Behavioural Analysis of Cognitive, Motivational, and Hedonic Aspects of Reward Processing

Lucy Rose Lewis

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In loving memory of Heather Lewis, Sylvia Hunter, and Ewart Lewis.

Summary

Processing of rewards involves three major mechanisms of cognition, motivation, and hedonic response, and reward-related deficits seen in psychiatric disorders encompass disruptions in these aspects of reward processing. Heterogeneity and poor treatment efficacy in psychiatric patients highlight the need to elucidate the neurobiological mechanisms underpinning these deficits as isolated mechanisms, rather than as a single entity. To understand the neurobiology of individual aspects of reward processing, preclinical rodent assays need to be capable of dissociating these. Thus, this thesis evaluated three assays aiming to measure cognitive, hedonic, or motivational deficits separately. Here, I also investigated the neurobiological mechanisms underpinning these aspects through the application of these assays to preclinical models of psychiatric disease.

The flavour modified affective bias test (m-ABT) was evaluated as an automated version of an established assay for cognitive bias deficits, but despite showing initial promise in being capable of measuring positive biases induced by reward value (Chapter 2), this assay did not reliably measure deficits in preclinical models of psychiatric disease (Chapter 3). However, the use of this assay in the transgenic *CACNA1C* heterozygous knockout rat model highlighted some possible cognitive bias deficits that warrant further investigation in alternative assays. In contrast, lick cluster analysis (LCA) was used to measure hedonic response to rewards (Chapter 4) and was able to demonstrate a lack of hedonic deficit in the chronic IFN-α model, as well as the rat chronic corticosterone (CORT) model which showed a deficit in other literature using alternative, less-specific methods, indicating this LCA assay could be used to dissociate hedonic response from other aspects of reward processing more reliably.

Furthermore, the effort-related choice paradigm (EfR) was applied to acute and chronic IFN-α and CORT models, as well as the transgenic *CACNA1C* heterozygous knockout rat model (Chapter 5). Acute treatment with IFN-α and CORT did not impair motivation for reward, whereas chronic treatment indicated a potential deficit that was not clearly demonstrated possibly due to experimental errors. The *CACNA1C* model showed no deficit in motivation for reward, but potential influence on habitual responding or impulse control, thus indicating the EfR assay could be used to dissociate motivational deficits from other aspects of reward processing as well as influences from other mechanisms.

Neurobiological characterisation of the chronic CORT and IFN-α models (Chapter 6) found no clear impact of CORT on neurogenesis or glutamatergic neurotransmission, but IFN-α resulted in elevated neurogenic markers and NR2B expression in the hippocampus.

Publications and Conference Presentations

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Contents

List of Abbreviations

ABT	Affective bias test
ACTH	Adrenocorticotropic hormone
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
CACNA1C	Calcium channel, voltage-dependent, L-type, alpha 1C subunit
CORT	Corticosterone
CRF	Corticotropin releasing factor
CS	Conditioned stimulus/stimuli
CSF	Cerebrospinal fluid
DCX	Doublecortin
DG	Dentate gyrus
EEfRT	Effort Expenditure for Rewards Task
EfR	Effort-related choice paradigm
ELISA	Enzyme-linked immunosorbent assay
GrDG	Granular cell layer of the dentate gyrus
HPA	Hypothalamic-pituitary-adrenal
i.p.	intraperitoneal
IFN-α	Interferon-alpha
IL-1	Interleukin-1
IL-6	Interleukin-6

ILI	Inter-lick interval
JBT	Judgement Bias Task
LCA	Lick cluster analysis
LCS	Lick cluster size
m-ABT	Modified affective bias test
MDD	Major Depressive Disorder
NMDA	*N*-methyl-D-aspartate
PFC / m-PFC	Prefrontal cortex / medial prefrontal cortex
PR	Progressive ratio
PRL	Response-bias probabilistic reward task
PRT	Probabilistic reversal learning task
SEM	Standard error of the mean
SPT	Sucrose preference test
STT	Sweet Taste Test
TNF-α	Tumour necrosis factor-alpha
Vol/lick	Volume per 1000 licks
WT	Wild type

Chapter 1 – General Introduction

1.1. Theories and mechanisms of reward processing

Natural rewards are stimuli or events essential to the survival of an organism (e.g. food, water, social interactions) and activate the brains' reward system (Kelley & Berridge, 2002; Rømer Thomsen, Whybrow, & Kringelbach, 2015). Early definitions from animal learning theories, such as Thorndike (1911) and Skinner (1938), collectively define rewards as stimuli with a positive valence capable of strengthening a stimulus-response (S-R) relationship, where the reward is contingent upon the response. Thus, partaking in vital behaviours such as food seeking, and consumption are rewarding to the organism to ensure these behaviours are continually reproduced.

More recently, 'reward' has been defined as a complex process dependent upon at least three psychological mechanisms. Berridge and Robinson (1998) describe these as activation of hedonic responses, associative learning between such responses and a stimulus, and the subsequent incentive salience attributed to these stimuli. Thus, rewards induce 'positive emotions', which stimulate learning about the reward and its environment, as well as encouraging approach and consummatory behaviours (Schultz, 2005).

This thesis will focus on the three main mechanisms of reward processing, hedonic responses ('liking'), motivation ('wanting') and learning (including decision-making capability) (Berridge & Robinson, 2003; Lewis, Benn, Dwyer, & Robinson, 2019).

1.1.1. Hedonic responses

Most commonly, rewards are associated with a conscious feeling of pleasure and enjoyment. The word 'hedonic' derives from the ancient Greek term for pleasure (Berridge & Kringelbach, 2015) and today hedonic refers to the sensation of pleasure experienced from a rewarding stimulus. Hedonic responses to rewards are an integral part of reward processing, and can be either objective (i.e. an unconscious physical reaction to a reward) or subjective (i.e. the conscious feeling of pleasure), or most often both (Berridge & Kringelbach, 2008a).

'Anhedonia' was first defined as "the inability to experience pleasure" by French psychologist Ribot (1896). This definition has been challenged more recently due to a lack of consistent evidence for traditional views of 'consummatory anhedonia', the lack of pleasure from engaging in a rewarding activity, in psychiatric patients (Strauss, Wilbur, Warren, August, & Gold, 2011). Anticipatory anhedonia, on the other hand, refers to a reduction in the anticipation of future pleasure (Winer, Jordan, & Collins, 2019). There is evidence for multiple mechanisms behind reward processing deficits rather than simply a

lack of pleasure (Berridge, Robinson, & Aldridge, 2009), thus leading to a re-conceptualization of the term 'anhedonia' to refer to an "impaired ability to pursue, experience and/or learn about pleasure" (Rømer Thomsen et al., 2015, p. 2).

Assessments of anhedonia in patients are often measured using self-report questionnaires (see Leventhal, Chasson, Tapia, Miller, & Pettit, 2006), which are restricted to the subjective component of experiencing pleasure and often do not dissociate the multiple components of reward processing recently recognised within the wider context of 'anhedonia'.

1.1.2. Motivation

Processing rewards is initially dominated by the motivation for reward due to innate appetitive behaviour, followed by consummatory behaviour and learning (Kringelbach & Berridge, 2016). This integrates a biological need with learning and memory of reward-related stimuli in order to drive goal-directed actions required for reward acquisition (Berridge, 2012; Lewis et al., 2019). Berridge et al. (2009) describes reward-related motivation as comparable to incentive salience, in which a motivational component is attributed to reward that drives acquisition behaviour, or 'wanting'.

Motivational deficits can present as symptoms seen across many psychopathologies, such as avolition, a reduced motivation to engage in goal-directed activities (Blanchard & Cohen, 2006). Deficits in motivation can also be linked to 'anticipatory anhedonia', whereby the motivation to engage in potentially rewarding events is attenuated due to a lack of reward anticipation. Alternatively, reduced motivation could lead to reduced anticipation of future rewards (Der-Avakian, Barnes, Markou, & Pizzagalli, 2015; Sherdell, Waugh, & Gotlib, 2012).

However, for several decades motivation and hedonic response have been confounded when assessing clinical populations using typical self-report measures that do not adequately separate the motivational 'wanting' of reward from unconscious 'liking' (Olney, Warlow, Naffziger, & Berridge, 2018). Although, it should be noted that more recently developed questionnaires include aspects of 'wanting' with the aim of differentiating anticipatory from consummatory anhedonia (Rømer Thomsen, 2015). Anticipation for future rewards may also rely on cognitive capabilities for prediction and expectations (Winer et al., 2019), thus accurately dissociating these reward processing mechanisms is challenging.

1.1.3. Cognition

The third aspect of reward processing relates to cognitive processing of rewards, such as learning, memory, and decision-making. Two main types of learning include associative and non-associative learning, such as habituation and sensitization. Reward-related learning

mostly involves associative learning, including Pavlovian associations formed following repeated pairings between a neutral stimulus and an unconditioned stimulus (US, i.e. a reward; e.g. Pavlov, 2003). Once associations are formed, the neutral stimulus (now referred to as the conditioned stimulus, CS) can trigger expectations of reward and thus activate motivational processes to continue producing reward acquisition behaviours (Berridge et al., 2009). In contrast, instrumental associations can form between a stimulus and a response required in order for the US to be delivered (Thorndike, 1898), or between the response and the outcome of US delivery (Tolman, 1949). The term "goal-directed actions" refer to the responses produced following learning this latter response-outcome association, either following presentation of a Pavlovian CS or simply in a primary motivational state (see Dickinson & Balleine, 1994).

Impairments in various types of cognitive processes including associative learning can be categorised by the type of information/stimuli processed, e.g., emotion-related information ("hot") such as facial expressions, or information without emotional influence ("cold") such as verbal learning or working memory (e.g. Grafman & Litvan, 1999; Roiser & Sahakian, 2013). Current theories suggest some "cold" processing deficits stem from feedback in cognitive tasks inducing negative emotions (Roiser & Sahakian, 2013), whilst "hot" processing can be directly applied to cognitive processing of reward-related stimuli, given the emotional value of rewards (Robinson & Roiser, 2016). These deficits can be seen in several psychiatric disorders, such as major depressive disorder (MDD) or psychosis (e.g. MacKenzie et al., 2017; Roiser et al., 2009; Roiser & Sahakian, 2013).

1.1.4. Interactions between reward-related processing mechanisms

Psychological models of learning suggest the value of reward is one determinant for the degree and strength of associative learning (Rescorla & Wagner, 1972). If hedonic responses are reduced then the value of a reward may also be reduced, hence, changes in hedonic responses could alter the strength of associations formed between the CS and US. Similarly, instrumental cues for rewards triggering motivation processes are shown to modulate cognitive processes such as attention in healthy mice, but not transgenic schizophrenia models (Ward et al., 2015), whilst learning stimulus-reward associations drives motivated movements such as lever pressing and approach (Madan, 2013). Thus, there are clear interactions between hedonic responses, motivation, and reward learning, demonstrating reward processing is multifaceted.

Despite this clear inter-related nature of these aspects, deficits in reward processing mechanisms may not be seen all at the same time, nor all within the same individual. This issue of heterogeneity across psychiatric populations has consistently been identified,

whereby one individual patient may not share a single symptom with another patient given the same diagnosis (Monroe & Anderson, 2015). Furthermore, differences in the neurobiology of these components in reward processing have become increasingly recognised (Berridge et al., 2009), e.g., some pharmacological interventions are shown to influence either 'wanting' or 'liking' separately, or in opposite directions (Olney et al., 2018).

Therefore, it is suggested that the neurobiological mechanisms underlying hedonic responses, motivation and cognitive processing can be dissociated from one another, and reward-related deficits are unlikely to be reduced to a single cause or set of causes. Despite this, common methods for measuring reward-related deficits in both humans and animal models (see Table 1.1) do not focus on dissociating these mechanisms, so there is a lack of understanding of the neurobiological underpinnings from each individual symptom and subsequently pharmacological interventions are not personalised to match patients' symptoms. Thus, each processing mechanism should be investigated individually to progress our understanding of reward processing, how deficits may arise, and future treatment targets (Lewis et al., 2019).

This thesis will address this need to elucidate the neurobiological underpinnings of individual reward-related deficits through the development and application of sensitive behavioural assays, which will evaluate changes in affective bias - one type of cognitive deficit - in Chapters 2 and 3; hedonic deficits in Chapter 4; and motivation for reward in Chapter 5, as separate aspects of reward processing.

Table 1.1. Summary of current methods to assay reward-related deficits in humans and rodent models

Reward-related deficit	Human assay	Rodent assay
Consummatory anhedonia	Self-report questionnaires, e.g., Fawcett-Clark Pleasure Scale (FCPS), Snaith-Hamilton Pleasure Scale (SHAPS), Revised Chapman Physical Anhedonia Scale (CPAS)	
	'Sweet Taste Test' (STT) (Kampov-Polevoy, Garbutt, & Janowsky, 1997)	Sucrose Preference Test (Willner, Towell, Sampson, Sophokleous, & Muscat, 1987)
	Orofacial reactivity (Berridge & Kringelbach, 2008a)	Orofacial reactivity (Grill & Norgren, 1978) Lick Cluster Analysis (Davis, 1973)
Reward learning / Cognitive biases	Response Bias Probabilistic Reward Task (PRT) (Pizzagalli, Jahn, & O'Shea, 2005)	PRT (Der-Avakian, D'Souza, Pizzagalli, & Markou, 2013)
	Probabilistic stimulus selection task (PSST) (Pechtel & Pizzagalli, 2013)	
	Back-translated Judgement Bias Task (Aylward, Hales, Robinson, & Robinson, 2017)	Judgement Bias Task (JBT) (Harding, Paul, & Mendl, 2004; Jones et al., 2018)
	Go/No-Go Task (Donders, 1969)	
	Probabilistic Reversal Learning (PRL) (Cools, Clark, Owen, & Robbins, 2002)	PRL (Bari et al., 2010)
		Affective bias test (ABT) (Stuart, Butler, Munafo, Nutt, & Robinson, 2013)
Motivation (including anticipatory anhedonia)	Self-report questionnaires for anticipatory anhedonia, e.g., Temporal Experience of Pleasure Scale (TEPS), Anticipatory and Consummatory Interpersonal Pleasure Scale (ACIPS)	
	Self-report questionnaires of motivation, e.g., Behavioural Activation System (BAS), Motivation and Pleasure Scale-Self Report (MAP-SR)	
	Effort Expenditure for Rewards Task (EEfRT) (Treadway, Buckholtz, Schwartzman, Lambert, & Zald, 2009)	Effort-related choice paradigm (Salamone et al., 1991)
	Progressive Ratio (McLeod & Griffiths, 1983)	Progressive Ratio (Randall et al., 2012)
		Intracranial self-stimulation (ICSS) (Olds & Milner, 1954)

1.2. Psychiatric disorders

Reward-related deficits have been observed in a number of psychiatric disorders, including affective disorders (e.g. Nusslock & Alloy, 2017; Whitton, Treadway, & Pizzagalli, 2015), psychosis (e.g. Barch, Pagliaccio, & Luking, 2016; Dowd & Barch, 2010), anxiety (e.g. Carlton, Sullivan-Toole, Ghane, & Richey, 2020; Morris, Bylsma, Yaroslavsky, Kovacs, & Rottenberg, 2015), and neurodegenerative conditions (e.g. Muhammed et al., 2016; Perry & Kramer, 2015).

Since these deficits are reported predominantly in patients with depression or schizophrenia, and one or more reward-related deficits are classed as 'core symptoms' of these disorders (Der-Avakian et al., 2015), this thesis will focus on the literature and empirical investigation of preclinical models for these two psychiatric conditions. However, it is important to note the relevance of understanding the underlying neurobiology of reward-related deficits for the wide range of other conditions presenting related symptoms.

1.2.1. Overview of Major Depressive Disorder

Clinical depression, or MDD, is a serious mood disorder and one of the leading causes of disability worldwide (WHO, 2018). Diagnosis is based on the presence of one of two core symptoms, a persistent low mood and anhedonia. Patients must also present several additional symptoms including fatigue, bradyphrenia, sleep disturbances, learning and memory deficits and a diminished ability to think or concentrate (*Diagnostic and Statistical Manual of Mental Disorders (DSM-5)*, 2013).

The global prevalence of MDD is higher amongst women, a difference tending to emerge during adolescence where hormonal differences begin to elevate (e.g. Salk, Petersen, Abramson, & Hyde, 2016). Further, cases of female-specific disorders of clinical depression involving hormonal fluctuations, such as postpartum or postmenopausal depression (Albert, 2015), suggests some biological sex differences may underlie this differential risk for developing depression. However, stressful life events involving social or economic disruption pose as additional risk factors for the development of MDD (e.g. Shapero et al., 2014), and these factors have also been associated with the gender gap in depression symptoms (e.g. Van de Velde, Bracke, & Levecque, 2010).

Aetiology of depression

The aetiology of MDD is poorly understood, and several hypotheses have been proposed to explain its pathophysiology (see Figure 1.1). The monoamine hypothesis was first introduced in the 1960's (Schildkraut, 1965) following evidence that an adrenergic antagonist used to treat hypertension subsequently induced symptoms of depression (e.g.

Freis, 1954), which suggests clinical depression is due to a deficiency in monoamine neurotransmitters (Delgado, 2000). Evidence for this comes from reduced serotonin (e.g. Mann, McBride, & Stanley, 1986; Owens & Nemeroff, 1994), and dopamine (e.g. Bonci & Hopf, 2005; Pizzagalli, 2014) found in patients with MDD, whilst typical pharmacological antidepressants increase monoaminergic neurotransmission (e.g. Hirschfeld, 2000).

However, despite increased monoamine release detected immediately, the therapeutic effects of monoaminergic antidepressants can take several weeks to become effective (Frazer & Benmansour, 2002), and one third of patients still remain unresponsive following several courses of antidepressant therapy (Trivedi et al., 2006). Thus, limitations in the monoamine hypothesis highlight the need for development of other possible theories for depression pathophysiology.

The therapeutic effects of antidepressants can take 2-4 weeks to take effect (Boku, Nakagawa, Toda, & Hishimoto, 2018), whilst chronic antidepressant therapy for this same time period has shown to increase the generation of new neurons, known as neurogenesis (e.g. Hanson, Owens, & Nemeroff, 2011). This potentially implicates downstream changes in neuroplasticity resulting from increases in monoaminergic neurotransmission may underlie the efficacy of typical antidepressants (Manji et al., 2003). Thus, the neurotrophic hypothesis postulates that reduced neurotrophic factors can lead to the development of MDD through neuronal atrophy and impaired neurogenesis (Duman & Li, 2012), as well as reducing antidepressant efficacy (Duman, Heninger, & Nestler, 1997). This has been supported through evidence of reduced synaptic plasticity (e.g. Bremner et al., 2000; MacQueen, Yucel, Taylor, Macdonald, & Joffe, 2008) and neurotrophic factors, such as Brain-Derived Neurotrophic Factor (BDNF), in MDD patients (e.g. Huang, Lee, & Liu, 2008; Jiang & Salton, 2013). However, transgenic BDNF knockout models failed to show a depressive-like phenotype, though BDNF was shown to be essential for antidepressant action (e.g. Monteggia et al., 2004), highlighting potential flaws in this hypothesis. In addition, the presence of neurogenesis in humans is still an ongoing debate where it is argued that adults do not retain this ability to generate new neurons (e.g. Sorrells et al., 2018), yet other evidence indicates this is possibly due to limited ability to measure neurogenesis in humans (e.g. Flor-García et al., 2020) and the use of novel methods have indeed identified immature neurons in the brains of healthy human adults (e.g. Moreno-Jiménez et al., 2019).

Finally, another hypothesis suggests that a dysregulation of glutamatergic neurotransmission can lead to structural and functional changes in neuronal networks implicated in the neurocircuitry of MDD (Sanacora, Treccani, & Popoli, 2012). Supporting this, elevated glutamate levels have been shown in depressed patients (e.g. Altamura et

al., 1993; Kim, Schmid-Burgk, Claus, & Kornhuber, 1982; Mitani et al., 2006) whilst some antidepressant therapies including ketamine, an *N*-methyl-ᴅ-aspartate (NMDA) receptor antagonist, reduce glutamate (e.g. Musazzi, Treccani, Mallei, & Popoli, 2013).

However, current evidence is still unclear on the exact neurobiological factors and mechanisms contributing to the pathophysiology of MDD and treatment efficacy.

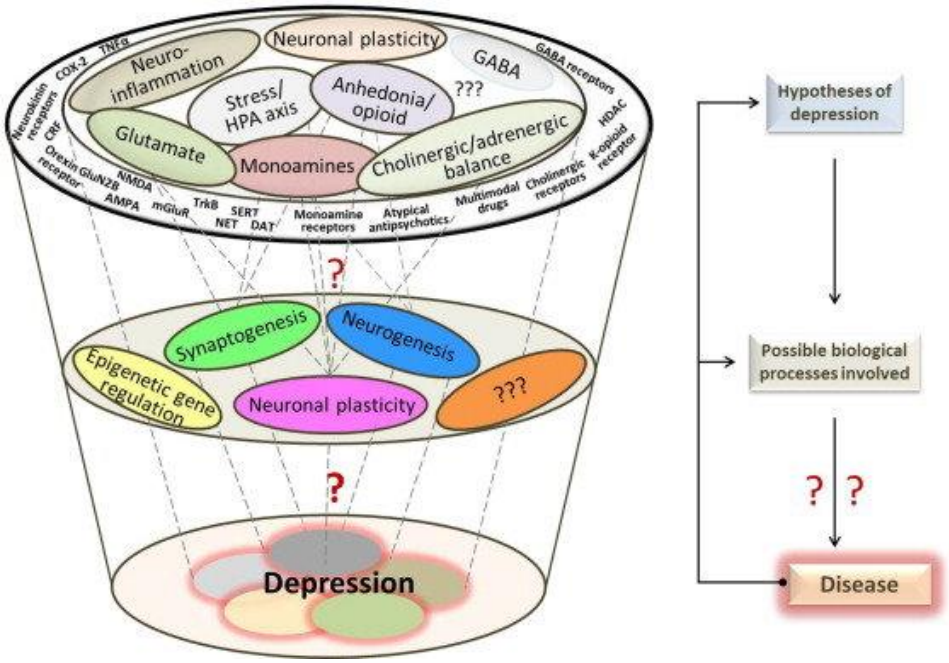


Figure 1.1. A schematic overview of proposed hypotheses for the pathophysiology of depression and the biological processes implicated in these. Taken from Dale, Bang-Andersen, and Sánchez (2015).

1.2.2. Overview of Schizophrenia

Schizophrenia is a chronic, debilitating neuropsychiatric condition characterised by three broad categories of symptoms (Arango & Carpenter, 2011): 1) Positive, the addition of experiences or thoughts that should not be present (e.g. hallucinations, delusions, detachment from reality); 2) Cognitive (e.g. disruption to normal cognitive processes such as memory, attention, decision-making); and 3) Negative, a lack of characteristics that should be present (e.g. emotional blunting, avolition, anhedonia).

The lifetime prevalence of schizophrenia in the general population is approximately 1% (Mueser & McGurk, 2004), with males at increased risk compared to females (McGrath, Saha, Chant, & Welham, 2008). Patients with schizophrenia show increased rates of

mortality, associated with poor lifestyle habits such as smoking, or increased risk of suicide (e.g. Brown, 1997; Oakley et al., 2018).

Aetiology of Schizophrenia

Like MDD, and many other psychiatric disorders, the exact aetiology and pathophysiological mechanisms behind schizophrenia are unknown (see Figure 1.2). One of the original hypotheses' dates to the discovery that neuroleptic drugs attenuating dopaminergic neurotransmission can reduce psychotic symptoms, whereas psychostimulants activating dopaminergic neurotransmission can induce a psychotic state (see Baumeister & Francis, 2002; Howes, McCutcheon, & Stone, 2015). Thus, the dopamine hypothesis suggests that the positive symptoms of schizophrenia may be caused by hyperactive dopaminergic neurotransmission.

However, patients also experience negative and cognitive symptoms, yet evidence indicates attenuated dopamine in depressed patients. Thus, a 'revised' dopamine hypothesis suggests hyperactivity of dopaminergic neurotransmission occurs mainly in mesolimbic areas, e.g. the striatum, and this localised dysregulation of dopamine may be responsible for the positive symptoms of schizophrenia (Howes & Kapur, 2009). Alternatively, hypoactive dopamine neurotransmission within the prefrontal cortex (PFC), a region linked to cognitive function, has been identified in patients (e.g. Walter, Kammerer, Frasch, Spitzer, & Abler, 2009). This, along with evidence for dopamine involvement in cognitive processes implicates dopaminergic hypoactivity in the PFC in the development of cognitive deficits in schizophrenia, although direct evidence here is somewhat limited (Brisch et al., 2014; Howes et al., 2015).

However, not all patients are responsive to dopaminergic antipsychotics (Howes & Kapur, 2014; Howes et al., 2015). The NMDA receptor antagonists, phencyclidine (PCP) and ketamine, have psychotomimetic properties (Moghaddam & Javitt, 2012), whilst NMDA receptor agonists have shown improvements in negative symptoms of schizophrenia (see Coyle, 2012). This, along with evidence of aberrant glutamate levels (e.g. Bustillo et al., 2014; Kim, Kornhuber, Schmid-Burgk, & Holzmüller, 1980) and glutamatergic receptor expression (e.g. Meador-Woodruff & Healy, 2000) in schizophrenia patients, led to the formation of the theory that NMDA receptor hypofunction leading to disrupted glutamatergic neurotransmission is involved in the development of schizophrenia.

However, this hypothesis was reconceptualised to implicate that NMDA receptor hypofunction specifically on γ-aminobutyric acid (GABA)-ergic interneurons reduces their activity, in turn disinhibiting glutamatergic pyramidal neurons and enhancing glutamatergic neurotransmission (see Heckers & Konradi, 2015). In addition, animal models of NMDA

receptor hypofunction have shown variations in dopaminergic neurotransmission (e.g. Kokkinou, Ashok, & Howes, 2018; Nakao et al., 2019). Thus, an integrated model of the dopamine and glutamatergic hypotheses in schizophrenia implicates that hypofunctional NMDA receptors on GABAergic interneurons cause disinhibition of pyramidal neurons projecting to midbrain dopaminergic neurons causing their activation (Howes et al., 2015; Lisman et al., 2008).

Finally, the neurodevelopmental hypothesis suggests environmental challenges in early life, combined with genetic predisposition and additional later life risks, alters neuronal development to increase risk of developing schizophrenia in later life (McGrath, Féron, Burne, Mackay-Sim, & Eyles, 2003), supported by evidence of neuroanatomical differences (e.g. Nour & Howes, 2015) and reduced synaptic plasticity (e.g. Fernandes et al., 2015).

These three hypotheses implicate a neurodevelopmental deficit contributing to grey matter loss and dysfunctional synaptic plasticity, further sensitizing dopaminergic activity (Howes & Murray, 2014). However, how these changes come together is not fully understood, nor how these contribute to the variety of symptoms experienced by schizophrenic patients.

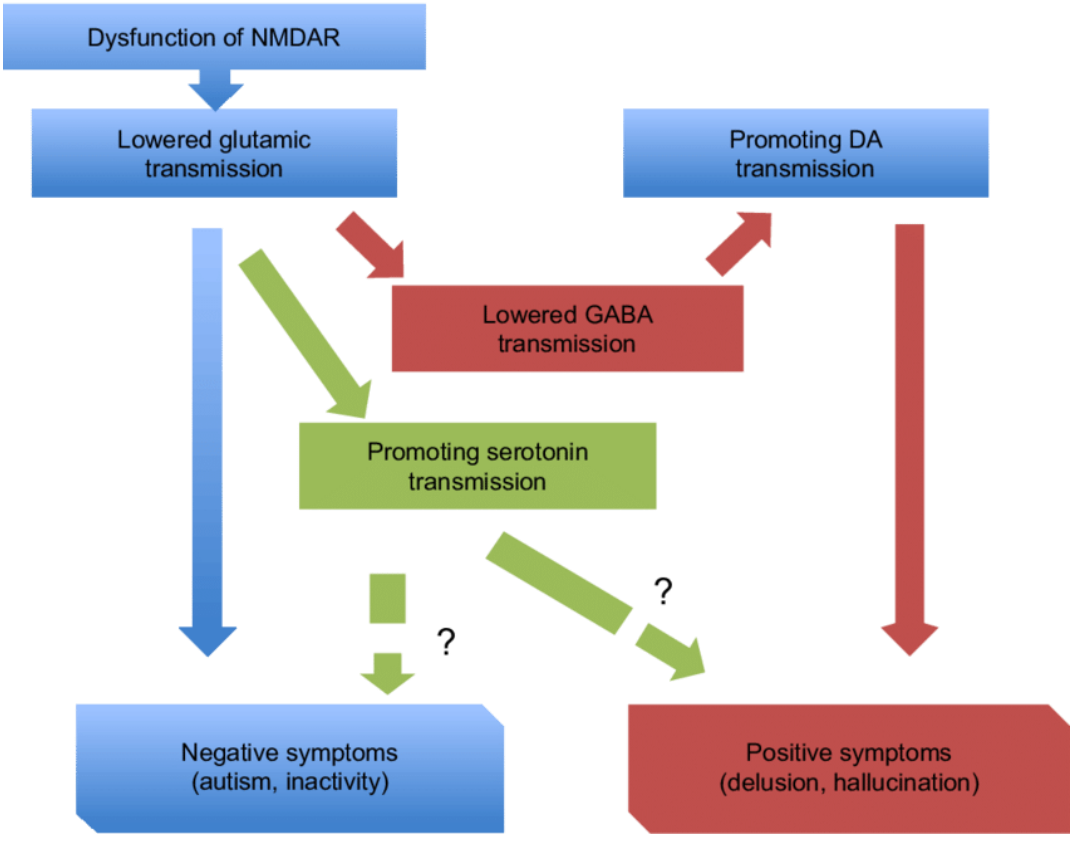


Figure 1.2. A schematic overview of the glutamate and dopamine hypotheses for the pathophysiology of schizophrenia and the deficits related to neurobiological dysfunction. Taken from Tsutsui et al. (2017).

1.2.3. Psychiatric diagnostic criteria

Whilst classical diagnosis of psychiatric disorders tends to rely on criteria outlined by the Diagnostic and Statistical Manuals (e.g. *Diagnostic and Statistical Manual of Mental Disorders (DSM-5)*, 2013) developed over a number of decades, a more recent framework was developed to aide this diagnosis by incorporating biological underpinnings with behaviours, and takes into account the heterogeneity and comorbidity across psychiatric patients.

The Research Domain Criteria (RDoC, Insel et al., 2010) was developed by the National Institute of Mental Health and focuses on understanding the psychological and physiological basis of separate 'domains' for mental health, to aid development of targeted treatments (see NIMH). One major domain within RDoC covers 'Positive Valence Systems', which include behavioural responses to positive stimuli, i.e. rewards. This domain therefore encompasses motivational actions, consummatory behaviours, and reward learning. However, within this domain these different aspects of the positive valence systems are indeed distinguished from one another into constructs that can translate to the reward processing mechanisms discussed in this thesis. From the research gathered to date, specific molecules, neural circuits, and behaviours can be categorised as relevant to these distinct constructs, forming more comprehensive links between brain, physiology, and the deficits seen in psychiatric disease. However, the ability to form these connections again relies upon the ability to reliably measure individual constructs in humans and animal models.

1.2.4. Anhedonia in psychiatric disorders

Anhedonia is a core feature of both MDD and schizophrenia (Whitton et al., 2015), reported in approximately one third of depressed patients (e.g. Pelizza & Ferrari, 2009). Self-report questionnaires (see Table 1.1) have measured impaired consummatory and anticipatory anhedonia in depressed patients (e.g. Rizvi et al., 2015; Wu et al., 2017), with reduced pleasure from both physical (i.e. eating) and social activities in schizophrenia patients compared to healthy controls (e.g. Blanchard, Bellack, & Mueser, 1994; Blanchard, Mueser, & Bellack, 1998). Reduced hedonic capacity has also been observed as a predictor of both depression severity and poor treatment outcome (see Craske, Meuret, Ritz, Treanor, & Dour, 2016; e.g. Vrieze et al., 2014), as well as greater risk of suicide (e.g. Fawcett et al., 1990). However, some measures have indicated that anticipatory anhedonia is present in schizophrenia but consummatory is not (e.g. Gard, Kring, Gard, Horan, & Green, 2007).

Limitations in self-report measures have been discussed previously, thus, objective methods to measure the 'unconscious' component of consummatory anhedonia have been

developed, which have given variable findings in humans. The 'sweet taste test' (STT) where participants are given varying concentrations of sweet solutions and rate their pleasantness/liking on a self-report scale have shown responses appeared unaltered in both MDD (Dichter, Smoski, Kampov-Polevoy, Gallop, & Garbutt, 2010) and schizophrenia patients (Berlin, Givry-Steiner, Lecrubier, & Puech, 1998). Schizophrenic patients who self-report anhedonia also do not appear to differ from healthy controls in their self-reported emotional responses to pleasant foods (e.g. Horan, Green, Kring, & Nuechterlein, 2006) or simulated social interactions (e.g. Aghevli, Blanchard, & Horan, 2003). However, it is arguable that these tasks still rely heavily on subjective self-report of pleasure so do not reliably assay consummatory anhedonia.

Alternatively, the assessment of natural orofacial reactions to the taste of rewarding or unpleasant solutions has been developed as a more selective and objective assay for consummatory hedonic response (Berridge & Kringelbach, 2008b; Dwyer, 2012). Although this method has shown distinguishing orofacial reactions in new-born infants, there is little evidence showing altered reactivity in MDD patients (e.g. Scinska et al., 2004), possibly due to learning to control and mimic orofacial reactions over time (Rømer Thomsen, 2015), and no literature available in schizophrenic patients.

The current lack of reliable evidence for a consummatory hedonic deficit in psychiatric patients could reflect difficulties in objective measurement of consummatory hedonic responses in humans, where patterns of eating/drinking are highly complex. Many studies use monetary rewards that are 'secondary', i.e. the reward value of these are learned and not inherent, rather than 'primary' rewards such as food/water that are natural and intrinsically rewarding, which may limit the unconscious 'liking' of secondary rewards (Rizvi, Pizzagalli, Sproule, & Kennedy, 2016). The heterogeneity of patients' symptoms could also cause variation in these findings, highlighting a need for more sensitive methods that can reliably isolate consummatory anhedonia from other reward-related deficits.

However, there remains the possibility that patients could retain this unconscious 'liking' response to reward, with deficits in the conscious reporting of hedonic response resulting from altered cognitive or motivational processes (Rømer Thomsen, 2015). Discrepancies in the measurement of anhedonia in schizophrenia patients led to the 'anhedonia paradox', where patients report reduced experiences of pleasure but behaviours measured in the laboratory are not representative of this (Pizzagalli, 2010). Instead, it is argued that self-reported anhedonia traits are reflective instead of reduced anticipation and estimation of past or future pleasure, i.e., more representative of anticipatory anhedonia than consummatory (see Strauss, 2013; Strauss & Gold, 2012).

1.2.5. Motivational deficits in psychiatric disorders

'Wanting' and anticipatory anhedonia have been linked to symptoms of apathy, the lack of interest or enthusiasm in activities, seen across both psychiatric and neurological conditions (Jordan, Zahodne, Okun, & Bowers, 2013; Simon et al., 2010). Anticipatory anhedonia is well reported in depressed populations according to self-reported questionnaires (e.g. Chentsova-Dutton & Hanley, 2010; Wu et al., 2017), while evidence in schizophrenia has again been mixed (see Barch et al., 2016).

As the dissociation between reward mechanisms becomes increasingly recognised, methods to objectively measure reward motivation deficits have been developed (see Der-Avakian et al., 2015; Groeneweg-Koolhoven et al., 2017). The 'Effort Expenditure for Rewards Task' (EEfRT) is a computer game-based method in which subjects choose between participating in a low difficulty task for a small monetary reward or a more difficult task for greater reward value, thus exerting more 'effort' to gain the greater reward (Treadway et al., 2009).

Reduced effort expenditure, i.e. less participation in the more difficult task, has been observed across both healthy subjects with high anhedonia ratings (e.g. Geaney, Treadway, & Smillie, 2015; Treadway et al., 2009), and patients with MDD (e.g. Treadway, Bossaller, Shelton, & Zald, 2012). Those with schizophrenia do not appear to show an overall reduction in effort expenditure in the EEfRT, but instead are less likely to select a higher effort option when it would be most advantageous to do so (e.g. Treadway, Peterman, Zald, & Park, 2015; Whitton et al., 2015).

Further, elevated anticipatory anhedonia has been shown to predict impairments in the EEfRT (e.g. Bryant, Winer, Salem, & Nadorff, 2017). Although, Sherdell et al. (2012) found no overall difference between control and MDD patients in effort expenditure yet still demonstrated this prediction of reduced motivation for reward in patients with greater anticipatory anhedonia scores. Similar measures using increasing button presses to gain monetary reward show an attenuated increase in effort expenditure when either reward value or probability of reward increases, a deficit that has been associated with greater presentation of negative symptoms (see Barch et al., 2016).

