acquisition (used as ‘baseline’).

Strain	Stress	Devaluation Condition	Mean Mag Entry/min (\pm SEM)
Wistar	No-stress	Valued	18.115(\pm 1.756)
		Devalued	15.955(\pm 1.844)
	Stress	Valued	16.399(\pm 2.529)
		Devalued	14.777(\pm 2.704)
WKY	No-stress	Valued	9.358(\pm 1.150)
		Devalued	9.620(\pm 1.174)
	Stress	Valued	10.575(\pm 1.716)
		Devalued	11.399(\pm 2.741)

ANOVA revealed a main effect of strain ($F(1, 40) = 17.698, p < .001, MSE = 442.654$). There was no main effect of stress treatment ($F < 1$) and no interaction between strain and stress ($F(1, 40) = 1.040, p = .314, MSE = 26.018$). Critically, there was no main effect of devaluation condition and no interaction between this and any other factor ($F_s < 1$).

Extinction – Lever Press Performance. The mean lever press response rates as a proportion of baseline for the 10 min extinction test are displayed in Figure 35 panel A. The performance of Wistar rats, regardless of stress condition, appeared to be sensitive to the current value of the goal. That is, Wistar No-stress and Wistar Stress rats performed fewer lever presses as a proportion of their baseline rates after the outcome had been paired with LiCl-induced nausea (Devalued – grey bars) compared to those animals which had not received this pairing (Valued – white bars). Conversely, the performance of the WKY rats was not goal-directed. This is demonstrated by their failure to show sensitivity to the reward devaluation procedure with rats in the devalued group pressing the lever at a nearly equivalent rate (WKY No-stress) or

at a greater rate (WKY Stress) to the valued group. This suggests that the responding of WKY rats after limited training is insensitive to changes in goal value and habitual.

The description of the data was confirmed by statistical analysis. An ANOVA yielded no main effect of strain ($F(1, 40) = 1.989, p = .166, MSE = .241$), stress ($F < 1$), nor an interaction between these two factors ($F(1, 40) = 1.809, p = .186, MSE = .219$). It did, however, reveal a main effect of devaluation condition ($F(1, 40) = 4.706, p = .036, MSE = .571$), and critically a significant strain \times devaluation condition interaction ($F(1, 40) = 4.161, p = .048, MSE = .505$). There was no stress \times devaluation condition interaction ($F(1, 40) = 1.543, p = .221, MSE = .187$) and no three-way interaction between strain, stress and devaluation condition ($F < 1$). Simple effect analysis of the strain \times devaluation condition interaction revealed that performance of rats in the devalued and valued groups differed in the Wistar rats ($F(1, 40) = 8.858, p = .005, MSE = .215$) but not in the WKY animals ($F(1, 40) = .008, p = .928, MSE = .000$).

Extinction – Magazine approach behaviour. Figure 35 Panel B shows magazine approach behaviour as a proportion of baseline during the 10 minute extinction test. Inspection of this figure reveals that the animals with an aversion to the reinforcer (Devalued – grey bars), regardless of strain, performed fewer magazine entries compared to the non-devalued animals (Valued –white bars). ANOVA yielded a main effect of devaluation condition ($F(1, 40) = 5.537, p = .024, MSE = .597$). There was no main effect of strain ($F(1, 40) = 2.221, p = .144, MSE = .239$), or stress ($F < 1$) and no interaction between these factors ($F < 1$). The ANOVA also yielded no strain \times devaluation ($F < 1$), stress \times devaluation ($F(1, 40) = 2.426, p = .127, MSE = .261$) or strain \times stress \times devaluation interactions ($F < 1$). Thus, WKY rats' instrumental

performance was insensitive to the effects of LiCl devaluation whereas LiCl was able to produce a devaluation of magazine approach behaviour.⁵

Outcome Devaluation. Figure 36 shows the consumption of the instrumental outcome across the two conditioning days and the consumption test (which followed the extinction test). Inspection of this figure suggests that taste aversion learning was not affected by strain or stress. Rats in all four conditions exhibited a strong aversion to the instrumental outcome in the devalued group. In contrast, all rats in the valued group (where the outcome had been paired with a saline injection) continued to consume the outcome across sessions. An ANOVA with factors of session (conditioning day one, conditioning day two and test), strain, stress and devaluation condition (devalued vs. valued) revealed an effect of devaluation condition ($F(1, 40) = 311.950, p < .001, MSE = 1425.692$), and session ($F(1.624, 64.979) = 111.210, p < .001, MSE = 999.147$), as well as an interaction between these two factors ($F(2, 80) = 61.321, p < .001, MSE = 447.483$). The ANOVA results also revealed a day \times strain interaction ($F(2, 80) = 3.622, p = .031, MSE = 26.431$) with WKY rats consuming significantly more than Wistar animals on conditioning day one but not on day two or during the consumption test (Day 1: $F(1, 40) = 6.352, p = .016, MSE = 60.077$).

⁵ Although there was no effect of stress condition in the main ANOVA, inspection of the figure does suggest numerically different levels of goal-directed behaviour in the stress and no-stress groups. In order to examine whether the overarching strain effects had obscured the stress effect in the main ANOVA analysis, both strains were analysed in a separate ANOVA. For Wistar rats an ANOVA yielded no main effect of stress treatment ($F < 1$), no main effect of devaluation condition ($F(1, 20) = 2.959, p = .101, MSE = .362$) and no interaction between these two factors ($F(1, 20) = 1.710, p = .206, MSE = .210$). For WKY rats, again there was no effect of stress treatment ($F < 1$), no effect of condition ($F(1, 20) = 2.585, p = .124, MSE = .240$) and no interaction ($F < 1$).

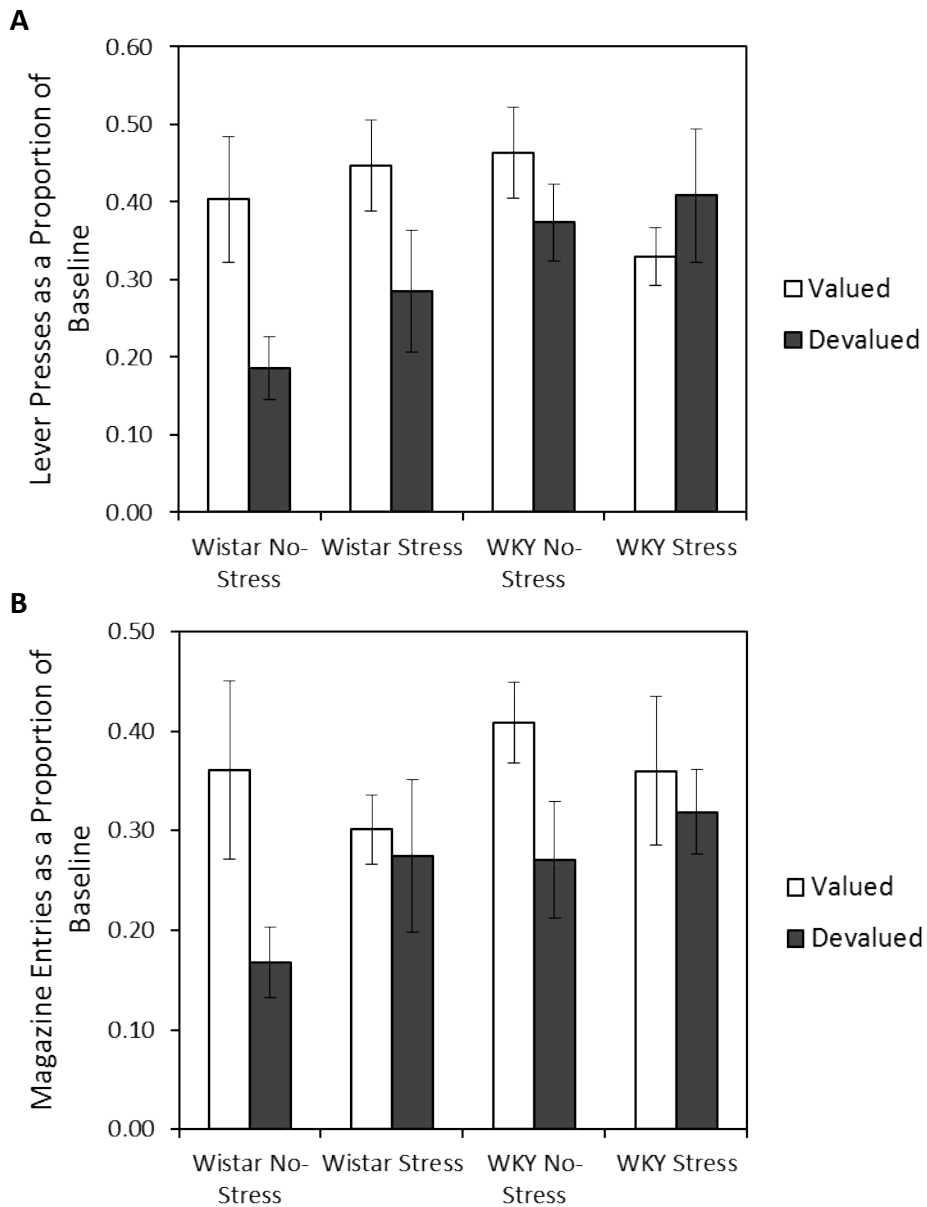


Figure 35 Effect of strain (WKY vs Wistar) and stress condition (No-Stress vs. Stress) on sensitivity of lever pressing (Panel A) and magazine entries (Panel B) to reward devaluation by LiCl-induced nausea. Mean lever presses per minute as a proportion of baseline (+SEM) in the extinction test are shown, where grey bars represent responses after devaluation by LiCl and white bars represent responses after no devaluation.

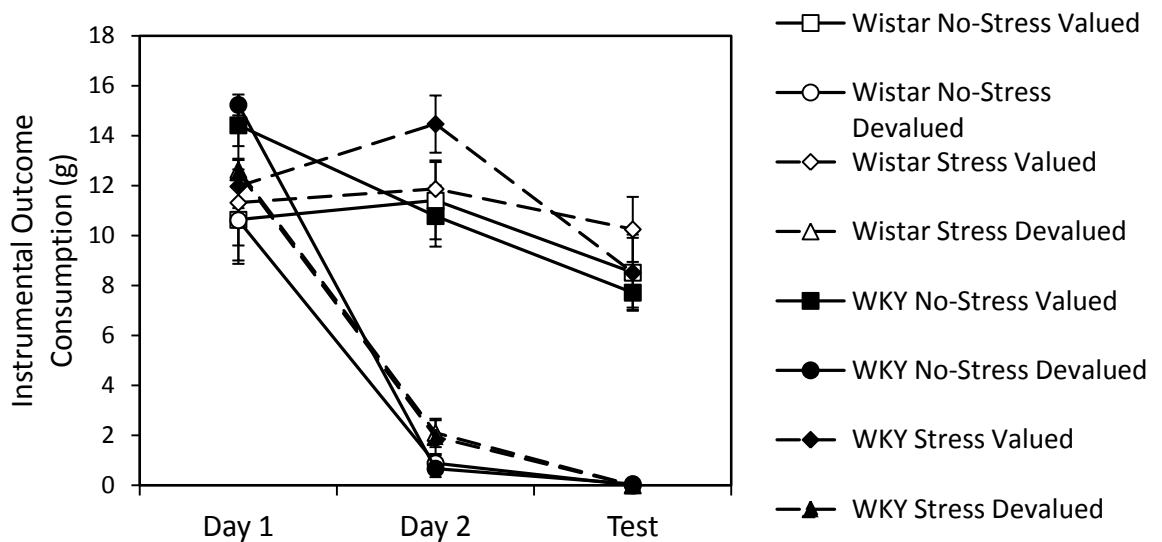


Figure 36 Mean chocolate pellet consumption (\pm SEM) over 2 days of taste aversion training and 1 post-extinction consumption test for WKY (Stress vs. No-stress) and Wistar rats (Stress vs. No-Stress). Rats received LiCl injections (Devalued) or saline injections (Valued) after 30 min free access to the instrumental outcome. The test phase also took place in a 30 min period immediately after the 10-min extinction test.

Reacquisition Test – Lever Press Performance. The results of the reacquisition test provided further confirmation that the LiCl injections had successfully devalued the instrumental outcome under the devaluation condition of all groups, and that this taste aversion had effectively transferred to the instrumental chamber. The mean lever presses per minute for the rewarded 20 min reacquisition test are presented in Figure 37 Panel A. This indicates that the devalued group, regardless of strain and stress, performed considerably fewer lever presses compared to the valued group. Statistical analysis by ANOVA revealed a significant main effect of devaluation group ($F(1, 40) = 26.798, p < .001, MSE = 1275.756$). The trend towards higher levels of responding for the WKY strain was maintained in the reacquisition test ($F(1, 40) = 3.075, p = .087, MSE = 146.394$) but importantly the level of devaluation was comparable for all groups as there was no strain \times devaluation, stress \times devaluation or strain \times stress \times devaluation interaction (all $F_s < 1$). Together with magazine approach behaviour during extinction, these data indicate that the devaluation procedure was just as effective in all strain and stress groups.