In addition, when rewarding stimuli are devalued, such as pairing with feelings of disgust, it has been shown that patients with schizophrenia do not reduce responding to gain the devalued reward, whereas healthy controls will avoid responding for this reward (e.g. Morris, Quail, Griffiths, Green, & Balleine, 2015). Thus, schizophrenia appears to disrupt the integration of experienced value of reward and production of goal-directed actions.

These findings suggest schizophrenia patients show a deficit in their general motivation for gaining a reward, even if it has a high value, which may differ from the deficit seen in MDD patients. However, these findings are not always consistent and mostly rely again on the processing of secondary rewards that, as mentioned previously, are not intrinsically rewarding and thus may not provide accurate measures of processing mechanisms.

1.2.6. Cognitive biases in psychiatric disorders

According to early theories of cognitive dysfunction in MDD, negative stimuli/events have greater salience in patients compared to healthy individuals, which Beck (1967) attributed to negative self-schema caused by past experiences, leading to biases in how patients process their environment. These biases can affect cognitive domains, such as learning, memory, and decision-making, and therefore are referred to as “cognitive biases”. Cognitive biases can be induced by changes in emotional/affective states - known as “affective biases”. Rewards also induce cognitive biases, known as “reward-induced biases”, which can also be influenced by affective state.

Depressed patients demonstrate enhanced recall of negative stimuli compared to positive (e.g. Matt, Vazquez, & Campbell, 1992), and assign more negative connotations to ambiguous stimuli, whereas healthy individuals are more likely to create positive associations (e.g. Everaert, Podina, & Koster, 2017; Mathews & Macleod, 2005). These processing biases induce negative expectations of future events, altering other cognitive domains such as decision-making and judgements (Beck, 2008; Blanchette & Richards, 2010).

Despite cognitive deficits being a common feature of schizophrenia, including general learning and memory, problem solving, and attention (Sheffield, Karcher, & Barch, 2018), cognitive biases are less commonly reported. Unlike MDD patients who show a bias in responding to positive/neutral facial expressions as negative (e.g. Bourke, Douglas, & Porter, 2010), patients with schizophrenia show a general deficit in the ability to recognise emotions (e.g. Hagiya et al., 2015; Kohler, Bilker, Hagendoorn, Gur, & Gur, 2000). Impairments in social cognition are often reported in schizophrenia, presenting as difficulties in identifying emotions, or inferring thoughts/emotions from others (see Green, Horan, & Lee, 2015).

In emotional memory tasks, schizophrenic patients with high negative symptom scores show reduced attention to emotional vs neutral facial expressions, and make more errors in recall of happy faces (e.g. Jang, Park, Lee, Cho, & Choi, 2016), which could indicate reduced positive biases perhaps due to attentional deficits. In contrast, memory recall for threat-related and depression-related words are shown to be enhanced for schizophrenia

patients with persecutory delusions, compared to healthy controls (e.g. Bentall, Kaney, & Bowen-Jones, 1995).

Patients with MDD not only show biases towards negative stimuli, but have shown reduced biases toward positive stimuli, including reduced recognition or interpretation of positive emotions, attenuated memory for positively associated words, and blunted responses to reward (e.g. Dean, Horndasch, Giannopoulos, & McCabe, 2016; Rzepa, Fisk, & McCabe, 2017). Acute antidepressant treatment enhanced these positive biases in healthy volunteers and MDD patients (e.g. Harmer et al., 2010; Harmer, Shelley, Cowen, & Goodwin, 2004; Scholl et al., 2017).

One task used to measure reward-related biases in humans is the 'Response Bias Probabilistic Reward Task' (PRT), where subjects are presented with two ambiguous stimuli that they must discriminatively respond to in order to gain a reward (Pizzagalli et al., 2005). One stimulus is more frequently rewarded, so the expected response of healthy subjects would be to develop a bias for responding to the more frequently rewarded stimulus even when the reward is no longer present. This indicates intact reward learning and decision-making and represents a reward-induced positive bias.

Patients with MDD consistently show reduced reward-induced biases in this task compared to healthy controls (e.g. Liu et al., 2011; Pizzagalli, Iosifescu, Hallet, Ratner, & Fava, 2008; Vrieze et al., 2013; Whitton et al., 2016), suggesting depressed patients have impaired learning and decision-making biases for reward-associated stimuli. Evidence of deficits in schizophrenia is much less consistent. Heerey, Bell-Warren, and Gold (2008) found no difference between schizophrenics and healthy controls in reward-induced bias. However, Taylor et al. (2018) did observe a deficit in forming this positive bias in their cohort of schizophrenia patients, which they attributed to higher levels of negative symptoms in this cohort.

Alternatively, in the 'probabilistic reversal learning' task (PRL), participants learn the value and required response for a reward but must re-learn new reward associations when the required responses are reversed (Cools et al., 2002). Thus, PRL evaluates the ability to detect changes in reward sensitivity and alter decision-making in response to positive and negative feedback. In depressed patients, misleading negative feedback disrupted reversal learning (e.g. Murphy, Michael, Robbins, & Sahakian, 2003) and enhanced switch rates without reversal having occurred (e.g. Taylor Tavares et al., 2008). In patients with schizophrenia, reversal learning also appears to be disrupted (e.g. Waltz & Gold, 2007), associated with neurocognitive impairments and poor learning from both positive and negative feedback (e.g. Reddy, Waltz, Green, Wynn, & Horan, 2016). Similarly, disruptions

are seen in attentional set-shifting tasks, where a change in behavioural response is required after learning an initial stimulus-response association (e.g. Tyson, Laws, Roberts, & Mortimer, 2004).

Emotional decision-making biases in humans have been measured in a Go/No-Go task, where subjects are presented with positive or negative stimuli, such as images of facial expressions, and are required to respond to these stimuli but withhold from responding to distractors. Patients with MDD show biases in increased attention toward negative stimuli in this task (e.g. Murphy et al., 1999) as well as a tendency to bias toward withholding responses with negative outcomes (e.g. Mkrtchian, Aylward, Dayan, Roiser, & Robinson, 2017). In contrast, patients with schizophrenia showed a general inhibitory deficit and increased responding to non-rewarding stimuli (e.g. Waltz, Frank, Wiecki, & Gold, 2011), with some evidence demonstrating faster responses to negative stimuli (e.g. Krakowski et al., 2016), and contradictory evidence showing longer reaction times (e.g. Egashira et al., 2015).

Thus, various assays consistently report deficits in biases of learning and memory, and decision-making, in clinically depressed patients, with limited and variable evidence in schizophrenia. Attenuated reward learning has been linked to elevated anhedonia scores in patients (e.g. Vrieze et al., 2013), whilst self-judgement biases decreased with increasing anhedonia severity in healthy populations (e.g. Dunn, Stefanovitch, Buchan, Lawrence, & Dalgleish, 2009). This may suggest deficits in cognitive biases depend upon the severity of negative symptoms and general cognitive deficits across patients.

1.3. Risk factors for psychopathologies

Current evidence pertaining to reward processing deficits are mostly assessed in MDD, however, MDD and schizophrenia share several common risk factors contributing to their aetiology, and depression is present in approximately 40% of schizophrenic patients (Upthegrove, Marwaha, & Birchwood, 2016). Therefore, the empirical investigations in this thesis will use manipulations of affective state that aim to mimic risk factors pertinent to one or both major psychiatric disorders.

While there are several possible risk factors for these psychopathologies (see Figures 1.3 and 1.4), this thesis will focus on preclinical models of stress, neuroinflammation, and genetic variability. Thus, the following section will discuss evidence for the contribution of these risk factors to psychiatric disorders and current animal models.

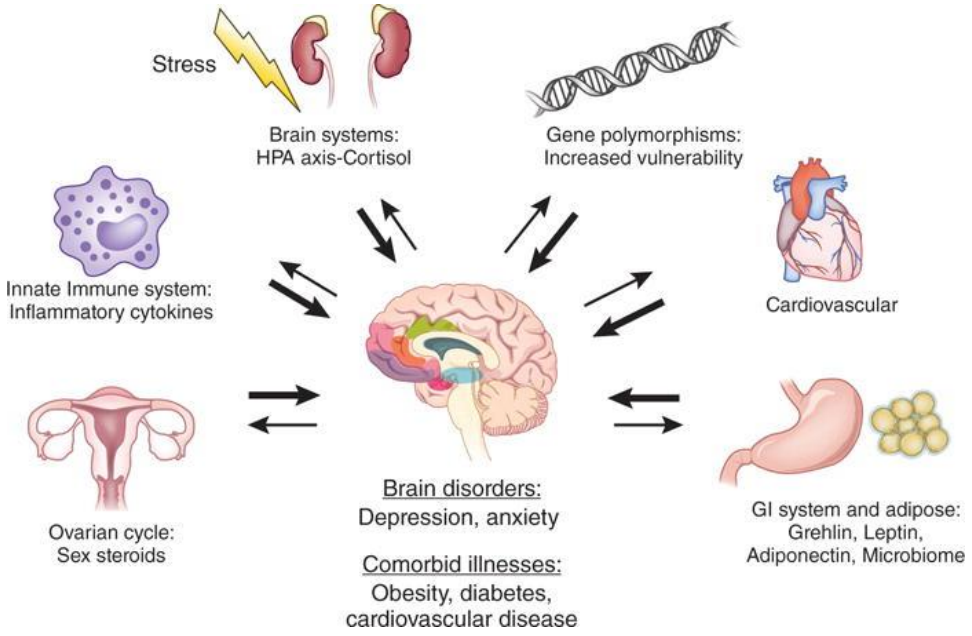


Figure 1.3. A schematic overview of risk factors for MDD, and other psychiatric/physiological disorders. Taken from Duman, Aghajanian, Sanacora, and Krystal (2016).

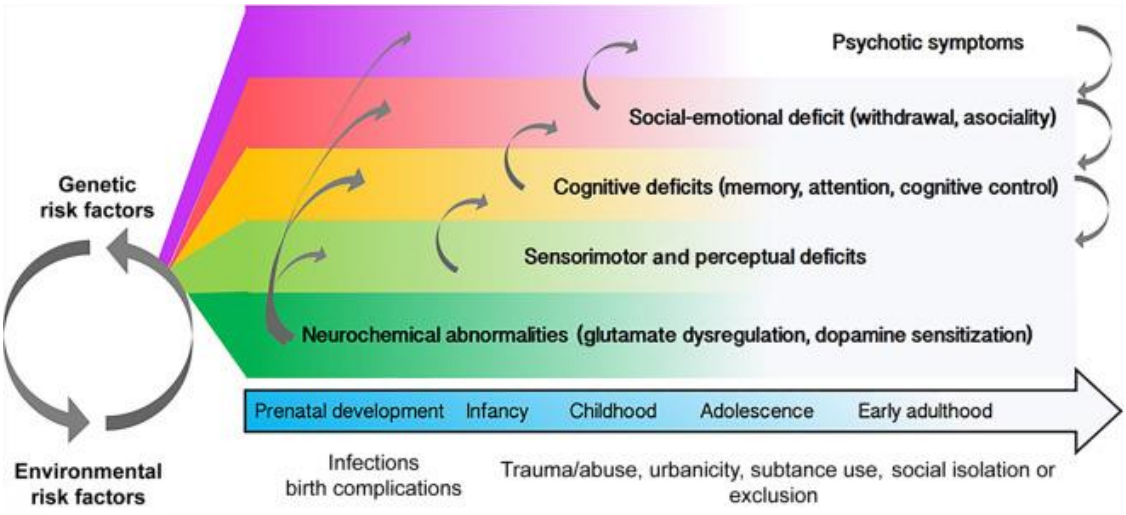


Figure 1.4. A schematic overview of risk factors and development of symptoms in schizophrenia. Taken from Ichinose and Park (2020).

1.3.1. Stress

Stress is a common risk factor implicated in the pathogenesis of depression (Krishnan & Nestler, 2008) and the onset or relapse of schizophrenia (McDonald & Murray, 2000). Early life adversity, including maternal/parental neglect and childhood trauma, have been suggested to contribute to the negative self-schema discussed previously, which may increase vulnerability to stressors in later life (Willner, Scheel-Krüger, & Belzung, 2013).

Under normal circumstances, stress activates the hypothalamic-pituitary-adrenal (HPA) axis (Smith & Vale, 2006), whereby corticotropin-releasing factor (CRF) is released from the hypothalamus to stimulate the production of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, which in turn results in the release of glucocorticoids, e.g. cortisol in humans, from the adrenal glands. The presence of glucocorticoids feeds back to the hypothalamus to reduce CRF production, regulating this physiological response to stress and maintaining homeostasis.

Evidence of altered HPA axis activity has been implicated in depressed patients (e.g. Binder & Nemeroff, 2010; Hayes & Ettigi, 1983; Sapolsky & Plotsky, 1990), with some alterations reported in schizophrenia depending upon stage of illness and treatment (e.g. Cesková, Kaspárek, Zourková, & Prikryl, 2006; Ismail, Murray, Wheeler, & O'Keane, 1998). In addition, cortisol levels have been negatively correlated with cognitive function in healthy, schizophrenic, and MDD subjects (e.g. Cherian, Schatzberg, & Keller, 2019; Hinkelmann et al., 2009), with some studies suggesting a positive relationship between cortisol and anhedonia in depression (e.g. Ahmed, Moussa, Moustafa, & Ayoub, 2016; Veen et al., 2011), however, given difficulties in measuring anhedonia in patients, this evidence is limited. Relationships between cortisol and motivation are less commonly reported, with some evidence indicating no correlation in healthy individuals (e.g. Rahe, Rubin, & Gunderson, 1972), but to my knowledge, this relationship has not been assessed with motivation for reward in patients.

Some animal models also focus on recapitulating this over-activation of the stress response, including exposure to environmental stressors including early life adversity or chronic mild stress (CMS), which elevate levels of the major stress hormone corticosterone (CORT) (e.g. Kvarta, Bradbrook, Dantrassy, Bailey, & Thompson, 2015; Macrì, Chiarotti, & Würbel, 2008), as well as direct exogenous administration of CORT. These models have shown “depression-like” phenotypes including classical immobility in the forced swim test (FST), impaired learning and memory (e.g. Bonapersona et al., 2019; Frisbee, Brooks, Stanley, & d'Audiffret, 2015), and reduced sucrose preference as a measure of anhedonia (e.g. Birnie et al., 2020; Ding et al., 2018; Frisbee et al., 2015). In addition, evidence of disruptions to

BDNF production, hippocampal cell proliferation, and synaptic plasticity has been reported (e.g. Khemissi, Farooq, Le Guisquet, Sakly, & Belzung, 2014; Liu et al., 2014; Willner et al., 2013).

Thus, stress-induced depression-like behaviours appear to be mediated by overproduction of glucocorticoids from aberrant HPA axis activity, which in turn could impact neurogenesis and neurodevelopment. For the empirical work in this thesis, exogenous CORT administration was used as the method for mimicking stress activation of the HPA axis to focus on the effects of this biological pathway.

1.3.2. Neuroinflammation

In the presence of pathogens, the innate immune system activates white blood cells to drive a non-specific inflammatory response, as well as targeted interaction with foreign bodies (Parkin & Cohen, 2001). Within the nervous system, immune-activating stimuli such as pathogens, neurotoxins, infection and injury can activate microglia, the macrophage cells of the brain, which release a variety of cytokines and proteins along with several other processes to clear harmful pathogens and debris (see Shabab, Khanabdali, Moghadamtousi, Kadir, & Mohan, 2017).

However, excess inflammation can lead to death of healthy cells. Since pro-apoptotic pathways are regulated through signalling molecules including cytokines produced by neuroinflammation, it can be suggested that neuroinflammation itself may damage neurons and contribute to several neurological and neuropsychiatric conditions (Shabab et al., 2017).

Patients with chronic inflammatory disorders, such as rheumatoid arthritis, develop depression at higher rates than the general population (see Felger & Lotrich, 2013), whilst some inflammatory cytokines have shown to be elevated in patients with depression (e.g. Köhler et al., 2017) and schizophrenia (e.g. Miller, Buckley, Seabolt, Mellor, & Kirkpatrick, 2011). Furthermore, in some MDD patients' anti-inflammatory drugs have an antidepressant effect (see Ménard, Hodes, & Russo, 2016). However, in other studies, findings of increased cytokines in MDD have been inconsistent (e.g. Krishnan & Nestler, 2008), highlighting the heterogeneity of these disorders.

Maternal infection during pregnancy has been associated with increased risk for schizophrenia in offspring (e.g. Brown et al., 2001; Brown & Patterson, 2011), as well as symptoms of depression in adolescence (e.g. Murphy et al., 2017). In contrast, Al-Haddad et al. (2019) found *in utero* exposure to any maternal infection increased risk of depression, but not psychosis, in offspring. The rodent maternal immune activation (MIA) model was developed to replicate this risk by administering an immunogen to pregnant dams, and their

offspring display some depression-like and schizophrenia-related phenotypes (e.g. Knuesel et al., 2014; Ronovsky, Berger, Molz, Berger, & Pollak, 2016). Despite some inconsistencies, a clear association can be made between maternal infection and offspring psychopathologies later in life, implicating some immunological influence on brain development.

Further evidence comes from exogenous administration of cytokines to treat other illnesses. Interferon-alpha (IFN-α) is a pro-inflammatory cytokine used in the treatment of viral diseases such as Hepatitis C, however, recent literature indicates that longitudinal treatment with IFN-α is associated with the development of clinical depression in 30 – 50% of patients (e.g. Hoyo-Becerra, Schlaak, & Hermann, 2014; Udina et al., 2012). Administration of IFN-α to rodents also induces some depression-like phenotypes (e.g. Callaghan et al., 2018; Callaghan, Rouine, & O'Mara, 2017).

Administration of IFN-α and other inflammatory agents can induce sickness behaviours (e.g. Krishnan & Nestler, 2008), an adaptive response to infection with similar behavioural phenotypes to psychiatric symptoms seen in humans, giving rise to the long-debated argument that depression results from, or is a form of, sickness behaviours (see Maes et al., 2012). In addition, evidence suggests cytokines including IL-1β, IL-6, and TNF-α modulate HPA axis activity (e.g. Kim, Na, Myint, & Leonard, 2016; Turnbull & Rivier, 1995), although this appears to be a bi-directional relationship (e.g. Chen et al., 2017; Waterman et al., 2006).

Thus, neuroinflammation induced by maternal infection and/or cytokine production appears to increase risk of developing psychopathologies, potentially via the HPA axis. Exogenous IFN-α administration is a translational preclinical model of neuroinflammation with little behavioural and neurobiological characterisation relating to depression-like phenotypes, therefore the empirical experiments in this thesis will apply sensitive behavioural assays to the evaluation of this neuroinflammatory model.

1.3.3. Genetic architecture

Early evidence of a genetic contribution to risk of developing psychiatric disease comes from twin studies demonstrating high concordance of schizophrenia in monozygotic twins (e.g. Hilker et al., 2018; Petronis, 2004), though the lack of 100% concordance indicates genetic architecture cannot be the sole contributor.

Recent developments in genomic technology enable detection of potential genetic risk variants, including single nucleotide polymorphisms (SNPs) that are common within the human population, and copy number variants (CNVs) that involve variation within larger portions of the genome and rarer. SNPs tend to contribute very low risk to development of

disease, with CNVs contributing a greater risk, and the accumulation of SNPs and CNVs within an individuals' genome contributes to their 'polygenic risk' (Khera et al., 2018).

To date, genome-wide association studies (GWASs) have identified 145 genetic risk loci, and subsequently 33 risk genes, associated with the presence of schizophrenia (e.g. Pardiñas et al., 2018), as well as 87-102 potential risk loci in MDD patients (e.g. Howard et al., 2019). Some common risk variants associated with schizophrenia overlap with those found in MDD (Anttila et al., 2018), indicating shared genetic risk for different psychiatric disorders.

Most risk genes associated with schizophrenia so far match the hypotheses for pathophysiology discussed in section 1.2.2. For example, SNPs have been identified within or near to the *DRD2* gene coding for D2 dopamine receptors (e.g. Edwards, Bacanu, Bigdeli, Moscati, & Kendler, 2016), and *GRIN2A* encoding the NMDA receptor 2A subunit (NR2A) (e.g. Harrison, 2015). The immune system, synaptic signalling, and neurotrophic factors have also been implicated in psychiatric disease from identifying genetic risk variants (Consortium, 2015), as well as genes encoding proteins involved in the HPA axis (Juruena, Bocharova, Agustini, & Young, 2018).

Voltage-gated calcium channels have also been implicated in the potential pathogenesis of schizophrenia since the identification of several SNPs within the genes *CACNA1C*, *CACNB2*, and *CACNA1I*, each coding for a different subunit (Andrade et al., 2016; Consortium, 2013). Although several genetic variants are associated with psychiatric disease, experiments in Chapters 3 and 5 of this thesis will focus on the *CACNA1C* gene, which codes for the α-1c subunit of $Ca_v1.2$ L-type calcium channels (LTCCs). *CACNA1C* has not only been associated with schizophrenia but also with increased risk for MDD, and bipolar disorder (e.g. Wray et al., 2012), however, this has been inconsistent with some evidence suggesting only an involvement of *CACNA1C* SNPs in treatment resistance (e.g. Calabrò et al., 2019). One study found a gene-environment interaction between the presence of a SNP in *CACNA1C* and previous exposure to life-threatening events in MDD (Zhao et al., 2019), suggesting an enhanced risk from this variant, alongside exposure to environmental factors.

It is important to note that whilst these risk factors have been implicated in psychopathologies, not all individuals exposed to the same stressor, expressing the same genetic mutation, or with the same indicators of neuroinflammation, will go on to experience a depressive episode or schizophrenia. Rather, each factor experienced by an individual accumulates a level of risk which may result in the development of psychiatric symptoms (Krishnan & Nestler, 2008).

1.4. Measurement of reward-related deficits in rodents and their neurobiological underpinnings

Reliable behavioural assays for animal models can help to parse the underlying neurobiological mechanisms of reward-related deficits seen across psychopathologies, as well as how they interact, and provide clear targets for treating individual symptoms (Berridge et al., 2009).

Traditional assays for depression-like symptoms have focused on phenotypes akin to 'behavioural despair' or 'learned helplessness' such as the FST (Porsolt, Anton, Blavet, & Jalfre, 1978), tail suspension test (Steru, Chermat, Thierry, & Simon, 1985), or inescapable shock (Maier, 1984). Although widely used in both fundamental biology research and drug development, the validity of these methods has been questioned, particularly given evidence of false positive and negative findings in disease models (e.g. Borsini & Meli, 1988; Steru et al., 1985; Vollmayr & Henn, 2001), and a lack of sensitivity to atypical antidepressants (e.g. Wesolowska et al., 2011). Given this, these methods cannot be said to accurately model the depressive phenotypes that would be seen in patients (Commons, Cholanians, Babb, & Ehlinger, 2017), and do not measure the reward-related deficits of the positive valence system domain.

The following section will discuss direct behavioural assays of reward-related deficits, focusing on those used empirically in this thesis, which can be translated to symptoms seen across psychiatric patients. It is important to note there are many limitations in the current assays of reward deficits in animal models, and a need to improve these such that they specifically measure the symptoms they claim to parse the neurobiological mechanisms underlying complex psychiatric disorders.

1.4.1. Anhedonia

Rodent behavioural assays

For decades, the most commonly used assay claimed to measure consummatory anhedonia in rodents is the sucrose preference test (SPT), where overall consumption of a sucrose solution is compared to consumption of plain water, measured via a choice test (Willner et al., 1987). A reduced preference for sucrose is commonly assumed to indicate consummatory anhedonia (e.g. Eagle, Mazei-Robison, & Robison, 2016; Papp, Willner, & Muscat, 1991).

However, the direct link between sucrose preference and anhedonia has been questioned (e.g. Anisman & Matheson, 2005; Der-Avakian & Markou, 2012; Dwyer, 2012). Although reduced hedonic response to sucrose is expected to lower sucrose preference, general

consumption of reward also relies heavily on motivation to attain it, and choice tests require intact cognitive processes to learn where the rewarding solution is. Thus, it cannot be concluded that differences from general consumption of sucrose specifically reflect hedonic deficits alone.

Berridge and Kringelbach (2015) emphasise the analysis of orofacial reactions to sweet solutions as a measure of hedonic response (see section 1.2.4). Rodents elicit 'positive' facial reactions to sweet tastes (e.g. tongue protrusions), and 'negative' aversion reactions to bitter tastes (e.g. gaping), similar to reactions portrayed by new-born babies (Grill & Norgren, 1978). In addition, rats have shown these negative reactions towards rewarding stimuli if previously associated with induced nausea (e.g. Pelchat, Grill, Rozin, & Jacobs, 1983). Thus, taste reactivity can provide a measure of the objective 'liking' hedonic responses of rodents to rewards, however, this requires filming and scoring individual animals behaviours making this task labour intensive and time-consuming.

Another selective measure of objective consummatory behaviour focuses on the microstructure of licking, which will be used to assay hedonic responses in Chapter 4 of this thesis. This is based on observations that rodents consume carbohydrate solutions of varying concentrations, such as sucrose, in an inverted U-shape manner (see Figure 1.5A), such that consumption of higher concentrations reduces as satiety is reached (Austen, Strickland, & Sanderson, 2016; Dwyer, 2012).

Davis (1973) reports the use of analysing the microstructural patterns in which rodents consume these solutions, and found they drink in 'bouts' consisting of a series of licks separated from other bouts by a pause. The average number of licks within each bout, known as lick cluster size (LCS), is proportional to the palatability of a solution (Figure 1.5B), regardless of overall consumption (e.g. Davis, 1989, 1996; Davis & Smith, 1992). Increasing concentrations of palatable solutions such as sucrose shows a positive monotonic relationship with LCS, whereas less palatable solutions such as bitter-tasting quinine have a negative monotonic relationship (e.g. Davis & Levine, 1977; Spector, Klumpp, & Kaplan, 1998; Spector & St John, 1998).

Thus, LCS may represent how palatable a solution is, and could therefore measure hedonic responses. Inducing pain or nausea also reduce LCS to palatable solutions, indicating that negative experiences can influence this behavioural response and thus the perception of reward (Dwyer, Gasalla Canto, Bura, & Lopez, 2017).

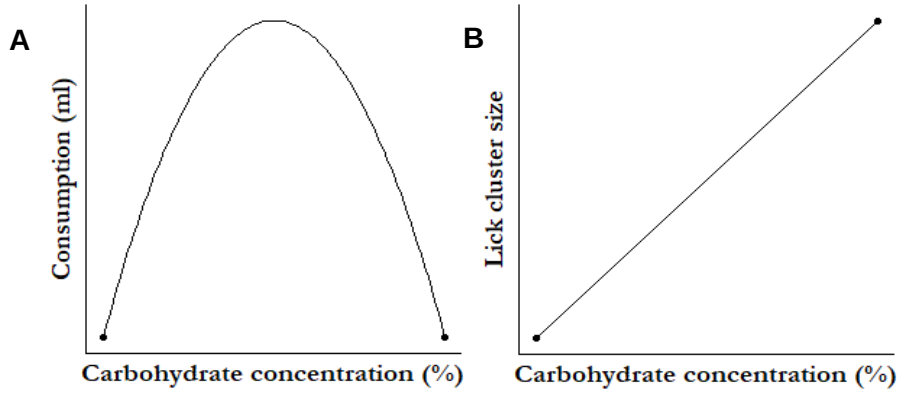


Figure 1.5. A schematic diagram of the relationships between increasing palatable carbohydrate concentration, e.g., sucrose, in a solution and consumption (A) or mean number of licks per bout (lick cluster size, B).

However, as discussed in 1.2.4, little evidence supports taste reactivity deficits in psychiatric patients which might reflect difficulties objectively measuring consummatory behaviour in humans, and thus questions the translatability to patient symptomology of taste reactivity and lick cluster analysis (LCA). Nevertheless, reduced LCS and reduced positive orofacial reactions to sucrose solutions in rodents represent a functional analogue of anhedonia, i.e. a reduced response to normatively rewarding events, regardless of the subjective experience itself (Dwyer, 2012). Although, it should be acknowledged that simple measures of consummatory behaviour may not accurately reflect the complexity of hedonic experience in humans.

Neurobiological underpinnings of anhedonia

Early theories for the neuropharmacology of anhedonia implicated dopamine as a core mediator given evidence of dopaminergic receptor antagonists reducing preference in the SPT (e.g. Muscat & Willner, 1989). The application of more sensitive analyses such as taste reactivity indicate that dopamine plays a greater role in assigning 'incentive salience' to stimuli (see Berridge, 2007) with only indirect effects on hedonic response (e.g. Peciña, Berridge, & Parker, 1997).

Serotonergic manipulation had no effect on LCS but appeared to influence overall consumption (e.g. Clifton, Lee, & Dourish, 2000; Lee & Simansky, 1997), and a serotonin receptor antagonist was not able to reverse anhedonia following haloperidol treatment (e.g. Galistu et al., 2011), suggesting this monoamine also does not directly underpin hedonic responses.

In contrast, opioid receptor stimulation in the nucleus accumbens (NAc) and ventral pallidum (VP) elevated positive orofacial reactions to reward (e.g. Peciña & Berridge, 2000,

2005), however, neither opioid agonists nor antagonists influenced LCS (e.g. Frisina & Sclafani, 2002; Higgs & Cooper, 1998). Taste reactivity studies have indicated 'hotspots' in the reward system mediate different mechanisms, where opioid stimulation in some regions enhance hedonic response (e.g. Castro & Berridge, 2014) whereas in other regions enhance motivation behaviours (e.g. Richard & Fields, 2016), which could explain these contradictory findings.

Endocannabinoids also enhance positive orofacial reactions to reward (e.g. Mahler, Smith, & Berridge, 2007), which depends upon opioid signalling (e.g. Mitchell, Berridge, & Mahler, 2018). In addition, benzodiazepines increase positive orofacial reactions to reward (e.g. Berridge & Treit, 1990), and increase LCS (e.g. Pittman et al., 2012). Blocking opioid receptors also attenuates the effects of benzodiazepines (e.g. Richardson, Reynolds, Cooper, & Berridge, 2005), suggesting endocannabinoids and benzodiazepines may influence hedonic response through opioid transmission.

There is limited literature evaluating models of psychiatric disease with LCA or taste reactivity. Rodents treated with phencyclidine (PCP) mimic negative, cognitive, and positive symptoms of schizophrenia, but sub-chronic PCP was found to have no effect on LCS without impacting inter-lick intervals (ILIs), suggesting any LCS alteration could be due to motor deficits (e.g. Lydall, Gilmour, & Dwyer, 2010). Alternatively, Lin, Arthurs, and Reilly (2017) found ketamine and pentobarbital reduced LCS to saccharin, however, ILIs were not reported here.

The AMPA receptor subunit, GluA1, has also been linked to the pathophysiology of schizophrenia and other nervous system disorders (Zhang & Abdullah, 2013). GluA1 knockout mice ($GluA1^{-/-}$) show attenuated LCS (e.g. Austen, Sprengel, & Sanderson, 2017; Strickland, Austen, Sprengel, & Sanderson, 2021), implicating the glutamatergic system in hedonic response.

For rodent models of MDD, literature is sparse. Mice exposed to tail handling, a more stressful handling technique, were found to have lower LCS compared to those exposed to tunnel handling (Clarkson, Dwyer, Flecknell, Leach, & Rowe, 2018). Wistar-Kyoto rats also display several phenotypes comparable to symptoms of MDD, in particular those seen in other stress models (Aleksandrova, Wang, & Phillips, 2019). Evidence of a hedonic deficit in this model has been inconsistent in the SPT, but Wright, Gilmour, and Dwyer (2020) recently demonstrated attenuated LCS in male Wistar-Kyoto rats. Thus, findings implicate the role of stress in inducing hedonic deficits, but evidence here is limited and LCA should be used in more direct models to improve this.

Unpublished research has also applied LCA to a *CACNA1C* heterozygous knockout (CACNA1C$^{+/-}$) model of psychiatric disease (Gasalla Canto, Hall, Thomas, Wilkinson, & Dwyer, 2019) that will be examined in Chapter 3 and 5 of this thesis. This model uses Zinc Finger Nuclease (ZFN) technology to produce a 4 base-pair deletion in Exon 6 of the *CACNA1C* gene, shifting the genetic framework to produce an early stop codon, thus disrupting transcription. In this study, wild type (22 male and 18 female) and CACNA1C$^{+/-}$ (29 male and 21 female) rats received pre-training with 8% sucrose, followed by 4 rounds of testing with 4% and 16% sucrose on alternate days, with an average for each variable calculated at the end of the experiment. All pre-training and testing was conducted in 6 automated drinking chambers described elsewhere (Wright et al., 2020), and data collection/analysis were as described in section 4.2.5 of this thesis. This experiment found CACNA1C$^{+/-}$ rats had significantly reduced LCS to both 4% and 16% sucrose compared to wild type controls, with no impact on overall sucrose consumption. Thus, CACNA1C$^{+/-}$ appear to show a hedonic deficit, which can be compared with other potential reward-related deficits investigated in this thesis.

These findings suggest an involvement of opioid signalling in the mediation of hedonic responses to reward, directly or indirectly through endocannabinoid or GABAergic system modulation. Stress, glutamatergic transmission, and genetic architecture may also be implicated in impaired hedonic responses.

1.4.2. Motivational deficits

Rodent behavioural assays

Intracranial self-stimulation (ICSS), where electrodes are surgically implanted into regions of the limbic system, had been used for decades as a measure of anhedonia. Rodents self-stimulate these electrodes by responding on a manipulandum, and 'anhedonia' was scored by altering reward stimulation frequency, and evaluating their change in response (Olds & Milner, 1954). However, this task is now thought to measure willingness to work for a reward, i.e., motivational processing rather than hedonic (e.g. Carlezon Jr & Chartoff, 2007; Der-Avakian et al., 2015; Der-Avakian & Markou, 2012).

Another common method for examining motivation is the progressive ratio (PR) operant task, where the number of lever presses required to obtain a fixed reward increases progressively, and motivation is assessed by the 'breakpoint' of the rodents, i.e. the effort point at which they stop responding (Randall et al., 2012). Thus, this can be interpreted as the amount of effort willing to put in to gain a reward, i.e., indicating a level of incentive motivation. Although motivational deficits are present in psychiatric patients, evidence of deficits in the PR task has been inconsistent in rodent models of MDD and schizophrenia

(e.g. Amitai, Powell, & Young, 2017; Barr & Phillips, 1998; Leventopoulos, Russig, Feldon, Pryce, & Opacka-Juffry, 2009).

However, there are limitations to PR tasks used to represent motivational deficits, including difficulty dissociating motivational from motor impairments, as well as arguments that PR tasks can be influenced by habitual responding or impulse control deficits (see Salamone, Koychev, Correa, & McGuire, 2015; Slaney, Hales, & Robinson, 2018).

As such, the effort-related choice paradigm (EfR) was developed by Salamone et al. (1991) which is comparable to the human EEfRT. Here, rodents are trained to press a lever on a fixed ratio schedule for a high valued reward, e.g., a fixed ratio of 5 lever presses produces one reward pellet. Once this is well established, they are given a choice between pressing the lever on this fixed ratio and freely consuming their usual chow from a bowl, which has a lower reward value. Thus, rodents are required to produce more effort for the higher valued reward, and alterations in effortful motivation for reward can be evaluated. The EfR will be the focus of empirical work investigating motivational deficits in Chapter 5 of this thesis.

Neurobiological mechanisms underpinning motivational deficits

Motivation and effort have become increasingly recognised as processes requiring an intact dopaminergic system (see Salamone et al., 2018; Salamone et al., 2016). Instrumental responding for rewards is altered by manipulations of dopamine (e.g. Salamone, Kurth, McCullough, Sokolowski, & Cousins, 1993; Salamone et al., 1991), and self-stimulation in ICSS also appears to be mediated by dopaminergic changes (e.g. Markou & Koob, 1992; Paterson, Myers, & Markou, 2000). Dopamine antagonists also increase chow consumption and reduce lever responding in the EfR task (e.g. Randall et al., 2012; Salamone et al., 1991), demonstrating dopamine's involvement in initiating and maintaining effort for reward retrieval, rather than lack of appetite (Salamone & Correa, 2002).

PR tasks also implicate the involvement of opioids in maintaining high effort to attain rewards (e.g. Zhang, Balmadrid, & Kelley, 2003). Both dopamine and opioid administration appear to increase incentive salience for Pavlovian-associated cues, suggesting their involvement in multiple types of associative motivation for reward (e.g. DiFeliceantonio & Berridge, 2016).