Reacquisition Test – Magazine Approach Behaviour. The effectiveness of the devaluation procedure is further supported by analysis of magazine approach behaviour during the 20 min reacquisition test (See Figure 37 Panel B). All devalued groups showed a marked suppression in their magazine approach behaviour compared with their appropriate valued group. In support of this, ANOVA yielded a main effect of devaluation ($F(1, 40) = 26.902, p < .001, MSE = 356.709$). The WKY animals again displayed lower levels of magazine approach behaviour compared to Wistar rats ($F(1, 40) = 22.838, p < .001, MSE = 302.824$). ANOVA revealed a significant strain \times devaluation condition interaction ($F(1, 40) = 4.536, p = .039, MSE = 60.140$). However, further simple effects analysis revealed a significant difference between valued and devalued groups for Wistar rats ($F(1, 40) = 26.765, p < .001, MSE = 354.891$) and the WKY strain ($F(1, 40) = 4.673, p = .037, MSE = 61.958$).

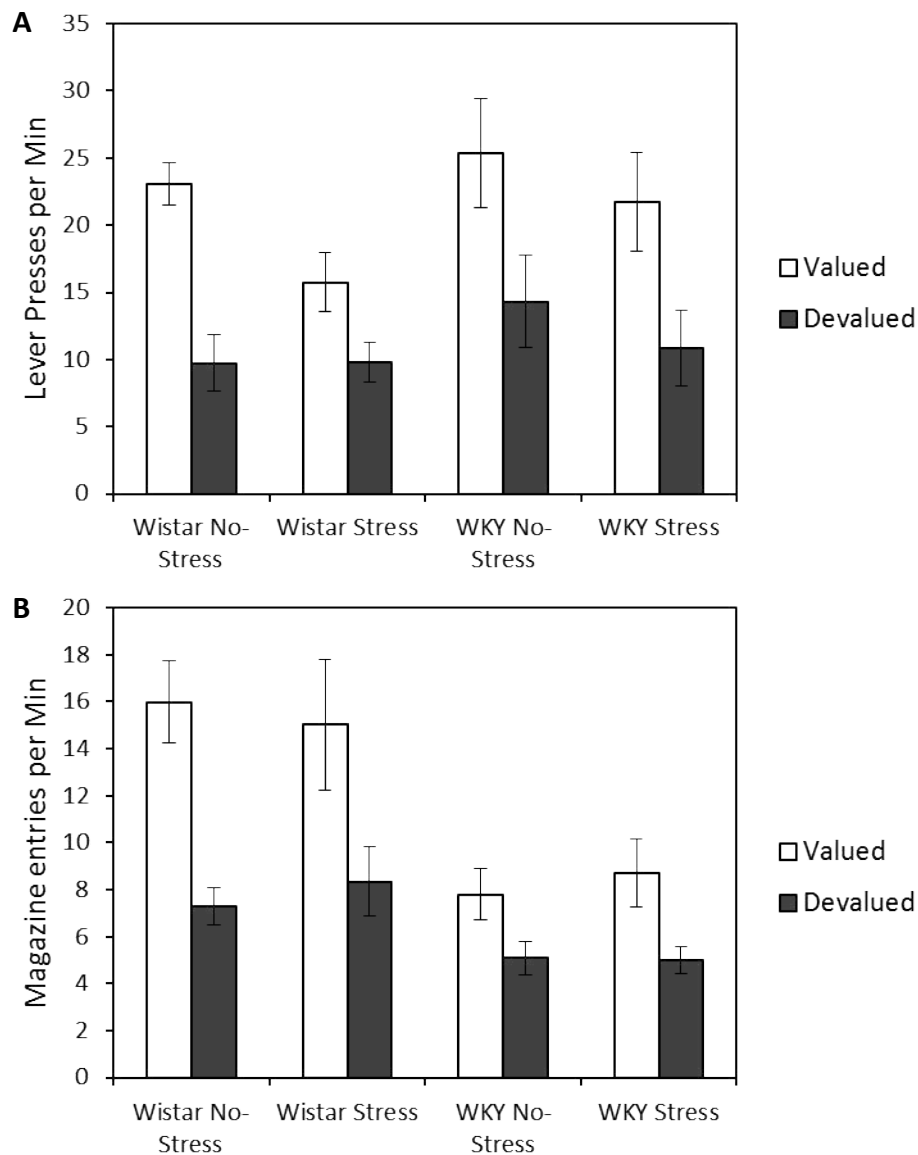


Figure 37 Effect of strain (WKY vs. Wistar) and stress condition (Stress vs. No-stress) on lever press reacquisition (Panel A) and magazine entries (Panel B) after reward devaluation by LiCl-induced nausea. Mean lever presses and mean magazine entries per minute (\pm SEM) in the rewarded reacquisition test after devaluation with LiCl (grey bars) or no devaluation (white bars) are shown. The reacquisition test was given 24 hours after the extinction and consumption tests.

6.4 Summary

Based on the results from the negative anticipatory contrast procedure (Experiment eight), it would appear that WKY rats under certain circumstances can adjust their current (consummatory) behaviour in light of future rewarding events, suggesting that WKY rats can mentally represent or predict future rewards.

However, in Experiment nine, WKY rats regardless of stress condition, were unable to use changes in

experienced reward value to modify their instrumental behaviour. Although WKY animals showed a reduced intake of pellets after LiCl-induced nausea, demonstrating that they had effectively acquired the taste aversion, they failed to modify their lever-press behaviour in response to this changed outcome value. That is, WKY rats in the devalued group responded at equivalent levels to those seen in the non-devalued group. This suggests that the control of responding in the WKY rats was not dependent on the expected outcome but instead was dominated by reflexive S-R habits, even after only limited levels of training.

As planned, Experiment ten used the differential outcomes procedure to further investigate the deficit in reward representation as it presents itself in the WKY inbred rat strain. If WKY animals cannot form or cannot use associations between the discriminative stimuli and the different sensory properties of the two reinforcers, or indeed if they are entirely stimulus bound, they should be unable to show any benefit from having differential outcomes during the acquisition of a conditional discrimination. Based on the apparent dominance of habitual S-R behaviour in Experiment nine, I would expect that the performance of the WKY rats will be equivalent across acquisition training of the conditional discrimination, regardless of training conditions (differential vs. non-differential outcomes).

6.5 Experiment 10 - Materials and Methods

6.5.1 Apparatus

The same eight experimental chambers used in Experiment five (section 4.4.2) were used. Two panel lights, 2 cm in diameter, were located at the top left and right of the right-hand wall above the two levers. A white LED light located in the top of the magazine could also be illuminated. The discriminative

stimuli consisted of flashing panel lights (stimulus 1) and steady panel lights together with illumination of the magazine light (stimulus 2) - this was to prevent contamination with planned experiments using auditory stimuli that are not reported in this thesis.

6.5.2 Procedure

Each rat was assigned to one of the eight experimental chambers, and thereafter always trained in the same chamber. Training consisted of three stages: magazine training, lever press training and acquisition training on a continuous performance conditional discrimination task (See Figure 38). Subsequently, rats underwent two tests, one in extinction and one in which the visual stimuli were not presented (reinforcer-only test). One test session was run on each day, interspersed with an additional training session (RT).



Figure 38 Schematic depicting the experimental protocol for the differential outcomes procedure. RT = Reacquisition Training.

Pre-training. Full details of the pre-training stages can be found in section 4.4.3 (Experiment five).

Conditional Discrimination Training. Instrumental training of the conditional discrimination occurred across ten days. Each daily session lasted 40 min and consisted of eight 5 min presentations of the visual

stimuli. The identity of the first stimulus (flashing vs. steady light) was randomly allocated, and the stimulus identities were then strictly alternated thereafter. All other aspects of conditional discrimination training were as described in Experiment five. An equal number of rats from each strain and each stress condition were assigned to the differential and non-differential groups, for which stimulus-outcome contingencies were manipulated. All contingencies were fully counterbalanced across the cohort.

Extinction test and reinforcer only test. The extinction test was carried out after the final day of acquisition training. Parameters were the same as during the training phase, except that rewards were not delivered after each appropriate lever press response. The rats then underwent a single day of reacquisition training, followed by a reinforcer-only test in which rewards were available but in the absence of the visual stimuli. See Experiment five (section 4.4.3) for further details.

6.5.3 Data analysis

For all analysis, correct and incorrect lever press responses were converted into discrimination ratios (as previously described in Experiment five). Data were analysed via a four-way ANOVA with factors of session (days 1 – 10), strain (Wistar vs. WKY), stress (stress vs. No-stress) and group (differential vs. non-differential).

6.6 Results

Acquisition of conditional discrimination. Figure 39 displays the mean discrimination ratios for the continuous performance conditional discrimination task across ten days of acquisition. Panel A of Figure 39 shows the performance of the Wistar animals and Panel B shows the performance of the WKY animals. For both panels, the filled symbols represent the rats in the differential group (with stimulus-correlated outcomes), whereas open symbols represent the rats in the non-differential group (where a correct response was reward with pellets and sucrose at equal probability).

As can be observed from the Panel A, Wistar rats in both differential and non-differential groups learnt the discrimination as training proceeded. However, acquisition was slower in the non-differential group, who learnt the discrimination at a reduced rate and reached a lower asymptote by day ten. Better performance for Wistar rats in the differential group is indicative of a differential-outcomes effect. As can be seen in Panel B, WKY rats in both groups learnt the conditional discrimination across training. There was, however, no difference in the performance of rats which experienced differential outcomes and those which did not. This suggests that while WKY rats were unimpaired in acquiring the basic instrumental discrimination, they did not exhibit a differential outcomes effect.

ANOVA analysis revealed a main effect of session ($F(4.696, 187.850) = 113.191, p < .001, MSE = .213$) and group ($F(1, 40) = 23.099, p < .001, MSE = .231$) with rats under the differential condition generally outperforming those in the non-differential condition. There was also a strain effect ($F(1, 40) = 15.009, p < .001, MSE = .150$) with WKY rats demonstrating higher discrimination ratios, collapsed across groups, compared to the Wistar strain. The ANOVA also revealed an interaction between strain and session ($F(9, 360) = 2.614, p = .006, MSE = .005$), with the strain differences present on days 1-5 and day

7 (smallest $F(1, 40) = 6.247, p = .017, MSE = .018$) but not on day 6 or on the last three days of training (largest $F(1, 40) = 2.563, p = .117, MSE = .007$). No main effect of stress was yielded by the analysis ($F(1, 40) = 1.136, p = .293, MSE = .011$) nor was there a strain \times stress interaction ($F(1, 40) = 1.841, p = .182, MSE = .018$). Importantly, the ANOVA did yield a strain \times group interaction ($F(1, 40) = 4.605, p = .038, MSE = .046$). Simple effect analysis subsequently revealed that Wistar rats in the differential group had higher discrimination ratios than those in the non-differential group ($F(1, 40) = 24.165, p < .001, MSE = .024$). In contrast, the discrimination ratios for WKY rats in the differential group were not significantly higher than WKY rats in the non-differential group ($F(1, 40) = 3.538, p = .067, MSE = .004$). Consistent with the description of the data, the ANOVA confirmed that WKY animals show less benefit from receiving stimulus-contingent outcomes during the training of a conditional discrimination task.

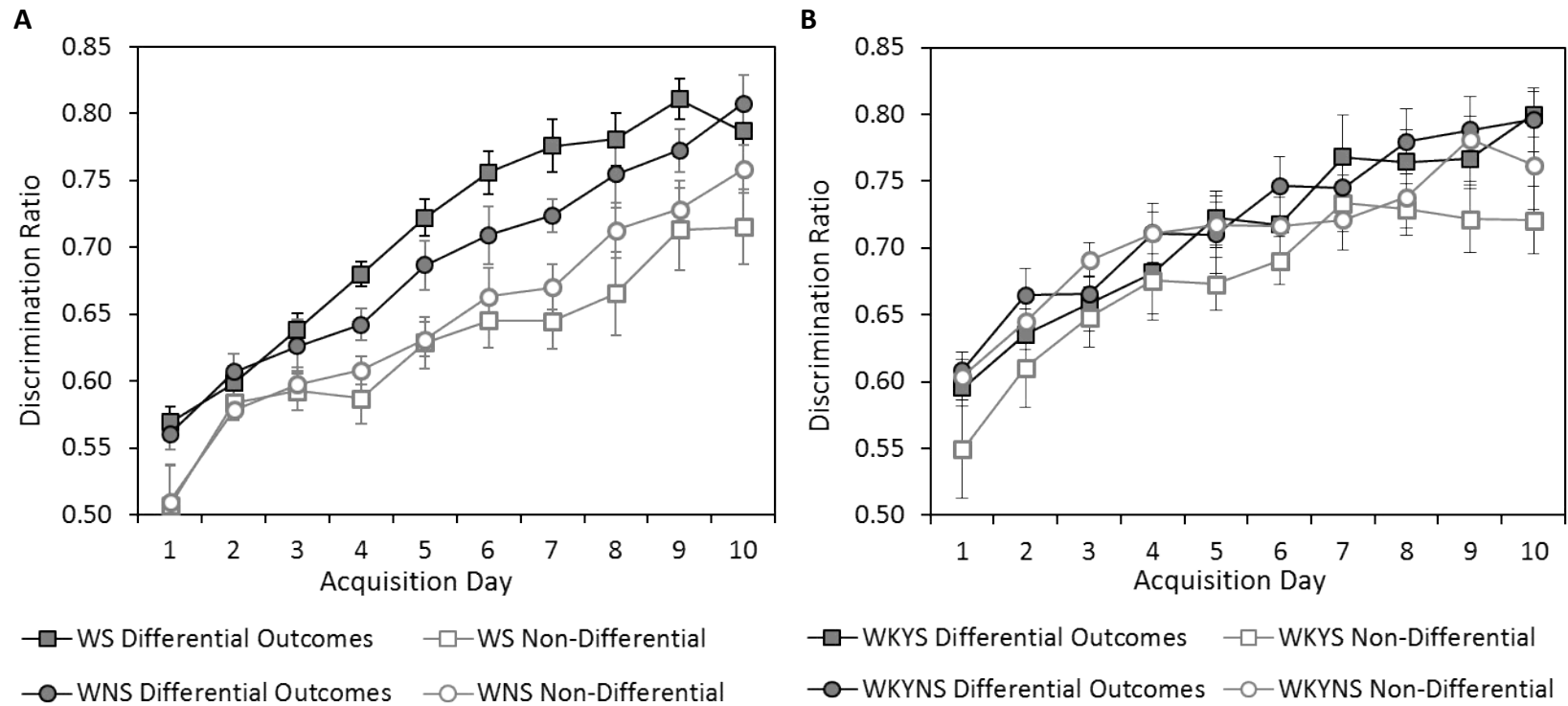


Figure 39 Discrimination ratios (correct responses/ total responses) during acquisition of a conditional discrimination task for Wistar rats (Panel A) and WKY rats (Panel B). WS denotes Wistar Stress rats (represented by filled squares for the Differential group and open squares for the Non-Differential group) and WNS denotes Wistar No-stress rats (represented by filled circles for the Differential group and open circles for the Non-Differential group). WKYS denotes WKY Stress rats (represented by filled squares for the Differential group and open squares for the Non-Differential group) and WKYNS denotes WKY No-stress rats (represented by filled circles for the Differential group and open circles for the Non-Differential group). Error bars represent \pm SEM.

Extinction Test. Figure 40 displays the mean discrimination ratios (\pm SEM) for the Wistar and WKY strains across the 40 min extinction test for Stress and No-stress conditions. Wistar rats appear to have maintained the discrimination during the unrewarded extinction test with discrimination ratios above .50 (95% CIs: Wistar No-stress Differential, .598 - .732; Wistar No-stress Non-Differential, .579 - .713; Wistar Stress Differential, .589 - .724; Wistar Stress Non-Differential, .546 - .681). The performance of the WKY animals was generally lower than the Wistar strain, but again discrimination ratios were above .50 (95% CIs: WKY No-Stress Non-Differential, .530 - .664; WKY Stress Differential, .517 - .651; WKY Stress Non-Differential, .539 - .674) - with the exception of WKY No-stress animals in the differential outcomes group (95% CI: .445 - .579). Taken together, it appears that the animals' performance during acquisition, particularly for Wistar rats, was not controlled exclusively by cues provided on reward delivery.

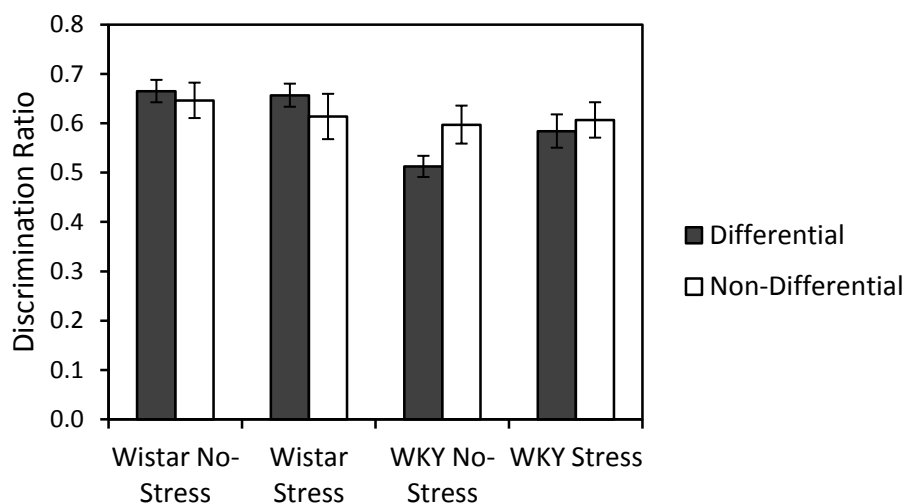


Figure 40 Discrimination ratios during the 40 min extinction test. Data is shown for Wistar and WKY rats separated into the No-stress and Stress conditions. Data displayed in dark grey represents animals in the Differential condition (where during training they had received stimulus contingent outcomes) and data displayed in white represent animals in the Non-Differential group (where animals behaviour was reinforced by random rewards during training). Error bars represent \pm SEM.

ANOVA analysis on the data summarised in Figure 40 revealed a main effect of strain ($F(1, 40) = 8.975, p = .005, MSE = .059$), no main effect of group ($F < 1$) and no strain \times group interaction ($F(1, 40) = 3.241, p = .079, MSE = .021$). The ANOVA also yielded no main effect of stress condition ($F < 1$), no strain \times stress interaction ($F(1, 40) = 1.690, p = .201, MSE = .011$) and no interaction between stress and any other factor (all $F_s < 1$).

Reinforcer-Only Test. Figure 41 depicts the mean discrimination ratios (\pm SEM) for Wistar and WKY rats during the 40 min reinforcer-only test, where visual stimuli were not presented, for both Stress and No-stress conditions. All Wistars (95% CIs: No-stress Differential, .650 - .734; No-stress Non-Differential, .563 - .647; Stress Differential, .622 - .706; Stress Non-Differential, .585 - .669) and WKY rats (95% CIs: No-stress Differential, .694 - .778; No-Stress Non-Differential, .618 - .702; Stress Differential, .674 - .758; Stress Non-Differential, .610 - .693) displayed discrimination ratios above .50 suggesting that the presentation of the reinforcer alone could indicate which lever press was the appropriate response to make in the absence of additional discriminative cues. As suggested for Experiment five (section 4.5), this was likely achieved via a win-stay strategy, together with O-R associations accounting for the persistent differential outcomes effect. With generally higher discrimination ratios for WKY animals, perseverative responding and/or backward associations between the outcome and the response appears to be more pronounced for this strain.

ANOVA analysis revealed a main effect of strain ($F(1, 40) = 8.948, p = .005, MSE = .023$) with WKY rats displaying higher discrimination ratios overall. There was no main effect of stress ($F <$

1) and no strain \times stress interaction ($F < 1$). Consistent with the description of the data, the ANOVA yielded a main effect of group ($F(1, 40) = 20.279, p < .001, MSE = .052$) which was present for both strains and stress conditions (strain \times group: $F < 1$; stress \times group, $F(1, 40) = 1.101, p = .300, MSE = .003$). There was also no strain \times stress \times group interaction ($F < 1$).

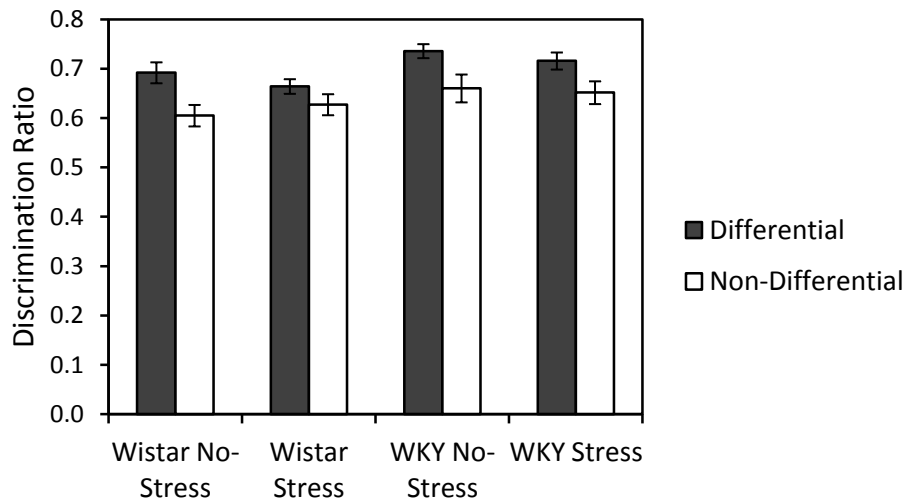


Figure 41 Discrimination ratios during the 40 min Reinforcer only test. Data is shown for Wistar and WKY rats separated into No-stress and Stress conditions. Data in dark grey represents the performance of animals in the Differential group whereas data in white represents the performance of animals in the Non-Differential group. During trials, no visual stimuli were provided to aid responding. Error bars represent \pm SEM.

6.7 Summary

Experiment ten used the differential outcomes effect to examine whether WKY rats could use differences in the sensory properties of two reinforcers to better learn a conditional discrimination. The outbred Wistar control strain trained on a discrimination in which specific rewards were contingent on the stimulus identity (e.g. flashing light \rightarrow pellet; steady light \rightarrow sucrose) better learnt the discrimination compared to rats for which reward type and stimulus identity were not correlated (i.e. where correct responses were rewarded with sucrose and pellets with a 50:50 probability). WKY rats

also learnt the discrimination, performing more on the correct than incorrect lever, but failed to show the differential outcomes effect. That is, WKY rats did not use the distinct sensory properties of the reward to aid their learning when the visual discrimination cues were present. However, the fact that the WKY rats did show better performance in the differential as compared to non-differential outcomes condition in the reinforcer-only test suggests that they may well be forming sensory specific R-O associations to some degree. The implications of this pattern of results will be considered below (section 6.8), and in the general discussion.

6.8 Discussion of Chapters 5 and 6

The set of experiments reported in Chapters five and six investigated potential reward-processing deficits in the WKY rat, a putative depression model, using the same techniques adopted for investigation of MAM-treated animals. Chapter five specifically investigated hedonic deficits in the model, whereas Chapter six investigated reward-related processes beyond anhedonia.

Experiment seven detected the presence of consummatory hedonic deficits in the WKY rat strain by monitoring their licking patterns during the voluntary consumption of three different sucrose concentrations. This was demonstrated by the lower palatability responses exhibited by WKY animals to the sweet solutions compared to an outbred Wistar control strain. Lower lick cluster sizes were also mirrored by generally lower consumption levels by WKY rats compared to Wistar animals, but only when consumption was not expressed relative to the rats' body weight. This suggests that lick cluster size is a more sensitive and selective measure of hedonic changes than consumption

measures alone (as will be further discussed in relation to stress in the general discussion section of this thesis, section 7.6).

The presence of an anhedonic-like profile in the WKY model is in accordance with previous work (e.g. De La Garza, 2005; Malkesman et al., 2005; Paré, 2000); however, the current experiment is the first to demonstrate its presence using a measure unaffected by potential motivational changes in the strain. Furthermore, from analysing ILI the possibility that the reduced palatability responses are an artefact of motoric or postural problems with the animal can be ruled out from the current dataset. In addition to previous work, the application of a chronic mild stress procedure does not appear to be a necessary precursor for hedonic deficits to manifest in this strain (stress effects for both strains will be considered fully in the general discussion, section 7.6).

To detect the presence of anticipatory hedonic deficits in the WKY strain, Experiment eight used lick analysis to assess the hedonic component of the reward in conjunction with a negative anticipatory contrast procedure. It was demonstrated that, as WKY rats learn to expect a more palatable solution will be made available, they suppress their consumption but not their palatability responses towards the currently available solution. This dissociation between the consumption and LCS parameters suggests that WKY animals are able to form some expectations of future events but are unable to adjust their affective responses in light of these expectations. Whilst potential floor effects must be considered, this provides the novel suggestion that WKY rats display behaviours analogous to anticipatory anhedonia.

With consideration of potential floor effects, the low LCSs exhibited by the WKY strain in both Experiment seven and Experiment eight may give little scope for a functional devaluation of the initial solution due to the expectation of a more palatable reward. This is unlikely to reflect an insensitivity of the techniques used overall. Indeed, conditioned taste aversion studies (e.g. Dwyer, 2009; Dwyer et al., 2008) show that LCSs can be considerably lower than those seen for WKY animals in Experiment eight. Furthermore, returning to Experiment seven, the LCSs for WKY animals were modulated by solution concentration to some degree. This demonstrates that this parameter is not completely fixed at a low level but can be modulated by external factors in a way that would be expected for healthy rats.

Chapter six investigated other reward-related deficits, beyond anhedonia, in the WKY model. Using an outcome devaluation procedure, Experiment nine demonstrated that WKY rats, given limited instrumental training for a food reward, are insensitive to post-conditioning changes in outcome value: WKY rats averted to a reinforcer responded just as much as animals not averted to the reinforcer. This result, considered in isolation, suggests either that WKY animals are unable to encode outcomes or that these animals undergo a faster transition to S-R mechanisms, with three days of training being sufficient to produce habit-based behaviour in these animals.