Some rodent models of stress have shown mixed results for its effect on responding in PR tasks (e.g. Koob, 2008; Rüedi-Bettschen, Pedersen, Feldon, & Pryce, 2005; Shalev & Kafkafi, 2002). Whereas, adenosine, acetylcholine, and $GABA_A$ receptor agonists also reduce lever responding and increase chow consumption in the EfR task (e.g. Farrar et al., 2008; Font et al., 2008; Nunes, Randall, Podurgiel, Correa, & Salamone, 2013). In contrast,

serotonergic neurotransmission appeared to have no impact on effort-related decision-making in modified EfR tasks (e.g. Denk et al., 2005; Izquierdo et al., 2012). This is supported by evidence that dopamine enhancing drugs appear to reverse amotivational shifts in the EfR (e.g. Yohn, Lopez-Cruz, Hutson, Correa, & Salamone, 2016), whereas serotonin-targeting typical antidepressants do not (e.g. Yohn, Collins, et al., 2016).

Thus, these findings suggest a major involvement of dopamine in motivation for reward, and dysfunctional dopaminergic transmission may result in deficits seen across psychiatric disorders. However, other factors also appear to influence effortful behaviour, such as stress, GABAergic transmission, and adenosine receptor antagonism.

1.4.3. Cognitive processing biases

Rodent behavioural assays

Several behavioural assays have been developed to investigate changes in cognitive processing of rewarding stimuli in rodents following manipulations of affective state. Decision-making and interpretation biases can be measured using the judgement bias task (JBT), in which rodents are trained to produce a response to the presentation of a 'positive' stimulus, and a different response to the presentation of a 'negative' or less positive stimulus (Harding et al., 2004). When ambiguous stimuli are presented, healthy rodents produce more positive responses, whereas those with a hypothesised negative affective state produce more negative responses (e.g. Anderson, Munafo, & Robinson, 2013; Hales, Houghton, & Robinson, 2017; Hales, Robinson, & Houghton, 2016; Mendl, Parker, & Burman, 2009). Another version of this task relies on natural investigative behaviour, with rats trained to nose poke and either keep their nose inserted in the food trough in response to a tone indicating reward, or withdraw in response to a tone indicating punishment (Jones et al., 2018), similar to the human Go/No-Go task.

A rodent version of the PRL described in section 1.2.6 was developed to detect changes in reward sensitivity by measuring altered decision-making following positive and negative feedback (Bari et al., 2010). Rats are trained to nose poke in an illuminated hole to gain a reward, then are presented with two illuminated holes where one is more frequently rewarded. The probability of reward from nose poking is then reversed, thus, this task measures rodents' ability to alter their response biases and decision-making following positive (i.e., receiving a reward when it is least expected) and negative (i.e., not receiving a reward when it is more expected) feedback.

Similarly, the PRT is used to assess reward-induced response biases in patients, leading to the development of a translational rodent PRT (Der-Avakian et al., 2013). Here, rats are trained to discriminate between two auditory stimuli each requiring a specific operant

response to gain a reward. They are then presented with similar tones, where a correct discriminative response to one tone is reinforced more frequently than a correct response to the other. Healthy rats develop a positive bias toward the more frequently rewarded stimulus, demonstrating intact reward-induced biases.

The affective bias test (ABT) examines alterations in learning and memory driven by changes in affective state in rodents (for general reviews of the ABT see Robinson, 2018; Robinson & Roiser, 2016). In brief, rodents learn to associate digging substrates with a single reward pellet, and their affective state is manipulated during pairing sessions with one substrate, whilst a vehicle is given alongside the second substrate. If their affective state is manipulated with a 'pro-depressant', this is expected to induce a negative affective state and thus result in a negative bias for the manipulation-paired substrate. For example, pairing the CS^+ with the immunomodulator LPS in the ABT results in a reduced preference for this CS^+ compared to vehicle-paired CS^+ when these two are presented together in a choice test (Stuart, Wood, & Robinson, 2017). In contrast, if their affective state is manipulated with an 'antidepressant', this is expected to induce a positive affective state and result in a positive bias for the manipulation-paired substrate. For example, pairing one digging substrate with fluoxetine administration results in increased preference for this substrate compared to the vehicle-paired substrate, indicating a positive affective bias (Stuart et al., 2013).

Alternatively, the modified ABT (m-ABT) investigates the influence of affective state manipulations on reward-induced biases and will be the focus of empirical Chapters 2 and 3. Here, one digging substrate is paired with two reward pellets, while the second is paired with one. Thus, healthy animals are expected to form a reward-induced positive bias toward the former substrate when both substrates are presented having equal value, based on their previous expectations. Pharmacological or environmental manipulations to induce a putative negative affective state, for example chronic treatment with retinoic acid, prior to learning these reward-stimulus associations reduce this positive bias for the substrate paired with the higher reward value (Stuart et al., 2017). Thus, the m-ABT can measure deficits in forming reward-induced biases caused by prior manipulation of affective state. A full description of the ABT and m-ABT is provided in Chapter 2.

Importantly, rodents displaying deficits in the ABT or m-ABT have not shown consistent impairments in the SPT (e.g. Stuart et al., 2017) or in progressive ratio tasks, (e.g. Stuart, Hinchcliffe, & Robinson, 2019), highlighting that cognitive biases may not be mediated by the same underlying neurobiological mechanisms as reward consumption and motivation, and thus would not result from a change in either of these aspects.

Neurobiological underpinnings of cognitive biases

In humans, reductions in monoamines including serotonin, dopamine and noradrenaline have been linked to impaired reward learning (e.g. Rogers et al., 1999) and negative processing biases of rewarding stimuli (e.g. McLean, Rubinsztein, Robbins, & Sahakian, 2004; Murphy, Smith, Cowen, Robbins, & Sahakian, 2002; Rogers et al., 2003; Roiser et al., 2008).

In the PRL, serotonergic modulation impaired shifting of decision-making behaviours (e.g. Bari et al., 2010; Chamberlain et al., 2006), whereas reversal learning was enhanced following oxytocin treatment with no impact on motivation (e.g. Roberts et al., 2019). Dopamine elevation also impaired probabilistic reversal learning (e.g. Alsiö et al., 2019; Vo, Seergobin, Morrow, & MacDonald, 2016).

Studies investigating neurobiological mechanisms in the JBT are limited, but suggest some involvement of monoamines in mediating positive interpretation biases (e.g. Rygula, Papciak, & Popik, 2014), as well as endocannabinoids (e.g. Kregiel, Malek, Popik, Starowicz, & Rygula, 2016). However, the dopamine-enhancing drug cocaine had no effect on judgement bias whereas an alternative dopamine modulating drug, mazindol, induced a negative bias (e.g. Rygula, Szczech, Papciak, Nikiforuk, & Popik, 2014), although it is suggested cocaine can have anxiogenic properties, and both cocaine and mazindol also influence the noradrenergic system. Furthermore, elevated noradrenaline and CORT appeared to reduce positive interpretation biases (e.g. Enkel et al., 2010; Rygula, Papciak, et al., 2014).

However, some rodents demonstrate innate 'pessimistic' or 'optimistic' traits that can be identified with the JBT, which can influence the effects of antidepressants on other cognitive processes such as the PRL (e.g. Drozd, Rychlik, Fijalkowska, & Rygula, 2019). Elevations in dopamine and serotonin appear to cause a shift in optimistic rats to behaving in a more pessimistic manner (e.g. Golebiowska & Rygula, 2017).

In humans and rodents, reward-induced biases in the PRT were impaired by dopamine receptor agonists, and psychosocial stress (e.g. Bogdan & Pizzagalli, 2006; Der-Avakian et al., 2013; Der-Avakian et al., 2017; Pizzagalli, Goetz, Ostacher, Iosifescu, & Perlis, 2008). In addition, genetic variants associated with impaired metabolism have been related to differing response biases (e.g. Yapici-Eser et al., 2020), indicating response biases to reward may be mediated by various systems including dopamine, stress, and metabolism.

In the ABT, negative learning and memory biases have been found following acute endocannabinoid antagonists, $GABA_A$ receptor agonists, and psychosocial stress, whereas drugs of abuse do not influence these (e.g. Stuart et al., 2013). Monoamine depletors,

CORT, and immunomodulators also induce negative biases (e.g. Hinchcliffe, Stuart, Mendl, & Robinson, 2017; Stuart et al., 2017), whilst antidepressants targeting monoaminergic systems induce positive biases (e.g. Refsgaard et al., 2016). A recent investigation of hormone-induced changes found oestradiol induced positive affective biases, whilst testosterone and progesterone induced negative affective biases (Hinchcliffe, Mendl, & Robinson, 2020).

The NMDA receptor antagonist ketamine, which has rapid-acting antidepressant effects in MDD patients (Berman et al., 2000), had no overall effect on producing an affective bias alone in the ABT. However, when administered prior to learning, ketamine improved negative affective biases induced by FG7142 and psychosocial stress, whereas the delayed-onset antidepressant venlafaxine did not (Stuart, Butler, Munafo, Nutt, & Robinson, 2015). This effect of ketamine was specific to the medial prefrontal cortex (mPFC), whereas venlafaxine appeared to act upon the amygdala, suggesting the amygdala plays a role in forming affective biases whereas the mPFC could be involved in storage of learned biases (Robinson, 2018). The similar NMDA receptor antagonist, PCP, impaired formation of reward-induced positive biases in the m-ABT (Sahin, Doostdar, & Neill, 2016), which could indicate that forming reward-induced biases in the m-ABT involves the mPFC whilst affective biases in the ABT involves the amygdala, or possibly that PCP and ketamine act upon different neurocircuits. However, this may also reflect different dosing regimens, as the therapeutic effects of ketamine were demonstrated using acute dosing of low concentrations, while PCP was administered as sub-chronic dosing. In addition, chronic treatment with immunomodulators or retinoic acid has shown to impair the formation of reward-induced positive biases in the m-ABT (Stuart et al., 2017).

Together, these findings suggest that reward-related cognitive biases can be influenced by several biological pathways, including monoaminergic transmission, immunomodulation hormonal changes and stress. The amygdala is implicated in the formation of affective biases, whereas recall of biases is thought to be mediated through higher cortical and hippocampal regions. The combination of other reward-related assays has also indicated that some neurobiological mechanisms underpinning affective biases and reward-induced biases are separate from other reward-related deficits such as consummatory anhedonia.

1.5. Summary and thesis aims

The literature discussed here demonstrate a clear clinical need to understand the symptoms of psychiatric disorders, where there is currently a lack of effective treatments. Clinical assessments of patient symptomology often cannot differentiate between multiple components, yet high heterogeneity across patients indicate the importance of this.

For several decades, preclinical assays did not focus on dissociating these symptoms in animal models, and as such, have not commonly been used to distinguish between deficits. However, recent advances reveal complex neurobiological pathways involved in the presentation of specific reward-related deficits, highlighting important differences between these behaviours, although there are many overlaps and interactions between these.

The aim of this thesis is to address current limitations in preclinical research of cognitive, motivational, and hedonic aspects of reward processing. Firstly, reliable behavioural assays of reward processing mechanisms are important to this research area, thus, Chapter 2 reports the development of a novel version of the ABT/m-ABT. Second, the neurobiological mechanisms underpinning each of these reward processing mechanisms is still unknown in relation to the pathophysiology of psychiatric disorders, thus sensitive behavioural assays measuring individual symptoms will be applied to models of these disorders. Chapter 3, 4, and 5 will assess reward-induced biases, hedonic response, and motivational deficits respectively, with various manipulations mimicking risk factors pertinent to MDD and/or schizophrenia.

Finally, Chapter 6 aims to elucidate the neurobiological impact of such risk factors and manipulations through biochemical and histological analyses, to understand how these deficits may arise and how they interact. Therefore, this thesis focuses on the development of preclinical behavioural assays for phenotypes matching to symptoms that may be present in several psychopathologies, including MDD and schizophrenia, and their application to rodent models of these disorders, to further understanding of the neurobiological mechanisms behind reward-related deficits.

Chapter 2 – Evaluation of novel flavour preference-based affective bias tests

2.1. Introduction

2.1.1. Current cognitive bias assays in rodents

As discussed in section 1.4.3, current methods developed to probe affective biases and reward-induced biases in rodents include the affective bias test (ABT) and the modified-ABT (m-ABT), respectively, which examine the influence of negative or positive affective states on learning and memory of reward-paired digging substrates.

In the ABT, rodents are presented with one of two digging substrates that contain a reward (the conditioned stimulus, CS^+), alongside one different substrate not containing a reward. In one pairing session, one CS^+ is presented after acute pharmacological or environmental intervention to manipulate the rodents' affective state, and in an independent session the second CS^+ is presented following a vehicle/control. In this task, each CS^+ contains the same value of reward, but one digging substrate will be coupled with the rodents' manipulated affective state at the point of learning the CS-US association, whilst the other substrate should not be affected (Stuart et al., 2013).

In the m-ABT, rodents learn to associate a higher value reward with one digging substrate and a lower valued reward with a different substrate, where one of each substrate is presented alongside an unrewarded substrate during pairing sessions. In this task, the two CS^+ contain different values of reward during pairing but with animals in the same affective state for both experiences (Stuart et al., 2017).

Several pharmacological and environmental manipulations have demonstrated the use of these tasks in measuring biases in rodent models. Pro- or antidepressants induce expected negative or positive affective biases, respectively, in the ABT (Hinchcliffe et al., 2017; Stuart et al., 2013), whilst pro-depressants also reduce formation of reward-induced positive biases in the m-ABT (Stuart et al., 2017), demonstrating high predictive validity. The same antidepressant therapies used in the ABT show similar effects on measures of emotional processing when given to human participants (Harmer et al., 2010; Harmer, Duman, & Cowen, 2017; Harmer et al., 2009; Harmer et al., 2004), indicating translational validity of this task.

Despite the high validity of these tests, the reliability of this method could be potentially limited by its design. The bowl-digging nature of the test increases labour and time consumption for the researcher. In addition, all aspects of the task are manual, giving a greater risk of subconscious researcher bias or human error. Therefore, to increase the

reliability and reproducibility of the ABT, it would be advantageous to develop an automated version with the same validity.

During the development of the digging ABT, control experiments showed that pairing both digging substrates with a vehicle did not result in any biases, and animals are usually tested in the m-ABT without prior manipulation to provide proof of concept that the animals can form cognitive biases (Stuart et al., 2013). Although this increases validity of the digging task, it does not clearly indicate whether altered biases from pharmacological or environmental intervention are due solely to changes in the affective state and not a result of induced cognitive deficits. It is possible that if the manipulation used induces a general cognitive deficit, such as in the ability to retrieve the memory of stimulus-reward associations, then this could present as a deficit. Furthermore, despite the assumption that affective state manipulations would simply alter forming a positive bias for the higher valued CS^+, it is possible these manipulations could alter the value of both the higher reward and lower reward such that their relative difference in value remains the same.

Thus, the aim of this chapter was to evaluate novel assays for affective and reward-induced biases, hereby known as the flavour ABT or flavour m-ABT, respectively, by replacing digging substrates with flavoured solutions paired with sucrose solutions and measuring biases through consumption. In addition, I also aim to improve upon the design of the original assays by including additional choice tests to control for general cognitive deficits and understand the mechanisms by which learning biases might take place.

2.1.2. Design of a flavour ABT/m-ABT

Rodents can acquire preferences for flavours paired with a palatable substance (e.g. Dwyer, Haselgrove, & Jones, 2011; Holman, 1975; Sclafani & Ackroff, 1994). Further, pairing these flavours with aversive sensations, such as nausea, have shown to reduce hedonic responses to that flavour (e.g. Dwyer et al., 2017), indicating flavour preferences can be influenced by experiences during learning. This therefore suggests flavour preferences could also be influenced by manipulations to affective state, and thus could be used to measure changes in affective bias and reward learning.

In these flavour preference-based assays, I replaced digging substrates with flavoured solutions, and reward pellets with sucrose solution, with the underlying principles of the ABT / m-ABT remaining the same. In the flavour ABT, one flavoured solution containing sucrose is paired with a manipulation of affective state (CS-A), whilst another flavoured solution containing the same amount of sucrose is paired with the vehicle/control (CS-B). During pairing sessions, each rewarded flavour is presented alongside a ‘neutral’ flavour with no reward (CS-C), then CS-A and CS-B are presented together in a choice test with neither

solution containing the reward. A negative or positive affective bias could therefore be measured from this choice test.

Additional choice tests were added to examine preferences for CS-A/B vs CS-C, as well as preference for a novel unrewarded flavoured solution (CS-D) vs CS-C. The former tests investigate general memory recall of the flavour-reward association to control for cognitive deficits that may influence reward learning. In addition, since depression may involve biases toward focusing on negative events (see section 1.2.6), rodents in a putative negative affective state may focus on learning to avoid the unrewarded flavour, CS-C, rather than learning the value of the rewarded flavours. Thus, the latter additional choice test investigated whether rats learned to avoid the unrewarded flavour. To investigate the application of this flavour ABT design to measuring changes in affective bias, one positive and one negative affective state-inducing drug previously used in the digging ABT were tested in Experiment 1.

Experiment 2 describes the development of the flavour m-ABT, in which a variety of training length and sucrose concentrations were assessed to determine the optimal design conditions for this task. During independent pairing sessions, one flavoured solution contained a high concentration of sucrose (CS-A^{++}), and another flavoured solution contained a lower concentration of sucrose (CS-B^{+}), where each solution was presented alongside CS-C. A choice test was then conducted to assess preference for CS-A^{++} vs CS-B^{+}, without sucrose, where a positive bias for the CS-A^{++} would be expected. Thus, chronic manipulation to induce a putative model of depression prior to pairing sessions would be expected to result in a deficit in forming this reward-induced positive bias. The same additional choice tests described above were also conducted to evaluate whether this deficit was due to affective state or general cognition.

2.1.3. Summary

In this chapter, the effects of the established pro-depressant, CORT, and antidepressant, venlafaxine, were examined on inducing negative and positive biases, respectively, in a novel flavour ABT assay described in Experiment 1. Healthy rats were then used in Experiment 2 to evaluate the behavioural design of a novel flavour m-ABT assay, compared with the same subjects in a replication of the digging m-ABT in a novel facility.

2.2. Experiment 1 – Flavour ABT pilot

Experiment 1 evaluates the novel flavour ABT as an alternative assay of affective bias to address limitations associated with a manual digging version of this task. Total consumption of a flavour-sucrose solution previously paired with either the antidepressant Venlafaxine or the pro-depressant CORT compared with another flavour-sucrose solution paired with a vehicle were examined to determine preferences for the drug-paired flavour. If the flavour ABT would be capable of detecting affective biases similarly to the digging ABT, then the outcomes of this experiment would demonstrate a significant preference for the drug-paired flavour with Venlafaxine, indicative of positive affective bias, and a significant preference for the vehicle-paired flavour with CORT, indicative of negative affective bias.

2.2.1. Methods

2.2.1.1. Subjects

Throughout experiments using the apparatus described below (in Chapter 2, 3 and 4), the sample size required per group was n = 16 – 24 based on previous literature with this same apparatus (Dwyer et al., 2017; Riordan & Dwyer, 2019; Wright, Gilmour, & Dwyer, 2013; Wright et al., 2020). The expected sample size for the ABT is n = 12 – 16 per group based on previous literature (Hinchcliffe et al., 2020; Hinchcliffe et al., 2017; Stuart et al., 2013; Stuart et al., 2017), but given the novelty of this flavour preference version it was reasonable to undertake a more cautious approach to ensure statistical power.

Male Sprague-Dawley rats (n = 48, Charles River, UK) were housed three per conventional cage, with a wooden chewing block, cardboard tube, and nesting material as enrichment. Rats were maintained on a 12h light/dark cycle with all experimental work was conducted during the light cycle, and temperatures within the home room environment were maintained between 20-24°C. All rats were acclimatised to the environment for at least one week, followed by one week of handling before any procedures began. All rats were maintained between 85 – 90% of their free-feeding weights prior to commencing pre-training, allowing moderate weight gain expected in free-feeding rats, as determined by growth charts. Food rations were given in the rats' home cages at least 1 hour after the end of the session. All rats were exposed to two consecutive 22-hour periods of water restriction at the start of pre-training.

All experiments were conducted in compliance with the UK Animals (Scientific Procedures) Act 1986 and with local University Ethics Review Committee approval.

2.2.1.2. Apparatus

12 white plastic boxes (32x15x12cm) were used for all stages of the experiment, containing metal grid floors and wire mesh lids. Drinking solutions were given in 50ml falcon tubes with stainless steel drinking spouts, where each spout is positioned in the left- and/or right-hand access hole within the box lid at the beginning of each experimental session.

2.2.1.3. Pre-training

On the first day of pre-training, the length of the drinking spouts reached inside the box for easy detection. This can usually result in noisy data from rats touching parts of the metal spout other than the ball bearing, so once drinking from the spout had begun, its position was then retracted to be flush with the edge of the box for the remainder of the experiment.

All animals were given access to two bottles of 0.1% saccharin (w/w) for 10 minutes daily whilst on water restriction on days one and two, followed by access to two bottles of 10% sucrose with 0.1% saccharin (w/w) until they reached a steady level of consumption from both spouts.

2.2.1.4. Flavour ABT design

All solutions used during the flavour ABT consisted of distilled water, 0.05% Kool-Aid and 0.1% saccharin (w/w). The Kool-Aid flavours used in this study included grape, tangerine, cherry, and tamarind. CS-A and CS-B solutions additionally contained 8% sucrose during pairing sessions only.

For all flavour ABT experiments, the reward-paired flavours (CS-A and CS-B) and unrewarded flavours (CS-C and CS-D) were counterbalanced between Grape/Cherry or Tangerine/Tamarind, such that solutions were presented equally often in left/right bottles over training.

All rats received four daily pairing sessions. Two sessions consisted of a two-bottle choice between one flavour containing 8% sucrose, CS-A, vs a different flavour without added sucrose, CS-C. The other two sessions consisted of a two-bottle choice between a different flavour containing 8% sucrose, CS-B, vs CS-C. The order in which rats received CS-A or CS-B pairing sessions was counterbalanced across all groups. One group of rats (n=24) received a single i.p. injection of Venlafaxine (3mg/kg) prior to each pairing session with CS-A, and saline prior to each pairing session with CS-B. A second group (n=24) received a single subcutaneous injection of CORT (10mg/kg) prior to pairing with CS-A, and vehicle (10% DMSO, 20% Cremaphor and 70% saline) prior to pairing with CS-B. All rats were given their respective drug ~30 minutes prior to the pairing session. Data from training sessions can be seen in Appendix E.

Following the pairing sessions, all rats were given five choice test sessions for 10 minutes each day, where no solutions had added sucrose and no drugs/vehicle were administered. The first two sessions consisted of two-bottle choice tests between CS-A and CS-B without added sucrose, counterbalanced for left and right spout holes to reduce any side bias noise. The remaining sessions consisted of a counterbalanced order of three choice tests between CS-A vs CS-C, CS-B vs CS-C, and CS-C vs CS-D, in which D was a novel flavour introduced only in this testing session (see Figure 2.1 for full design outline).

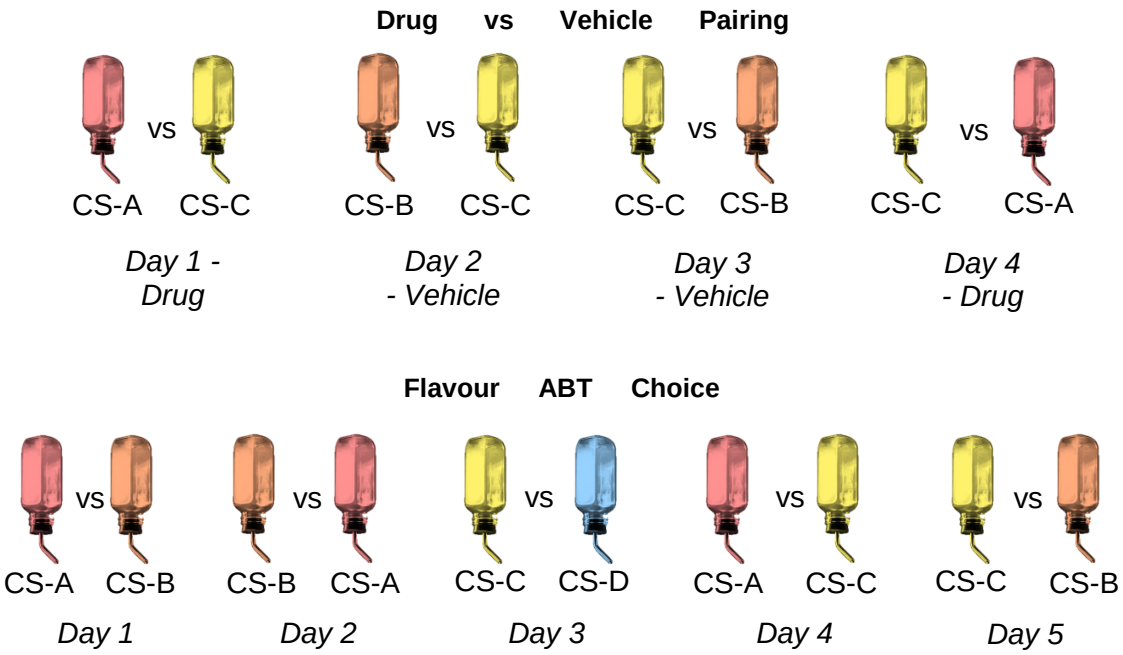


Figure 2.1. A schematic diagram of the flavour ABT design.
All rats were given four days of pairing sessions with CS-A after pre-treatment with a pro-depressant (CORT) or antidepressant (venlafaxine), referred to as 'drug', or CS-B after pre-treatment with a vehicle solution, followed by five choice tests, without any pre-treatment, to assess learning and memory. CS-A and CS-B contained 8% sucrose during pairing sessions but were without added sucrose during choice tests. The order that pairing sessions and choice tests were presented were counterbalanced across different animals – this diagram shows an example of this order.

2.2.1.5. Data Analysis

Drinking bottles were weighed before and after each session to calculate total volume of each solution consumed. This volume was then averaged for each group to compare consumption of each solution between groups in each choice test. Percentage preferences were also calculated for individual choice tests and averaged for each group to compare. For the CS-A vs CS-B choice tests, data from day one and day two were averaged together for each rat before calculating an overall average for both consumption and percentage preferences. Side biases were checked at the end of the experiment and any animal consuming greater than 90% from either the left or the right bottle, averaged across all

choice test days, would be removed from analysis. In this experiment, no animal was removed from analysis.

An alpha level of 0.05 was used as the level of significance, and all analyses were conducted using choice test data on SPSS software (v23, IBM). Overall consumption data for each choice test were analysed using repeated measures ANOVA's, where the within-subjects factor 'flavour' was compared with fixed factor 'group'. All ANOVAs were compared using Fisher's Least Significant Difference (LSD) post-hoc analysis. Individual group percentage preferences were analysed separately using one-sample t-tests, with 0.5 as the test value, and compared using independent samples t-tests.

The Shapiro-Wilk test for normality was conducted on all data but was only presented where the data was not normally distributed. Where data was not normally distributed, an alternative non-parametric test was conducted (i.e. the Mann-Whitney U test for independent t-tests or Kruskal-Wallis test for one-way ANOVAs). Where this was not possible, such as in the analyses of two-way or repeated measures ANOVAs, then a statement highlighting this issue would be made.

All graphs throughout this thesis were produced in R (v4.0.2).

2.2.1.6. Bayes analysis

Standard null hypothesis statistical testing (NHST) provides a *p* value denoting the likelihood of a result occurring by chance (when the null hypothesis is assumed to be true) and determines whether a hypothesis is accepted or rejected. However, NHST does not provide evidence for a null effect and by only providing NHST, data interpretation is more open to false conclusions (see Bendtsen, 2018). In this thesis, Bayesian analysis was used as a supplementary method in situations where it is important to know if the data supports conclusions about the absence of an effect directly relating to the key experimental hypotheses.

The Bayes factor (BF) is a calculated ratio of the likelihood of the observed data given one hypothesis being true compared to another. This represents the strength of evidence in favour of one theory, as it enables the evaluation of data in favour of a null hypothesis (Rouder, Morey, Verhagen, Swagman, & Wagenmakers, 2017).

BF's were interpreted as described by Jeffreys (1961), with principles outlined in Table 1.2. BF_{10} denotes evidence supporting the alternate hypothesis and BF_{01} denotes evidence supporting the null hypothesis.

Table 1.2. Evidence categories for Bayes interpretation, as defined by Jeffreys (1961)

Bayes Factor	Strength of Evidence
> 100	Decisive
30 – 100	Very strong
10 – 30	Strong
3 – 10	Moderate
1 – 3	Anecdotal
1	No evidence

All Bayesian statistics were conducted using JASP (v0.11.1.0). For main effects, BFs were taken direct from the model comparison output. For interactions, the inclusion BF's were calculated from the 'Effects' output in JASP, which is an automated method implemented in JASP in which BF's are determined by averaging the effects "across all models" containing the given interaction and comparing this with the models that do not contain the interaction (see Rouder et al., 2017; van den Bergh et al., 2019 for further details).

2.2.2. Results

2.2.2.1. Measuring affective bias in the CS-A vs CS-B choice tests

Neither CORT nor venlafaxine induced an affective bias in the flavour ABT, however, preferences were numerically higher for CS-A in the CORT-treated group and the opposite in venlafaxine-treated rats despite previous evidence in the digging ABT showing a clear negative and positive affective bias, respectively. Bayes analysis provided little evidence in support of this, indicating the data here was inconclusive.

A repeated measures ANOVA revealed no difference in overall consumption of CS-A vs CS-B, $F_{1, 46} = 0.57$, MSE = 8.67, $p = 0.45$, $\eta_p^2 = 0.012$, nor any effect of group on overall consumption, $F_{1, 46} = 1.34$, MSE = 3.05, $p = 0.25$, $\eta_p^2 = 0.028$.

There was a significant interaction between flavour * group, $F_{1, 46} = 4.73$, MSE = 8.67, $p = 0.035$, $\eta_p^2 = 0.093$ (Figure 2.2A). Pairwise comparisons revealed that rats pre-treated with Venlafaxine consumed significantly more CS-B compared to CS-A, t(46) = 2.07, $p = 0.044$, whereas rats treated with CORT showed no difference in consumption of the two flavours, t(46) = 1.002, $p = 0.321$.

A Bayesian repeated measures ANOVA revealed moderate evidence to support no difference in overall consumption of either flavour, $BF_{01} = 3.28$, and for no difference between the groups, $BF_{01} = 3.34$.

Analysis of overall percentage preference for CS-A vs CS-B in CORT-treated rats (mean = 54.5%) using a one-sample t-test revealed no significant bias for either flavour, t(23) = 1.08, $p = 0.29$. Analysis of percentage preference in venlafaxine-treated rats (mean = 42.3%) also showed no significant bias for either flavour, t(23) = -1.81, $p = 0.083$. An independent-samples t-test revealed a significantly higher CS-A vs CS-B preference in CORT-treated rats compared to venlafaxine-treated rats, t(46) = 2.05, $p = 0.046$ (Figure 2.2B).

Bayesian one-sample t-tests were conducted on these data and revealed only anecdotal evidence to support the lack of CS-A vs CS-B bias in CORT-treated rats, $BF_{01} = 2.77$. Almost no evidence was found to support a lack of bias in venlafaxine-treated rats, $BF_{01} = 1.18$.

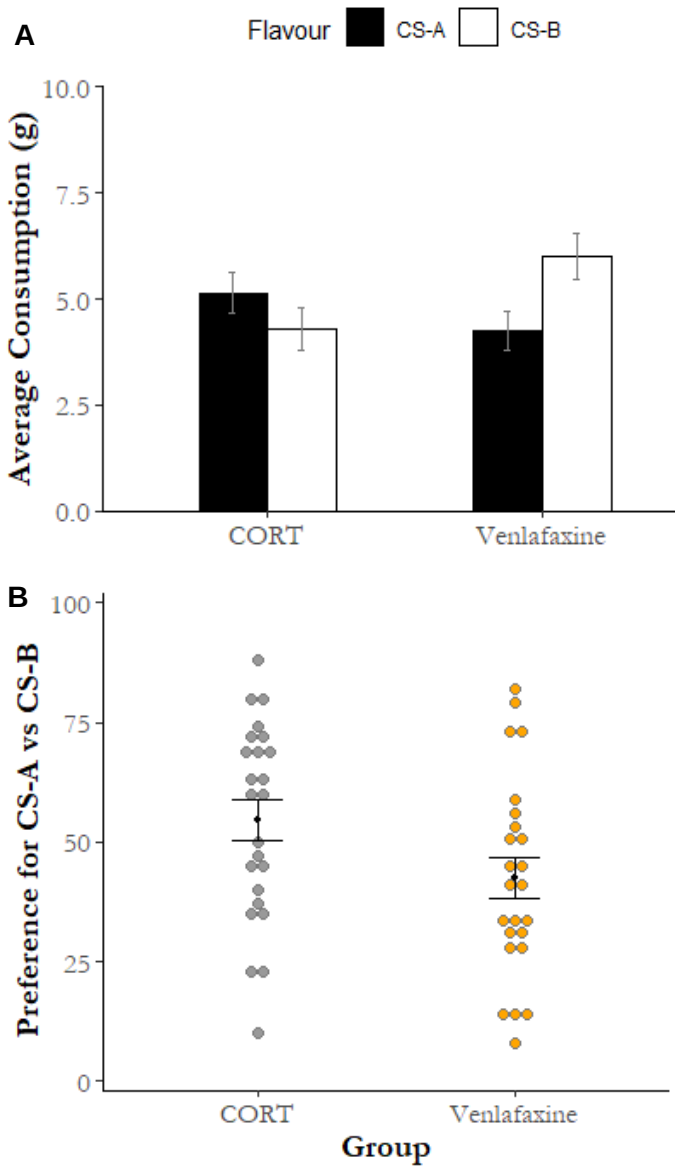


Figure 2.2. Averaged data from the CS-A vs CS-B choice test in the flavour ABT pilot, Experiment 1.
There was no difference in overall mean consumption of CS-A vs CS-B by either groups (A), and neither group showed a significant mean percentage preference for CS-A vs CS-B (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

2.2.2.2. Assessing avoidance of the unrewarded flavour

In this choice test, these rats showed a preference for consuming the novel unrewarded flavour compared to the previously unrewarded flavour, indicating they learned to avoid the unrewarded flavour in this experiment, and neither CORT nor venlafaxine appeared to disrupt this avoidance.

A repeated measures ANOVA found a main effect of flavour where consumption was significantly greater for CS-D vs CS-C, $F_{1, 46} = 13.29$, MSE = 13.35, $p = 0.001$, $\eta_p^2 = 0.22$, with no effect of group on consumption of either flavour, $F_{1, 46} = 0.31$, MSE = 3.21, $p = 0.58$, $\eta_p^2 = 0.007$. There was no interaction between flavour * group, $F_{1, 46} < 0.001$, MSE = 13.35, $p = 0.99$, $\eta_p^2 = 0.00$ (Figure 2.3A). Shapiro-Wilks test of normality revealed that consumption data for CS-C was not normally distributed for either the CORT, W(24) = 0.85, $p = 0.002$, nor venlafaxine groups, W(24) = 0.91, $p = 0.029$. However, a suitable alternative statistical test for this type of analysis was not available.

Percentage preference for CS-D vs CS-C was not normally distributed for either CORT-treated rats, W(24) = 0.86, $p = 0.003$, nor for venlafaxine-treated rats, W(24) = 0.91, $p = 0.028$. The one-sample Wilcoxon signed rank test found the median preference for CS-D vs CS-C in CORT-treated rats was significantly greater than 50%, Z = 245, $p = 0.007$. This was also true for venlafaxine-treated rats, Z = 235, $p = 0.015$, demonstrating both groups preferred CS-D over CS-C.

A Mann-Whitney U test found no significant difference between CORT and venlafaxine groups in preference for CS-D vs CS-C, U = 262, $p = 0.59$.