Several aspects of the data presented in Experiment nine deserve comment. Firstly, the lack of a stress effect on the balance between goal-directed and habitual systems appears to be at odds with previous demonstrations that stress attenuates goal-directed responding (see section 1.3.6). The general implications of stress for both WKY and Wistar strains will be discussed fully in the

general discussion of this thesis (section 7.6). For now, one possibility is that the stress manipulation used for the current experiments was simply less severe than in previous studies.

Secondly, the dissociation observed between the effects of reward devaluation on magazine approach and instrumental lever pressing in the WKY strain has been previously demonstrated in the literature. Similarly to the current findings, Nelson and Killcross (2006) found that an amphetamine challenge made lever-press behaviour but not magazine approach behaviour insensitive to reward devaluation. This finding is consistent with the idea that magazine approach is under the control of different psychological and neural processes to lever-press performance (e.g. Killcross & Coutureau, 2003). One line of thought is that responses proximal to the reward, such as magazine entry, may be more sensitive to devaluation procedures than responses, such as lever pressing, which are more distal to reward delivery (Balleine, Garner, Gonzalez & Dickinson, 1995). Alternatively, it is possible that magazine approach behaviour is under greater control by Pavlovian, as opposed to instrumental, contingencies (Balleine et al., 1995; Nelson & Killcross, 2006). Indeed, unlike for instrumental behaviour, reward delivery is independent of magazine approach responses (see Killcross & Blundell, 2002, for further discussion). Regardless of the underlying mechanisms, the magazine approach behaviour (together with the reacquisition test) in this experiment provides unequivocal evidence that the devaluation procedure was equally as effective in the WKY groups.

Turning to Experiment ten, the presence of a reward processing deficit was reinforced by the fact that WKY rats did not display the typical DOE seen in the control groups. That is, although the WKY rats successfully acquired the general instrumental discrimination, their performance was not

influenced by whether or not each action was selectively paired with a unique reward. Taken together, the results of Experiments nine and ten show that the current value of a reward does not drive instrumental behaviour in the WKY rat, nor do they use the specific properties of rewards to direct the acquisition of an instrumental discrimination. While these results clearly indicate some deficits in reward processing in WKY rats, it should be noted that they are clearly sensitive to at least some properties of rewards under some circumstances. In particular, they show an anticipatory contrast effect on intake measures in Experiment eight, their magazine entry behaviour in Experiment nine is sensitive to outcome devaluation, and they were sensitive to the type of reward in the reinforcer-only test in Experiment ten. In short, although the WKY rats clearly show deficits in both hedonic and instrumental responses to rewards, this does not reflect a complete failure to process or encode rewarding stimuli. I will consider the characterisation of the nature of the WKY strain deficit in the general discussion (section 7.7).

Chapter Seven

7. General discussion

7.1 Summary of results

The general theme of this thesis has been the analysis of hedonic and instrumental responses to rewarding stimuli. Experiment one developed a within-subject anticipatory contrast procedure, affording the examination of hedonic responses through the examination of licking microstructure. This demonstrated that, in a context where access to a dilute sucrose solution was followed by a more concentrated solution, both consumption and hedonic responses to dilute sucrose were suppressed.

Experiment two and three investigated the hedonic responses of animals prenatally treated with MAM, a putative schizophrenia model, in both anticipatory contrast and simple consumption situations. MAM treated animals displayed no deficits that were indicative of either consummatory or anticipatory anhedonia. Experiments four and five investigated reward processing, beyond anhedonia, in this model and also found no evidence of impaired instrumental behaviour in response to either post-training outcome devaluation or the provision of differential outcomes in a discrimination task. Experiment six investigated the suggestion from prior experiments that MAM-treated rats might be less prone to habit-based behaviours through over-training the instrumental response. Whilst inconclusive, the results did not rule out an over-reliance on A-O associations in MAM-treated animals. In short, MAM treated animals did not display any hedonic deficits or impairments in

instrumental behaviour indicative of the sort of reward processing problems that might be expected of a comprehensive animal model of schizophrenia.

Experiments seven and eight investigated consummatory and anticipatory hedonic responses in the WKY inbred rat strain, a putative depression model. WKY rats displayed lower consumption and lick cluster sizes when exposed to sucrose, consistent with a consummatory hedonic deficit in these animals. Furthermore, in the negative anticipatory contrast procedure, a contrast effect developed in their consumption, but not in their hedonic responses, to a dilute sucrose solution that was presented in a context where it was to be followed by a more concentrated solution. The absence of suppressed lick cluster sizes in this contrast procedure is possibly indicative of an anticipatory hedonic deficit in this strain.

Experiments nine and ten further examined the instrumental behaviour of WKY rats in response to reward devaluation and the differential outcomes procedure. Instrumental lever press responses after minimal training were insensitive to reward devaluation in the WKY rats. In addition, WKY rats showed no benefit (unlike controls) from the presence of differential outcomes during the acquisition of a conditional discrimination task. However, the performance of WKY rats in the differential and non-differential groups mirrored those of controls in both a subsequent extinction test (where only visual cues could direct performance), and in a reinforcer-only test (where responding could only be directed by the presence or nature of the outcome). While the application of chronic mild stressors influenced some of the behaviours examined in Experiments seven to ten (in particular those relating to the amount of consumption), these effects did not differ between the WKY rats and

their Wistar controls. Considered together, these results for the WKY inbred rat strain are consistent with the reward-related deficits that feature in depression.

The results of these experiments speak to a number of issues relating to the nature of hedonic and instrumental responses to rewards and the ways in which these are affected (or not) in preclinical models of human psychiatric disorders. These will be considered below.

7.2 Negative Anticipatory Contrast as a measure of Anticipatory Anhedonia

Experiment one combined a negative anticipatory contrast procedure with microstructural analysis of licking to serve as a sensitive test of anticipatory hedonic behaviour. It was found that, in 'normal' animals, expectation of a more palatable solution in the near future caused a suppression in consumption and lick cluster size measures to a currently available solution of lesser value. This result was replicated in Experiments three and eight, showing the robustness of the negative anticipatory contrast effect (both in terms of consumption and LCS) across Lister-Hooded, Sprague-Dawley and Wistar outbred strains. Critically, in this procedure, the central focus of analysis is the response to a currently available solution as a function of what is expected to occur in the future. As such, the use of licking microstructure in the context of an anticipatory contrast procedure is a potentially valuable means to assay anticipatory hedonic responses.

Taken at face value, the fact that anticipatory contrast influences both consumption and hedonic responses is consistent with the idea that the suppression of consumption by contrast is a direct product of the devaluation of a currently available reward by the expectation of a preferred

reward in the future. However, WKY rats displayed the typical contrast-produced change in consumption, which was not mirrored by a similar change in hedonic reactions. While the implications of this dissociation for the WKY rat as a possible depression model will be considered below, it is important to note that the dissociation also questions whether anticipatory contrast is actually a direct product of reward devaluation, or whether reward devaluation and consumption suppression are two separate products of experiencing contrast. Other things being equal, such a dissociation would be clear evidence against the idea that contrast is produced by reward devaluation. However, the generally low level of hedonic responses displayed through lick cluster sizes in the WKY rats allows for the possibility that the apparent dissociation was the result of a restriction in the range of hedonic responses displayed by WKY animals. As a result, the relationship between consumption and hedonic responses in the contrast situation remains to be determined. Regardless, the fact that both consumption and hedonic responses depend on an anticipation of future rewards means that this procedure still affords a behavioural assay of anticipatory hedonic processes.

Unlike for the outcome devaluation and differential outcome techniques used in this thesis, the neural circuits that underpin the contrast effect have undergone little investigation. Barbano and Cador (2006) used conditioned locomotor activity prior to expected palatable foods as a measure of anticipation and found that the systemic administration of a dopamine receptor antagonist (flupenthixol), but not an opioid receptor antagonist (naloxone - an antagonist with high affinity for mu opioid receptors), decreased the expression of anticipatory activity in food restricted rats. Conversely, Katsuura and Taha (2014) used an anticipatory contrast paradigm and found that the increased

consumption that usually occurs for a non-contrasted solution (i.e. 4% sucrose followed by plain water) relative to a contrasted solution (i.e. 4% sucrose followed by 20% sucrose) was prevented by the administration of non-specific opioid receptor antagonists (naltrexone) and mu opioid antagonists (beta-fundaltrexamine, but not delta opioid receptor antagonist, naltrindole) infused into the NAc shell. As beta-fundaltrexamine had no significant effects on the same 4% sucrose in the contrast condition, the authors suggest that the anticipation of a preferred solution reduces mu opioid signalling-dependent consumption of a less preferred solution. In light of these contradictory findings, and given the role of dopamine in reward 'wanting' (thought to be closely linked to reward anticipation), it would be informative to investigate both dopaminergic and opioidergic manipulations in relation to my negative anticipatory contrast procedure. Moreover, in light of the dissociation of consumption and hedonic responses observed in the WKY rats and the fact that the prior investigations of the neurobiology of anticipatory contrast were restricted to consumption measures alone, any future study would benefit from utilising the licking microstructure techniques exemplified here. For the moment, it suffices to say that neither the basic behavioural mechanisms, nor the underpinning neurobiology, of anticipatory contrast have been conclusively established.

7.3 Reward Processing in MAM-treated Rats

Using microstructural analysis of licking in both simple exposure and contrasted situations, no evidence for either consummatory or anticipatory hedonic deficits were found in animals prenatally exposed to MAM. The implications for these observations for MAM treatment as a schizophrenia

model will be considered in the next section, but to put it succinctly, MAM treatment appears to have no effect on the hedonic processing of rewards.

Aside from the potentially contentious hedonic component of schizophrenia, Chapter four of this thesis demonstrates that the MAM model is also unable to elicit behavioural deficits related to reward processing that might reflect symptoms associated with schizophrenia. Behavioural and neuroimaging studies have suggested that alterations in the schizophrenic brain cause patients to rely predominately on reflexive habits (see Griffiths et al., 2014, and section 1.3.3 of the general introduction). However, using an outcome devaluation task together with a differential outcomes procedure, it was shown that MAM-treated rats are able to form, maintain and update representations of reward value and use these representations to both motivate and direct their behaviours. Moreover, the MAM-treated rats' superior reversal learning, together with the trend towards persistent goal-directed performance, despite extended instrumental training, suggests that MAM animals may be particularly sensitive to A-O contingencies. Given that this is essentially the opposite of the expected pattern of habit-driven behaviour, it would appear that MAM-treated rats are unimpaired with respect to their instrumental responses to rewards, or at least they are not impaired in the way that that would be expected from a schizophrenia model.

The absence of the predicted behavioural deficits requires further comment. Firstly, the absence of anhedonia in the model may not be surprising when we consider that hedonic reactions appear to be controlled by discreet hedonic hotspots in the rodent brain (see section 1.2). Not only are these hotspot regions very small (often 1mm³) but only the caudal hotspot of the ventral pallidum

has been shown to be necessary for hedonic responses, with damage to this area actually replacing hedonic liking reactions with disgust reactions (e.g. Ho & Berridge, 2014). Moreover, these hedonic hotspots are located in close proximity to 'coldspots', the stimulation of which suppresses positive hedonic reactions (e.g. Castro & Berridge, 2014a). Given the diffuse effects of MAM throughout the frontal cortex, the overall balance between subregions which enhance and diminish hedonic reactions might be relatively unchanged, even if both sets of subregions are individually disrupted to some degree by MAM treatment.

Secondly, the lack of habitual behaviour in MAM-treated rats appears to be inconsistent with reports of prefrontal impairments and a hyperactivity of subcortical dopamine systems in these animals (e.g. Moore et al., 2006). However, in as-yet-unpublished experiments performed during my PhD, sub-chronic PCP treatment, a manipulation that also produces 'hypofrontality' and increased mesolimbic dopaminergic tone in rats (e.g. Jones et al., 2011; Neill et al., 2014), also failed to bias behaviours towards habitual systems.

Finally, while the importance of cortico-striatal systems in the control of instrumental behaviour is well established (see section 1.6.1b), it is important to remember that the balance between S-R and A-O control appears to reflect the interaction of prelimbic PFC and dorsomedial striatum (promoting A-O control of behaviour) and infralimbic PFC and dorsolateral striatum (promoting S-R control of behaviour). Again, given the diffuse effects of MAM throughout the frontal cortex, it is possible that the balance between these two systems is not grossly impaired despite partial disruption of both. While the suggestion of a potential bias to A-O control of behaviour in

MAM-treated animals might appear to be consistent with a disruption of either infralimbic PFC or dorsolateral striatum, any such disruption cannot be complete, because lesions of these regions (see section 1.6.1b) result in the clear maintenance of goal-directed responding even after extended instrumental training (which was not seen with MAM-treated animals in Experiment six).

In short, in the experiments reported here, MAM treated animals do not show any evidence for impaired hedonic responses or impairment in the control of instrumental behaviour that would reflect a deficit in reward processing.

7.4 MAM Treatment in the Context of Modelling Schizophrenia

Given that MAM-treated animals do not appear to show a general deficit in reward processing, either in terms of hedonic reaction or the control of instrumental responding, it is important to reconsider the validity and utility of MAM treatment in the context of providing an animal model of schizophrenia.