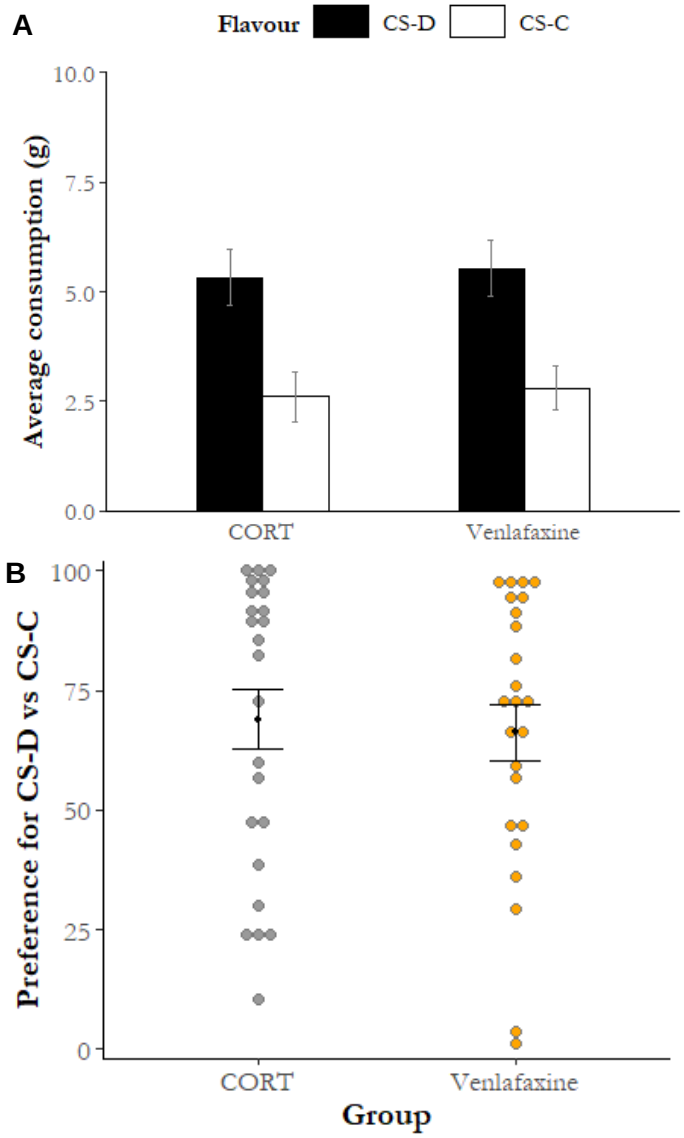


Figure 2.3. Averaged data from the CS-D vs CS-C choice test in the flavour ABT pilot. The mean consumption for both groups was significantly greater for CS-D than CS-C (A). Mean percentage preference for CS-D vs CS-C was significantly above 50% for both CORT- and venlafaxine-treated rats (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

2.2.2.3. Examining deficits in general cognitive processes

CORT-treated rats showed a significant preference for the higher valued flavour compared to the unrewarded flavour, indicating intact recall of this flavour-reward association, whereas venlafaxine-treated rats did not show this preference. In addition, both CORT-treated and venlafaxine-treated rats showed a significant preference for the lower valued flavour over the unrewarded flavour, indicating intact recall of this flavour-reward association.

Repeated measures ANOVA found a main effect of flavour where consumption was significantly greater for CS-A vs CS-C, $F_{1, 46} = 7.34$, MSE = 20.39, $p = 0.009$, $\eta_p^2 = 0.14$. There was no effect of group, $F_{1, 46} = 1.072$, MSE = 2.98, $p = 0.31$, $\eta_p^2 = 0.023$, and no interaction between flavour * group, $F_{1, 46} = 1.48$, MSE = 20.39, $p = 0.23$, $\eta_p^2 = 0.031$ (Figure 2.4A). Shapiro-Wilks test of normality revealed that consumption data for CS-C was not normally distributed for either the CORT, W(24) = 0.88, $p = 0.008$, nor venlafaxine groups, W(24) = 0.87, $p = 0.005$. However, a suitable alternative statistical test for this type of analysis was not available.

Percentage preference for CS-A vs CS-C was not normally distributed for either CORT-treated rats, W(24) = 0.87, $p = 0.005$, nor for venlafaxine-treated rats, W(24) = 0.88, $p = 0.01$. Therefore, the one-sample Wilcoxon signed rank test was conducted on these data. The one-sample Wilcoxon signed rank test found the median preference for CS-A vs CS-C in CORT-treated rats was significantly greater than 50%, Z = 241, $p = 0.009$. However, this was not significantly different to 50% for venlafaxine-treated rats, Z = 180, $p = 0.39$.

A Mann-Whitney U test found no significant difference between CORT and venlafaxine groups in preference for CS-A vs CS-C, U = 235, $p = 0.27$.

A main effect of flavour was also found where consumption was significantly greater for CS-B vs CS-C, $F_{1, 46} = 33.10$, MSE = 13.08, $p < 0.001$, $\eta_p^2 = 0.42$. There was no overall effect of group, $F_{1, 46} = 1.83$, MSE = 3.30, $p = 0.18$, $\eta_p^2 = 0.038$, and no interaction between flavour * group, $F_{1, 46} = 1.87$, MSE = 13.08, $p = 0.18$, $\eta_p^2 = 0.039$ (Figure 2.5A). Shapiro-Wilks test of normality revealed that consumption data for CS-C was not normally distributed for either the CORT, W(24) = 0.87, $p = 0.006$, nor venlafaxine groups, W(24) = 0.86, $p = 0.004$. However, a suitable alternative statistical test for this type of analysis was not available.

Percentage preference for CS-B vs CS-C was not normally distributed for either CORT-treated rats, W(24) = 0.84, $p = 0.002$, nor for venlafaxine-treated rats, W(24) = 0.83, $p = 0.001$. Therefore, the one-sample Wilcoxon signed rank test was conducted on these data. The one-sample Wilcoxon signed rank test found the median preference for CS-B vs CS-C in CORT-treated rats was significantly greater than 50%, Z = 222, $p = 0.011$. This was also true for venlafaxine-treated rats, Z = 268, $p < 0.001$.

A Mann-Whitney U test found no significant difference between CORT and venlafaxine groups in preference for CS-A vs CS-C, U = 332, p = 0.36.

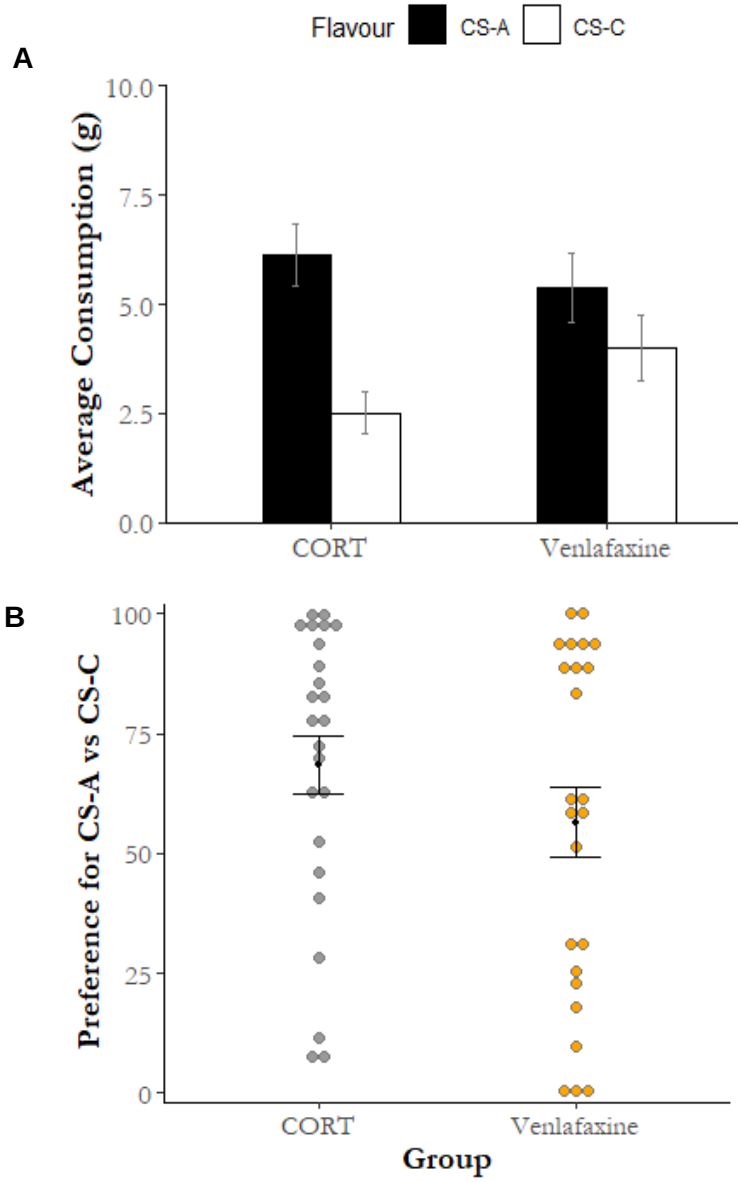


Figure 2.4. Averaged data from the CS-A vs CS-C choice test in the flavour ABT pilot.
Mean consumption of CS-A was greater than CS-C for both CORT and venlafaxine-treated groups (A). Mean percentage preference for CS-A vs CS-C was significantly above 50% for CORT-treated animals, but not for those treated with venlafaxine (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

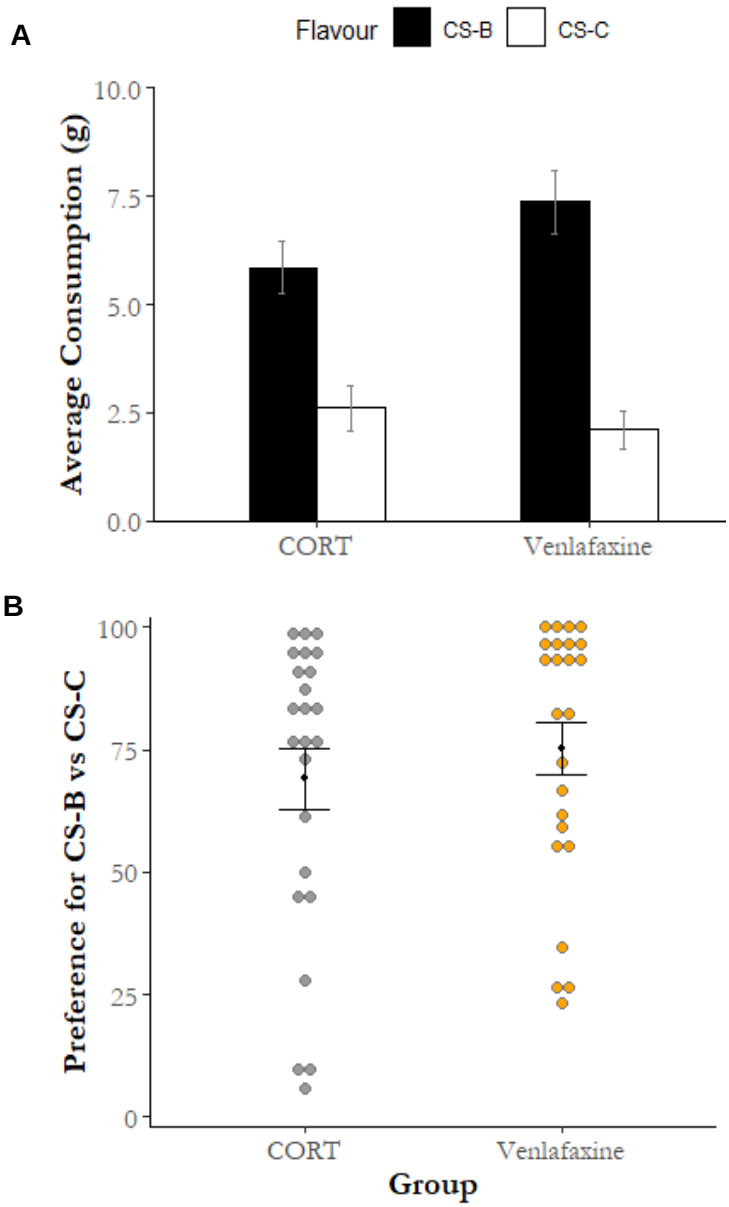


Figure 2.5. Averaged data from the CS-B vs CS-C choice test in the flavour ABT pilot. Mean consumption for both groups was greater for CS-B than CS-C, with no difference between CORT or Venlafaxine-treated rats (A). Mean percentage preference for CS-B vs CS-C was significantly above 50% in both CORT and venlafaxine groups (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

2.2.3. Summary

Experiment 1 aimed to evaluate the use of a flavour version of the ABT using an established pro-depressant, CORT, to induce a negative bias toward the flavour paired with drug administration, and an established antidepressant, venlafaxine, to induce a positive bias toward the flavour paired with drug administration.

The results from this experiment did not establish any affective biases, negative or positive, from the administration of these drugs. Bayesian analyses confirmed this lack of bias in the direction of my hypothesis. Although these results were inconclusive, they showed a trend in the opposite directions to those predicted by my hypothesis and thus showed the opposite pattern to the findings from the digging ABT in previous literature.

Unlike the digging ABT, this flavour version included additional choice tests to assess general cognitive processing of the stimuli. The findings from these tests showed that all rats had a significant preference for the novel flavour compared to the unrewarded flavour used throughout the pairing sessions, suggesting they do learn to avoid this unrewarded flavour, which may contribute to their learning about the reward-paired flavours.

These results also showed that although all rats appeared to consume more of the flavour previously paired with drug administration, only CORT-treated rats had a significant positive bias for this flavour compared to the previously unrewarded flavour. Venlafaxine-treated rats did not show a positive bias for this flavour compared to the unrewarded flavour, despite it being expected that all rats show this preference, regardless of group, to demonstrate intact learning about the flavour-reward association. Alternatively, all rats from both groups showed a significant positive bias toward the flavour previously paired with the vehicle, indicating there were no deficits in learning this reward-flavour association.

2.3. Experiment 2 – Digging vs flavour m-ABT pilot

Experiment 2 aims to evaluate the novel flavour m-ABT as an alternative assay for measuring the formation of reward-induced positive biases to address the limitations associated with the manual digging version of this task. Total consumption of a flavoured solution previously paired with a high concentration of sucrose compared with a different flavoured solution previously paired with a low concentration of sucrose was examined to establish whether preferences for the high-reward flavour would be formed, indicating a reward-induced positive bias. The digging m-ABT will also be conducted to compare against the novel flavour version, and is expected to demonstrate a preference of the animals to dig in a substrate previously paired with two reward pellets compared with a substrate previously paired with one reward pellet.

2.3.1. Methods

2.3.1.1. Subjects

48 male Sprague-Dawley rats (Charles River, UK) were used in the flavour m-ABT section of Experiment 2. 16 of these rats were then randomly selected for the digging m-ABT section. Husbandry details were as described for Experiment 1.

2.3.1.2. Apparatus

For the flavour m-ABT, the same drinking apparatus used is described previously.

For the digging m-ABT, an arena measuring 70x40x18cm with three compartments was used, described by Tait, Bowman, Neuwirth, and Brown (2018). The largest compartment, roughly measuring two-thirds of the arena, was used as a holding area. The remaining third was split into two chambers in which a glass bowl containing the digging substrate was placed. Access to these two chambers could be manually blocked by individual opaque black barriers entering through the top of the arena.

2.3.1.3. Flavour m-ABT design

All solutions used during the flavour m-ABT consisted of distilled water, 0.05% Kool-Aid and 0.1% saccharin. The Kool-Aid flavours used in this study included Grape, Tangerine, Cherry, and Tamarind. CS-A^{++} and CS-B^{+} solutions additionally contained a specified concentration of sucrose during pairing sessions only.

For all flavour m-ABT experiments, the reward-paired flavours (CS-A^{++} and CS-B^{+}) and unrewarded flavours (CS-C and CS-D) were counterbalanced between Grape/Cherry or Tangerine/Tamarind, such that solutions were presented equally often in left/right bottles over training.

All rats were first pre-trained to consume 0.1% saccharin with and without sucrose from the drinking apparatus, as described previously.

Following pre-training, all rats received four days of 10-minute pairing sessions. One session consisted of a two-bottle choice test between a flavour containing a high concentration of sucrose, CS-A^{++}, vs a different flavour without added sucrose, CS-C. Another session consisted of a two-bottle choice test between a separate flavour containing a low concentration of sucrose, CS-B^{+}, vs CS-C. Rats were split into four training conditions (n=12 per condition) to evaluate the optimal concentrations for running this flavour m-ABT (see Table 2.1), in which the sucrose concentrations of CS-A^{++} and CS-B^{+} varied, as well as the number of pairing sessions within one day. Rats that received two pairing sessions per day would receive their first session in the morning, then their second session at least 4 hours later.

Table 2.1. Training conditions for the flavour m-ABT. Table showing counterbalanced sucrose concentrations and number of training sessions per day for Experiment 2.

Group	CS-A^{++} (High reward)	CS-B^{+} (Low reward)	Number of pairing sessions per day
1	15%	5%	2
2	16%	8%	2
3	15%	5%	1
4	16%	8%	1

Following the pairing sessions, all rats were given four choice test sessions for 10-minutes each. Firstly, they received a two-bottle test between CS-A^{++} and CS-B^{+}, without added sucrose, which was followed by a counterbalance between CS-A^{++} vs CS-C, CS-B^{+} vs CS-C, and CS-C vs CS-D, in which D was a novel flavour only introduced in this session (see Figure 2.6).

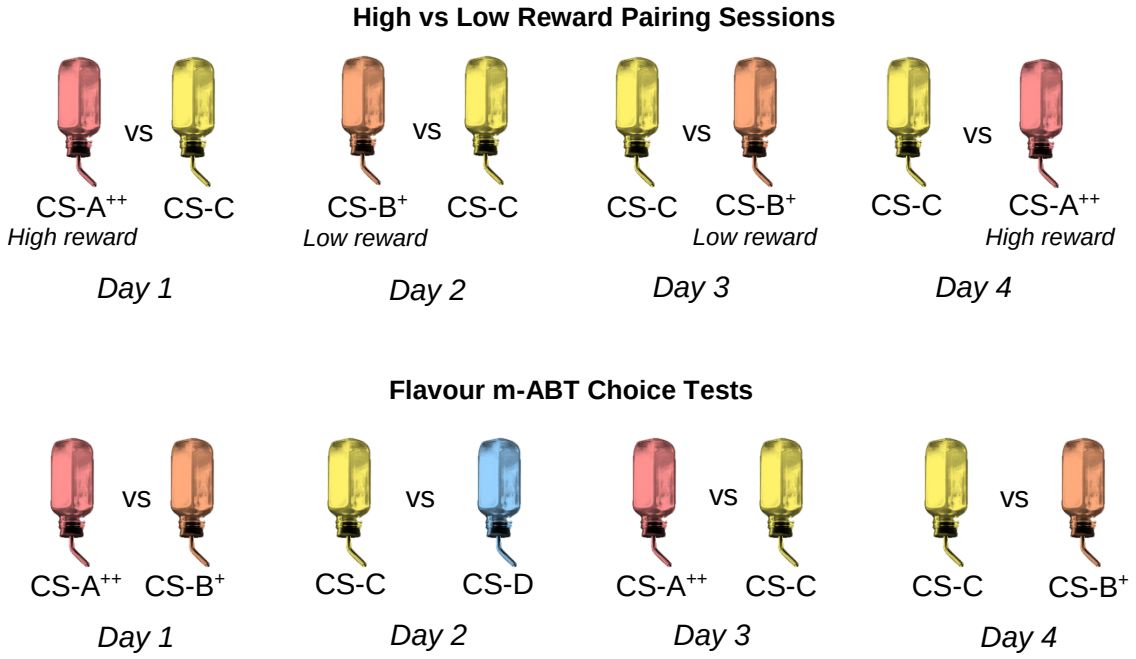


Figure 2.6. A schematic diagram of the flavour m-ABT design, with example counterbalance order. All rats were given four days of pairing sessions with CS-A^{++} containing a high concentration of sucrose, or CS-B^{+} containing a low concentration of sucrose, vs an unrewarded flavour solution, CS-C. This was followed by four choice tests, without any added sucrose, to assess reward-induced learning and memory.

2.3.1.4. Digging m-ABT

All digging substrates used in this experiment consisted of sawdust for training purposes only, then shredded cloth, cotton balls, hairbands, or pipe cleaners, counterbalanced across all animals. The design for the digging m-ABT follows that described in Stuart et al. (2013).

One week following the flavour m-ABT, 16 rats from the flavour m-ABT cohort underwent two days of pre-training for the digging m-ABT. In this, rats were placed in the digging arena for 10 minutes in pairs, with two bowls filled with pellets easily accessible in the open chambers.

On the third day of pre-training, rats were placed in the arena individually for 10 minutes with two full bowls, then on day four only two pellets were placed in each bowl and the rat was placed in the arena for up to 30 seconds or until all pellets had been consumed, repeated for 12 trials.

Following pre-training, all rats were trained to dig for their sucrose pellet reward. On day one of digging training, one pellet was placed in one of three locations in one bowl and covered with 1cm of sawdust. This was repeated with both bowls for 12 trials. On days two

and three, 2cm of sawdust was used in this same manner but when the reward had been located, access to the other bowl was blocked with a barrier.

Once all rats were successfully able to dig for their rewards, they were given a discrimination task to ensure they had the ability to discriminate between the substrates used. 2cm of one of two substrates was placed into each bowl, and only one was rewarded. The animal was first allowed to explore both bowls but once they began to dig in one substrate, access to the other substrate was blocked. This was repeated until the animal chose the rewarded substrate in 6 consecutive trials.

After all rats showed clear abilities to discriminate between substrates, they received four daily pairing sessions, in which one day they were given the choice to dig in one substrate containing two reward pellets (CS-2) vs a different substrate with no pellets (CS-0), then another day they were given a new substrate containing one reward pellet (CS-1) vs CS-0. This was repeated for two pairing sessions per rewarded substrate.

Following pairing sessions, all rats received a choice test between CS-2 vs CS-1, where bowls were rewarded using a random reinforcement protocol in which each substrate had a 1 in 3 probability of containing the reward. On subsequent days, they received a counterbalanced order of choice tests between CS-2 vs CS-0, CS-1 vs CS-0, and CS-N vs CS-0, in which CS-N was a novel substrate introduced only in this choice test.

Throughout pairing sessions and choice tests, once the rat had made a choice of which substrate to dig, access to the other bowl was blocked.

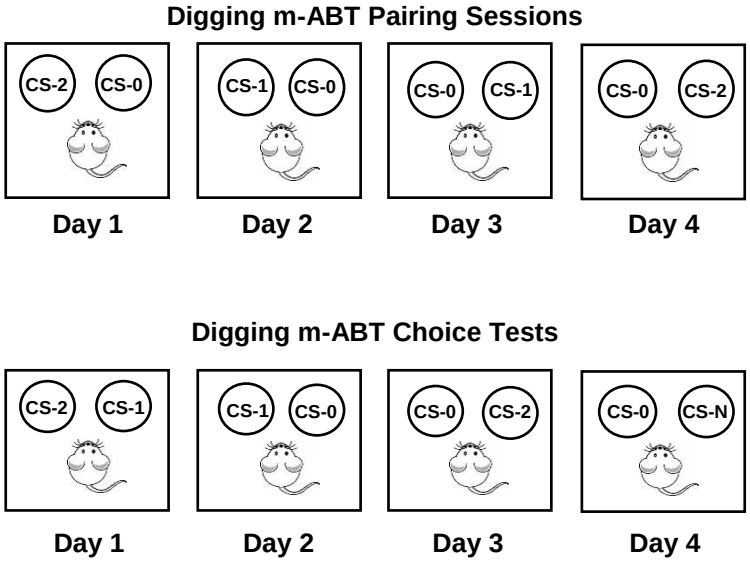


Figure 2.7. A schematic diagram of the digging m-ABT design, with example counterbalance order.
All rats were given four days of pairing sessions between substrate, CS-2, containing two reward pellets, or CS-1 containing one reward pellet, vs an unrewarded substrate, CS-0. This was followed by four choice tests, with a random reinforcement order, to assess learning and memory. CS-N refers to a novel substrate introduced only in one choice test.

2.3.1.5. Data Analysis

Consumption data was analysed, and removal of animals based on side biases, as described in section 2.2.1.5. In this experiment no animal was removed from analysis. For the flavour m-ABT, repeated measures ANOVAs were used to compare fixed factors of 'training length' and 'sucrose concentration' with overall consumption of each flavour in the choice tests. Univariate ANOVAs were used to compare these same fixed factors against percentage preferences.

For the digging m-ABT, the total number of choices for each substrate in the choice tests was recorded and a preference percentage was calculated. One-sample t-tests with 50% as the test value were analysed.

Bayes analysis was conducted as described in section 2.2.1.6.

2.3.2. Results

2.3.2.1. Measuring reward-induced positive bias using the flavour m-ABT

Overall, these rats did form a positive bias for the flavour previously paired with a high value reward vs the flavour previously paired with a low value reward, regardless of the amount of training received. The sucrose concentrations used for the high vs low rewards also did not influence this preference, though evidence for this was inconclusive and overall consumption was greater in the 15 vs 5% group.

A repeated measures ANOVA found a main effect of flavour where mean consumption was significantly greater for CS-A^{++} compared to CS-B^{+} in the choice test (Figure 2.8A), $F_{1, 44} = 20.37$, MSE = 8.55, $p < 0.001$, $\eta_p^2 = 0.32$. There was no interaction between flavour * training length, $F_{1, 44} = 0.48$, MSE = 8.55, $p = 0.49$, $\eta_p^2 = 0.011$, between flavour * sucrose concentration, $F_{1, 44} = 1.99$, MSE = 8.55, $p = 0.17$, $\eta_p^2 = 0.043$, nor between flavour * sucrose concentration * training length, $F_{1, 44} = 0.061$, MSE = 8.55, $p = 0.81$, $\eta_p^2 = 0.001$.

There was no overall effect of training length on consumption, $F_{1, 44} = 2.11$, MSE = 2.19, $p = 0.15$, $\eta_p^2 = 0.046$. However, there was a significant effect of sucrose concentration, $F_{1, 44} = 14.50$, MSE = 2.19, $p < 0.001$, $\eta_p^2 = 0.25$, where overall rats appeared to consume more when previously exposed to 15% vs 5% compared to 16% vs 8% sucrose.

A univariate ANOVA comparing percentage preferences for CS-A^{++} vs CS-B^{+} found no overall effect of training length, $F_{1, 44} = 1.24$, MSE = 0.13, $p = 0.27$, $\eta_p^2 = 0.027$, nor sucrose concentration, $F_{1, 44} = 0.39$, MSE = 0.13, $p = 0.39$, $\eta_p^2 = 0.017$, on percentage preference (Figure 2.8B). There was also no interaction between training length * sucrose concentration, $F_{1, 44} = 0.41$, MSE = 0.13, $p = 0.53$, $\eta_p^2 = 0.009$. Shapiro-Wilks test of normality revealed that percentage preference for CS-A^{++} vs CS-B^{+} was not normally distributed for rats in the group trained with short training length and 16% vs 8% sucrose, $W(12) = 0.86$, $p = 0.047$. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA found only anecdotal evidence for no effect of sucrose concentration on preferences, $BF_{01} = 2.78$, and moderate evidence for no effect of training length, $BF_{01} = 3.29$. Strong evidence was found to support no interaction between concentration * training length, $BF_{01} = 10.16$.

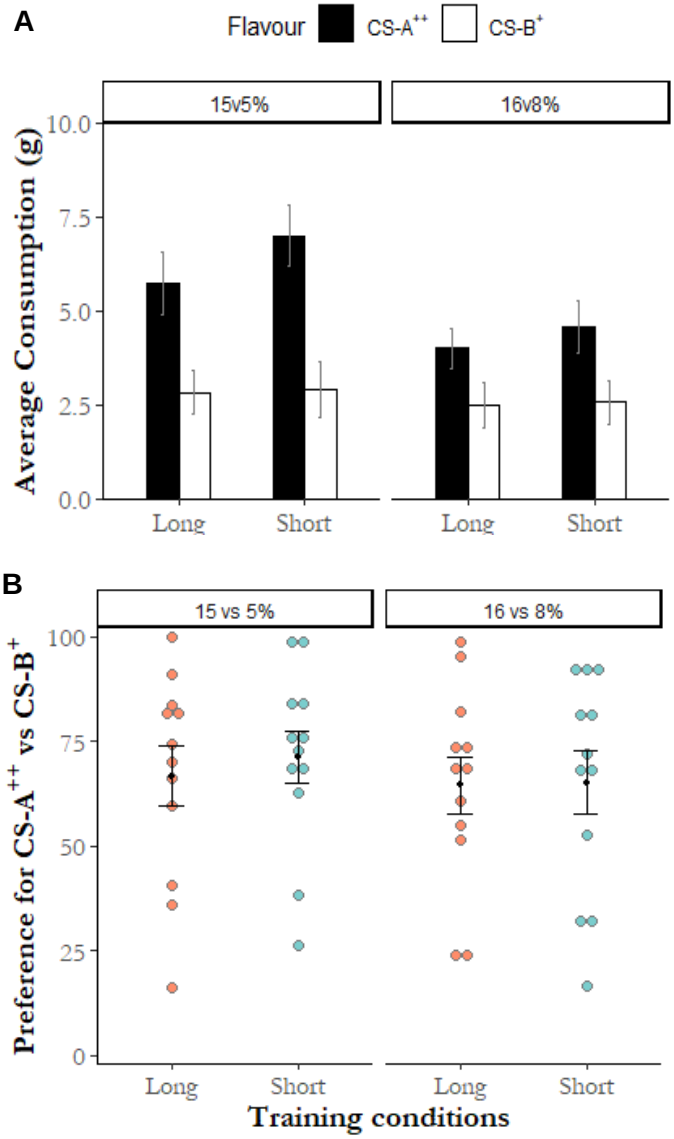


Figure 2.8. Averaged data from the CS-A^{++} vs CS-B^{+} choice test in the flavour m-ABT pilot. Graphs show the mean consumption of both CS-A^{++} and CS-B^{+} (A) and mean percentage preferences for CS-A^{++} vs CS-B^{+} rounded to the nearest whole number (B) in rats receiving either one (short) or two (long) training sessions per day, plus either 15 vs 5% sucrose or 16 vs 8% sucrose as the reward.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

2.3.2.2. Assessing flavour avoidance in the flavour m-ABT

These rats did not show a preference for consuming the novel unrewarded flavour compared with the familiar unrewarded flavour and thus did not show avoidance of the familiar unrewarded flavour, and this lack of avoidance was not influenced by training conditions, however the latter was found to be inconclusive following Bayes analysis.

A repeated measures ANOVA revealed no overall bias for consumption of CS-D compared to consumption of CS-C (Figure 2.9A), $F_{1, 44} = 0.32$, MSE = 19.72, $p = 0.58$, $\eta_p^2 = 0.007$. There was no interaction between flavour * training length, $F_{1, 44} = 0.75$, MSE = 19.72, $p = 0.39$, $\eta_p^2 = 0.017$, between flavour * sucrose concentration, $F_{1, 44} = 0.42$, MSE = 19.72, $p = 0.52$, $\eta_p^2 = 0.009$, nor between flavour * training length * sucrose concentration, $F_{1, 44} = 0.53$, MSE = 19.72, $p = 0.47$, $\eta_p^2 = 0.012$.

No overall effect of sucrose concentration on consumption was found, $F_{1,44} = 0.04$, MSE = 2.84, $p = 0.84$, $\eta_p^2 = 0.001$. However, there was a significant effect of training length, $F_{1, 44} = 4.95$, MSE = 2.84, $p = 0.031$, $\eta_p^2 = 0.101$, in which those animals that received only one session per day consumed significantly greater overall compared to those that received two sessions per day. There was no overall interaction between training length and sucrose concentration, $F_{1,44} = 0.016$, MSE = 2.84, $p = 0.90$, $\eta_p^2 < 0.001$. Shapiro-Wilks test of normality found that consumption data for CS-D was not normally distributed in the group with short training length and 16% vs 8% sucrose, W(12) = 0.86, $p = 0.044$. Consumption data for CS-C was also not normally distributed for the group with the long training length and 15% vs 5% sucrose, W(12) = 0.84, $p = 0.026$. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA comparing percentage preferences for CS-D vs CS-C found no overall effect of training length, $F_{1, 44} = 1.24$, MSE = 0.13, $p = 0.27$, $\eta_p^2 = 0.027$, nor sucrose concentration, $F_{1, 44} = 0.77$, MSE = 0.13, $p = 0.39$, $\eta_p^2 = 0.017$, on percentage preference (Figure 2.9B). There was also no interaction between training length * sucrose concentration, $F_{1, 44} = 0.41$, MSE = 0.13, $p = 0.53$, $\eta_p^2 = 0.009$. Shapiro-Wilks test of normality found that percentage preference for CS-D vs CS-C was not normally distributed in the group with long training length and 15% vs 5% sucrose, W(12) = 0.85, $p = 0.039$, nor the group with short training length and 16% vs 8% sucrose, W(12) = 0.85, $p = 0.035$. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA found only anecdotal evidence to support the lack of effect of sucrose concentration, $BF_{01} = 2.54$, or training length, $BF_{01} = 2.08$, on preference for CS-D. Moderate evidence was found to support the lack of interaction between concentration * training length, $BF_{01} = 6.28$. A Bayesian one-sample t-test found moderate evidence to support the lack of overall preference for CS-D, $BF_{01} = 4.99$.

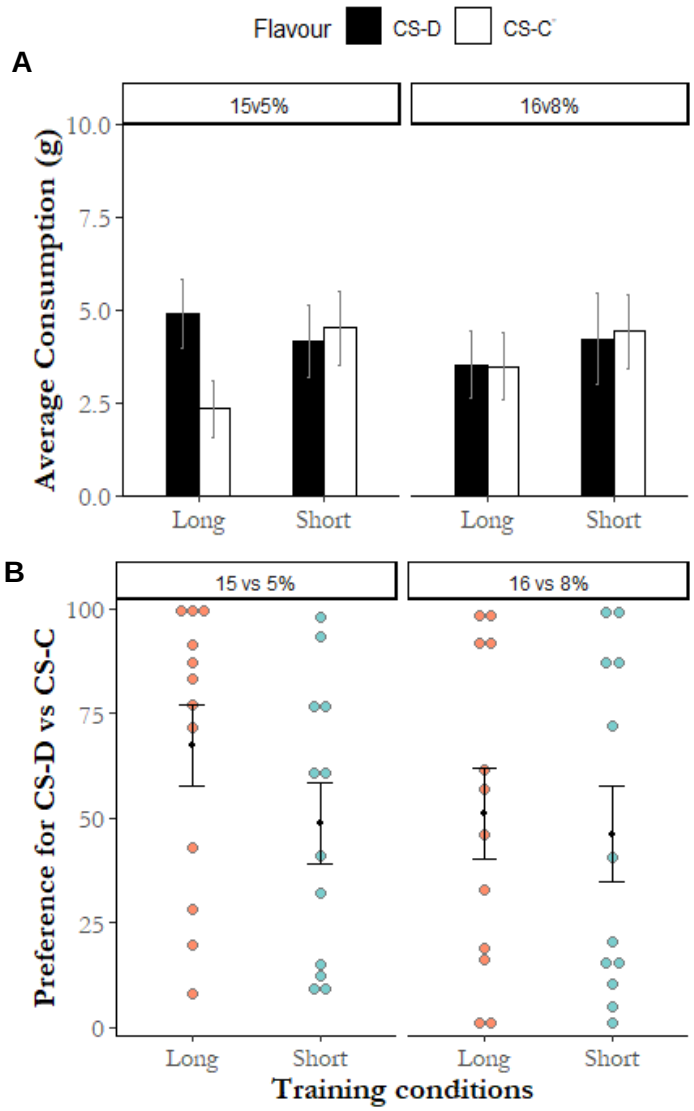


Figure 2.9. Averaged data from the CS-D vs CS-C choice test in the flavour m-ABT pilot. Graphs show the mean consumption of both CS-D and CS-C (A) and mean percentage preferences for CS-D vs CS-C rounded to the nearest whole number (B) in rats receiving either one (short) or two (long) training sessions per day, plus either 15 vs 5% sucrose or 16 vs 8% sucrose as the reward.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

2.3.2.3. Assessing deficits in general cognitive processes

A mean preference for consumption of both the high and low reward-paired flavour compared to the previously unrewarded flavour was found in this experiment, indicating these rats had intact recall of these flavour-reward associations. The number of training sessions received did not appear to influence this learning, but the chosen sucrose concentrations did influence learning the flavour-high reward association with higher preferences seen with a greater difference between the high and low reward.

A repeated measures ANOVA showed a main effect of flavour where consumption was significantly greater for CS-A^{++} compared to CS-C, $F_{1, 44} = 70.21$, MSE = 4.65, $p < 0.001$, $\eta_p^2 = 0.62$. There was no overall effect of training length, $F_{1, 44} = 1.65$, MSE = 6.90, $p = 0.21$, $\eta_p^2 = 0.036$, nor sucrose concentration, $F_{1, 44} = 0.11$, MSE = 6.90, $p = 0.74$, $\eta_p^2 = 0.002$, on overall consumption (Figure 2.10A).