The first relevant issue is that of anhedonia. The presence of consummatory anhedonia in schizophrenia has been a topic of debate in the literature, but an overwhelming majority of the laboratory-based assessments suggest that schizophrenia patients do experience in-the-moment pleasure which is indistinguishable from healthy controls (section 1.3.2 of the general introduction). As such, the absence of a simple lowering of hedonic reactions in the MAM model may be entirely consistent with the disorder. However, to reconcile the apparent paradox between self-report measures and laboratory-based assessments, one theory is that schizophrenia patients are unable to

appropriately experience retrospective or prospective pleasure (Strauss & Gold, 2012). The literature remains conflicted: use of both the experience sampling method and the Temporal Experience of Pleasure Scale have highlighted a deficit in patients' ability to predict pleasure from future events (e.g. Gard et al., 2007); while Strauss and colleagues (2011) were unable to replicate these findings. If anticipatory anhedonia indeed features in the disorder, an anticipatory hedonic deficit, as revealed by a lack or attenuation of the negative anticipatory contrast effect, would be expected in any complete model of schizophrenia. No such attenuation was observed in the work reported here, implying that prenatal MAM treatment is not a comprehensive model of schizophrenia in terms of anhedonia – whether characterised in terms of consummatory or anticipatory processes. Indeed, others such as Foussias and Remington (2010) go so far as to suggest that all negative symptoms of schizophrenia should be reconceptualised as secondary to a primary motivational deficit. However, unpublished work performed within the lab of my industrial supervisor at Eli Lilly (Gary Gilmour, personal communication, 2015) show that MAM-treated animals lack motivational deficits when given the opportunity to lever press for rewards under a progressive-ratio schedule of reinforcement. That is, even if amotivation is more central to schizophrenia symptomatology than anhedonia, the MAM model still appears incomplete.

The second relevant issue is that of reward processing in the context of instrumental behaviours. As noted above, there was no evidence for excessive reliance on habitual responding in MAM-treated rats, and, if anything, there was a suggestion that MAM rats may display some bias towards A-O control of behaviour. This does not reflect the observation in clinical samples of a bias to

habitual responses (see Griffiths et al., 2014, and section 1.3.3 of the general introduction).

Moreover, the fact that the outcome devaluation and DOE tasks depend on the cortico-striatal networks that had been thought to be disrupted by MAM treatment also appears to be contradictory to other findings. For example, Moore et al (2006) found a behavioural phenotype in MAM-treated animals (i.e. increased NMDA-induced orofacial dyskinesias and impaired reversal learning on a Y-maze task) that is consistent with a dysfunctional frontal cortex, while impaired performance on the attentional-set shifting task (another task that depends on intact frontal systems) is a well replicated finding for this model (e.g. Gastambide et al., 2012). That said, there is some evidence to suggest that deficits in MAM-exposed rats are limited to only certain aspects of cortico-striatal function.

Featherstone et al. (2007) studied the performance of MAM-exposed rats on a 5-choice serial reaction time task which is analogous to the continuous performance task in humans. Here, rats are required to attend to a light stimulus which is displayed in one of five locations, thus requiring continued and divided attention from the animal. Despite performance on this task being known to depend, at least partially, on the prefrontal cortex (Christakou, Robbins & Everitt, 2001; Chudasama, Passetti, Rhodes et al., 2003; Passetti, Chudasama & Robbins, 2002) and dorsal striatum (Rogers, Baunez, Everitt & Robbins, 2001), no deficit was found in the performance of MAM-treated rats.

Featherstone and colleagues suggest that the similarities in cognitive impairments seen between the MAM model and schizophrenia may occur at a purely superficial level, without the MAM behavioural deficits tapping into the same underlying neurobiological processes that are impaired in the disorder.

Alternatively, with neurogenesis disrupted at a late stage during gestation, the structural abnormalities

seen in the PFC (and the hippocampus) may be sufficient to cause behavioural impairments on only a limited range of tasks. For example, Flagstad et al. (2005) failed to show any evidence of working memory impairments in MAM-treated rats using a delayed non-match-to-position paradigm (a task which depends on prefrontal cortex integrity). The authors reason that as these memory systems rely on early maturation of the PFC, disturbances of PFC-dependent tasks may only relate to a later maturation of this structure (although the experimental parameters used in the task (e.g. first overtraining animals on a non-delay version of the task or allowing animals to adopt a movement-mediated strategy) may also explain their results). Further, the same authors found that disrupted hippocampal neurogenesis produced by prenatal MAM treatment was not gross enough (or did not target the critical areas of the structure) to see impairment on a reference memory version of the Morris Water-Maze task.

Reversal learning deficits, which are also thought to be partly attributable to perturbations in cortico-striatal functioning, were not observed in Experiment five. This also appears to be inconsistent with the fact that reversal learning deficits that have previously been reported in relation to the MAM model (e.g. Flagstad et al., 2005; Gastambide et al., 2012). However, in the reversal paradigm of the Morris Water-Maze task, MAM-treated rats performed as well as controls on the last day of testing, despite showing deficient reversal performance earlier in the task (days two and three of testing) (Flagstad et al., 2005). Discrepancies have also been seen across the three reversals typically used in the attentional set-shifting task, with Featherstone et al. (2007) reporting impairments during the first and third reversal, but Gastambide et al. (2012) reporting impairments during the first

and second reversal. Moreover, unpublished work performed at Eli Lilly (Gary Gilmour, personal communication, 2015) has suggested large discrepancies across different MAM-treated cohorts in the behavioural impairments displayed. For example, of the eight cohorts tested on a digging reversal task, only half showed a significant impairment, and of the eleven cohorts run through an instrumental reversal task, eight failed to show any significant differences between MAM-treated rats and their saline treated controls, despite all cohorts showing the expected alterations in brain weights. Similar levels of variability have also been seen across other tasks. It is not immediately clear what might be causing this heterogeneity in response to MAM treatment, though it is possible that it may relate to the difficulty in accurately timing the intervention at a precise gestational period (relying on the use of vaginal plugs). Regardless, whilst this sort of variability across the MAM model perhaps reflects the heterogeneous nature of schizophrenia, it is not conducive to drug discovery efforts - thus limiting the utility of MAM in this context.

In summary, MAM treatment has previously been reported to produce a variety of behavioural deficits and it clearly results in significant disruption of brain development. However these behavioural deficits do not appear to extend to the processing of rewards in ways that might mimic the negative symptoms of schizophrenia. Moreover, the deficits that have been reported to follow from MAM treatment are somewhat variable (to say the least), and may also relate to disruption of brain systems at least partially unrelated to schizophrenia. Thus, while MAM treatment might remain an interesting manipulation through which to examine the long-term effects of a time-focused disruption

of neural development, it does not appear to produce a particularly useful or consistent schizophrenia model when taken in isolation.

7.5 Reward Processing in the WKY Inbred Rat Strain

Chapter five investigated consummatory and anticipatory hedonic deficits in the WKY inbred rat strain, a putative model of comorbid depression and anxiety. Using the same methods employed for investigating the MAM model, WKY animals showed behaviours consistent with both consummatory and anticipatory hedonic deficits.

Turning first to Experiment seven, the results of consummatory hedonic deficits in the model are in accordance with previous research on the WKY strain. Using a wide variety of behavioural paradigms it has been shown that WKY rats demonstrate deficits in two-bottle preference tests (Malkesman et al., 2005), show less self-administration of pleasurable substances (De la Garza, 2005) and find a sexually receptive female less rewarding (Paré, 2000). The current experiment advances previous findings by directly demonstrating reduced hedonic tone in WKY rats using an appropriate measure that does not conflate hedonic and motivational impairments.

Considering the hedonic hotspots of the rodent brain and the importance of opioid systems in amplifying pleasure responses (section 1.2 of the general introduction), a reduction of opioid activity may feature in WKY rats at one or more of these hotspot regions. Indeed, reciprocal relationships have been seen between the VP and NAc, in that one cannot enhance 'liking' without the other (as reviewed by Berridge & Kringelbach, 2015), so increasing opioid levels in either of these two regions

may improve hedonic reactions in this strain. Alternatively, as has been briefly mentioned earlier, WKY rats may have altered kappa-opioid receptor systems in the brain with kappa-opioid antagonists reversing some of their depression-like behaviours (e.g. reversing increased immobility in the forced swim test as compared to a Sprague-Dawley control; Carr et al., 2010). Carr et al. (2010) found elevated baseline levels of Dynorphin A (an endogenous kappa-opioid receptor ligand) in the NAc of WKY rats compared to their Sprague-Dawley controls. Whilst increased kappa-opioid activity in the precise hotspot region of the NAc shell should increase hedonic tone (inconsistent with our findings), increased levels in regions outside the hotspot would be expected to suppress 'liking' responses (Castro & Berridge, 2014a). The systemic or regional application of a selective kappa-opioid receptor antagonist (e.g. U50488H) would determine whether reduced hedonic tone is due to increased kappa-opioid activity levels in the WKY rat strain.

Experiment eight demonstrated that WKY rats were able to display a contrast effect in terms of consumption, but not in terms of their palatability responses. The dissociation seen between consumption measures and palatability responses for the WKY strain sheds light on the cognitive/affective interactions found in the WKY model. The contrast-dependent changes in consumption may suggest that WKY rats are able to form some sort of expectation of the second solution in the pairing: they are adjusting their intake of the initial solution in light of this expectation. The lack of contrast-dependent changes in LCS suggests that WKY rats are unable to modulate their affective responses to the initial solution based on the expectation of the second solution. This blunted modulation of their affective responses in light of future events may reflect the presence of

anticipatory anhedonia in this rat strain. However, it should be remembered that the general reduction in hedonic tone in these animals might have produced a restriction of range in the critical LCS measure, obscuring any potential hedonic effects of contrast. Regardless, the results of Experiments seven and eight clearly demonstrate that WKY rats display an analogue of the anhedonia associated with depression in humans.

Chapter six investigated whether the WKY strain also includes reward-related deficits, beyond the narrow concept of anhedonia. WKY rats were unable to use reward representations to control their behaviours even after only minimal levels of training, consistent with habitual control of their performance. Furthermore, WKY rats did not benefit from differential outcomes during the acquisition of a conditional discrimination task, also suggesting that reward representations cannot be used by this strain to direct their choice behaviours. Both a reliance on stimulus-response associations and a lack of the Differential outcomes effect may be consistent with altered amygdala formation in the WKY inbred strain. For example, lesions (e.g. Balleine et al., 2003) and temporary inactivation (Parkes & Balleine, 2013) of the BLA render animals insensitive to the devaluation of particular reinforcing events. Furthermore, Blundell et al. (2001) demonstrated that BLA-lesioned animals could not make use of the distinct sensory properties of different reinforcers to aid their discrimination learning. Whilst impaired amygdala function would be entirely consistent with an anxious depressed phenotype (e.g. Sandi & Richter-Levin, 2009; Wolfensberger, Veltman, Hoogendijk, Boomsma, & de Geus, 2008), it cannot account for all WKY-related behaviours, particularly the reduced palatability responses to sweet tastes observed in Experiment seven. Indeed, Mahler and Berridge (2012) demonstrated that

DAMGO microinjection into either the BLA or CeA does not enhance the number of positive hedonic reactions to sweet tastes, and neuroimaging results suggests that amygdala activation does not reflect the subjective pleasantness of food cues (Small, Veldhuizen, Felsted, Mak & McGlone, 2008). Whilst WKY rats may suffer from deficits in amygdala-related behaviours, clearly deficits in other brain regions (perhaps the connected NAc) must account for their impaired hedonic processing.

The associations that can be formed in WKY animals will be discussed in section 7.7. Further work is needed to determine the precise impairment in reward representation in this strain, but based on current work, WKY rats appear to be impaired in multiple reward-related processes. Therefore, the results presented here reinforce the idea that the WKY rat is a valuable rodent model for the pre-clinical investigation of depression.

7.6 Stress Effects in the WKY Model and their Controls

Whilst the application of a chronic mild stress procedure generally exacerbated the behavioural deficits seen in the WKY strain, no interactions were seen between stress and strain for any of the experiments reported in this thesis. Taken at face value, this suggests that the explicit application of stressors is not a necessary antecedent for reward-processing deficits to manifest in the WKY rat.

Turning first to the results of Experiment seven, regardless of strain, stress appeared to reduce general consumption levels across the three concentrations of sucrose. This general stress effect on consumption confirms that the unpredictable chronic mild stress procedure adopted here

was effective. Moreover, the fact that these stress effects were maintained over both Experiments seven and eight suggests there was little or no overall habituation/de-sensitization to the procedure. The application of a chronic mild stress procedure, however, did not produce a decrease in the LCS measure in either the WKY rats or Wistar controls. The dissociation between consumption and LCS measures in the control strain is important when considering the fact that unpredictable chronic mild stress is a widely used depression model. Indeed, the primary impetus for developing the chronic mild stress procedure was to simulate anhedonia in rats (see Wiborg, 2013, for a review), and the stress-produced reduction in sucrose consumption has been interpreted to reflect abnormal hedonic reactions (e.g. Willner et al., 1987). Considering the consumption data alone from Experiment seven, the reduced intake for stressed Wistar rats across solutions is apparently consistent with this previous interpretation that chronic stress reduces in-the-moment pleasure. However, my results show that, at least after the stress procedure used here, a reduction in consumption does not necessarily mean a reduction in a rat's hedonic responses. Given that the licking microstructure measure is a more direct measure (and potentially less subject to confounding motivational influences) of hedonic responses than simple consumption measures, these results highlight that the reduction in sucrose consumption following chronic stress might not actually reflect the presence of anhedonia. More generally, the dissociation of consumption and LCS measures implies that both should be considered in investigating the presence of an anhedonic profile.

That said, the relationship between the current stress procedures and those used previously is somewhat questioned by the lack of a stress effect on the balance between goal-directed and

habitual systems. As discussed in section 1.3.6, it has previously been shown that both chronically and acutely stressed rats were insensitive to changes in outcome value, indicating that their behaviour was under habitual control (Braun & Hauber, 2013; Dias-Ferreira et al., 2009). The training schedule used in both of these experiments differed from the current study, making it hard to determine the equivalence of the training regimens. That said, both used ratio schedules in which a response is followed by a certain probability of a reward. With a high experienced contingency maintained between the action and the outcome, these schedules promote goal directed, as opposed to habitual, responding in healthy untreated rats. The lack of a stress effect for Experiment nine may suggest that the stress procedure adopted was not substantial enough to modify behaviour on all tasks.

Returning to the question of whether the application of external stressors is needed to elicit a behavioural profile in WKY rats, the fact that there was either no stress effect in the WKY rats, or that any stress effects were also reflected in the Wistar controls, certainly suggests that external stressors are not required. However, any interpretation of the stress effects in WKY animals requires the No-stress group to actually be non-stressed. Whilst every attempt was made to reduce stress in the No-stress WKY animals, this strain may well be hyper-responsive to stress effects. In light of this possibility, it is important to consider the possible effects of the experimental manipulations themselves. For example, food restriction (which was used in all the experiments reported here) has been used as part of chronic stress regimes used elsewhere (see Willner, 1997; Xu, Barish, Pan, Ogle & O'Donnell, 2012, for reviews), and the use of a shared holding room meant that the animals

were exposed to the presence of unfamiliar laboratory personnel and other rats. Furthermore, since taste aversion with LiCl was used to devalue the outcome in Experiment nine, all animals were given intraperitoneal injections, which again may have affected the WKY animals. Finally, going back as far as the early postnatal period, reduced parental care from a 'depressed' WKY dam may have induced stress effects in the WKY animals (see Ahmadiyeh, Slone-Wilcoxon, Takahashi & Redei, 2004; Cierpial, Shasby & McCarty, 1987).

Based on the current results alone, it is possible either that the WKY rats have a strong genetic diathesis that supersedes any environmental influences, or that only minor stressors are required to shape their behavioural profile. Thus, the current results do not definitively determine whether stress is required to elicit the WKY deficits seen across these experiments. That said, the fact that applying explicit external stressors, which were sufficient to influence the behaviour of the Wistar controls, did not produce any greater effects in the WKY model certainly implies that future consideration of the WKY rats can focus on strain effects alone.

7.7 Representations of Reward in the WKY Model

Experiment nine suggests that three days of training were sufficient to bias the WKY rats' behaviour towards habitual systems, controlled by S-R associations. Considered alone, this could reflect one of four possible alternatives: habitual behaviour in the WKY rat may be due to (1) a failure to form A-O associations (and thus the WKY rats might be S-R only); (2) a failure to form sensory specific (as opposed to general motivational) outcome representations; (3) a fast transition from A-O

to S-R controlled behaviours, or (4) that S-R and A-O associations might both form in the WKYs, but that S-R associations have the dominant effect on instrumental responses when both types of association are retrieved.

While all these possibilities are consistent with the results from Experiment nine, the results of the other experiments reported in this thesis suggest which of the four are perhaps more likely. The idea that WKY rats are entirely S-R organisms (possibility 1) is certainly consistent with the lack of a differential outcomes effect during task acquisition (Experiment ten). As lever pressing during this conditional discrimination task is reinforced in the presence of distinct visual stimuli, it is possible that the conditional discrimination can be solved by forming S-R associations alone. As the reward would not be included as part of an entirely S-R associative framework controlling behaviour, no performance benefit would be seen for animals in the differential as opposed to non-differential groups – which is exactly the pattern displayed by the WKY rats. However, the reinforcer only test in Experiment ten is not consistent with an S-R only analysis. The fact that WKY rats show better performance in the differential than non-differential conditions, when the visual discrimination stimuli are not present, implies that the WKY rats must have encoded at least some aspects of the different outcomes, even if these outcome representations did not influence behaviour when the visual discrimination stimuli were available.

Moreover, the idea of WKY rats being entirely stimulus-bound, unable to encode any part of a reward, is difficult to reconcile with the contrast effects in consumption seen in Experiment eight. This interpretation would require that the negative contrast effect in consumption can be explained by S-R

mechanisms. However, negative anticipatory contrast is not usually amenable to an S-R analysis: if a licking requirement (i.e. an instrumental contingency) is placed on the initial solution before access is given to the second solution, the negative anticipatory contrast effect disappears or reverses, demonstrating that it is not an instrumental (i.e. S-R) response (see Flaherty, 1996, for further discussion of this topic).

Moving on to the second possibility, that WKY rats are only capable of impoverished outcome representations, an inability to encode the sensory properties of different rewards could also explain the lack of a differential outcomes effect during the acquisition of a conditional discrimination. When a conditioned stimulus (CS: such as a light) is paired with an unconditioned stimulus (US: such as food), it is thought that several distinct associations can form (Konorski, 1967). Upon CS presentation an internal representation of the CS is evoked. US presentation is capable of evoking both sensory (USs) and motivational (USm) representations. In the normal animal, associations can be formed between the CS representation and the representations of the USs and USm and/or between the CS representations and the responses the USs and USm evoke (see Blundell et al., 2001). It should be noted at this stage that USs refers to the sensory and hedonic properties of the reinforcer that are specific to the individual outcome, whereas USm refers to the general arousing aspects of motivation for each outcome (Blundell et al., 2001). Similarly to S-R associations, associations formed between the discriminative stimuli and the general motivational aspects of the rewards (USm) would allow the WKY rats to solve a conditional discrimination, but would not allow differential outcomes to serve as additional stimuli to aid learning. As both discriminative stimuli (S), and indeed both responses (R),

would elicit the same outcome representation (O), differential S-O and R-O associations would not be formed and essentially the stimuli alone (as is the case in the non-differential group) would direct the rats' choice behaviour.

This 'motivation only' outcome idea is perhaps consistent with the negative anticipatory contrast data in terms of consumption. Insofar as consumption reflects the motivational aspects of the reward (USm), while LCS reflects the palatability or sensory aspects of the reward (USs), an ability to form USm representations but not USs representations in the WKY rat may promote contrast effects in consumption but not in palatability. That said, this interpretation does not fit with the reinforcer only test of Experiment ten where better performance is seen for WKY rats in the differential as opposed to non-differential groups. For this to be explained by the formation of USm associations, the sucrose and pellet reinforcers would need to have different motivational values. But if the USm representations were sufficiently different to control responding in the reinforcer-only test, then they should also be sufficiently different to support differential responding in the acquisition stage of this task.

When the results of Experiments eight, nine and ten are considered together, the data are probably not consistent with either possibility 1 or 2. If the rats do encode something about the reward under at least some circumstances then the issue may lie with when this learning is expressed.

A fast transition from A-O to S-R associations (possibility 3) is consistent with the WKY rats generally higher response rates during the acquisition of an instrumental task in Experiment nine (and

indeed in Experiment ten, although the raw lever-press data is not presented here). As mentioned previously, one potentially important determinant of the associative structure underlying instrumental behaviour is the experienced contingency between the response and its outcome (Dickinson, 1985). Dickinson proposed that both overtraining and interval schedules reduce the experienced correlation between response rate and reward rate. As awareness of the instrumental contingency is dependent on a variation in behaviour giving rise to a variation in the delivery of rewards, it is possible that WKY rats are responding at sufficiently high levels to reduce the effective correlation between response and reward rates, causing their responses to be driven by S-R associations.

This idea of a fast transition can be also be used to explain the reinforcer-only results of Experiment ten if the R-O associations can be expressed when S-R links are not retrieved (given that the S is not there in the reinforcer-only test). However, this also fits well with the idea that WKYs might form both A-O and S-R associations and that it is simply a matter of S-R associations dominating (possibility 4). Both these possibilities also fit with the neurobiological data on reward devaluation, and the idea that both A-O and S-R associations form, and that different parts of the cortico-striatal circuits alter the balance between which is expressed. That is, results showing that infralimbic cortex lesions after extended training cause animals to return to A-O dependent behaviours suggests that these two associations compete for their influence over instrumental responding, such that one can dominate but does not remove the other (Coutureau & Killcross, 2003). Finally, both possibilities are consistent with the results from the negative anticipatory contrast

procedure. When S-R associations cannot be formed, WKY rats can and do form associations between the stimuli (i.e. the context) and the outcome (i.e. the second solution's identity).

In summary, the most appropriate characterisation of the WKY deficit based on the current results is not a failure to process/encode the specific nature of rewards, but a dominance of habitual behaviours, even though A-O associations are formed. Whether this reflects a rapid transition to S-R, or is a general result of an imbalance to S-R control of behaviour, cannot be determined on the basis of the current data. Section 7.10 will highlight potential future investigations which would afford a better understanding of the precise reward processing deficits seen in the WKY model. But regardless of the exact characterisation, the dominance of S-R associations (be it overall or through a swift transition) suggests that WKY animals have a deficit in using, but not necessarily encoding, the nature and value of rewards. Thus the WKY inbred rat strain may provide a good model of the inflexible behaviour which features in depression.

7.8 WKY Model and Depression

Based on the current results, the WKY rat strain appears to provide a valuable rodent model of depression, at least in terms of the affective and reward processing deficits. However, a few factors require consideration. First, Experiment seven demonstrated that consumption was clearly modulated by solution concentration in WKY rats, whereas the modulation of their palatability responses was severely attenuated. Indeed, LCS was only marginally higher for 24% sucrose than 2% sucrose. As such, it is at least possible that the reduced LCSs seen could reflect a licking

behaviour that is so impaired that changes in affective responses are untraceable. That said, analysis of the WKY rats alone demonstrated that the differences between the high and low concentrations of sucrose were statistically significant, suggesting both that the WKY rats licking behaviour is not fixed by some motor impairment and that the WKYs affective responses to the solutions were severely blunted rather than entirely absent. Moreover, the analysis of the inter-lick intervals within licking clusters did not reveal a general motor impairment which could have produced all of the lick cluster size reductions observed in Experiments seven and eight.

Second, whilst the majority of laboratory-based studies have indicated that depressed individuals, compared to healthy controls, generally rate positive stimuli as less positive and/or arousing (e.g. Berenbaum & Oltmanns, 1992; Dunn et al., 2004; Sloan et al., 1997), inconsistencies do exist in the literature (see Treadway & Zald, 2011). Of particular relevance to the current investigation, the “sweet-taste test” in depressed individuals (a test that closely mirrors our animal measure of hedonic experience) has shown normative in-the-moment pleasure ratings across four separate studies (Amsterdam et al., 1987; Berlin et al., 1998; Dichter et al., 2010; Kazes, Danion, Grange et al., 1994 - see Treadway & Zald, 2011, for a review). While the sizeable individual differences in taste sensitivity found in human subjects may explain the lack of power to discern a depression-related difference in responses to sweet tastes (Duffy & Bartoshuk, 2000), it is also possible that hedonic deficits in depressed patients are stimulus specific. That is, patients experience deficient hedonic processing of some stimuli (i.e. pictures and film-clips) but a relatively unimpaired hedonic capacity to experience other stimuli (e.g. food and drink - although see

Berenbaum & Oltmanns, 1992). Perhaps it is simply the case that responses to sweet solutions may not be as sensitive to changes in hedonic perceptions in humans as is the case for animals. One important distinction that may also underlie these discrepancies between clinical and preclinical studies is that studies in the clinic rely on subjective ratings, whereas studies in rodents rely on objective measures. Regardless of these issues, the current results show that WKY rats model consummatory anhedonia, if not perhaps towards the same eliciting stimuli as in clinical depression.

Despite Experiment seven showing some concentration effects for LCS in the WKY strain, it must be conceded that the greatly blunted range of LCSs seen in these animals may give little scope for the typical lowering of hedonic responses that are seen for a contrasted solution (i.e. a 4% solution that is reliably followed by a 32% solution). As discussed in the summary section for Experiment eight, a potential floor effect for WKY animals during the negative anticipatory contrast procedure is unlikely to be due to a measurement problem, as taste aversion studies strongly indicate that LCSs are capable of going far lower than that seen in the current study. Furthermore, as alluded to earlier, the reduced hedonic range in WKY rats does not appear to be the result of gross disturbances in the general licking competency of these animals as other parameters such as ILI appear relatively normal. Regardless, it is clear that further work must also be carried out to confirm the presence of anticipatory hedonic deficits in the WKY rat strain.