There was no interaction between flavour * training length, $F_{1, 44} = 0.14$, MSE = 4.65, $p = 0.71$, $\eta_p^2 = 0.003$, between flavour * training length * sucrose concentration, $F_{1, 44} = 0.11$, MSE = 4.65, $p = 0.74$, $\eta_p^2 = 0.003$, nor between training length * sucrose concentration, $F_{1, 44} = 0.47$, MSE = 3.26, $p = 0.50$, $\eta_p^2 = 0.011$. However, there was a significant interaction between flavour * sucrose concentration, $F_{1, 44} = 17.06$, MSE = 4.65, $p < 0.001$, $\eta_p^2 = 0.28$. Fishers LSD post-hoc analysis revealed consumption of CS-A^{++} was highest for the 15 vs 5% condition ($p = 0.007$), whilst consumption for CS-C was highest in the 16 vs 8% condition ($p = 0.02$). Shapiro-Wilks test of normality found that percentage preference for CS-A^{++} vs CS-C was not normally distributed in the group with long training length and 15% vs 5% sucrose, W(12) = 0.52, $p < 0.001$, nor the group with short training length and 15% vs 5% sucrose, W(12) = 0.45, $p < 0.001$, nor the group with short training length and 16% vs 8% sucrose, W(12) = 0.86, $p = 0.046$. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA revealed no significant effect of training length on percentage preference for CS-A^{++} vs CS-C, $F_{1, 44} = 0.069$, MSE = 0.048, $p = 0.79$, $\eta_p^2 = 0.002$. There was a significant effect of sucrose concentration on preference, $F_{1, 44} = 10.80$, MSE = 0.048, $p = 0.002$, $\eta_p^2 = 0.20$, with a higher preference in the 15 vs 5% condition compared to 16 vs 8%. There was no interaction between training length * sucrose concentration on percentage preferences, $F_{1, 44} = 1.11$, MSE = 0.048, $p = 0.29$, $\eta_p^2 = 0.025$ (Figure 2.10B). Shapiro-Wilks test of normality found that percentage preference for CS-A^{++} vs CS-C was not normally distributed in the group with long training length and 15% vs 5% sucrose, W(12) = 0.48, $p < 0.001$, nor the group with short training length and 16% vs 8% sucrose, W(12) = 0.49, $p < 0.001$, nor the group with short training length and 15% vs 5% sucrose, W(12) =

0.85, p = 0.033. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA found moderate evidence to support the lack of effect of training length on preference for CS-A^{++} vs CS-C, BF_{01} = 3.40, and anecdotal evidence was found to support the lack of interaction between sucrose concentration * training length, BF_{01} = 2.07.

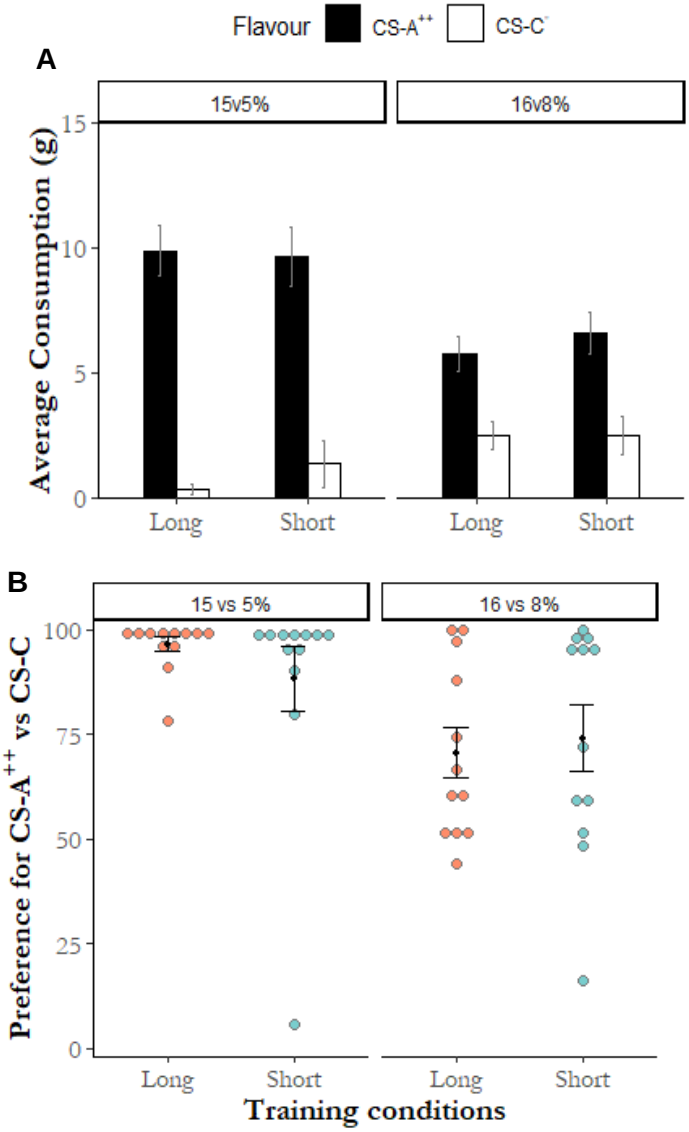


Figure 2.10. Averaged data from the CS-A^{++} vs CS-C choice test in the flavour m-ABT pilot. Graphs show the mean consumption of both CS-A^{++} and CS-C (A) and mean percentage preferences for CS-A^{++} vs CS-C rounded to the nearest whole number (B) in rats receiving either one (short) or two (long) training sessions per day, plus either 15 vs 5% sucrose or 16 vs 8% sucrose as the reward.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

Furthermore, a repeated measures ANOVA revealed a main effect of flavour where consumption was significantly greater for CS-B$^+$ compared to CS-C, $F_{1, 44}$ = 10.65, MSE = 2.84, p = 0.002, η_p^2 = 0.20. There was no overall effect of training length, $F_{1, 44}$ = 0.66, MSE = 6.24, p = 0.42, η_p^2 = 0.015, nor sucrose concentration, $F_{1,44}$ = 0.012, MSE = 6.24, p = 0.91, η_p^2 <0.001, on consumption (Figure 2.11A).

There was no interaction between training length * sucrose concentration, $F_{1, 44}$ = 0.48, MSE = 6.24, p = 0.49, η_p^2 = 0.011, flavour * training length, $F_{1, 44}$ = 1.28, MSE = 2.84, p = 0.26, η_p^2 = 0.028, flavour * sucrose concentration, $F_{1, 44}$ = 1.12, MSE = 2.84, p = 0.29, η_p^2 = 0.025, nor between flavour * training length * sucrose concentration, $F_{1, 44}$ = 0.65, MSE = 2.84, p = 0.43, η_p^2 = 0.015. Shapiro-Wilks test of normality found the consumption data for CS-B$^+$ was not normally distributed in the group with the long training length and 15% vs 5% sucrose, W(12) = 0.84, p = 0.027. Consumption of CS-C was also not normally distributed in this group, W(12) = 0.55, p <0.001, nor the group with long training length and 16% vs 8% sucrose, W(12) = 0.77, p = 0.004, the group with short training length and 15% vs 5% sucrose, W(12) = 0.79, p = 0.008, nor the group with short training length and 16% vs 8% sucrose, W(12) = 0.82, p = 0.016. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA revealed no overall effect of training length, $F_{1, 44}$ = 1.30, MSE = 0.052, p = 0.26, η_p^2 = 0.029, nor sucrose concentration, $F_{1, 44}$ = 1.94, MSE = 0.052, p = 0.17, η_p^2 = 0.042, on percentage preference for CS-B$^+$ vs CS-C (Figure 2.11B). Further, there was no interaction between training length * sucrose concentration, $F_{1, 44}$ = 1.03, MSE = 0.052, p = 0.32, η_p^2 = 0.023. Shapiro-Wilks test of normality found that percentage preference for CS-B$^+$ vs CS-C was not normally distributed in the group with long training length and 15% vs 5% sucrose, W(12) = 0.54, p <0.001, nor the group with long training length and 16% vs 8% sucrose, W(12) = 0.73, p = 0.001, nor the group with short training length and 15% vs 5% sucrose, W(12) = 0.81, p = 0.013. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA found only anecdotal evidence to support a lack of effect of sucrose concentration, BF_{01}= 1.60, and training length, BF_{01} = 2.07, on this preference. Moderate evidence was found to support the lack of interaction between concentration * training length, BF_{01} = 3.51.

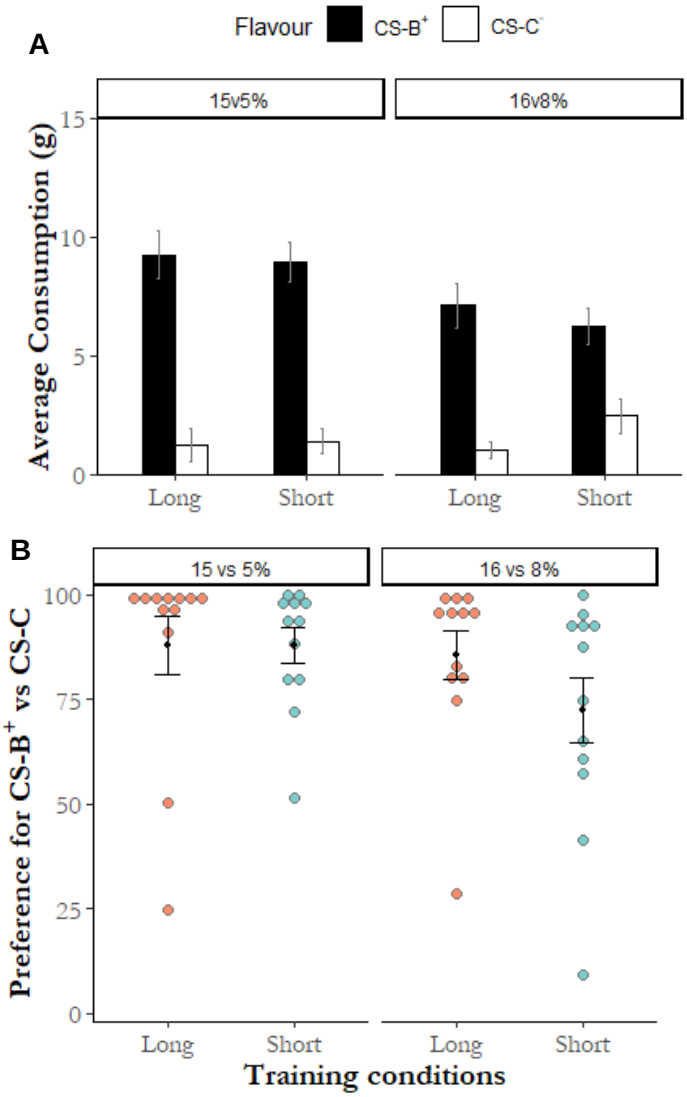


Figure 2.11. Averaged data from the CS-B⁺ vs CS-C choice test in the flavour m-ABT pilot. Graphs show the mean consumption of both CS-B⁺ and CS-C (A) and mean percentage preferences for CS-B⁺ vs CS-C rounded to the nearest whole number (B) in rats receiving either one (short) or two (long) training sessions per day, plus either 15 vs 5% sucrose or 16 vs 8% sucrose as the reward.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

2.3.2.4. Measuring reward-induced positive bias using the digging m-ABT

In the digging m-ABT, these rats did not form a reward-induced positive bias for the higher value reward-paired substrate, however this finding was inconclusive. In the additional choice tests, the rats were able form and recall the learned reward-substrate associations and also demonstrated avoidance of the familiar unrewarded stimulus compared to the novel unrewarded stimulus.

A one-sample t-test revealed there was no significant bias for CS-2 vs CS-1 in the digging choice test, $t(15) = 1.21$, $p = 0.24$, indicating a lack of reward-induced positive bias formed for the higher valued substrate during the digging m-ABT (Figure 2.12A).

Further one-sample t-tests found a significant positive bias for CS-N vs CS-0, $t(15) = 2.72$, $p = 0.016$ (Figure 2.12B), CS-2 vs CS-0, $t(15) = 2.85$, $p = 0.012$ (Figure 2.12C), and CS-1 vs CS-0, $t(15) = 2.73$, $p = 0.016$ (Figure 2.12D).

A Bayesian one-sample t-test found only anecdotal evidence to support the lack of bias in the CS-2 vs CS-1 choice test, $BF_{01} = 2.09$.

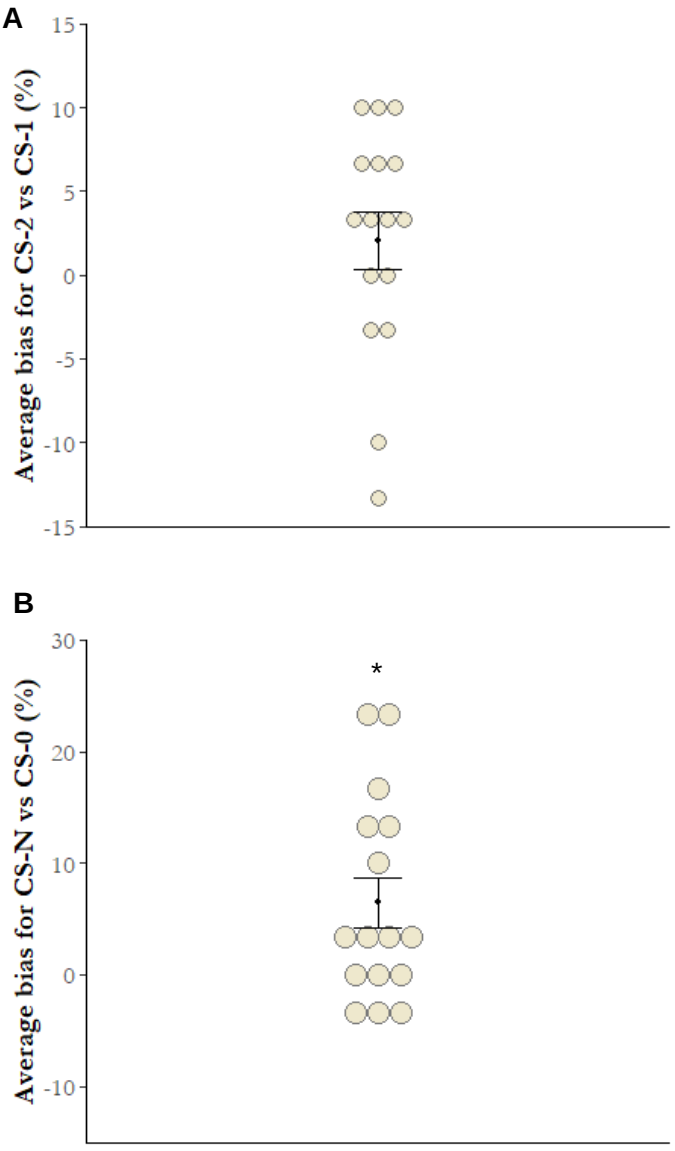


Figure 2.12. Average choice bias for the CS-2 vs CS-1, and CS-N vs CS-0 choice tests in the digging m-ABT.
There was no significant bias in the CS-2 vs CS-1 choice test (A), whereas a significant positive bias was found for CS-N vs CS-0 (B). Average bias represents the mean percentage above or below 50%, indicating a bias toward one substrate from the choice tests.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points. * = p <0.05.

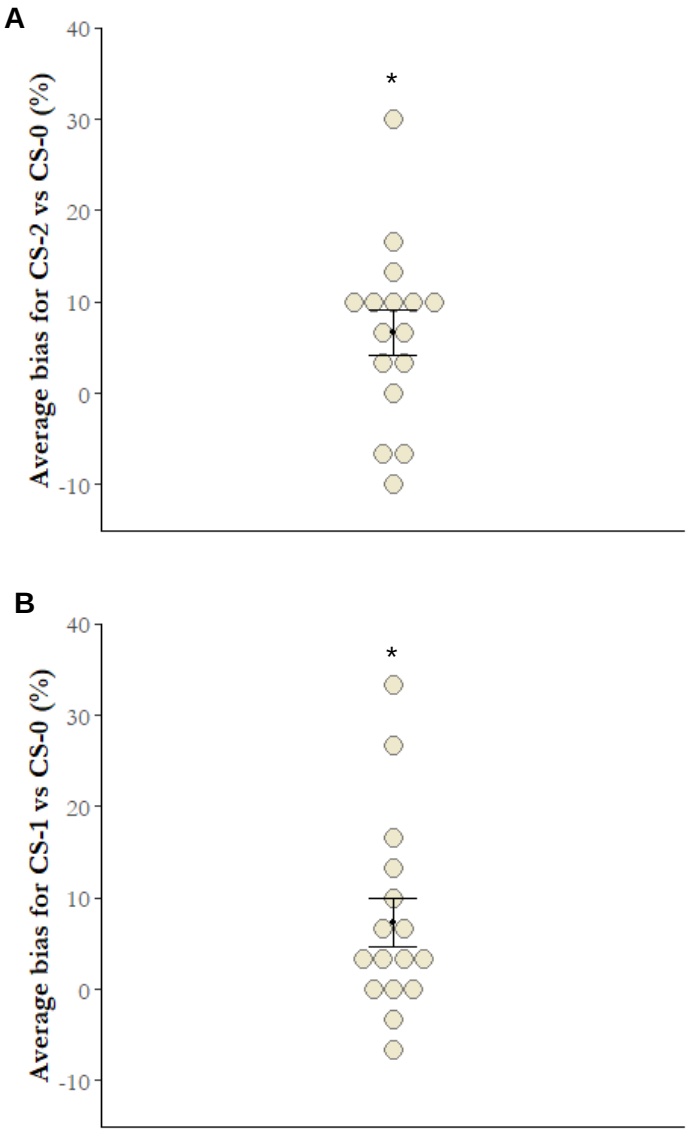


Figure 2.12. Average choice bias for the CS-2 vs CS-0, and CS-1 vs CS-0 choice tests in the digging m-ABT.
A significant positive bias was found for CS-2 vs CS-0 (A) and for CS-1 vs CS-0 (B). Average bias represents the percentage above or below 50%, indicating a bias toward one substrate from the choice tests.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points. * = p <0.05.

2.3.3. Summary

Experiment 2 aimed to evaluate a novel flavour version of the m-ABT in healthy rats and determine the optimal training conditions for this assay. Further, this experiment aimed to compare the flavour m-ABT against a pilot of the traditional digging m-ABT to investigate whether this digging version can be reproduced in an alternative facility to the one it was developed, and whether the flavour m-ABT shows similar findings.

These results showed that firstly, the design of a flavour m-ABT was able to measure positive biases for a flavour previously paired with a high reward compared to that previously paired with a low reward, as can be seen from the digging m-ABT in previous research (Stuart et al., 2013). Additional choice tests were utilized in the development of this flavour m-ABT, which in turn clearly showed that all rats could recall the general associations between the conditioned stimuli and the reward, indicated by positive biases toward the flavour paired previously with either a high or a low reward compared with the flavour previously paired with no reward. In addition, the choice test comparing consumption of a completely novel flavour against the previously unrewarded flavour found no general avoidance of the unrewarded stimulus.

To evaluate the optimal training conditions for the development of this assay, two different reward concentration ratios were used (3:1 as 15 vs 5% sucrose and 2:1 as 16 vs 8% sucrose) as well as two training lengths (either one or two training sessions per day). The results from Experiment 2 indicate that a 3:1 ratio increased general consumption of both flavours in the main choice test but had no impact on percentage preference. Training length had no impact on either consumption or percentage preference.

Furthermore, consumption of the flavour previously paired with a high reward compared to the unrewarded flavour was higher in those rats who received a 3:1 ratio during training; suggesting learning a positive bias in these groups was greater than those who received a 2:1 ratio.

Finally, the digging m-ABT was conducted in 16 randomly selected rats from the original cohort. The results from this showed that none of these rats appeared to have a positive bias for the digging substrate previously paired with two reward pellets compared to one. Despite this, all rats showed a positive bias for the either the two pellet-paired substrate or the one pellet-paired substrate when given the choice against the unrewarded substrate, showing recall of the association between the reward and substrate were intact. They also showed a positive bias for a novel substrate compared with the unrewarded substrate, indicating avoidance of the unrewarded substrate.

2.4. Discussion of Chapter 2

This chapter evaluated two novel flavour-preference assays aiming to measure either positive or negative affective biases in response to acute pharmacological intervention, Experiment 1, or measure reward-induced positive biases to a flavour associated with a higher reward value in Experiment 2.

2.4.1. Flavour ABT

The flavour ABT was not able to replicate negative and positive biases seen following association of a rewarding stimulus with CORT or venlafaxine, respectively. In fact, rats administered with venlafaxine appeared to consume overall more of the flavour paired with a vehicle compared to the flavour paired with venlafaxine, indicating a negative bias, which was unexpected. However, preference percentage for this flavour was not significant and Bayesian analysis suggested no firm conclusions could be made from these findings.

Given that these two drugs have been tested in this same acute dose procedure in the original ABT (Hinchcliffe et al., 2017; Stuart et al., 2013, 2015; Stuart et al., 2017), a lack of effect in the flavour ABT might suggest a lack of sensitivity in this assay to be able to detect affective biases at acute doses. Since the digging ABT uses reward pellets with various dietary components, using pure sucrose solutions in my flavour ABT might result in a different level of reinforcement for learning, which in turn makes a dissociation between two rewarded flavours associated with different affective states more challenging. Evidence suggests flavour preferences can be altered depending on nutritional values (e.g. Ackroff, 2008) and that sucrose is often less preferred to the glucose polymer, Polycose (e.g. Ackroff & Sclafani, 1991). However, it is also shown that sucrose is stronger than fat, fructose or ethanol alone at forming flavour preferences (e.g. Ackroff & Sclafani, 2004; Lucas, Ackroff, & Sclafani, 1998), though flavours paired with a combined liquid diet with high fat content elicited a stronger preference compared to the same liquid diet containing a high carbohydrate content (Lucas et al., 1998). These findings suggest that although the hypothesised changes in affective bias were not produced in my flavour ABT, sucrose should still be a strong reinforcer for this, though potentially not as reinforcing as a combined nutritional dietary solution.

If a lack of affective bias were a result of weak reinforcement, the additional choice tests would indicate this through a lack of preference for the previously rewarded flavours (CS^+) against the previously unrewarded flavour (CS). The findings in the flavour ABT showed that although general consumption of the CS^+ given alongside drug pre-treatment was higher than the CS, a significant preference for the CS^+ was only seen in the CORT-treated

group, not the venlafaxine-treated group. Furthermore, preference for the CS^+ given alongside vehicle pre-treatment was significantly greater than the CS in both groups.

These findings could indicate that acute venlafaxine might disrupt short-term memory processes, however, the effects of venlafaxine on learning and memory has been reported on numerous occasions to induce either a positive effect on learning with the ability to actually reverse deficits in rodent depression models, or had no effect at all (see Pehrson et al., 2015). In addition, a Bayesian analysis was performed on this lack of CS^+ preference in the venlafaxine group, which demonstrated weak support, and thus indicates this finding is inconclusive.

Results from the additional choice tests also showed an overall preference for the novel flavour when presented alongside the CS, despite the novel flavour also not being paired with a reinforcer. This suggests these rats learned to avoid the unrewarded CS from the pairing sessions, which may explain the lack of bias between the two rewarded CS' if their learning was focused on the unrewarded stimulus instead. However, given the lack of affective bias, this cannot add further explanation to the mechanisms underpinning affective bias deficits. Furthermore, the digging m-ABT in Experiment 2 also found avoidance of the unrewarded substrate, indicating that this finding does not explain the differences seen between the flavour and digging ABT.

2.4.2. Flavour m-ABT

The flavour m-ABT demonstrated that flavour preferences can be influenced by the absolute value of reward during CS-US learning, as indicated by a clear positive bias for the flavour previously paired with a higher value reward (CS^{++}) compared to a flavour previously paired with a lower value reward (CS^+), thus a flavour version of the m-ABT could be used to measure reward-induced biases between two differentially valued CS.

To evaluate the design of this flavour m-ABT, I compared several parameters of the CS-US pairing sessions. These findings show that reward-induced positive biases, as measured by preference for CS^{++} vs CS^+, was not influenced by either the number of pairing sessions in a day, or the ratio between sucrose concentrations used during pairing. The digging m-ABT uses a 2:1 ratio for reward pellets (Stuart et al., 2013), however, as discussed in section 2.5.1, there is potential for any flavour based assay using sucrose reinforcement to be less sensitive than the digging version due to only one nutrient present in the reward. Thus, to increase sensitivity of this flavour m-ABT, the optimal reward ratio for future flavour m-ABT work is suggested as 3:1 (e.g., 15 vs 5%). Furthermore, to maintain this task as a less laborious version of the digging m-ABT and to match the digging version as much as

possible, the optimal training length for future flavour m-ABT work was suggested as one pairing session per day, for four days.

I also explored the mechanisms of CS-US learning in this flavour m-ABT through the addition of the same choice tests used in the flavour ABT. These tests found clear preferences for CS^{++} or CS^{+} vs the unrewarded flavour, confirming the animals were able to successfully learn and recall these flavour-reward associations.

However, unlike the flavour ABT, I found no preference for a novel unrewarded flavour when presented alongside the flavour associated with no reward during pairing sessions. This would suggest that learning CS-US associations might occur prior to flavour avoidance learning, but particularly when the reward value is not constant. Although, if the missing calorie deficit results in flavour avoidance, then it should be expected here that the previously unrewarded flavour is avoided *more* since it was presented alongside a much more calorific reward during CS^{++} pairing sessions. Alternatively, the differences between the CS^{++} and CS^{+} that are specific to the flavour m-ABT could increase attention to these stimuli, rather than to the unrewarded flavour, thus resulting in the absence of avoidance here.

Following these findings of clear preferences for CS^{++} vs CS^{+} and thus learning reward value through flavour associations, the flavour m-ABT can be used to further explore whether the formation of positive biases is influenced by long-term changes to affective state prior to CS-US learning. However, these rats received pairing sessions with bottles counterbalanced for side and yet received a single CS^{++} vs CS^{+} choice test, which could have been subject to a general side bias. This could result in a flavour preference that reflects side preference; therefore, I aim to increase reliability of the flavour m-ABT by adding an additional CS^{++} vs CS^{+} choice test day, with bottles presented on the alternate side of the box to the previous day to reduce side biases. In addition, strong preferences were found using the 3:1 ratio with some animals showing 100% preference of CS^{++} over CS^{+}, which again could be due to side biases, and so this additional choice test should reduce possible ceiling effects.

2.4.3. Digging m-ABT

The same animals run in the flavour m-ABT were then tested in the traditional digging m-ABT. No preference for the higher valued substrate vs the lower valued substrate was found, indicating these rats did not form reward-induced positive biases as would be expected. However, there were positive biases formed for the novel substrate vs the unrewarded substrate, and both previously rewarded substrates vs the unrewarded substrate. This suggests these rats could form and recall reward-substrate associations and

appeared to learn through avoidance of the unrewarded stimulus, however, they were unable to distinguish reward value between the two rewarded substrates.

This contrasts with reports from the development of this digging task where healthy rats should show a clear positive bias (Stuart et al., 2013). Although a slightly different arena was used in this experiment to that reported previously, the ability to block access to the alternate bowl after rats had made a choice rather than reach into the cage to remove it is the only major difference and works on the same principle. It is possible that human errors may underlie this lack of positive bias, such as mistaking sniffing in the substrate for digging, and therefore prematurely recording a decision made. Alternatively, these animals were food restricted to 85% of their free-feeding weights, and perhaps this caused a motivational state that interfered with any reward learning biases such that they chose any substrate presented that was previously associated with food. Finally, another potential reason for this lack of positive bias could be due to side biases in the arena. As the position of the bowls were equally counterbalanced throughout the choice test session for each animal, if the rat chose one side continuously then this would result in no preference. However, these rats did show preferences in all other choice tests and therefore were clearly making decisions in these rather than relying on side biases.

Ultimately, these findings demonstrate the difficulties in replicating this hand-run design, reiterating the need to increase reliability of this task, possibly via an automated flavour preference version.

2.4.4. Conclusion

Chapter 2 shows the development of two novel flavour preference assays, the flavour ABT which aimed to measure negative and positive bias formation with acute drug administration, and the flavour m-ABT which aimed to measure the formation of a positive bias to a higher valued reward. The findings from these experiments indicate a potential lack of sensitivity in the flavour ABT to measure affective biases from acute drug administration; however, the latter experiment does demonstrate the ability of the flavour m-ABT to measure the formation of a reward-induced positive bias for a higher valued reward compared to a lower valued reward. Experiments in Chapter 3 will focus on the use of the flavour m-ABT to measure deficits in the formation of positive biases following long-term changes to affective state. Additional tests will provide further information on whether deficits occur from disruption to general cognitive processes or weaker avoidance of a non-rewarding stimulus.

Chapter 3 – Investigating reward-induced biases in putative models of psychiatric disease

3.1. Introduction

In Chapter 2, the development of a flavour preference version of the affective bias test (ABT) and modified-ABT (m-ABT) was discussed. From the evaluation of these novel versions, the 'flavour m-ABT' appeared to be able to measure the formation of a positive bias for a higher valued reward compared to lower valued reward in healthy rodents, however its application to preclinical models has not yet been assessed. Thus, this chapter aims to apply this newly developed flavour m-ABT method to evaluate positive bias formation in three preclinical models of psychiatric disease.

Evaluation of preclinical models in the original digging m-ABT has been discussed mostly in section 1.4.3. In the chronic interferon alpha (IFN-α) induced model of depression, rats receive chronic administration of the pro-inflammatory cytokine IFN-α. Stuart et al. (2017) previously demonstrated that acute IFN-α treatment induced a negative affective bias in the ABT, and chronic treatment impaired the formation of a reward-induced positive bias in the m-ABT. In humans, Cooper et al. (2018) found IFN-α treatment reduced vigilance to happy/positive facial expressions, and were more accurate at detecting facial expressions of disgust, i.e. they showed a negative bias and reduced positive bias to emotional stimuli, indicating some translational validity of this model in the ABT. Given chronic IFN-α had previously induced a deficit in the digging version, this model was chosen to evaluate the flavour version and is hypothesised to produce a deficit in forming a positive bias toward the flavour paired with a higher value reward.

Acute corticosterone (CORT) treatment has also been consistently shown to induce a negative affective bias in the ABT (Hinchcliffe et al., 2017; Stuart et al., 2019; Stuart et al., 2017). Whilst the effect of chronic CORT treatment in the digging m-ABT has not been reported, exposure to early life adversity did not appear to influence formation of a reward-induced positive bias in the m-ABT, but when acute CORT was administered these animals showed enhanced negative bias (Stuart et al., 2019). In addition, acute stress in humans has been shown to impair reward-induced positive biases and reward learning in the PRT (Bogdan, Perlis, Fagerness, & Pizzagalli, 2010; Bogdan, Santesso, Fagerness, Perlis, & Pizzagalli, 2011). Thus, to evaluate the impact of long-term stress on reward-induced biases, the chronic CORT model was also examined in the flavour m-ABT. Given this previous evidence of stress-induced deficits in reward learning and biases, this model is hypothesised to produce a deficit in forming a reward-induced positive bias.

Finally, the heterozygous *CACNA1C* knockout rat model (CACNA1C$^{+/-}$) model described in section 1.4.1 was used to evaluate the effects of this genetic risk variant on reward-induced positive bias. Studies have shown this model displays deficits in general associative memory (e.g. Sykes, Clifton, Hall, & Thomas, 2018), and cognitive flexibility and decision-making for reward (e.g. Koppe et al., 2017; Sykes, Haddon, et al., 2018). In humans, Lancaster, Heerey, Mantripragada, and Linden (2014) found that healthy participants with the *CACNA1C* risk variant associated with schizophrenia showed overall reduced response bias to reward-related stimuli on a probabilistic learning task, suggesting this variant may induce deficits in forming reward-induced positive biases. It can therefore be hypothesised that if the *CACNA1C* gene is involved in reward learning, this CACNA1C$^{+/-}$ model would also show a deficit in positive bias formation in the m-ABT.

Thus, Chapter 3 aims to evaluate the novel flavour m-ABT developed from Chapter 2 in its ability to detect changes in reward-induced biases following chronic treatment with two pharmacological pro-depressants, as well as following genetic manipulation associated with psychiatric disease. In Experiment 3, an initial pilot experiment was conducted to evaluate chronic CORT and IFN-α treatment in the flavour m-ABT, which was followed up in further separate experiments investigating effects of drug treatment and sex in Experiments 4 and 5. An initial pilot experiment with the CACNA1C$^{+/-}$ model was conducted in Experiment 6, which was followed up further to investigate the effects of genotype and sex in Experiment 7.

3.2. General flavour m-ABT and drug treatment methods

3.2.1. Subjects

For all experiments in Chapter 3, Sprague-Dawley rats were supplied by Charles River UK and housed in cages of two or three. CACNA1C$^{+/-}$ rats were bred on a Sprague-Dawley background in-house or at Charles River UK. Sample sizes were determined as described in section 2.2.1.1 and husbandry details were as described for Experiment 1.

All rats were acclimatised to the environment for at least one week, followed by one week of handling before any procedures began. All rats were maintained between 85 – 90% of their free-feeding weights prior to commencing pre-training, allowing moderate weight gain expected in free-feeding rats, as determined by growth charts. Food rations were given in the rats' home cages at least one hour after the end of the session. At the start of pre-training, all rats were additionally exposed to two consecutive 22-hour periods of water restriction.

Experiments were conducted in compliance with the UK Animals (Scientific Procedures) Act 1986) and with local Cardiff University Ethics Review Committee approval.

3.2.2. Experimental sessions

Throughout the experiments in Chapter 3, the apparatus and pre-training procedure described in section 2.2.1 was used.

Once all rats reached a steady level of consumption from each bottle, they were given 10 minutes access each day to a two-bottle choice between a flavour containing only 0.1% saccharin, known as the blank flavour or CS-C, alongside one of two other flavour solutions. One flavoured solution contained a high concentration of sucrose, hereby referred to as CS-A^{++}, while the other flavoured solution contained a lower concentration of sucrose, hereby referred to as CS-B^{+}. This was repeated twice for each solution, giving four total pairing sessions.

Following pairing sessions, rats were then given five choice test sessions for 10 minutes each day. For the first two days, all rats received a two-bottle choice between flavours CS-A^{++} and CS-B^{+} containing only 0.1% saccharin, where the solutions were positioned in the opposite access hole on the second day to reduce side biases. For the following three days, all rats received a counterbalanced order of choice tests between either CS-A^{++} vs CS-C, CS-B^{+} vs CS-C, or CS-C vs CS-D, in which D was a novel flavour introduced only in this choice test (see Figure 3.1). No solution during the choice test sessions contained any sucrose.

For all flavour m-ABT experiments, the reward-paired flavours (CS-A^{++} and CS-B^{+}) and unrewarded flavours (CS-C and CS-D) were counterbalanced between Grape/Cherry or Tangerine/Tamarind, such that solutions were presented equally often in left/right bottles over training.

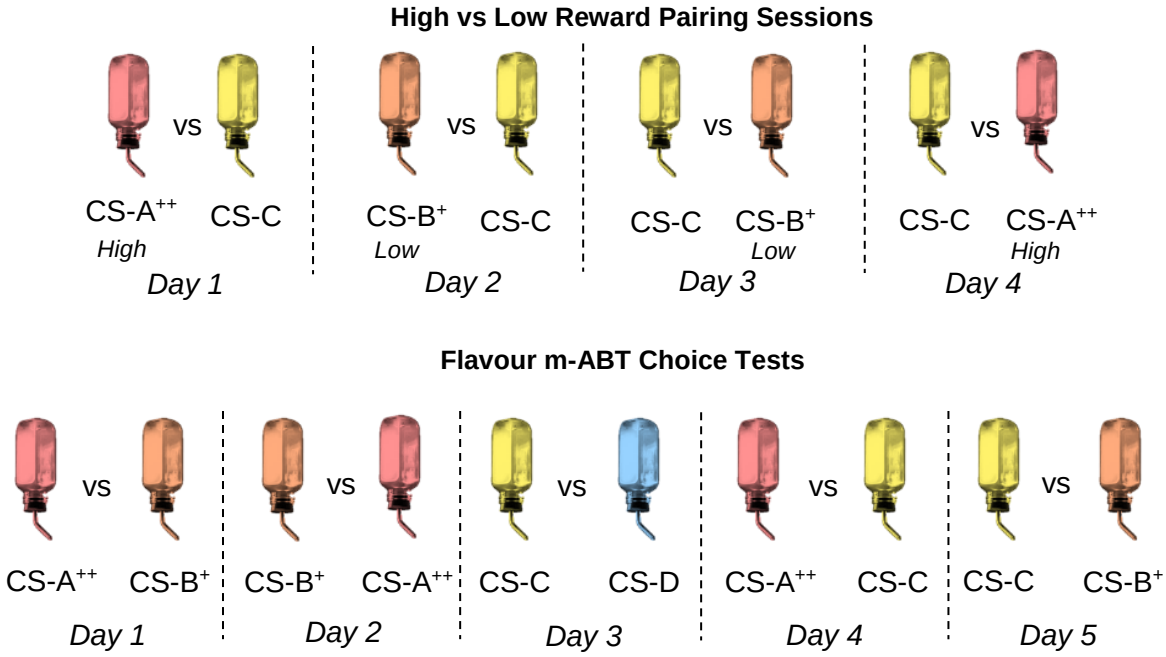


Figure 3.1. A schematic diagram of the flavour m-ABT design for all experiments in Chapter 3, with example counterbalance order. All rats were given four days of pairing sessions with CS-A^{++} containing a high concentration of sucrose, or CS-B^{+} containing a low concentration of sucrose, vs an unrewarded flavour solution, CS-C. This was followed by five choice tests, without any added sucrose, to assess learning and memory.