The presence of anticipatory anhedonia in depressed patients has not received much attention in the literature. McFarland and Klein (2009) found that individuals suffering from depression gave significantly lower ratings of positive emotions during the anticipation of monetary

rewards compared to never depressed control subjects. Similarly, fMRI studies have revealed diminished striatal responses to anticipation of reward (Forbes et al., 2009; Smoski et al., 2009). High self-report of anticipatory anhedonia has also been shown for depressed patients (Sherdell et al., 2012). Combined, these data provide some evidence for a deficit in experienced emotion during reward anticipation in depression. However, some contradictory findings also exist in the literature. Using the Monetary Incentive Delay task (that probes consummatory and anticipatory hedonics) no differences were found between depressed individuals and control individuals during reward anticipation (Knutson et al., 2008; Pizzagalli et al., 2009). The interpretation of these experiments is made difficult, however, as the period of reward anticipation was not passive, but instead required the subject to prepare for a speeded manual response. Clearly, the measure of anticipatory anhedonia may be confounded by the depressed subjects heightened punishment motivation (Treadway & Zald, 2011). Further work, specifically using the temporal-experience of pleasure scale and passive Pavlovian versions of the MID task (see Dowd & Barch, 2012), needs to be carried out before we can be certain as to how the WKY model relates to the hedonic processing deficits found in depression.

The demonstration that WKY rats' instrumental behaviour is controlled predominantly by S-R associations (Experiment nine and ten) is consistent with the cognitive and motivational deficits recognised in depression. As discussed in section 1.3.6, early descriptions of the disorder placed a large emphasis on cognitive inflexibility, while more recently it has been suggested that reward processing impairments may be central to these cognitive disturbances. In relation to depression, Griffiths et al. (2014) state that "as experienced rewards are no longer pleasurable, it is easy to

envisage how action control could become biased away from goal-directed actions" (p. 8). The idea of an over-reliance on habitual systems is also in line with the impaired decision-making abilities seen for depressed individuals together with the high comorbidity seen between depression and other habit-based disorders such as substance abuse. Moreover, structural and functional changes in the depressed brain show significant overlap with the core circuitry known to control the balance between A-O and S-R systems.

As acknowledged in section 7.6, it is currently unclear whether the strain alone or interactions between strain and stress was critical in biasing behaviour towards reflexive habits, primarily due to the increased stress vulnerability of the WKY rat. With both stress (Schwabe & Wolf, 2010) and pharmacological manipulations implicating cortisol (Schwabe et al., 2010) in increased habitual behaviours in human studies, it is possible that the hypercortisolaemia seen in the WKY strain induced the shift to habitual behaviours reported here. Moreover, it is likely that any neurochemical alterations in the strain are accompanied by structural or functional changes in the cortico-striatal circuits underlying goal-directed and habitual behaviours. The bias to habitual systems in rats after a stress procedure has been shown to be accompanied by atrophy of the medial prefrontal cortex and dorsomedial striatum (implicated in A-O control) and hypertrophy of the dorsolateral striatum (implicated in S-R control) (Dias-Ferreira et al., 2009; Schwabe & Wolf, 2011). While the stress manipulation used here was clearly not substantial enough to induce a similar behavioural profile in the control strain, it is possible that similar stress-induced or inherent structural abnormalities are present in WKY rats. Further work in the clinic is required to both confirm a dominance of S-R

associations in depressed patients (on a comparable outcome devaluation task) and to determine the neural mechanisms involved.

While the results here suggest that the WKY rat is a promising model for the pre-clinical investigation of depression, as with any modelling approach, there are some potential complicating factors that need to be considered in any future work. Most importantly, the breeding history of the WKY rat has resulted in some divergence between the genetic profiles of rats associated with different suppliers (e.g. Zhang-James, Middleton & Faraone, 2013). These genetic differences are reflected in phenotypic variability among WKY rats (Kurtz & Morris, 1987; Paré & Kluczynski, 1997) suggesting that there are actually a number of substrains of WKY animals. Genotyping suggests that WKY rats supplied by Charles River from UK (used here) or USA breeding stocks are the most relevant for studying depression-like behaviours in this rat strain (see Zhang-James et al., 2013).

Furthermore, WKY rats, alongside the spontaneously hypertensive rat (SHR) were originally developed from an outbred Wistar strain. As such, a large number of studies have used both Wistars (e.g. De La Garza et al., 2005; Malkesman et al., 2005; Malkesman, Braw, Maayam et al., 2006) and SHR rats (e.g. Paré, 1989a; Paré & Schimmel, 1986) as the control strain due to their common genetic backgrounds (e.g. Nam et al., 2014). However, in some studies, particularly concerning antidepressant effects and neurochemical characterisation, Sprague-Dawley comparison strains have been used (e.g. Carr et al., 2010; López-Rubalcava & Lucki, 2000; Rittenhouse et al., 2002; Tejani-Butt et al., 2003), despite their genetic divergence from the WKY strain (see Nam et al., 2014). Additionally, because the WKY rat was not completely inbred prior to initial distribution, this has led

some investigators to generate two fully inbred substrains of WKY ("WKY more immobile" and their "less immobile" controls) through bidirectional selective breeding, based on forced swim test mobility (e.g. Will et al., 2003; Williams, Mehta, Redej, Wang & Procissi, 2014). Regardless of the potential issues around control strains, the pattern of deficits seen here cannot simply be attributed to abnormally high levels of performance in the Wistar controls. For example, hedonic changes in the negative anticipatory contrast paradigm were seen in MAM, Lister Hooded, and Sprague-Dawley rats, but not WKYs, and the DOE and reward devaluation effects have been observed in multiple strains (including the Sprague-Dawleys and Wistars used here). Moreover, the WKY deficits are not simply a quantitative difference to controls, but in many cases reflect a qualitative difference between the behaviour observed in the WKY animals and that of controls (e.g. the absence of a DOE and devaluation effects, or the consumption/LCS dissociation in the negative anticipatory contrast paradigm).

7.9 Reward Related Processing in Psychiatric Disorders and their Animal Models

As supported by the results reported in Chapters five and six of this thesis, Ribot's original definition of anhedonia, as an inability to experience pleasure, appears to be outdated as it does not reflect the nuances that exist in reward processing nor how these might relate to different psychiatric and neurological disorders. Whilst understanding in this area is growing, many researchers still use anhedonia as a blanket term and use measuring techniques that could reflect an array of reward-related deficits alongside the intended hedonic measure. The picture in the clinic is further

complicated by a lack of clarity with the classification criteria used for the disorders. Indeed, as has been highlighted previously, the DSM-V includes a loss or reduction in interest or pleasure in usually enjoyable activities in relation to anhedonia in depression (American Psychiatric Association, 2013; Rømer-Thomsen et al., 2015). Yet, assuming equivalence between hedonic and motivational systems is clearly misguided given that these two processes are dissociable at both the behavioural and neural levels.

Based on the current experiments reported in this thesis it is clear that many different aspects of rewards should be considered using a battery of sensitive methods. Whilst translational assessment techniques (such as those reported here) are a critical first step, animal models need to reliably include symptom-like behaviours. In light of the heterogeneity of disorders such as schizophrenia and depression, it is unlikely that all behavioural impairments associated with these disorders will be replicated in a single model. Understanding the short-comings of a model (such as the MAM neurodevelopmental model) can be just as informative as finding positive results. Furthermore, it must be recognised that animal models of disorders are only as good as the knowledge in the clinical literature allows. More precise investigation into the affective and cognitive aspects of reward processing in disorders such as depression must be performed to inform the preclinical models of which face and construct validities they should encompass.

7.10 Future Directions

The presence of hedonic and other reward processing deficits in the WKY rat reinforces the idea that it might offer a useful pre-clinical depression model. This possibility would require further validation. One obvious possibility might be through the examination of the effects of established anti-depressant pharmacology on the sorts of deficits identified here. Another possibility is suggested by epidemiological studies which clearly demonstrate a strong gender bias for depression, with a higher likelihood of women developing depression, and indeed comorbid anxiety. Future work performed on the WKY strain should also consider comparing behavioural differences across genders. But prior to such model-validation research, the accurate characterisation of the precise reward-related deficits displayed by this model should be completed.

To date, I have demonstrated that the behaviour of WKY rats is controlled by S-R associations at the expense of A-O associations. To tease apart whether this is best characterised by a fast transition to S-R systems or a general imbalance to S-R control, future studies could adopt an outcome devaluation procedure after one day of instrumental training. By significantly reducing training length, an animal which is capable of goal-directed behaviour (but with a rapid transition to habitual control) should be sensitive to post-conditioning changes in reward value: reducing its responding on a lever paired with a devalued outcome compared to a non-devalued outcome. Similarly, instrumental tasks that employ two levers and two outcomes are also designed to promote goal-directed behaviours by maintaining a high experienced instrumental contingency (choosing one action completely stops reward delivery for the alternative action) regardless of training length. Using

these paradigms, a failure to detect suppression in lever press rates in WKY animals would provide cogent evidence that they cannot use reward value to guide their behaviours.

Whilst the current data reported here suggests that impoverished value representations (i.e. US_m but not US_s associations) is not the best characterisation of the WKYs behaviour (i.e. this does not allow for the differential outcomes effect during the reinforcer-only test of Experiment ten), the use of a differential outcomes procedure, with the two rewards varying in terms of their motivational rather than sensory properties, would provide the empirical evidence required to support this claim.

My work recently performed at Lilly UK has started to investigate these alternatives: In a two-lever, two-outcome paradigm WKY rats responded less on a lever associated with the devalued outcome (fed to satiety immediately before test) compared to the lever associated with the non-devalued outcome (not fed to satiety); moreover, while a marginal performance benefit was seen when WKY rats were trained on a conditional discrimination where stimulus-response contingencies were correlated with outcomes of different magnitudes (e.g. Flashing light: Left lever → 1 plain pellet; Steady light: Right lever → 5 plain pellets) than when the outcome magnitude was not consistently paired with different responses, this benefit was substantially less than for controls. Although preliminary, these results suggest that WKY animals may well form A-O associations relatively normally, but that they control instrumental behaviour only under restricted experimental conditions. One possible way to further probe these circumstances might be to put S-R and A-O associations into conflict – as has been attempted by using rewards as cues in an instrumental discrimination (e.g. Dwyer et al., 2010; de Wit, Kosaki, Balleine & Dickinson, 2006; Dickinson & de Wit, 2003)

In one sense, the results in this thesis raise as many questions about the nature of reward processing in the WKY rat as they provide answers. But regardless of the outcome of future attempts to characterise the precise reward processing deficits displayed by WKY rats, the fact that they clearly display a range of hedonic and cognitive deficits compared to controls suggests that any such future work should prove to be particularly valuable given the potential for the WKY rat to contribute to pre-clinical research. In contrast, the absence of any consistent reward processing deficits following MAM treatment suggests that the prime contribution of my research in this area is to question the utility of this treatment as a potential pre-clinical model for schizophrenia.

8. Appendices

8.1 Appendix A – MAM and WKY cohorts and testing order

Strain	Cohort	Experiment	Chapter	Start Date	
MAM	1 (<i>n</i> = 24 MAM, 18 Sham)	Consumption in MAM rats (Exp. 2)	3	June '12	
		Outcome devaluation procedure in MAM rats (Exp. 4)	4	July '12	
		Conditional discrimination and reversal	Not included in thesis	Aug '12	
		Hyper-locomotion in MAM rats after an MK-801 challenge	Included in appendix only	Sept '12	
MAM	2 (<i>n</i> = 24 MAM, 24 Sham)	Consumption in MAM rats (Exp. 2)	3	Feb '13	
		Outcome devaluation procedure in MAM rats (Exp. 4)	4	March '13	
WKY	1 (<i>n</i> = 12 KNS, 12 KS, 12 WNS, 12 WS)	<i>Chronic mild stress procedure commenced</i>		Oct '13	
		Negative anticipatory contrast in the WKY rat (Exp. 8)	5	Oct '13	
		Consumption in the WKY rat (Exp. 7)	5	Dec '13	
		<i>Stress procedure stopped - rats given environmental enrichment in home cages</i>			23rd Dec '13
		<i>Stress procedure reinstated</i>			Jan '14
		Outcome devaluation procedure in the WKY rat (Exp. 9)	6	Jan '14	
		Differential outcomes effect in the WKY rat (Exp. 10)	6	Feb '14	
MAM	3 (<i>n</i> = 16 MAM, 16 Sham, 16 Non-exposed)	Negative anticipatory contrast in MAM rats (Exp. 3)	3	Feb '14	
		Outcome devaluation procedure with extended training in MAM rats (Exp. 6)	4	March '14	
		Differential outcomes effect in MAM rats (Exp. 5)	4	April '14	
		Consumption in MAM rats (Exp. 3)	2	July '14	

8.2 Appendix B – MAM validation tests

To further validate the MAM model, Cohort one underwent a psychostimulant challenge with MK-801, see below. Brains for each of the three cohorts were also extracted and weighed after the completion of behavioural analyses. Evidence suggests that rats prenatally treated with MAM display an approximate 11% decrease in total brain weight (Flagstad et al., 2004). Animals were culled via a rising concentration of CO₂ and their brains extracted and weighed without fixation. The juvenile brain weights reported for cohort one were provided by Charles River, UK.