3.2.3. Data analysis

For all experiments in Chapter 3, consumption data collection, removal of animals based on side biases, and data analysis was as described in section 2.2.1.5. Bayesian analyses were also conducted, where indicated, as described in section 2.2.1.6.

3.2.4. Drug treatments

Chronic corticosterone

For all experiments, CORT (50µg/ml, Sigma Aldrich, UK) was dissolved in 1% ethanol and given to the treated group via their drinking water for 12 consecutive days, followed by dose-tapering with 3 days of 25µg/ml and 3 days of 12µg/ml, returning to plain water *ad libitum* before starting pre-training. Control (vehicle) animals were given only 1% ethanol in their drinking water for the same duration. Drugs were administered at the same time every day and prepared fresh. This dose was chosen based on previous, unpublished work in the

Robinson lab as well as published literature investigating reward-related deficits and depression-like phenotypes (e.g. Gourley, Kedves, Olausson, & Taylor, 2009; Gourley, Kiraly, Howell, Olausson, & Taylor, 2008; Gourley, Wu, & Taylor, 2008; Monsey et al., 2014; Olausson, Kiraly, Gourley, & Taylor, 2013).

Chronic IFN-α

IFN-α (I8657, Sigma Aldrich, UK) was first dissolved in distilled water and suspended in saline to create a stock solution of 10000 International Units (IU)/ml, stored at -80°C in aliquots. Each day, one aliquot was re-suspended in 0.9% saline to 100IU/ml, and 1ml/kg was injected intraperitoneal to the treatment group for 14 consecutive days, and at the end of each testing session thereafter, at the same time daily. Control animals were given intraperitoneal injections of 1ml/kg 0.9% saline in parallel to treated animals. This dosage of IFN-a was chosen from a dose-response study (Stuart et al., 2017) where this lower dose elicited a negative affective bias but did not induce sickness behaviours.

3.3. Experiment 3 – Investigating reward-induced positive bias formation in the chronic CORT and IFN-α models

Experiment 3 is an initial pilot to evaluate the validity of the novel flavour m-ABT in detecting deficits in forming reward-induced positive biases following two pharmacological manipulations of affective state in male rats. Chronic treatment with either CORT or IFN-α is expected to reduce the consumption preference for the high-reward flavour vs low-reward flavour compared to controls, indicating a deficit in forming a reward-induced positive bias similar to findings in the digging m-ABT.

3.3.1. Experimental design

48 male Sprague-Dawley rats weighing 240 – 306g on *ad libitum* food were used in this experiment. These rats were split into 4 treatment groups, chronic CORT (*n* = 16), chronic IFN-α (*n* = 16), vehicle-treated to match the CORT group (*n* = 8), and saline-treated to match the IFN-α group (*n* = 8), where treatments were as described in 3.2.4. Prior to any drug treatment, this cohort were subject to training in operant boxes and food restricted for Experiment 12 (see section 5.3 and Appendix A for experimental trajectory). Immediately following drug treatments, rats were run in the flavour m-ABT in this experiment before operant testing sessions began for Experiment 12.

Throughout Experiment 3 pairing sessions, flavour CS-A^{++} contained 15% sucrose, and CS-B^{+} contained 5% sucrose. 6 animals were removed from analysis due to side biases or consuming less than 1ml across each CS-A^{++} vs CS-B^{+} choice test day, leaving 6 saline controls, 15 IFN-treated rats and 7 vehicle controls. As described below, 1 rat was identified as an outlier based on their weight gain, and was removed from further analysis, leaving a final sample size of 14 CORT-treated rats.

Data from training sessions can be seen in Appendix E.

3.3.2. Results

Effects of chronic treatment on percentage weight gain

In addition to adapting stress responses, glucocorticoids are known to play a role in metabolism and energy regulation (Nieuwenhuizen & Rutters, 2008), and chronic CORT treatment in drinking water has shown to reduce weight gain in both rats (Boersma, Tamashiro, Moran, & Liang, 2016) and mice (Cassano et al., 2012).

Therefore, as a positive control, all rats were weighed daily. For CORT-treated rats and vehicle controls, the mean percentage increase in body weight from the start of full dose treatment (50μg/mL) to the end of full dose treatment was calculated (12 days). From this,

1 rat in the CORT treatment group was identified as an outlier with a percentage increase in weight of 19.4%, much greater than all other rats, and was removed from any further analysis[1]. For the IFN-treated and saline controls, the mean percentage increase in body weight from the start of IFN-α treatment up until food restriction began (8 days) was calculated.

An independent samples t-test showed that CORT-treated rats had significantly lower percentage increase in body weight compared to vehicle controls, $t(21) = 2.36$, $p = 0.028$ (Figure 3.2). This demonstrates that CORT treatment worked as expected. The percentage increase in body weight for saline-treated rats were not normally distributed, $W(8) = 0.79$, $p = 0.026$. An independent samples Mann-Whitney U test found no significant difference between saline and IFN-α treated rats as expected, $U(24) = 75$, $p = 0.53$ (Figure 3.2).

The lower overall percentage increases in bodyweight for the IFN-α and saline treated animals is due to the fact that this was calculated across a shorter period, as well as when the animals were older and not growing as rapidly. These data on weight gain after treatment are therefore not comparable across the vehicle/CORT vs saline/IFN groups.

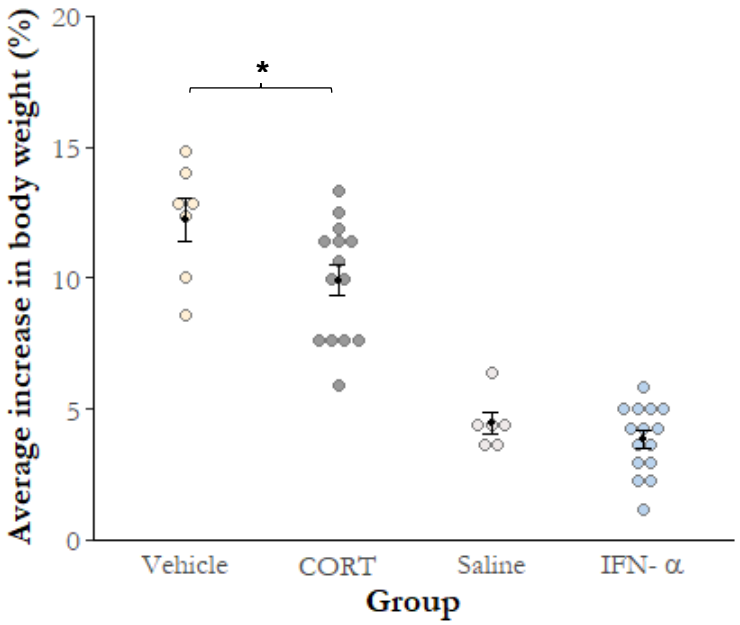


Figure 3.2. A graph demonstrating the average increase in body weight during the different treatment periods.
CORT rats had significantly lower percentage increase in body weight compared to vehicle controls during the CORT treatment period of 12 days ($p = 0.028$). No difference was found between IFN-α treated rats and saline controls during the IFN-α treatment period of 8 days ($p = 0.32$).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

[1] Removal of this rat altered the significance of the difference in overall weight gain of CORT-treated rats compared to vehicle-treated rats but made no impact on the general significance level of behavioural results reported later.

Effects of treatment on reward-induced positive bias

Saline vs ethanol controls

Since the vehicle and saline groups alone contained a very small sample size, I planned at the beginning of this experiment to combine these two control groups for analysis. However, it first needed to be established that there was no difference between these two control groups.

Overall, both saline- and vehicle-treated rats formed a positive bias for the flavour previously paired with a high value reward vs the flavour previously paired with a low value reward, with no difference between the two groups. However, Bayes analysis only provided anecdotal support for this.

A repeated measures ANOVA found a significant main effect of flavour with greater consumption of CS-A^{++} vs CS-B^{+}, $F_{1, 11} = 108.14$, MSE = 0.96, $p < 0.001$, $\eta_p^2 = 0.91$, and no interaction between flavour * group, $F_{1, 11} = 1.49$, MSE = 0.96, $p = 0.25$, $\eta_p^2 = 0.12$. There was no significant difference between saline and vehicle control rats in overall consumption, $F_{1, 11} = 2.35$, MSE = 1.72, $p = 0.15$, $\eta_p^2 = 0.18$.

An independent samples t-test found no significant difference between the two control groups in their percentage preference for CS-A^{++} vs CS-B^{+}, t(11) = -0.305, $p = 0.77$ (Table 3.1).

A Bayesian independent samples t-test provided anecdotal support for a lack of difference between these two control groups, $BF_{01} = 2.12$.

Table 3.1. A summary of results from vehicle and saline groups in the CS-A^{++} vs CS-B^{+} choice test of the flavour m-ABT in Experiment 3.

Group	Mean consumption (g) of CS-A^{++} (±SEM)	Mean consumption (g) of CS-B^{++} (±SEM)	Mean preference for CS-A^{++} vs CS-B^{+} (%)
Vehicle	3.96 (±0.32)	0.42 (±0.09)	90.0 (2.34)
Saline	5.23 (±0.85)	0.74 (±0.31)	88.8 (3.18)

Combined control vs CORT and IFN-α

As the control groups did not differ significantly, the two were combined for further analysis.

When comparing CORT, IFN-α, and control groups, results showed that all groups were able to form a reward-induced positive bias but this bias was significantly lower in the CORT and IFN-treated groups compared to controls.

A repeated measures ANOVA showed a main effect of flavour where consumption of CS-A^{++} was significantly greater than CS-B^{+}, $F_{1, 39}$ = 124.37, MSE = 1.78, p <0.001, η_p^2 = 0.76, with no significant interaction between group * flavour, $F_{2, 39}$ = 1.48, MSE = 1.78, p = 0.24, η_p^2 = 0.071 (Figure 3.3A). There was no significant difference between the three groups in overall consumption, $F_{1, 39}$ = 0.11, MSE = 2.23, p = 0.89, η_p^2 = 0.005. Shapiro-Wilks test of normality found that consumption of CS-A^{++} was not normally distributed in the CORT-treated group, W(14) = 0.83, p = 0.011. Consumption of CS-B^{+} was also not normally distributed in the control group, W(13) = 0.71, p <0.001, CORT-treated group, W(14) = 0.85, p = 0.023, nor IFN-treated group, W(15) = 0.87, p = 0.032. However, a suitable alternative statistical test for this type of analysis was not available.

Shapiro-Wilks test also demonstrated that percentage preference for CS-A^{++} vs CS-B^{+} in the control group was not normally distributed, W(13) = 0.84, p = 0.023. Thus, the Kruskal-Wallis one-way ANOVA was conducted to compare preference percentage for CS-A^{++} vs CS-B^{+} across the three groups. This found a significant overall difference between the groups, H(2) = 7.09, p = 0.029. Dunn's post-hoc pairwise comparisons showed that preference was significantly lower in the CORT-treated group compared to controls (p = 0.013) and in the IFN-treated group compared to controls (p = 0.035), with no difference between CORT and IFN-treated groups (p = 0.72).

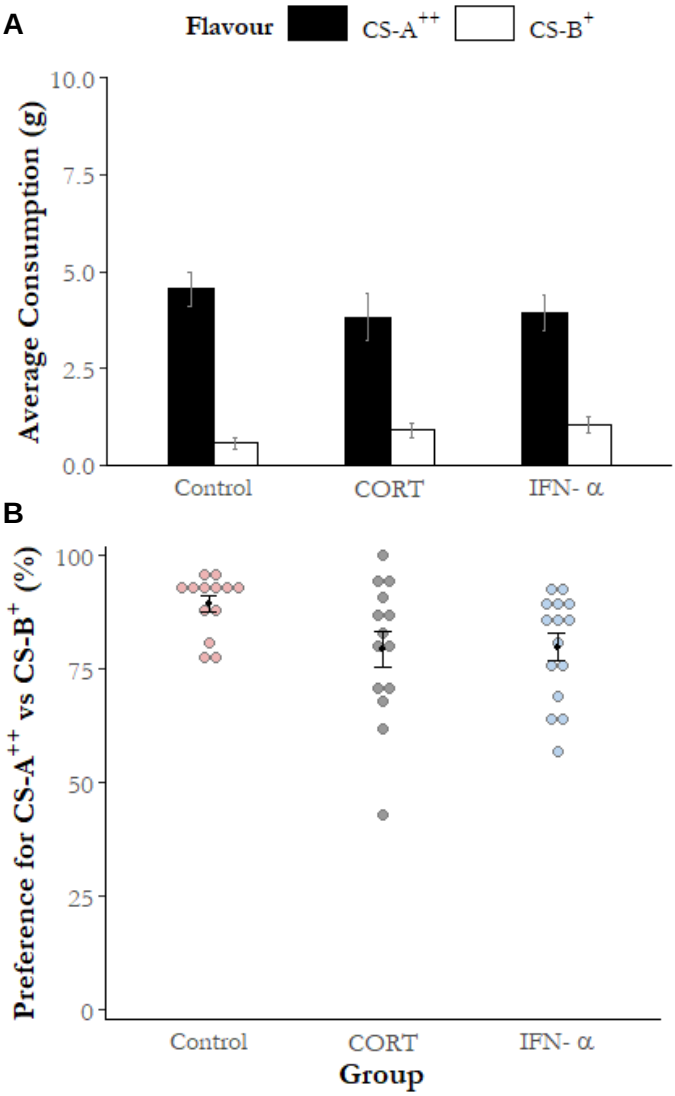


Figure 3.3. Graphs demonstrating CS-A^{++} vs CS-B^{+} choice test data in Experiment 3. No overall group effect was found in the overall consumption of either flavour (A). Both CORT-treated and IFN-treated rats showed a significantly reduced overall preference for CS-A^{++} vs CS-B^{+} compared to controls (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

Effects of treatment on additional choice tests

Learning and memory of flavour-high reward associations

Overall, rats showed a preference for the flavour previously paired with a high value reward vs the previously unrewarded flavour, indicating intact recall of the flavour-high reward association. There was no overall difference between the three groups in this recall and Bayes analysis provided anecdotal support for this lack of difference.

A repeated measures ANOVA found a main effect of flavour where consumption of CS-A^{++} was greater than CS-C, $F_{1, 39} = 24.18$, MSE = 2.61, $p < 0.001$, $\eta_p^2 = 0.38$, with no interaction between flavour * group, $F_{2, 39} = 1.04$, MSE = 2.61, $p = 0.36$, $\eta_p^2 = 0.051$ (Figure 3.4A). There was no significant effect of group on overall consumption, $F_{2, 39} = 1.21$, MSE = 1.23, $p = 0.31$, $\eta_p^2 = 0.058$. Shapiro-Wilks test of normality found that consumption of CS-C was not normally distributed in the control group, W(13) = 0.80, $p = 0.007$, CORT-treated group, W(14) = 0.79, $p = 0.003$, nor IFN-treated group, W(15) = 0.83, $p = 0.009$. However, a suitable alternative statistical test for this type of analysis was not available.

Shapiro-Wilks test also demonstrated that percentage preference for CS-A^{++} vs CS-C in the CORT-treated group was not normally distributed, W(14) = 0.81, $p = 0.006$, nor in the IFN-treated group, W(15) = 0.78, $p = 0.002$. Thus, the Kruskal-Wallis one-way ANOVA was conducted to compare preference percentage for CS-A^{++} vs CS-C across the three groups. There was no significant overall effect of group on preference for CS-A^{++} vs CS-C, K(2) = 0.97, $p = 0.62$ (Figure 3.4B).

A Bayesian univariate ANOVA provided only anecdotal evidence to support this lack of difference between combined control and CORT-treated rats, $BF_{01} = 1.58$, as well as between combined control and IFN-treated rats, $BF_{01} = 1.78$.

Learning and memory of flavour-low reward associations

Overall, rats showed a preference for the flavour previously paired with a low value reward vs the previously unrewarded flavour, indicating intact recall of the flavour-low reward association. There was no overall difference between the three groups in this recall, however, Bayes analysis showed this lack of difference was inconclusive between control and IFN-α rats.

A repeated measures ANOVA showed a main effect of flavour where consumption of CS-B^{+} was greater than CS-C, $F_{1, 39} = 21.67$, MSE = 1.67, $p < 0.001$, $\eta_p^2 = 0.36$, with no interaction between flavour * group, $F_{2, 39} = 1.24$, MSE = 1.67, $p = 0.30$, $\eta_p^2 = 0.06$ (Figure 3.5A). There was no significant effect of group on overall consumption, $F_{2, 39} = 0.27$, MSE = 1.22, $p = 0.77$, $\eta_p^2 = 0.013$. Shapiro-Wilks test of normality found that consumption of CS-C was not normally distributed in the control group, W(13) = 0.86, $p = 0.039$, CORT-treated group, W(14) = 0.77, $p = 0.002$, nor IFN-treated group, W(15) = 0.69, $p < 0.001$. However, a suitable alternative statistical test for this type of analysis was not available.

Shapiro-Wilks test also demonstrated that percentage preference for CS-B^{+} vs CS-C in the IFN-treated group was not normally distributed, W(15) = 0.66, $p < 0.001$. Thus, the Kruskal-

Wallis one-way ANOVA was conducted to compare preference percentage for CS-B^+ vs CS-C across the three groups. There was no significant overall effect of group on preference for CS-B^+ vs CS-C, K(2) = 2.54, p = 0.28 (Figure 3.5B).

A Bayesian univariate ANOVA found anecdotal support for a lack of difference between combined control and CORT-treated rats, BF_{01} = 2.78. Almost no evidence was found to support the lack of difference between combined control and IFN-treated rats, with evidence slightly in the direction of supporting a significant effect, BF_{10} = 1.02.

Avoidance of unrewarded stimuli

Overall, consumption of the novel unrewarded flavour was greater than the familiar unrewarded flavour, indicating these rats showed avoidance of the familiar flavour. There were no differences between the three groups in this avoidance, with anecdotal support from Bayes analysis.

A repeated measures ANOVA found a main effect of flavour where consumption of CS-D was greater than CS-C, $F_{1, 39}$ = 12.19, MSE = 2.44, p = 0.001, η_p^2 = 0.24, with no interaction between flavour * group, $F_{2, 39}$ = 0.49, MSE = 2.44, p = 0.62, η_p^2 = 0.025 (Figure 3.6A). There was no overall effect of group on consumption, $F_{2, 39}$ = 0.12, MSE = 1.68, p = 0.88, η_p^2 = 0.006. Shapiro-Wilks test of normality found that consumption of CS-D was not normally distributed in the CORT-treated group, W(14) = 0.67, p <0.001, or the IFN-treated group, W(15) = 0.82, p = 0.007. Consumption of CS-C was also not normally distributed in the control group, W(13) = 0.75, p = 0.002, CORT-treated group, W(14) = 0.71, p <0.001, nor the IFN-treated group, W(15) = 0.73, p <0.001. However, a suitable alternative statistical test for this type of analysis was not available.

Shapiro-Wilks test also demonstrated that percentage preference for CS-D vs CS-C in the control group was not normally distributed, W(13) = 0.82, p = 0.01, nor in the IFN-treated group, W(15) = 0.86, p = 0.027. Thus, the Kruskal-Wallis one-way ANOVA was conducted to compare preference percentage for CS-D vs CS-C across the three groups. There was no significant overall effect of group on preference for CS-D vs CS-C, K(2) = 0.59, p = 0.74 (Figure 3.6B).

A Bayesian univariate ANOVA found only anecdotal evidence to support this lack of difference between combined controls and CORT-treated rats, BF_{01} = 2.12, as well as between combined controls and IFN-treated rats, BF_{01} = 2.45.

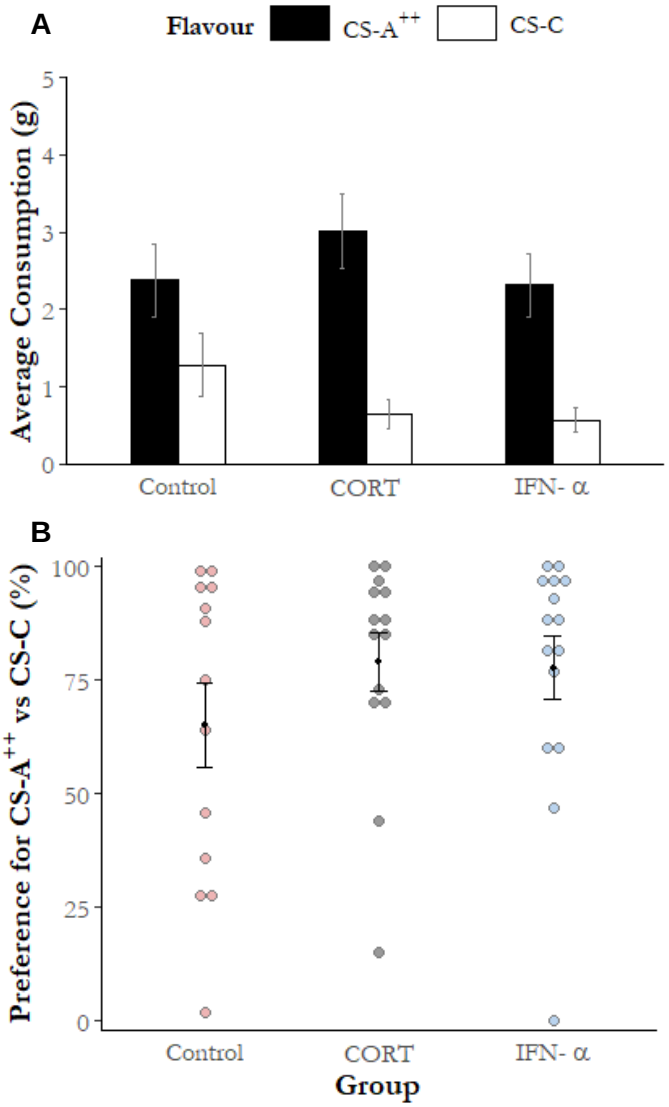


Figure 3.4. Graphs demonstrating CS-A^{++} vs CS-C choice test data in Experiment 3.
No significant difference was found between the combined control group and CORT or IFN-α groups in overall consumptions of either flavour (A), or percentage preference for CS-A^{++} (B). Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

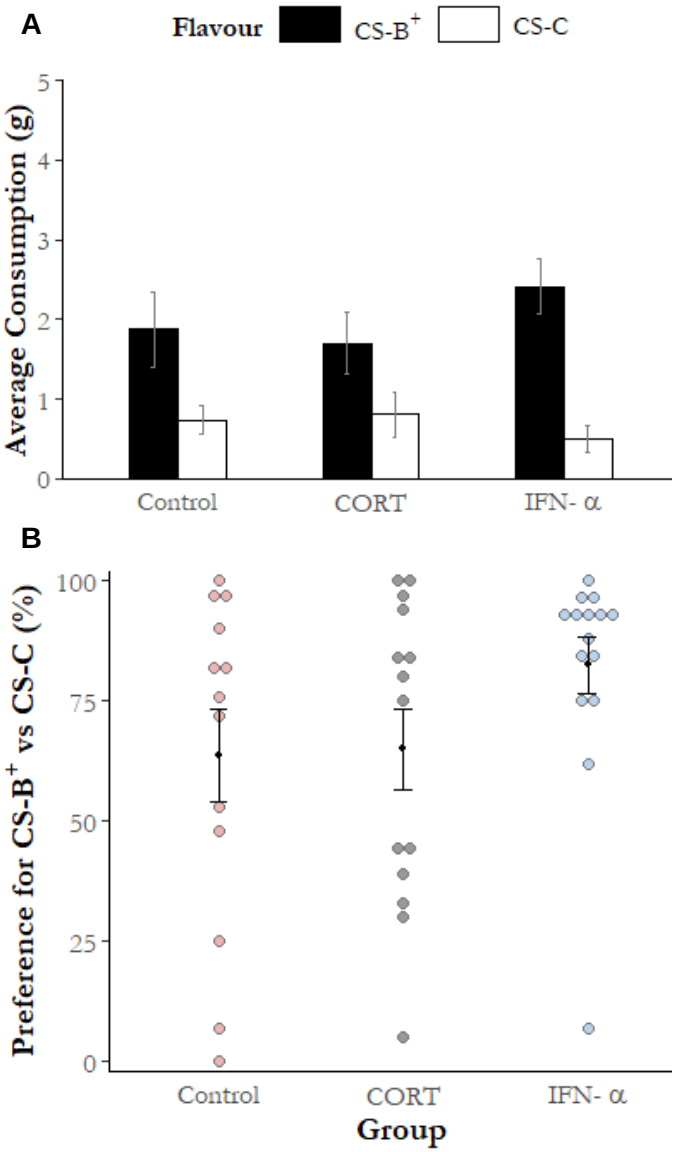


Figure 3.5. Graphs demonstrating CS-B$^+$ vs CS-C choice test data in Experiment 3.
No significant difference was found between the combined control group and CORT or IFN-α groups in overall consumptions of either flavour (A), or percentage preference for CS-B$^+$ (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

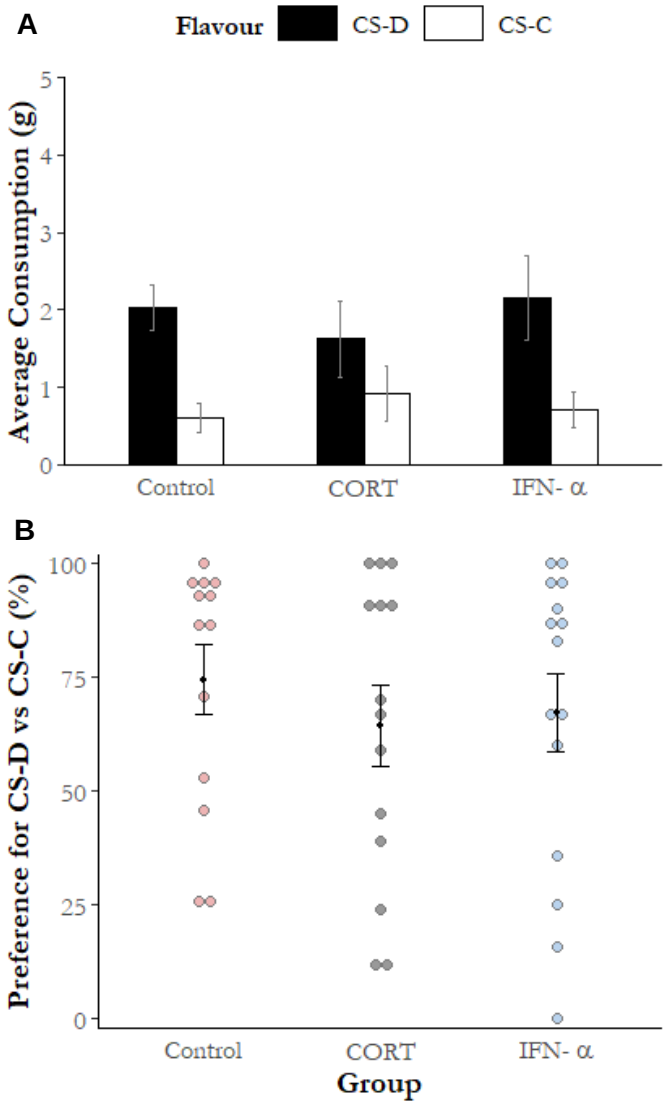


Figure 3.6. Graphs demonstrating CS-D vs CS-C choice test data in Experiment 3.
No significant difference was found between the combined control group and CORT or IFN-α groups in overall consumptions of either flavour (A), or percentage preference for CS-D (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

3.3.3. Summary

Experiment 3 was an initial pilot aiming to determine whether the novel flavour m-ABT developed in Chapter 2 could be applied to the chronic CORT and chronic IFN-α preclinical model of psychiatric disease to detect deficits in reward-induced positive biases.

Individual vehicle and saline control groups where treatment administration was matched to CORT and IFN-α respectively had small sample sizes and before the start of the experiment, it was planned to combine these two groups to create a larger control group for comparisons. An initial analysis of results between these two control groups found no differences and thus they were combined for this pilot experiment, however Bayesian analyses did not provide firm conclusive evidence to support this lack of differences and so results should be interpreted with caution.

After the removal of outliers, a significant reduction in weight gain was found in the chronic CORT treated group compared to the vehicle matched control group, which was expected due to the metabolic effects of CORT, thus indicating a positive control for the treatment. IFN-α is not suggested to influence weight gain and does not show an effect here.

Overall, there was a significant difference between the groups in reward-induced positive bias. Post-hoc analysis revealed that both chronic CORT and IFN-α treatment reduced positive bias toward the higher valued reward-paired flavour when compared to the combined control group.

Additional choice tests showed no significant differences between control and treatment groups in their general learning and memory about flavour-reward associations or avoidance of the unrewarded flavour, however, Bayes did not provide conclusive evidence for this. This may suggest that CORT or IFN-α does not significantly impact recall of these flavour-reward associations or avoidance learning.

3.4. Experiment 4 – Further evaluation of the chronic CORT model in the novel flavour m-ABT

Experiment 4 aims to further evaluate the validity of the novel flavour m-ABT in detecting deficits in forming reward-induced positive biases following manipulation of affective state using the chronic CORT model with a more robust design than Experiment 3. This experiment will also compare males and females to determine whether deficits are consistent across sexes and enable the generalisation of findings from this assay. Chronic CORT treatment is expected to reduce consumption preference for the high-reward flavour vs the low reward-flavour compared to controls in both sexes, indicating a deficit in forming a reward-induced positive bias, with no difference between males and females in this preference.

3.4.1. Experimental design

Experiment 4 used 32 naïve male and 32 naïve female Sprague-Dawley rats weighing between 298 – 469g and 199 – 273g respectively at 10 weeks of age on *ad libitum* food. These rats were either treated chronically with CORT in drinking water (*n*=16 per sex) or with the vehicle control (*n*=16 per sex) as described in section 3.3.2.

In this experiment, flavour CS-A^{++} contained 16% sucrose and flavour CS-B^{+} contained 8% sucrose during pairing sessions only. Two female rats were removed from analysis due to side biases greater than 90%, leaving n=15 CORT-treated and n=15 control. Repeated measures ANOVAs were used to analyse choice test consumption of each flavour, with 'group' and 'sex' as fixed factors. Fisher's LSD post-hoc analyses were conducted to analyse interactions. Univariate ANOVAs were conducted to compare percentage preferences across group and sex.

Data from training sessions can be seen in Appendix E.

3.4.2. Measurement of circulating CORT levels

Following the final testing of this cohort in Experiment 7 (described in section 4.3.4), between 25-27 days after the final CORT dosage was administered, all animals were terminally euthanized for tissue collection (described in section 6.2.1, see Appendix A). Immediately after termination, trunk blood was collected in heparin-coated tubes (BD 367869, Fisher Scientific UK) and centrifuged at 4000rpm for 10 minutes. Supernatant plasma was collected and stored at -20$^{\circ C}$ until analysis.

A competitive enzyme-linked immunosorbent assay (ELISA) kit (ab108821, Abcam) was used to analyse levels of circulating CORT in the plasma of 15 rats from each group, performed according to manufacturer's instructions. All samples were diluted 1:50 as

determined by optimisation. Prism v8 (GraphPad) was used to create a standard curve from standards of known concentrations provided within the kit and interpolate CORT concentrations for the samples. Interpolated sample concentrations were multiplied by the dilution factor and presented as ng/mL plasma.

3.4.3. Results

Effects of chronic CORT treatment on percentage weight gain

As described for Experiment 3, as a positive control all rats were weighed daily following the start of CORT treatment and their mean weight gain was analysed.

A univariate ANOVA found no overall effect of group on percentage body weight increase, $F_{1, 60} = 0.12$, MSE = 7.23, $p = 0.73$, $n_p^2 = 0.002$ (Figure 3.7). There was a significant effect of sex where males had a greater increase than females, $F_{1, 60} = 23.43$, MSE = 7.23, $p < 0.001$, $n_p^2 = 0.28$, with no significant interaction between group * sex, $F_{1, 60} < 0.001$, MSE = 7.23, $p = 0.99$, $n_p^2 < 0.001$.

A Bayesian univariate ANOVA provided moderate support for this lack of overall group effect on weight gain, $BF_{01} = 3.77$. Thus, these findings indicate that CORT treatment had no effect on weight gain, supported by Bayes analysis, and suggest it did not work as expected.

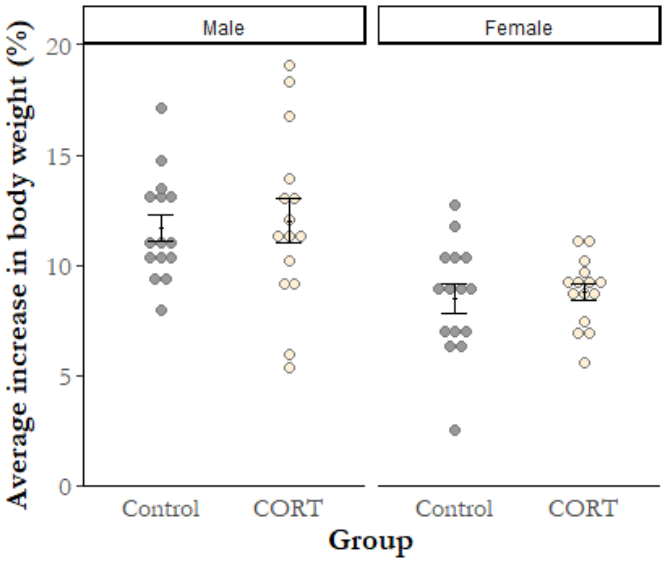


Figure 3.7. Monitoring of body weight changes as a positive control for CORT treatment.
There was no difference between control and CORT-treated rats in their body weight increase over the duration of CORT treatment.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

Effects of chronic CORT treatment and/or sex on reward-induced positive bias

Overall, rats showed a preference for consumption of the flavour previously paired with a high value reward vs the flavour previously paired with a low value reward, with no difference between CORT and control rats, indicating both groups were able to form a reward-induced positive bias. There was also no difference between males and females. However, Bayes analysis only provided anecdotal support for these lack of differences.

There was a significant main effect of flavour, with consumption greater for CS-A^{++} than CS-B^{+} (Figure 3.8A), $F_{1, 58}$ = 7.03, MSE = 2.62, p = 0.01, η_p^2 = 0.11, and no interaction between flavour * group, $F_{1, 58}$ = 1.34, MSE = 2.62, p = 0.25, η_p^2 = 0.023; flavour * sex, $F_{1, 58}$ = 1.04, MSE = 2.62, p = 0.31, η_p^2 = 0.018; nor between flavour * sex * group, $F_{1, 58}$ = 0.069, MSE = 2.62, p = 0.79, η_p^2 = 0.001.

There was no difference between control and CORT-treated rats in overall consumption, $F_{1, 58}$ = 3.34, MSE = 1.15, p = 0.073, η_p^2 = 0.054. Overall, male rats consumed significantly more than females, $F_{1, 58}$ = 9.66, MSE = 1.15, p = 0.003, η_p^2 = 0.14, with no interaction between group * sex, $F_{1, 58}$ = 0.26, MSE = 1.15, p = 0.61, η_p^2 = 0.004. Shapiro-Wilks test of normality found that consumption of CS-B^{+} was not normally distributed in the female control group, W(15) = 0.85, p = 0.019, female CORT-treated group, W(15) = 0.83, p = 0.008, nor male CORT-treated group, W(16) = 0.81, p = 0.04. Consumption of CS-A^{++} was also not normally distributed in male CORT-treated rats, W(16) = 0.86, p = 0.017. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA found no overall difference in preference percentage for CS-A^{++} vs CS-B^{+} between control and CORT-treated rats (Figure 3.8B), $F_{1, 58}$ = 1.43, MSE = 0.087, p = 0.24, η_p^2 = 0.024, nor between males and females, $F_{1, 58}$ = 1.25, MSE = 0.087, p = 0.27, η_p^2 = 0.021. There was also no interaction between group * sex here, $F_{1, 58}$ = 0.30, MSE = 0.087, p = 0.59, η_p^2 = 0.005. Shapiro-Wilks test found that percentage preference for CS-A^{++} vs CS-B^{+} in the male CORT-treated group was not normally distributed, W(15) = 0.85, p = 0.016, nor in the female CORT-treated group, W(16) = 0.85, p = 0.015. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA revealed only anecdotal support for a lack of overall group effect on percentage preference for CS-A^{++} vs CS-B^{+}, BF_{01} = 1.95, however, preference in the CORT treated group was numerically greater than the control group indicating no suggestion of a deficit. A Bayesian univariate ANOVA found anecdotal support for the lack of sex effect, BF_{01} = 2.39. Moderate evidence was found to support the lack of interaction between group * sex, BF_{01} = 4.09.

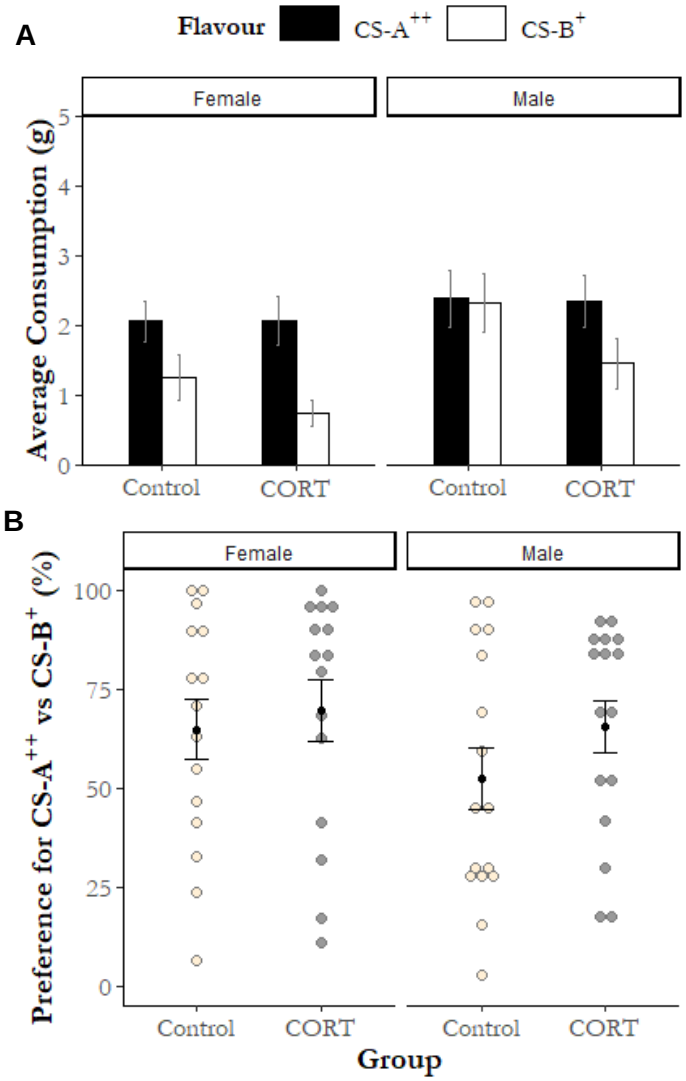


Figure 3.8. Graphs demonstrating CS-A^{++} vs CS-B^{+} choice test data for the control group and CORT-treated rats in Experiment 4.
No significant difference was found between the two groups in overall consumptions of either flavour (A) not percentage preference for CS-A^{++} vs CS-B^{+} (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

Effects of chronic CORT treatment on additional choice tests

Learning and memory of flavour-high reward associations

Overall, rats showed a preference for consumption of the flavour previously paired with a high value reward vs the previously unrewarded flavour, indicating intact recall of this flavour-high reward association. There were no differences between control and CORT treated rats, nor between males and females, with Bayes analysis providing only anecdotal support for this.

A repeated measures ANOVA found a significant main effect of flavour, with consumption of CS-A^{++} greater than CS-C, $F_{1, 58}$ = 13.95, MSE = 3.53, p <0.001, η_p^2 = 0.19. There were no interactions between flavour * group, $F_{1, 58}$ = 2.57, MSE = 3.53, p = 0.11, η_p^2 = 0.042; flavour * sex, $F_{1, 58}$ = 0.49, MSE = 3.53, p = 0.48, η_p^2 = 0.009; nor flavour * sex * group, $F_{1, 58}$ = 0.59, MSE = 3.53, p = 0.45, η_p^2 = 0.01.

Further, there was no significant difference between groups in overall consumption, $F_{1, 58}$ = 2.21, MSE = 1.77, p = 0.14, η_p^2 = 0.037, nor between males and female, $F_{1, 58}$ = 1.20, MSE = 1.77, p = 0.28, η_p^2 = 0.02, and no interaction between group * sex, $F_{1, 58}$ = 0.40, MSE = 1.77, p = 0.53, η_p^2 = 0.007 (Figure 3.9A). Shapiro-Wilks test of normality found that consumption of CS-A^{++} was not normally distributed in the female control group, W(15) = 0.86, p = 0.028, female CORT-treated group, W(15) = 0.87, p = 0.034. Consumption of CS-C was also not normally distributed in female CORT-treated rats, W(15) = 0.86, p = 0.023, male control rats, W(16) = 0.87, p = 0.02, nor male CORT-treated rats, W(16) = 0.84, p = 0.011. However, a suitable alternative statistical test for this type of analysis was not available.

Univariate ANOVAs showed there was no overall effect of group on consumption preference for CS-A^{++} vs CS-C, $F_{1, 58}$ = 0.13, MSE = 0.083, p = 0.21, η_p^2 = 0.027, nor an effect of sex, $F_{1, 58}$ = 0.24, MSE = 0.083, p = 0.63, η_p^2 = 0.004. There was no interaction between group * sex, $F_{1, 58}$ = 0.74, MSE = 0.083, p = 0.39, η_p^2 = 0.013 (Figure 3.9B). Shapiro-Wilks test also showed percentage preference for CS-A^{++} vs CS-C was not normally distributed in male control rats, W(16) = 0.89, p = 0.048, nor male CORT-treated rats, W(16) = 0.87, p = 0.025. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA provided anecdotal support for the lack of overall group effect on preference for CS-A^{++} vs CS-C, BF_{01} = 1.45, and the lack of sex effect, BF_{01} = 2.76. Moderate support was found for the lack of interaction between group * sex, BF_{01} = 4.02.

<u>Learning and memory of flavour-low reward associations</u>

Overall, rats showed a preference for consumption of the flavour previously paired with a low value reward vs the previously unrewarded flavour, indicating intact recall of this flavour-low reward association. There were no differences between control and CORT treated rats, nor between males and females, with Bayes analysis providing moderate support for this.

A main effect of flavour was found with greater consumption of CS-B^{+} than CS-C, $F_{1, 58}$ = 25.05, MSE = 3.37, p <0.001, η_p^2 = 0.30 (Figure 3.10A). There were no interactions between flavour * group, $F_{1, 58}$ = 0.06, MSE = 3.37, p = 0.82, η_p^2 = 0.001; flavour * sex, $F_{1, 58}$ = 0.35, MSE = 3.37, p = 0.56, η_p^2 = 0.006; nor flavour * group * sex, $F_{1, 58}$ = 0.11,

MSE = 3.37, p = 0.75, η_p^2 = 0.002. There was also no effect of group, $F_{1, 58}$ = 2.81, MSE = 2.09, p = 0.099, η_p^2 = 0.046, nor sex, $F_{1, 58}$ = 2.33, MSE = 2.09, p = 0.13, η_p^2 = 0.039, on overall consumption. Further, there was no significant interaction between group * sex on consumption, $F_{1, 58}$ = 1.29, MSE = 2.09, p = 0.26, η_p^2 = 0.022.

Shapiro-Wilks test of normality found that consumption of CS-B^+ was not normally distributed in the female CORT-treated group, W(15) = 0.83, p = 0.01. Consumption of CS-C was also not normally distributed in the female control group, W(15) = 0.86, p = 0.027, the female CORT-treated group, W(15) = 0.79, p = 0.002, male control group, W(16) = 0.85, p = 0.013, nor the male CORT-treated group, W(16) = 0.88, p = 0.036. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA showed no significant difference between control and CORT-treated rats preference for CS-B^+ vs CS-C, $F_{1, 58}$ = 0.11, MSE = 0.098, p = 0.75, η_p^2 = 0.002 (Figure 3.10B), nor between males and females, $F_{1, 58}$ = 0.67, MSE = 0.098, p = 0.42, η_p^2 = 0.011. There was no interaction between group * sex on this preference, $F_{1, 58}$ = 1.21, MSE = 0.098, p = 0.28, η_p^2 = 0.02. Shapiro-Wilks test found percentage preference for CS-B^+ vs CS-C was not normally distributed in female CORT-treated rats, W(15) = 0.81, p = 0.005, male control rats, W(16) = 0.87, p = 0.027, nor male CORT-treated rats, W(16) = 0.87, p = 0.026. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA provided moderate support for a lack of overall group effect on preference for CS-B^+ vs CS-C, BF_{01} = 3.91, and a lack of sex effect, BF_{01} = 3.22. Moderate support was also found for the lack of group * sex interaction, BF_{01} = 4.69.

<u>Effects of chronic CORT treatment on avoidance of unrewarded stimuli</u>

Overall, rats showed a preference for the novel unrewarded flavour vs the familiar unrewarded flavour, indicating avoidance of the latter. There were no differences between the groups nor sex in this avoidance, supported overall by Bayes analysis.

A repeated measures ANOVA showed a significant main effect of flavour, with overall consumption greater for CS-D than CS-C, $F_{1, 58}$ = 7.33, MSE = 3.62, p = 0.009, η_p^2 = 0.11 (Figure 3.11A). There was no interaction here between flavour * group, $F_{1, 58}$ = 2.62, MSE = 3.62, p = 0.11, η_p^2 = 0.043, nor between flavour * sex, $F_{1, 58}$ = 0.17, MSE = 3.62, p = 0.68, η_p^2 = 0.003.

There was a significant interaction between flavour * group * sex, $F_{1, 58}$ = 5.25, MSE = 3.62, p = 0.026, η_p^2 = 0.083. For this experiment, the most theoretically interesting investigation is whether there is a difference in consumption of either flavour in any of the sub groups.

Thus, Fisher's LSD post-hoc analysis investigated this and found only significantly greater consumption for CS-D vs CS-C in male control rats (p <0.001), but not for female controls (p = 0.43), male CORT-treated rats (p = 0.69) or female CORT-treated rats (p = 0.15).

Overall, there was a significant effect of group, whereby control rats consumed significantly greater than CORT-treated rats, $F_{1, 58}$ = 4.73, MSE = 2.22, p = 0.034, η_p^2 = 0.075. There was no overall effect of sex on consumption, $F_{1, 58}$ = 0.60, MSE = 2.22, p = 0.44, η_p^2 = 0.01, and no interaction between group * sex, $F_{1, 58}$ = 0.14, MSE = 2.22, p = 0.71, η_p^2 = 0.002. Shapiro-Wilks test found consumption data for CS-C was not normally distributed in female control rats, W(15) = 0.85, p = 0.017, female CORT-treated rats, W(15) = 0.86, p = 0.02, nor male control rats, W(16) = 0.69, p <0.001. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA found no overall significant difference in consumption preference of CS-D vs CS-C between control vs CORT-treated rats, $F_{1, 58}$ = 0.46, MSE = 0.11, p = 0.50, η_p^2 = 0.008, nor between males and females, $F_{1, 58}$ = 0.029, MSE = 0.11, p = 0.87, η_p^2 = 0.001. Overall, there was no interaction between group * sex on preference, $F_{1, 58}$ = 0.24, MSE = 0.11, p = 0.15, η_p^2 = 0.036 (Figure 3.11B). Shapiro-Wilks test found percentage preference for CS-D vs CS-C was not normally distributed for female CORT-treated rats, W(15) = 0.82, p = 0.006, nor for male control rats, W(16) = 0.79, p = 0.003.

A Bayesian univariate ANOVA provided moderate support for a lack of overall group effect on preference for CS-D vs CS-C, BF_{01} = 3.67, as well as a lack of sex effect, BF_{01} = 3.80. Only anecdotal support was found for the lack of interaction between group * sex, BF_{01} = 2.15.

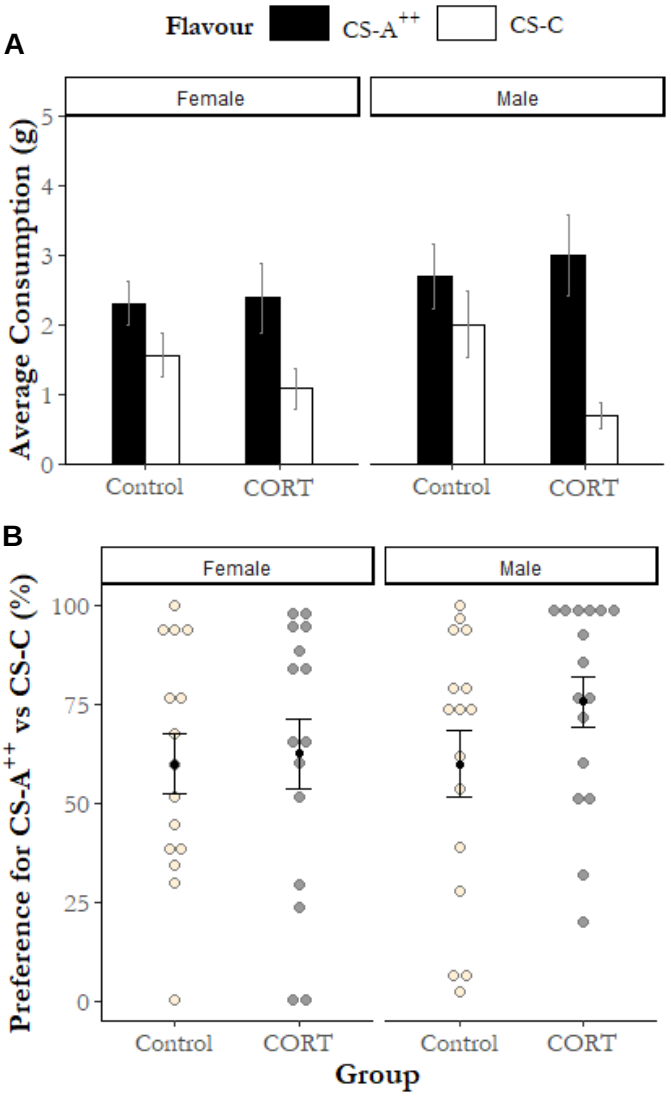


Figure 3.9. Graphs demonstrating CS-A^{++} vs CS-C choice test data for the combined control group and CORT-treated rats in Experiment 4.
No significant difference was found between the two groups in overall consumptions of either flavour (A) not percentage preference for CS-A^{++} vs CS-C (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

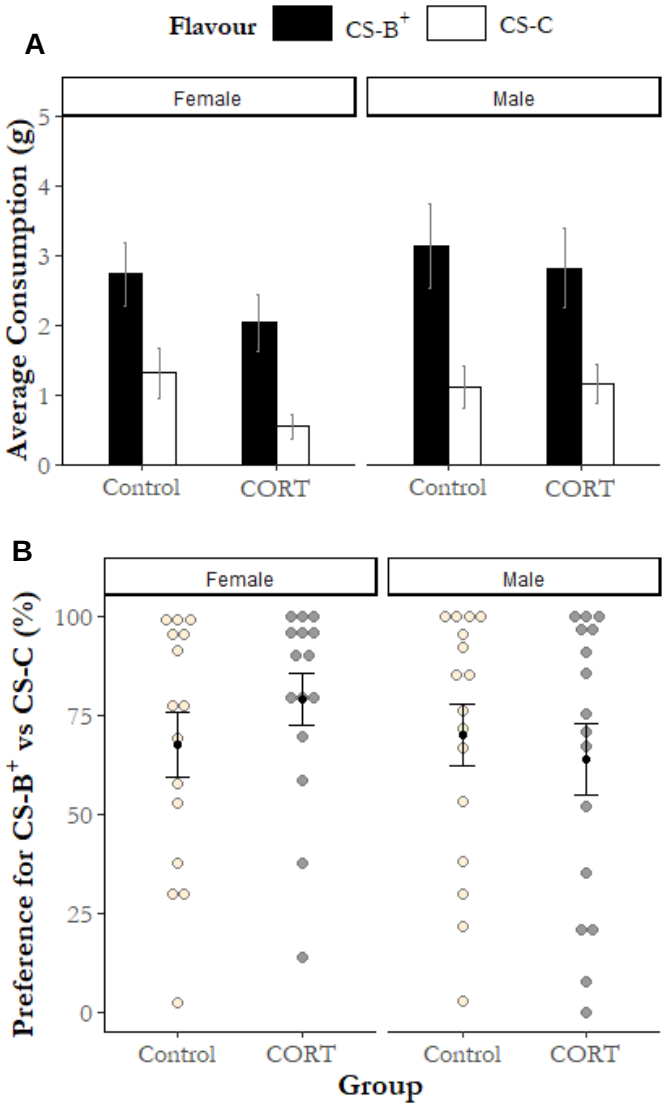


Figure 3.10. Graphs demonstrating CS-B$^+$ vs CS-C choice test data for the combined control group and CORT-treated rats in Experiment 4.
No significant difference was found between the two groups in overall consumptions of either flavour (A) or percentage preference for CS-B$^+$ vs CS-C (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

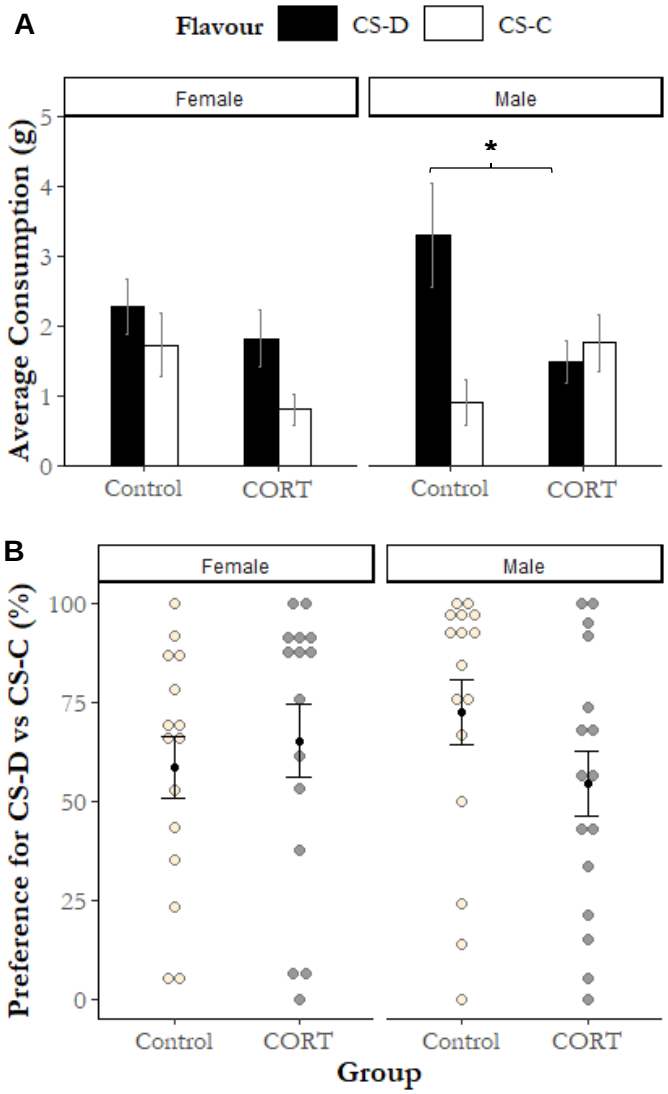


Figure 3.11. Graphs demonstrating CS-D vs CS-C choice test data for the combined control group and CORT-treated rats in Experiment 4.
No significant difference was found between the two groups in overall consumptions of either flavour (A); however, male control rats consumed significantly more CS-D compared to male CORT-treated rats. There was no overall difference in percentage preference for CS-D vs CS-C (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

Effects of chronic CORT treatment on plasma CORT levels

Since results from body weight changes could not be used as a positive control for CORT treatment, I sought to clarify whether CORT intake had been successful in this experiment through direct measurement of circulating CORT in blood plasma taken at the end of the experiment.

There was no change in plasma CORT level found following chronic CORT treatment, nor a difference between males and females, supported by Bayes analysis.

A univariate ANOVA revealed no significant effect of group on circulating CORT levels, $F_{1, 38} = 0.001$, MSE = 4124.88, $p = 0.97$, $\eta_p^2 < 0.001$, nor an effect of sex, $F_{1, 38} = 0.11$, MSE = 4124.88, $p = 0.74$, $\eta_p^2 = 0.002$. There was no interaction between group * sex, $F_{1, 38} = 0.007$, MSE = 4124.88, $p = 0.94$, $\eta_p^2 < 0.001$ (Figure 3.12).

A Bayesian univariate ANOVA provided moderate evidence to support a lack of overall group effect on plasma CORT concentration, $BF_{01} = 3.81$, as well as for a lack of overall sex effect, $BF_{01} = 3.63$. Strong support was also found for the lack of interaction between group * sex here, $BF_{01} = 16.70$.

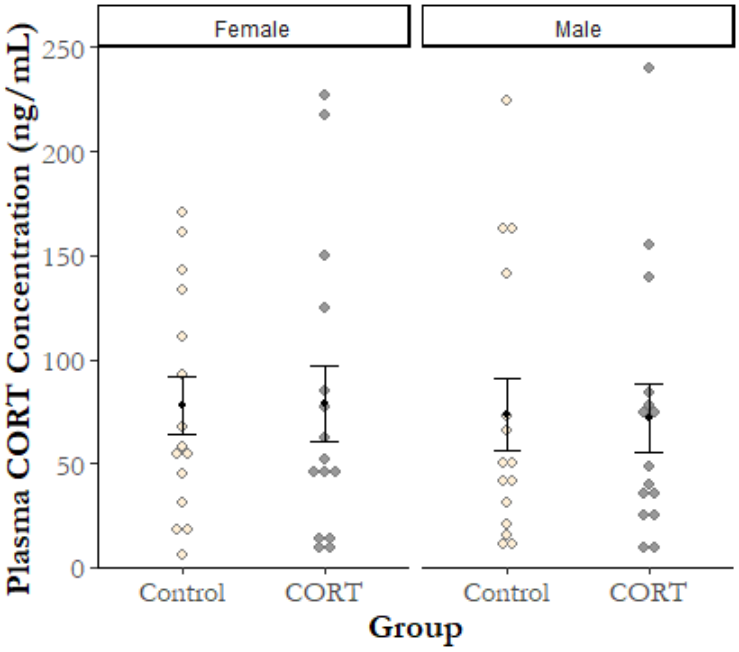


Figure 3.12. Circulating plasma CORT levels as measured by ELISA.
There was no difference between control and CORT-treated rats in their circulating CORT levels, in either males or females.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

3.4.4. Summary

Experiment 4 aimed to address limitations in the initial pilot Experiment 3 that evaluated the novel flavour m-ABT in both the chronic CORT and IFN-α models. This experiment applied a more robust experimental design focusing solely on the chronic CORT model to evaluate the novel flavour m-ABT as an assay for detecting deficits in forming reward-induced biases, as well as determine whether any detected deficits are conserved across males and females.

The behavioural findings from this experiment were unexpected, with no deficit in the formation of positive bias in the CORT-treated group compared to control group, regardless of sex. Although preferences were lower overall compared to experiment 3 as was the aim by changing the sucrose concentrations to 16% and 8%, it appeared this change may have generally reduced the ability to form a positive bias in control animals.

Due to the lack of a positive control for CORT treatment in body weight increase, an ELISA was conducted to measure circulating plasma CORT levels at the end of the experiment. As CORT had been administered to one group, these rats could be expected to have increased levels of circulating CORT with long-term treatment. Although, previous evidence has been variable due to the negative feedback of CORT on the HPA axis, such that some overproduction of CORT would increase overall concentrations but too much overproduction stops further production of CORT, a concept discussed more at the end of this chapter. In this experiment, no difference was found in CORT levels between control and CORT-treated rats. This might suggest issues with CORT intake in this experiment and therefore a lack of deficit in forming reward-induced positive biases may be due to this drug administration issue.

Furthermore, findings from the additional choice tests showed that overall rats showed intact learning and recall the flavour-reward associations, as well as avoidance of the unrewarded flavour. There were no significant differences between the groups in any additional tests.

3.5. Experiment 5 – Further evaluation of the chronic IFN-α model in the novel flavour m-ABT

Experiment 5 aims to further evaluate the validity of the novel flavour m-ABT in detecting deficits in forming reward-induced positive biases following manipulation of affective state using the chronic IFN-α model with a more robust design than Experiment 3. This experiment will also compare males and females to determine whether deficits are consistent across sexes and enable the generalisation of findings from this assay. Chronic IFN-α treatment is expected to reduce consumption preference for the high-reward flavour vs the low reward-flavour compared to controls in both sexes, indicating a deficit in forming a reward-induced positive bias, with no difference between males and females in this preference.

3.5.1. Experimental design

32 naïve male and 32 naïve female Sprague-Dawley rats (Charles River, UK), weighing 310g – 460g and 221g – 304g respectively on *ad libitum* food at approximately 12 weeks of age were used in Experiment 5. Treatments were as described in 3.2.4, where 16 rats of each sex were treated with IFN-α and 16 of each sex treated with saline control.

In Experiment 5, flavour CS-A^{++} contained 16% sucrose and CS-B^{+} contained 8% sucrose during pairing sessions. No animals were removed from analysis. Repeated measures ANOVAs were used to analyse choice test consumption of each flavour, with 'group' and 'sex' as fixed factors. Fisher's LSD post-hoc analyses were conducted to analyse interactions. Univariate ANOVAs were conducted to compare percentage preferences across group and sex.

Data from training sessions can be seen in Appendix E.

3.5.2. Results

Effects of chronic IFN-α treatment and/or sex on reward-induced positive bias

Overall, rats showed a preference for consumption of the flavour previously paired with a high value reward vs the flavour previously paired with a low value reward, with no difference between IFN-α and control rats, indicating both groups were able to form a reward-induced positive bias. There was also no difference between males and females. However, Bayes analysis only provided anecdotal support for these lack of differences.

A repeated measures ANOVA found a main effect of flavour with consumption of CS-A^{++} greater than CS-B^{+}, $F_{1, 60} = 30.53$, MSE = 3.72, $p < 0.001$, $\eta_p^2 = 0.34$. There was no overall effect of group, $F_{1, 60} = 0.036$, MSE = 1.99, $p = 0.85$, $\eta_p^2 = 0.001$, or sex, $F_{1, 60} = 0.62$,

MSE = 1.99, $p = 0.44$, $\eta_p^2 = 0.01$, on overall consumption (Figure 3.13A). There was also no significant interaction between flavour * group, $F_{1, 60} = 1.61$, MSE = 3.72, $p = 0.21$, $\eta_p^2 = 0.026$; flavour * sex, $F_{1, 60} = 3.68$, MSE = 3.72, $p = 0.06$, $\eta_p^2 = 0.06$; sex * group, $F_{1, 60} = 1.85$, MSE = 1.99, $p = 0.18$, $\eta_p^2 = 0.03$; or between flavour * sex * group, $F_{1, 60} = 2.03$, MSE = 3.72, $p = 0.16$, $\eta_p^2 = 0.033$.

Shapiro-Wilks test of normality found the consumption data for CS-B^+ was not normally distributed in female control rats, W(16) = 0.82, $p = 0.005$. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA was conducted to compare group/sex differences in percentage preferences for CS-A^{++} vs CS-B^+ (Figure 3.13B). This analysis found no overall significant effect of group, $F_{1, 60} = 0.96$, MSE = 0.044, $p = 0.33$, $\eta_p^2 = 0.016$, or sex, $F_{1, 60} = 2.68$, MSE = 0.044, $p = 0.11$, $\eta_p^2 = 0.043$. There was also no interaction between group * sex, $F_{1, 60} = 0.46$, MSE = 0.044, $p = 0.50$, $\eta_p^2 = 0.008$.

A Bayesian univariate ANOVA found only anecdotal support for a lack of group effect on this preference for CS-A^{++} vs CS-B^+, $BF_{01} = 2.60$, however, preferences were numerically higher in the IFN-α treated group compared to controls indicating no suggestion of a deficit. Anecdotal evidence was also found to support a lack of sex effect, $BF_{01} = 1.27$. Moderate evidence was found to support the lack of interaction between group * sex in preference for CS-A^{++} vs CS-B^+, $BF_{01} = 3.34$.

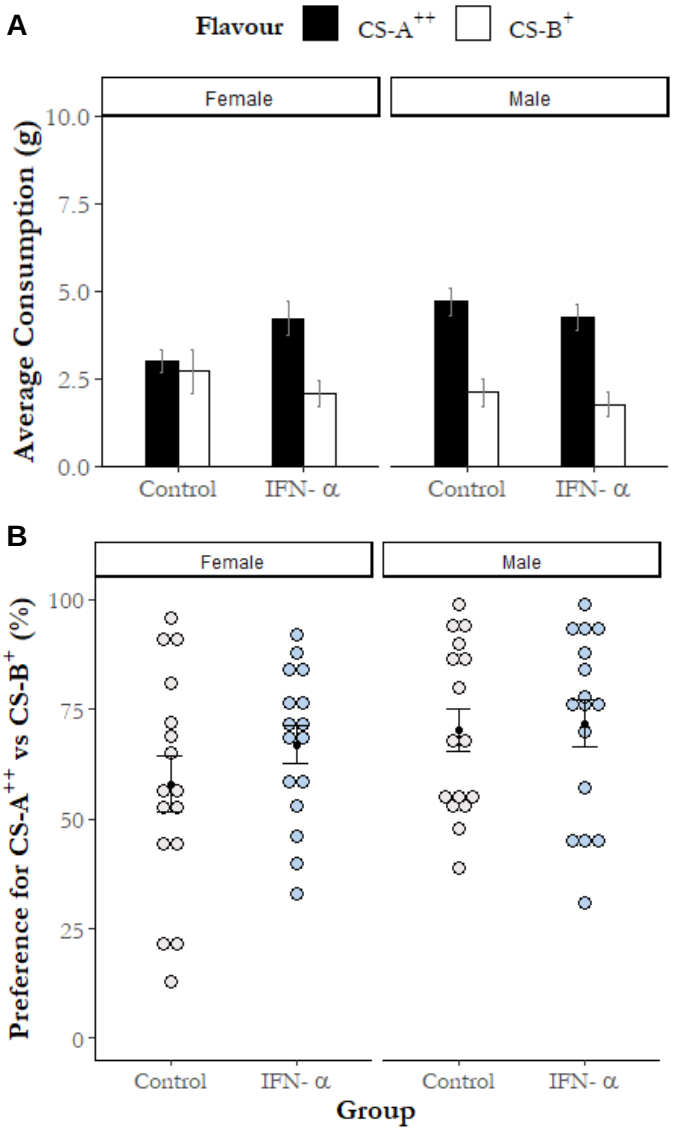


Figure 3.13. Graphs demonstrating CS-A^{++} vs CS-B^{+} choice test data for male and female control vs IFN-treated rats in Experiment 5.
No significant difference was found between the two groups, or males and females, in overall consumptions of either flavour (A) or percentage preference for CS-A^{++} (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

Effects of chronic IFN-α treatment and/or sex on additional choice tests

Learning and memory of flavour-high reward associations

Overall, rats showed a preference for consumption of the flavour previously paired with a high value reward vs the previously unrewarded flavour, indicating intact recall of this flavour-high reward association. There were no differences between control and IFN-α treated rats, nor between males and females, with Bayes analysis providing support for this.

A repeated measures ANOVA found a significant main effect of flavour, with consumption higher for CS-A^{++} vs CS-C, $F_{1, 60} = 96.48$, MSE = 5.22, $p < 0.001$, $\eta_p^2 = 0.62$. There was no overall effect of group, $F_{1, 60} = 0.20$, MSE = 2.71, $p = 0.65$, $\eta_p^2 = 0.003$, however there was a significant effect of sex, $F_{1, 60} = 9.31$, MSE = 2.71, $p = 0.003$, $\eta_p^2 = 0.13$, with overall consumption higher in females than males (Figure 3.14A). There was no significant interaction between flavour * group, $F_{1, 60} = 0.14$, MSE = 5.22, $p = 0.71$, $\eta_p^2 = 0.002$; flavour * sex, $F_{1, 60} = 3.03$, MSE = 5.22, $p = 0.087$, $\eta_p^2 = 0.048$; sex * group, $F_{1, 60} = 0.78$, MSE = 2.71, $p = 0.38$, $\eta_p^2 = 0.013$; or flavour * sex * group, $F_{1, 60} = 2.10$, MSE = 5.22, $p = 0.15$, $\eta_p^2 = 0.034$.

Shapiro-Wilks test of normality found consumption data for CS-A^{++} was not normally distributed in female control rats, W(16) = 0.86, $p = 0.015$. Consumption data for CS-C was also not normally distributed in female control rats, W(16) = 0.82, $p = 0.004$, female IFN-treated rats, W(16) = 0.78, $p = 0.002$, male control rats, W(16) = 0.68, $p < 0.001$, nor male IFN-treated rats, W(16) = 0.71, $p < 0.001$. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA found no overall effect of group, $F_{1, 60} = 0.008$, MSE = 0.064, $p = 0.93$, $\eta_p^2 < 0.001$, or sex, $F_{1, 60} = 0.65$, MSE = 0.064, $p = 0.42$, $\eta_p^2 = 0.011$, on preference percentages for CS-A^{++} vs CS-C (Figure 3.14B). There was also no interaction between group * sex, $F_{1, 60} = 2.07$, MSE = 0.064, $p = 0.16$, $\eta_p^2 = 0.033$. Shapiro-Wilks test of normality found percentage preference for CS-A^{++} vs CS-C was not normally distributed in female control rats, W(16) = 0.78, $p = 0.001$, female IFN-treated rats, W(16) = 0.81, $p = 0.004$, male control rats, W(16) = 0.72, $p < 0.001$, nor male IFN-treated rats, W(16) = 0.79, $p = 0.002$. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA provides moderate support for a lack of overall group effect on preference for CS-A^{++} vs CS-C, $BF_{01} = 3.90$, with only anecdotal support for a lack of overall sex effect on this preference, $BF_{01} = 2.97$. Anecdotal support was also found for the lack of group * sex interaction here, $BF_{01} = 2.48$.

Learning and memory of flavour-low reward associations

Overall, rats showed a preference for consumption of the flavour previously paired with a low value reward vs the previously unrewarded flavour, indicating intact recall of this flavour-low reward association. There were no differences between control and IFN-α treated rats, with moderate support from Bayes analysis. There was also no overall difference between males and females, however Bayes deemed this inconclusive.

A repeated measures ANOVA found a main effect of flavour with greater consumption of CS-B^+ compared to CS-C, $F_{1, 60} = 89.49$, MSE = 4.33, $p < 0.001$, $\eta_p^2 = 0.59$ (Figure 3.15A). There was no overall effect of group, $F_{1, 60} = 1.64$, MSE = 4.77, $p = 0.21$, $\eta_p^2 = 0.027$, however, there was a significant main effect of sex on overall consumption, $F_{1, 60} = 4.09$, MSE = 4.77, $p = 0.047$, $\eta_p^2 = 0.064$, where females consumed overall more compared to males.

There were no significant interactions between flavour * group, $F_{1, 60} = 0.069$, MSE = 4.33, $p = 0.79$, $\eta_p^2 = 0.001$; group * sex, $F_{1, 60} = 2.73$, MSE = 4.77, $p = 0.10$, $\eta_p^2 = 0.043$; or flavour * group * sex, $F_{1, 60} = 0.52$, MSE = 4.33, $p = 0.47$, $\eta_p^2 = 0.009$. There was a significant interaction between flavour * sex, $F_{1, 60} = 8.5$, MSE = 4.33, $p = 0.005$, $\eta_p^2 = 0.12$. Post-hoc analysis revealed females consumed significantly more of CS-B^+ compared to males ($p = 0.003$), with no difference in consumption of CS-C ($p = 0.53$). Shapiro-Wilks test of normality found consumption data for CS-C was not normally distributed in female control rats, W(16) = 0.67, $p < 0.001$, female IFN-treated rats, W(16) = 0.59, $p < 0.001$, male control rats, W(16) = 0.72, $p < 0.001$, nor male IFN-treated rats, W(16) = 0.81, $p = 0.004$. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA found no overall effect of group, $F_{1, 60} = 0.17$, MSE = 0.052, $p = 0.69$, $\eta_p^2 = 0.003$, or sex, $F_{1, 60} = 3.19$, MSE = 0.052, $p = 0.079$, $\eta_p^2 = 0.051$, on this percentage preference (Figure 3.15B). There was also no interaction between group * sex, $F_{1, 60} = 0.51$, MSE = 0.052, $p = 0.48$, $\eta_p^2 = 0.008$. Shapiro-Wilks test of normality found percentage preference for CS-B^+ vs CS-C was not normally distributed for female control rats, W(16) = 0.72, $p < 0.001$, female IFN-treated rats, W(16) = 0.61, male control rats, W(16) = 0.86, $p = 0.018$, nor male IFN-treated rats, W(16) = 0.87, $p = 0.024$. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA provided moderate evidence to support the lack of overall group effect on preference for CS-B^+ vs CS-C, $BF_{01} = 3.66$, with almost no evidence to support an effect in either direction for the overall difference between males and females, $BF_{01} = 0.963$. Moderate evidence was also found to support the lack of group * sex interaction, $BF_{01} = 3.19$.