Juvenile Brain Weights: MAM Cohort 1

1) Absolute Brain Weights:

The brains of pups prenatally treated with MAM were significantly lighter than saline-treated controls, $t(6) = -4.568$, $p = .004$

2) Relative Brain Weights:

When relative juvenile brain weights (brain weight/ body weight) were considered, there was no difference between MAM-treated and saline-treated animals, $t(6) = -.547$, $p = .604$

Adult Brain Weights: MAM Cohort 1-3

Table 16 Effect of prenatal MAM and saline treatment at GD17 on adult brain weights (g) for each of the three cohorts. Relative brain weights were calculated as Brain Weight/Body Weight.

Cohort	Prenatal Treatment	<i>n</i>	Mean Brain Weights (g)	SEM	% Difference	Mean Relative Brain Weights (g)	SEM	% Difference
1	<i>Saline</i>	18	2.242	0.022	12.391	0.357	0.008	0.615
	<i>MAM</i>	24	1.965	0.011		0.351	0.005	
2	<i>Saline</i>	24	2.297	0.023	11.657	0.368	0.007	10.965
	<i>MAM</i>	23*	2.023	0.097		0.328	0.029	
3	<i>Saline</i>	16	2.216	0.021	M v. S 12.211	0.415	0.010	M v. S 13.329
	<i>MAM</i>	16	1.946	0.021	M v. N 12.137	0.360	0.008	M v. N 7.729
	<i>Non-exposed</i>	16	2.214	0.023	S v. N 0.085	0.390	0.009	S v. N 6.069

*one saline-treated animal died after testing and it's brain was not harvested

Independent 2-tailed t-test:

Cohort 1:

1) *Absolute Brain weights*: $t(26.037) = -11.219, p < .001$

2) *Relative Brain weights*: $t(36) = -.648, p = .521$

Cohort 2:

1) *Absolute Brain weights*: $t(45) = -8.968, p < .001$

2) *Relative Brain weights*: $t(45) = -4.445, p < .001$

ANOVA analysis:

Cohort 3:

1) *Absolute Brain weights*:

- Main effect of treatment ($F(2, 45) = 50.780, p < .001, MSE = .388$).
- Simple effect analyses:
 - MAM-brains were significantly lighter than brains from both saline-treated ($F(1, 45) = 76.421, p < .001, MSE = .001$) and non-exposed animals ($F(1, 45) = 75.298, p < .001, MSE = .001$).
 - No significant difference in brain weights for saline and non-exposed control groups ($F < 1$).

2) *Relative Brain weights*:

- Main effect of treatment ($F(2, 45) = 9.647, p < .001, MSE = .012$).
- Simple effect analyses:
 - MAM-brains were significantly lighter than for saline-treated ($F(1, 45) = 17.899, p < .001, MSE = .0002$) and non-exposed controls ($F(1, 45) = 5.325, p = .021, MSE = .0002$).
 - No significant difference in brain weights between saline and non-exposed control groups ($F(1, 45) = 3.698, p = .052, MSE = .0002$).

Cohort 1: MK-801 Challenge:

Hypersensitivity to the locomotor enhancing effects of MK-801 (a NMDA-R antagonist) has previously been found to be a robust behavioural feature of prenatal MAM treatment (e.g. Le Pen et al., 2006; 2011).

Apparatus

Activity testing took place in eight white plastic boxes, measuring 48 × 31 × 18 cm, with metal grid floors and lids. Two infrared beams spanned the boxes. Interruption of the beam by the movement of the animal generated a signal that was recorded by a linked PC.

Protocol

Upon completion of behavioural analysis, rats in cohort one received locomotor activity assessments to test the effectiveness of the prenatal MAM treatment. MAM-induced augmented hyperlocomotion following MK-801 treatment was assessed. Rats were placed in LMA cages and left for a 30 min habituation period. Animals were then given 0.1 mg/kg MK-801 sub-cutaneously and returned to the cages for a further 60 min. Across the 90 min session, the number of interruptions that occurred to infrared beams spanning the cages recorded the activity of the animals.

Measurements

The apparatus used for locomotor activity testing counted the number of movements made and grouped them into ten-minute bins. The data were analysed using mixed ANOVA with 'bin' as a within-subject factor, and 'drug treatment' as a between-subject factor.

Results

Data for the first MAM cohort are shown in Figure 42. Rats prenatally exposed to MAM were hypersensitive to the locomotor effects of MK-801 (given at 30 min).

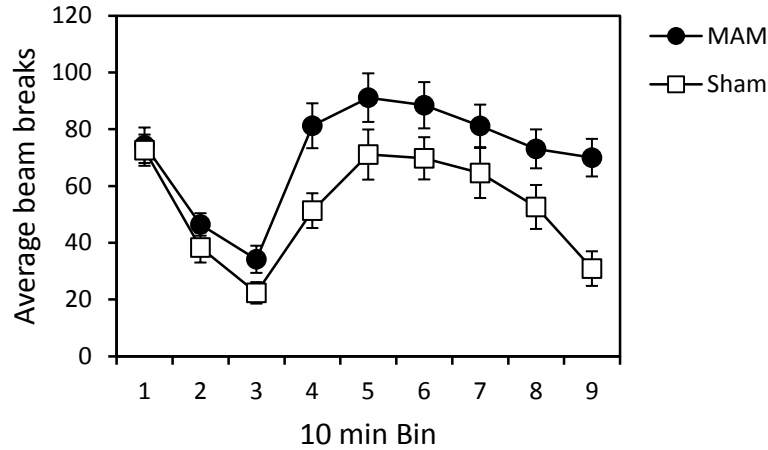


Figure 42 Locomotor activity for saline-treated and MAM-treated rats before and after an MK-801 challenge. MK-801 was given at a 0.1 mg/kg dose at bin 3. Beam breaks were recorded for 30 min before drug administration and 60 min after. N = MAM, 20; Sham, 14. 8 animals are missing from the data set due to a recording error. Error bars represent \pm SEM.

ANOVA revealed significant effects of bin ($F(3.448, 110.348) = 21.017, p < .001, MSE = 24668.780$) and an interaction between bin and prenatal MAM treatment ($F(8, 256) = 2.028, p = .044, MSE = 1025.920$). There was also main effect of MAM treatment ($F(1, 32) = 7.464, p = .010, MSE = 25366.765$). Simple effect analyses of the bin \times treatment interaction revealed no significant difference between prenatal treatment for blocks 1 – 3 or 5-8 (*Largest* $F(1, 32) = 3.836, p = 0.59, MSE = 3450.038$). A significant difference between treatment groups was seen for blocks 4 and 9 (smallest $F(1, 32) = 7.706, p = .009, MSE = 7364.194$).

8.3 Appendix C - Stress procedure

Forced swim tests performed on 07/02/2014 and the 27/02/2014 were not recorded due to equipment problems.

<i>Day</i>	<i>Date</i>	<i>Stress /Husbandry</i>	<i>Experiment details</i>
Thur	17/10/2013	Arrival - rats weight-matched and separated into Stress and No-Stress conditions	
Fri	18/10/2013		
Sat	19/10/2013		
Sun	20/10/2013		
Mon	21/10/2013		
Tue	22/10/2013		
Wed	23/10/2013		
Thur	24/10/2013	Cage Swap	
Fri	25/10/2013	Pair Up	
Sat	26/10/2013	Overnight Illumination	
Sun	27/10/2013		
Mon	28/10/2013		Habituate to test equipment (all)
Tue	29/10/2013		Habituate to test equipment (all)
Wed	30/10/2013	Overnight Illumination	Habituate to test equipment (all)
Thur	31/10/2013	Forced Swim test	PT_NAC
Fri	01/11/2013		NAC_1
Sat	02/11/2013		NAC_2
Sun	03/11/2013	Pair up	NAC_3
Mon	04/11/2013	Overnight Illumination	NAC_4
Tue	05/11/2013		NAC_5
Wed	06/11/2013		NAC_6
Thur	07/11/2013	Wet Cage	NAC_7
Fri	08/11/2013	Wet Cage	NAC_8
Sat	09/11/2013		NAC_9
Sun	10/11/2013		Rest
Mon	11/11/2013		NAC_10
Tue	12/11/2013	Pair up	NAC_11
Wed	13/11/2013		NAC_12
Thur	14/11/2013	Cage Swap	NAC_13
Fri	15/11/2013		NAC_14
Sat	16/11/2013	Forced Swim Test	Rest
Sun	17/11/2013		NAC_15
Mon	18/11/2013		NAC_16
Tue	19/11/2013	Overnight Illumination	NAC_17
Wed	20/11/2013		NAC_18
Thur	21/11/2013	Forced Swim test	NAC_19
Fri	22/11/2013	Overnight Illumination	NAC_20
Sat	23/11/2013		NAC_21
Sun	24/11/2013		Rest
Mon	25/11/2013		NAC_22
Tue	26/11/2013	Cage Swap	NAC_23
Wed	27/11/2013	Wet Cage	NAC_24
Thur	28/11/2013	Pair up	NAC_25
Fri	29/11/2013		NAC_26
Sat	30/11/2013		Rest
Sun	01/12/2013		Rest
Mon	02/12/2013	Overnight Illumination	NAC_27
Tue	03/12/2013		NAC_28
Wed	04/12/2013	Cage Swap	NAC_29

Thur	05/12/2013		NAC_30
Fri	06/12/2013	Forced Swim test	NAC_31
Sat	07/12/2013		NAC_32
Sun	08/12/2013		
Mon	09/12/2013	Pair Up	
Tue	10/12/2013		
Wed	11/12/2013	Wet cage	Cons_T1
Thur	12/12/2013	Wet cage	Cons_T2
Fri	13/12/2013		Cons_T3
Sat	14/12/2013		
Sun	15/12/2013		
Mon	16/12/2013	Pair Up	Cons_T4
Tue	17/12/2013	Cage Swap	Cons_T5
Wed	18/12/2013		Cons_T6
Thur	19/12/2013		
Fri	20/12/2013	Forced swim Test	
Sat	21/12/2013		Cons_T7
Sun	22/12/2013		Cons_T8
Mon	23/12/2013		Cons_T9
Mon	06/01/2014		
Tue	07/01/2014	Environmental Enrichment removed	
Wed	08/01/2014		
Thur	09/01/2014	Overnight illumination	
Fri	10/01/2014	Cage Swap	
Sat	11/01/2014		
Sun	12/01/2014		
Mon	13/01/2014		
Tue	14/01/2014		
Wed	15/01/2014	Wet-Cage	
Thur	16/01/2014	Pair-Up, Food deprivation	Food deprivation
Fri	17/01/2014	Wet-Cage	
Sat	18/01/2014		
Sun	19/01/2014		
Mon	20/01/2014	Forced Swim test	
Tues	21/01/2014	Overnight Illumination	
Wed	22/01/2014		
Thur	23/01/2014	Pair-Up	Habit - Magazine train 1
Fri	24/01/2014		Magazine train 2
Sat	25/01/2014		
Sun	26/01/2014		
Mon	27/01/2014		Lever Press pretrain 1
Tue	28/01/2014	Cage Swap	Lever Press pretrain 2
Wed	29/01/2014	Overnight illumination	Lever Press Train 1
Thur	30/01/2014	Forced Swim test	Lever Press Train 2
Fri	31/01/2014		Lever Press Train 3
Sat	01/02/2014		LiCl Devaluation
Sun	02/02/2014		LiCl Devaluation
Mon	03/02/2014	Overnight illumination	Extinction and consumption test
Tue	04/02/2014		Reinforced test

Wed	05/02/2014	Pair Up	
Thur	06/02/2014		
Fri	07/02/2014	Forced Swim test	
Mon	10/02/2014	Cage Swap	DOE - Magazine training for reward one
Tue	11/02/2014		Magaine training for reward two
Wed	12/02/2014	Wet Cage	Lever Press Training - Left Lever
Thur	13/02/2014	Wet Cage	Lever Press Training - Right Lever
Fri	14/02/2014		Additional training on lever if necessary
Sat	15/02/2014		
Sun	16/02/2014		
Mon	17/02/2014		R130
Tues	18/02/2014		R130
Wed	19/02/2014	Cage swap	Discrimination Training1
Thur	20/02/2014	Pair Up	Discrimination Training2
Fri	21/02/2014	Wet Cage	Discrimination Training3
Sat	22/02/2014		DiscriminationTraining4
Sun	23/02/2014		DiscriminationTraining5
Mon	24/02/2014		DiscriminationTraining6
Tue	25/02/2014	Moved cages to different room	DiscriminationTraining7
Wed	26/02/2014	Moved cages back	DiscriminationTraining8
Thur	27/02/2014	Forced Swim test	DiscriminationTraining9
Fri	28/02/2014	Overnight illumination	DiscriminationTraining10
Sat	01/03/2014		Extinction
Sun	02/03/2014	Pair Up	Reacquisition
Mon	03/03/2014	Overnight illumination	Reinforcer Only Test
Tue	04/03/2014	Cage Swap	
Wed	05/03/2014		
Thur	06/03/2014	Overnight Illumination	
Fri	07/03/2014		
Sat	08/03/2014		
Sun	09/03/2014		

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