Effects of chronic IFN-α on avoidance of an unrewarded stimulus

Overall, rats showed a preference for the novel unrewarded flavour vs the familiar unrewarded flavour, indicating avoidance of the latter. There were no differences between the groups nor sex in this avoidance, with only anecdotal support from Bayes analysis.

A main effect of flavour was found with greater consumption of CS-D compared to CS-C, $F_{1, 60}$ = 14.28, MSE = 8.73, p <0.001, η_p^2 = 0.19 (Figure 3.16A). There was no effect of group, $F_{1, 60}$ = 0.38, MSE = 5.36, p = 0.54, η_p^2 = 0.006; or sex, $F_{1, 60}$ = 0.001, MSE = 5.36, p = 0.97, η_p^2 <0.001, on overall consumption. There were no interactions between flavour * group, $F_{1, 60}$ = 2.16, MSE = 8.73, p = 0.15, η_p^2 = 0.035; flavour * sex, $F_{1, 60}$ = 0.23, MSE = 8.73, p = 0.64, η_p^2 = 0.004; group * sex, $F_{1, 60}$ = 0.45, MSE = 5.36, p = 0.51, η_p^2 = 0.007; or flavour * group * sex, $F_{1, 60}$ = 1.54, MSE = 8.73, p = 0.22, η_p^2 = 0.025.

Shapiro-Wilks test for normality found consumption data for CS-D was not normally distributed in male control rats, W(16) = 0.84, p = 0.009, nor male IFN-treated rats, W(16) = 0.84, p = 0.009. Consumption data for CS-C was not normally distributed in female control rats, W(16) = 0.63, p <0.001, female IFN-treated rats, W(16) = 0.82, p = 0.006, male control rats, W(16) = 0.85, p = 0.013, nor male IFN-treated rats, W(16) = 0.88, p = 0.034. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA found no overall effect of group, $F_{1, 60}$ = 1.24, MSE = 0.096, p = 0.27, η_p^2 = 0.02, or sex, $F_{1, 60}$ = 2.14, MSE = 0.096, p = 0.15, on this preference for CS-D (Figure 3.16B). There was also no interaction between group * sex, $F_{1, 60}$ = 0.88, MSE = 0.096, p = 0.35, η_p^2 = 0.014. Shapiro-Wilks test for normality found percentage preference for CS-D vs CS-C was not normally distributed in female control rats, W(16) = 0.71, p <0.001. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA found anecdotal support for the overall lack of group effect on preference for CS-D vs CS-C, BF_{01} = 2.34, and lack of sex effect, BF_{01} = 1.59. Moderate evidence was found to support the lack of interaction between group * sex here, BF_{01} = 3.25.

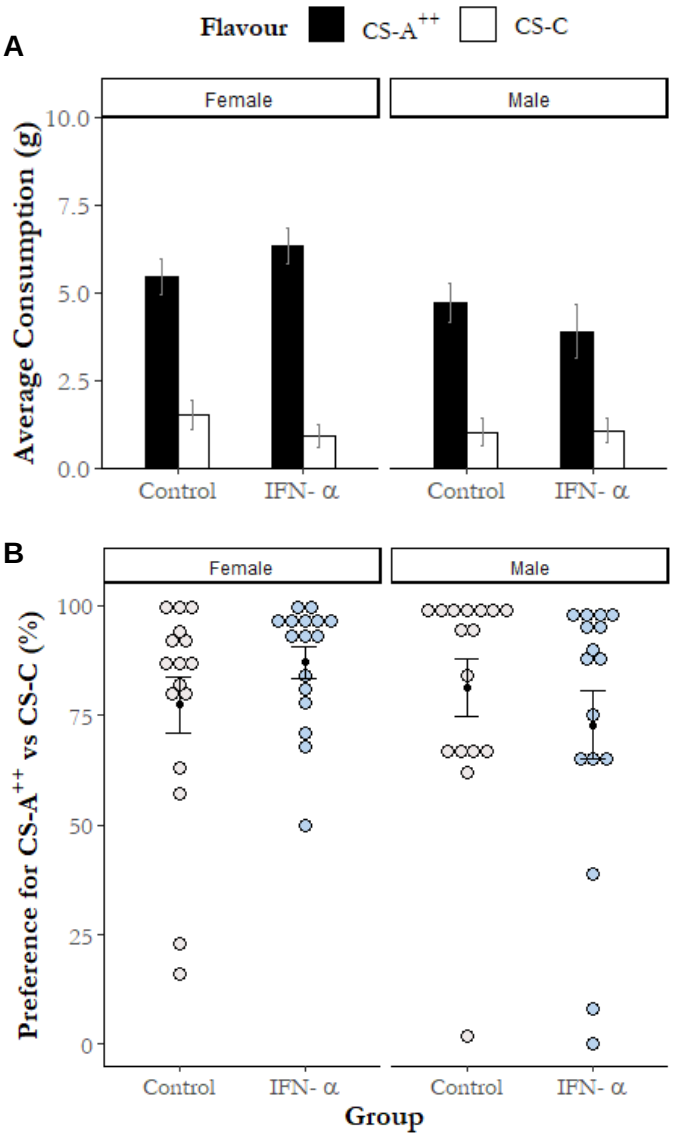


Figure 3.14. Graphs demonstrating CS-A^{++} vs CS-C choice test data for male and female control vs IFN-treated rats in Experiment 5.

No significant difference was found between the two groups, or males and females, in overall consumptions of either flavour (A) or percentage preference for CS-A^{++} (B).

Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

Bars represent means ±SEM. Dots represent individual data points.

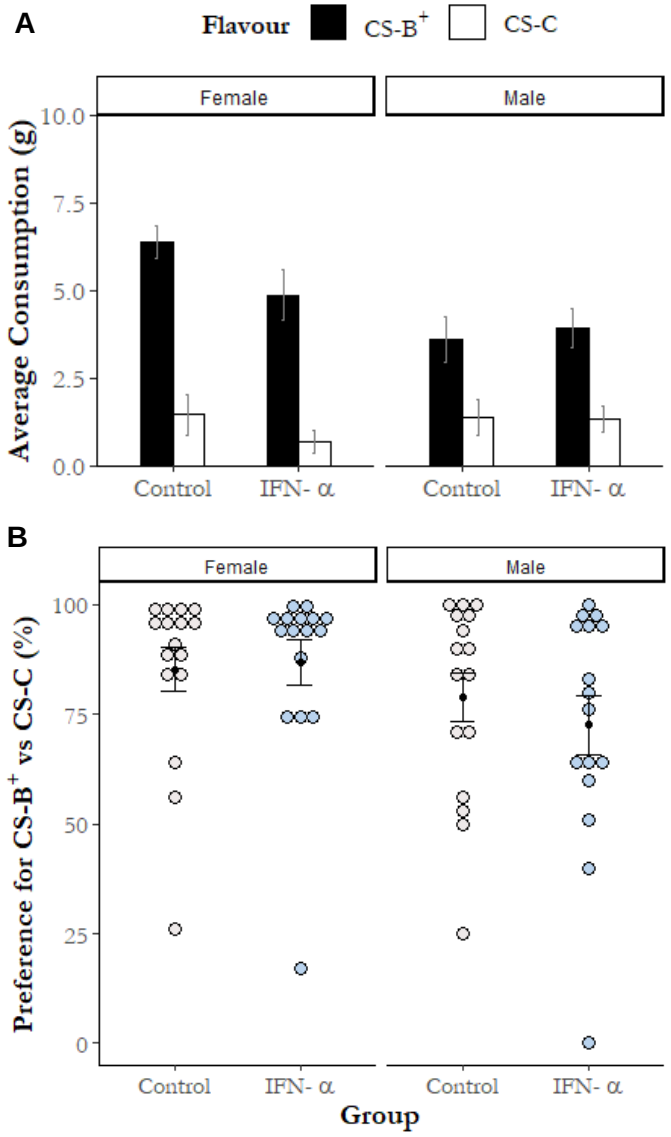


Figure 3.15. Graphs demonstrating CS-B$^+$ vs CS-C choice test data for male and female control vs IFN-treated rats in Experiment 5.
No significant difference was found between the two groups, or males and females, in overall consumptions of either flavour (A) or percentage preference for CS-B$^+$ (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

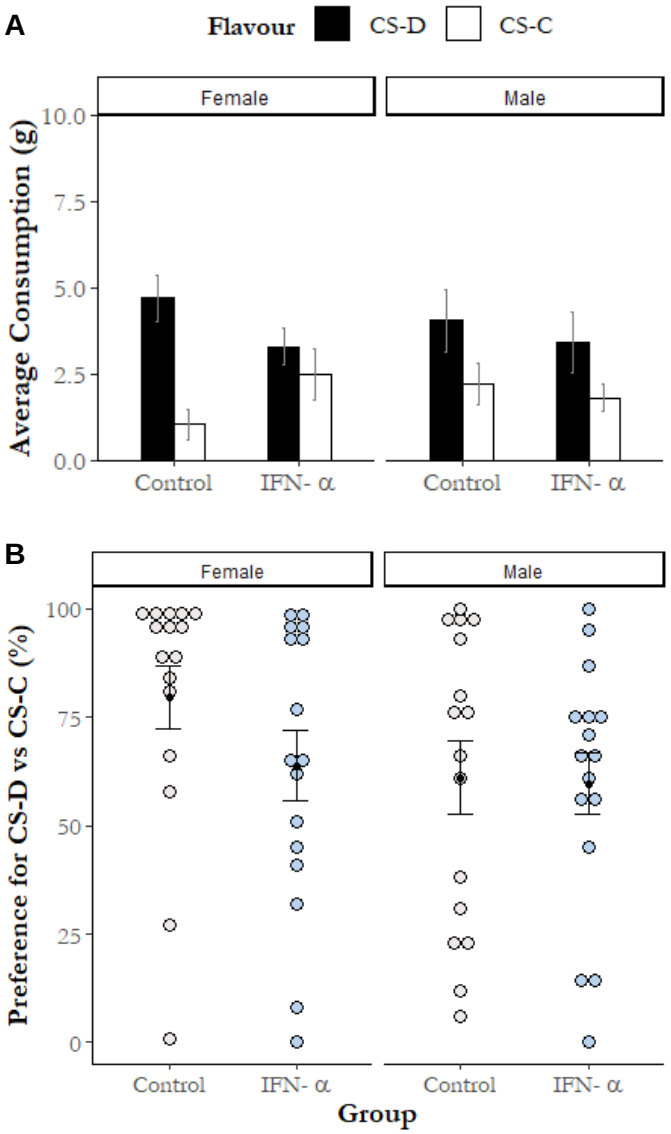


Figure 3.16. Graphs demonstrating CS-D vs CS-C choice test data for male and female control vs IFN-treated rats in Experiment 5.
No significant difference was found between the two groups, or males and females, in overall consumptions of either flavour (A) or percentage preference for CS-D (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

3.5.3. Summary

Experiment 5 aimed to address some limitations in the initial pilot Experiment 3 to evaluate the novel flavour m-ABT as an assay for measuring deficits in reward-induced positive biases in the chronic IFN-α model, as well as determine whether any detected deficits are conserved across males and females.

Like Experiment 4, this experiment was not able to replicate the deficit in positive bias formation observed in Experiment 3. Bayesian analysis found moderate support for a lack of interaction between group and sex in the formation of a positive bias here, and anecdotal support for a lack of overall group effect, indicating a conclusive lack of deficit measured by this novel flavour m-ABT. One possibility for this lack of deficit contradicting the findings of Experiment 3 is that the IFN-α treatment did not work, however, histological analyses of these brains later in this thesis reveal significant group differences that indicate a heightened immunological response in IFN-treated rats, suggesting this treatment did indeed work as expected (see section 6.4).

Furthermore, findings from the additional choice tests showed that overall rats showed intact learning and recall the flavour-reward associations, as well as avoidance of the unrewarded flavour. There were no significant differences between the groups in any additional tests.

3.6. Reward-induced biases in the heterozygous *CACNA1C* knockout model

Deficits in reward-induced bias *CACNA1C* heterozygous knockout rats (CACNA1C$^{+/-}$) were explored in the following experiments. Experiment 6 was an initial experiment in females aiming to determine whether CACNA1C$^{+/-}$ rats showed a deficit in forming reward-induced positive biases following the same flavour m-ABT design as Experiments 4 and 5. After Experiments 4 and 5 failed to recapitulate the deficits seen in Experiment 3, and Experiment 6 showed inconclusive findings, then Experiment 7 was conducted to further examine deficits in the CACNA1C$^{+/-}$ model following the flavour m-ABT design originally used in Experiment 3. Experiment 7 also compared males and females to determine whether deficits are consistent across sexes and enable the generalisation of findings from this assay. The CACNA1C$^{+/-}$ model was expected to show reduced consumption preference for the high-reward flavour vs the low reward-flavour compared to wild type rats in both sexes, indicating a deficit in forming a reward-induced positive bias, with no difference between males and females in this preference.

3.6.1. Transgenic heterozygous *CACNA1C* knockout model

CACNA1C$^{+/-}$ were bred on a Sprague Dawley background. Breeding of CACNA1C$^{+/-}$ rats resulted in litters with a distribution of wild type (WT) and heterozygote knockout pups, as the homozygote deletion is embryonically lethal. A selection of WT and heterozygote rats bred from an in-house breeding stock were used for Experiment 6, whilst Experiment 7 used a selection of animals bred from an outsourced colony at Charles River, UK.

3.6.2. Experiment 6 – Investigating a deficit in reward-induced positive bias

3.6.2.1. Experimental design

40 naïve female rats (WT *n*=17, CACNA1C$^{+/-}$ *n*=23), weighing 208g – 282g on *ad libitum* food at approximately 14-15 weeks of age were used in this experiment. Genotyping of rats was completed by Dr Patricia Gasalla Canto or Jennifer Carter of Cardiff University.

In Experiment 6, flavour CS-A^{++} contained 16% sucrose and CS-B^{+} contained 8% sucrose during pairing sessions. No animals were removed from analysis. Repeated measures ANOVAs were used to analyse choice test consumption of each flavour, with ‘genotype’ as a fixed factor. Fisher’s LSD post-hoc analyses were conducted to analyse interactions. An independent samples t-test was conducted to compare percentage preferences across genotypes.

Data from training sessions can be seen in Appendix E.

3.6.2.2. Results

Effects of CACNA1C heterozygous knockout on reward-induced positive bias

Overall, rats showed a preference for consumption of the flavour previously paired with a high value reward vs the flavour previously paired with a low value reward, indicating formation of a reward-induced positive bias, with no difference between wild type and CACNA1C$^{+/-}$ rats. However, Bayes analysis provided no support for this lack of difference between the genotypes.

A repeated-measures ANOVA found a significant effect of flavour, with consumption of CS-A^{++} greater than CS-B^{+}, $F_{1, 38} = 4.74$, MSE = 4.28, $p = 0.036$, $\eta_p^2 = 0.11$. There was no significant effect of genotype on overall consumption of CS-A^{++} and CS-B^{+}, $F_{1, 38} = 0.15$, MSE = 2.59, $p = 0.70$, $\eta_p^2 = 0.004$ (Figure 3.17A), and no significant interaction between genotype * flavour, $F_{1, 38} = 1.08$, MSE = 4.28, $p = 0.31$, $\eta_p^2 = 0.028$.

Shapiro-Wilks test for normality found consumption data for CS-A^{++} was not normally distributed in CACNA1C$^{+/-}$ rats, W(23) = 0.85, $p = 0.003$, nor in WT rats, W(17) = 0.79, $p = 0.001$. Consumption data for CS-B^{+} was also not normally distributed in WT rats, W(17) = 0.79, $p = 0.001$. However, a suitable alternative statistical test for this type of analysis was not available.

An independent samples t-test found no significant difference between wild type and CACNA1C$^{+/-}$ rats in their preference for CS-A^{++} vs CS-B^{+}, t(38) = 1.65, $p = 0.11$ (Figure 3.17B).

A Bayesian independent samples t-test revealed almost no evidence to support this lack of difference in preference, $BF_{01} = 1.12$.

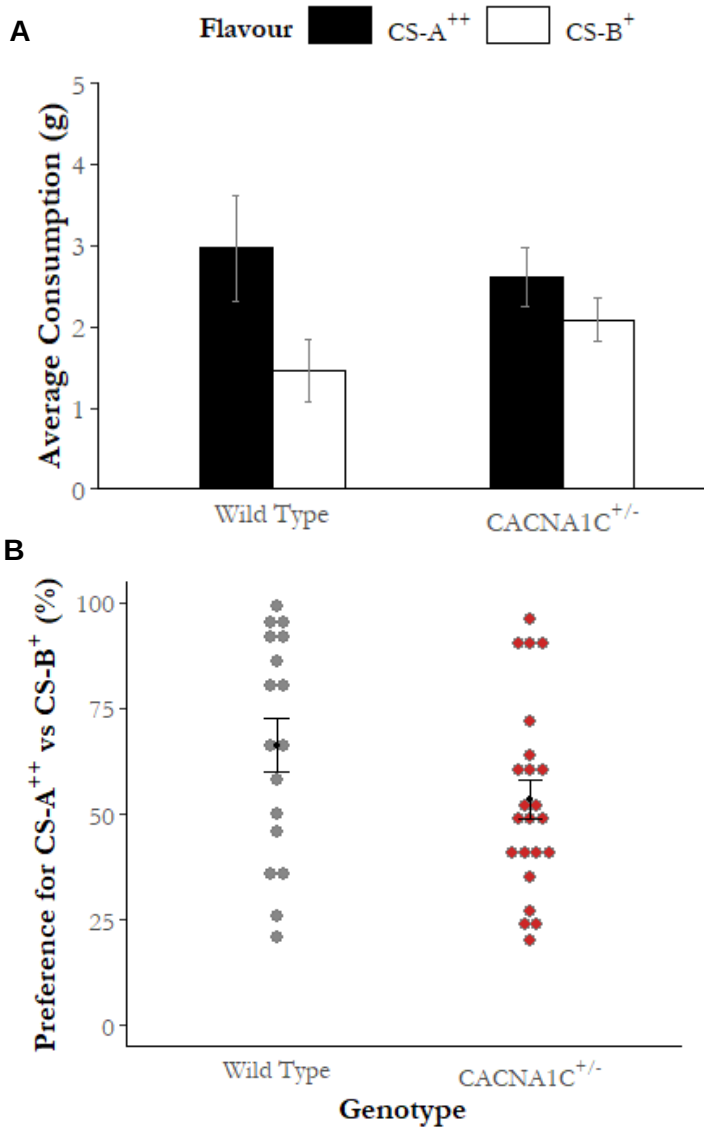


Figure 3.17. Graphs demonstrating CS-A^{++} vs CS-B^{+} choice test data for wild type and CACNA1C heterozygous knockouts (CACNA1C$^{+/-}$) in Experiment 6.
No significant difference was found between the two genotypes in overall consumptions of flavours (A), nor percentage preference for CS-A^{++} vs CS-B^{+} (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

Effects of *CACNA1C* heterozygous knockout on additional choice tests

Learning and memory of flavour-high reward associations

Overall, there was no preference for consumption between the flavour previously paired with a high value reward vs the previously unrewarded flavour, with no difference between the genotypes. However, Bayes analysis only provided anecdotal support for this lack of difference.

There was no overall significant difference in consumption of CS-A^{++} compared to CS-C, $F_{1, 38}$ = 4.02, MSE = 2.74, p = 0.052, η_p^2 = 0.096. There was also no significant effect of genotype, $F_{1, 38}$ = 0.51, MSE = 1.40, p = 0.48, η_p^2 = 0.013 (Figure 3.18A), with no interaction between genotype * flavour, $F_{1, 38}$ = 0.29, MSE = 2.74, p = 0.59, η_p^2 = 0.008.

Shapiro-Wilks test for normality found consumption data for CS-A^{++} was not normally distributed in CACNA1$C^{+/-}$ rats, W(23) = 0.85, p = 0.002, nor in WT rats, W(17) = 0.88, p = 0.029. Consumption data for CS-C was also not normally distributed in CACNA1$C^{+/-}$ rats, W(23) = 0.80, p <0.001, nor WT rats, W(17) = 0.66, p <0.001. However, a suitable alternative statistical test for this type of analysis was not available.

Percentage preference for CS-A^{++} vs CS-C was not normally distributed in either CACNA1$C^{+/-}$ rats, W(23) = 0.85, p = 0.003, nor in WT rats, W(17) = 0.75, p <0.001. Therefore, the Mann-Whitney U test was conducted to compare these two groups. This found no significant difference between WT and CACNA1$C^{+/-}$ rats in their preference for CS-A^{++} vs CS-C, U = 265, p = 0.057 (Figure 3.18B).

A Bayesian independent samples t-test found only anecdotal evidence to support this lack of difference in preference, BF_{01} = 1.56.

Learning and memory of flavour-low reward associations

Overall, rats showed a preference for the flavour previously paired with a low value reward vs the previously unrewarded flavour, indicating intact recall of this flavour-low reward association. There was no difference found between wild type and CACNA1$C^{+/-}$ rats, with only anecdotal support from Bayes analysis.

There was a significant effect of flavour, with overall consumption greater for CS-B^{+} vs CS-C, $F_{1, 38}$ = 8.65, MSE = 5.58, p = 0.006, η_p^2 = 0.19. There was no significant effect of genotype on consumption, $F_{1, 38}$ = 0.13, MSE = 3.67, p = 0.72, η_p^2 = 0.003 (Figure 3.19A), and no interaction between genotype * flavour, $F_{1, 38}$ = 0.043, MSE = 5.58, p = 0.84, η_p^2 = 0.001.

Shapiro-Wilks test for normality found consumption data for CS-B^+ was not normally distributed in CACNA1$C^{+/-}$ rats, $W(23) = 0.89$, $p = 0.019$, nor in WT rats, $W(17) = 0.71$, $p < 0.001$. Consumption data for CS-C was also not normally distributed in CACNA1$C^{+/-}$ rats, $W(23) = 0.48$, $p < 0.001$, nor WT rats, $W(17) = 0.69$, $p < 0.001$. However, a suitable alternative statistical test for this type of analysis was not available.

Percentage preference for CS-B^+ vs CS-C was not normally distributed in either CACNA1$C^{+/-}$ rats, $W(23) = 0.74$, $p < 0.001$, nor in WT rats, $W(17) = 0.87$, $p = 0.024$. Therefore, the Mann-Whitney U test was conducted to compare these two groups. This found no significant difference between WT and CACNA1$C^{+/-}$ rats in their preference for CS-B^+ vs CS-C, $U = 155$, $p = 0.27$ (Figure 3.19B).

A Bayesian independent samples t-test found anecdotal evidence to support this lack of difference, $BF_{01} = 1.98$.

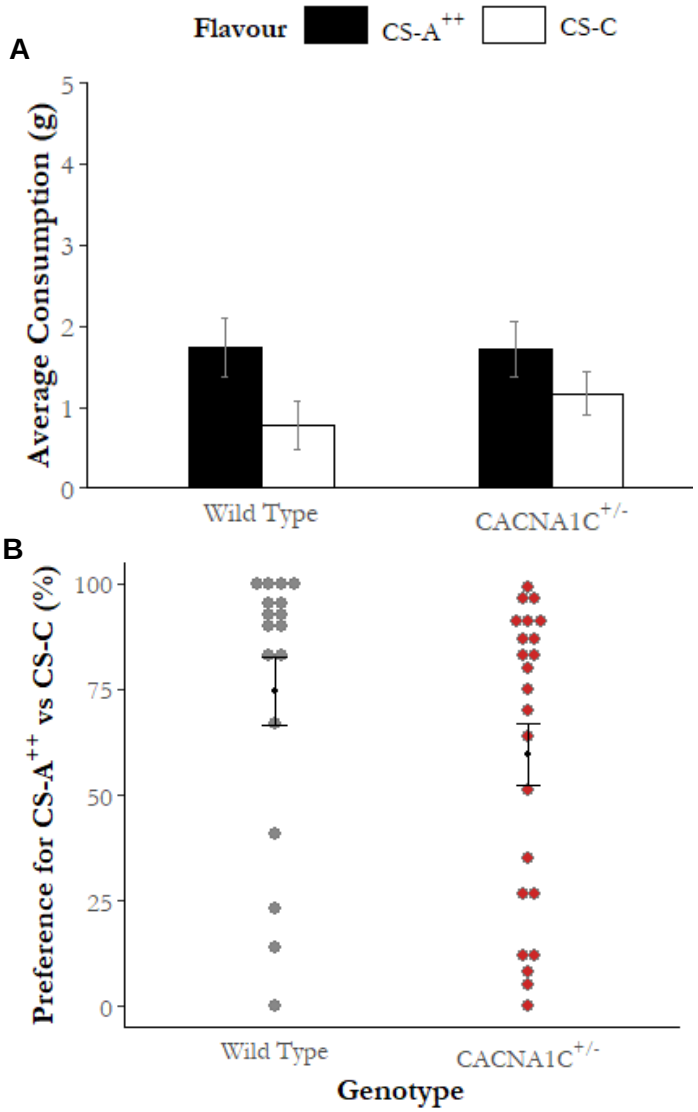


Figure 3.18. Graphs demonstrating CS-A^{++} vs CS-C choice test data for wild type and CACNA1C heterozygous knockouts (CACNA1C$^{+/-}$) in Experiment 6.
No significant difference was found between the two genotypes in overall consumptions of flavours (A), nor percentage preference for CS-A^{++} vs CS-C (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

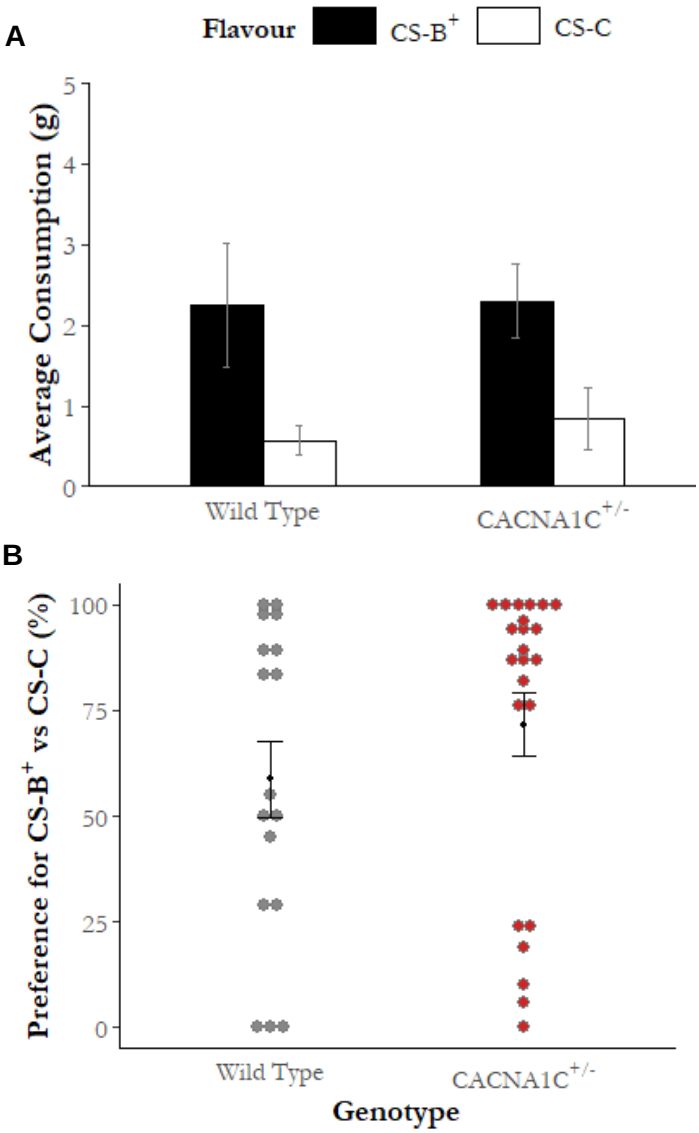


Figure 3.19. Graphs demonstrating CS-B^{+} vs CS-C choice test data for wild type and CACNA1C heterozygous knockouts (CACNA1C$^{+/-}$) in Experiment 6.
No significant difference was found between the two genotypes in overall consumptions of flavours (A), nor percentage preference for CS-B^{+} vs CS-C (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

Effects of CACNA1C heterozygous knockout on avoidance of an unrewarded stimulus

Overall, these rats did not show a preference for the novel unrewarded flavour vs the familiar unrewarded flavour, with no difference between genotypes, indicating they did not learn to avoid the familiar flavour.

A repeated measures ANOVA showed no significant overall difference in consumption of CS-D vs CS-C, $F_{1, 38} = 0.22$, MSE = 2.12, $p = 0.64$, $\eta_p^2 = 0.006$. There was also no significant effect of genotype, $F_{1, 38} = 0.014$, MSE = 2.01, $p = 0.91$, $\eta_p^2 < 0.001$ (Figure 3.20A), nor an interaction between genotype * flavour, $F_{1, 38} = 0.67$, MSE = 2.12, $p = 0.42$, $\eta_p^2 = 0.017$.

Shapiro-Wilks test for normality found consumption data for CS-D was not normally distributed in CACNA1C$^{+/-}$ rats, W(23) = 0.80, $p < 0.001$, nor in WT rats, W(17) = 0.84, $p = 0.008$. Consumption data for CS-C was also not normally distributed in CACNA1C$^{+/-}$ rats, W(23) = 0.81, $p < 0.001$, nor WT rats, W(17) = 0.77, $p < 0.001$. However, a suitable alternative statistical test for this type of analysis was not available.

Percentage preference for CS-D vs CS-C was not normally distributed in either CACNA1C$^{+/-}$ rats, W(23) = 0.89, $p = 0.013$, nor in WT rats, W(17) = 0.86, $p = 0.017$. Therefore, the Mann-Whitney U test was conducted to compare these two groups. This found no significant difference between WT and CACNA1C$^{+/-}$ rats in their preference for CS-A^{++} vs CS-C, U = 200.5, $p = 0.89$ (Figure 3.20B).

A Bayesian independent samples t-test found moderate evidence to support this lack of difference in preference between the two genotypes, $BF_{01} = 3.21$.

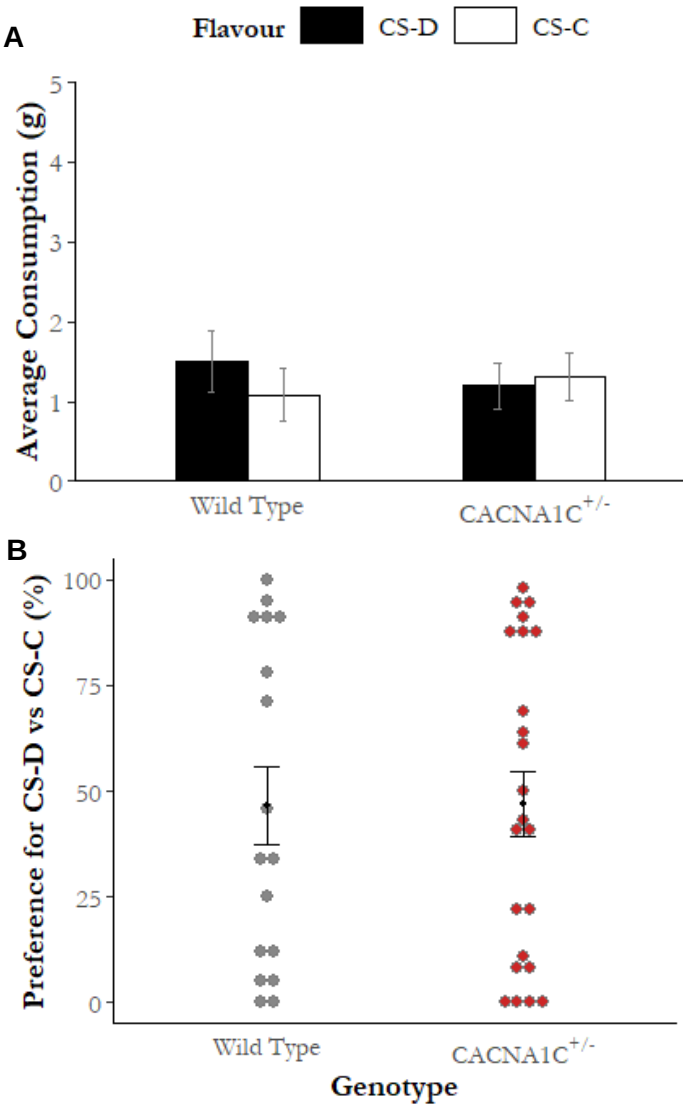


Figure 3.20. Graphs demonstrating CS-D vs CS-C choice test data for wild type and CACNA1C heterozygous knockouts (CACNA1C$^{+/-}$) in Experiment 6.
No significant difference was found between the two genotypes in overall consumptions of flavours (A), nor percentage preference for CS-D vs CS-C (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

3.6.2.3. Summary

Experiment 6 aimed to investigate the application of the flavour m-ABT to a genetic model of psychiatric disease, the *CACNA1C* heterozygous knockout rat. Following on from limitations identified in the initial flavour m-ABT pilot Experiment 3, 16 vs 8% sucrose were used as the high vs low rewards for this experiment as an initial pilot investigation in this model with only female rats.

While there was no significant difference between the CACNA1C$^{+/-}$ and control rats in preference for the higher value flavour compared to the lower value flavour, the Bayesian analysis for this suggested this evidence was not conclusive in supporting a true absence of an effect. The fact that only the control group showed a significant preference for this higher value flavour may suggest a more powerful assay could reveal a deficit in this formation of a reward-induced positive bias in CACNA1C$^{+/-}$ rats.

Similarly, additional choice tests found that wild type rats showed a preference for the high reward flavour vs the unrewarded flavour, but the CACNA1C$^{+/-}$ rats did not show this preference. In contrast, the opposite was found when assessing preference for the low reward flavour vs the unrewarded flavour. These findings suggest that there were differences in learning and memory of the reward-flavour associations between the two genotypes, and that perhaps any deficit in the ability to form a reward-induced positive bias in the CACNA1C$^{+/-}$ rats was due to an inability to form this association. However, again, direct comparison between the two genotypes did not reveal any significant differences. Finally, neither genotype showed any preference for the novel unrewarded flavour compared to the familiar unrewarded flavour, suggesting that these rats did not learn to avoid this unrewarded flavour during pairing sessions.

Considering the inconclusive results of Experiment 6, Experiment 7 aimed to re-examine the effects of *CACNA1C* in the flavour m-ABT with altered sucrose concentrations and including males and females.

3.6.3. Experiment 7 – Further evaluation of reward-induced positive bias in the CACNA1C$^{+/-}$ model

3.6.3.1. Experimental design

39 male rats (WT *n* = 30, CACNA1C$^{+/-}$ *n* = 9) weighing 190g – 400g and 31 female rats (WT *n* = 24, CACNA1C$^{+/-}$ *n* = 7) weighing 150g – 257g on *ad libitum* food varying from approx. 7 – 13 weeks of age were available. These animals were first run in the effort-related choice test (see section 5.5) whilst I was blind to their genotypes. Automated genotyping was then performed by TransnetYX, Memphis, USA. It was noted that there were very few CACNA1C$^{+/-}$ knockouts – after an investigation into this lack of heterozygotes, it appeared a technician had incorrectly genotyped some of the parent breeders as CACNA1C$^{+/-}$ when they were WT, thus resulting in several litters of WT only.

As a result, only a random selection of WT animals were taken from the full cohort to run in the flavour m-ABT assay of Experiment 7, alongside all available CACNA1C$^{+/-}$ knockouts. The cohort used here were 28 male (WT *n*=19, CACNA1C$^{+/-}$ *n*=9) rats weighing 190g – 400g and 24 female (WT *n*=17, CACNA1C$^{+/-}$ *n*=7) rats weighing 150g – 257g on *ad libitum* food varying from approx. 7 – 13 weeks of age.

Data from training sessions can be seen in Appendix E.

3.6.3.2. Results

<u>Effects of *CACNA1C* heterozygous knockout on reward-induced positive bias</u>

Overall, rats showed a preference for consumption of the flavour previously paired with a high value reward vs the flavour previously paired with a low value reward, indicating formation of a reward-induced positive bias. There was no overall difference between the two genotypes, nor between males and females. However, there was a significant difference in males, with CACNA1C$^{+/-}$ rats showing reduced preference compared to wild type rats and thus indicating reduced ability to form a reward-induced positive bias.

A repeated measures ANOVA found a significant main effect of flavour, with consumption of CS-A^{++} overall higher than CS-B^{+}, $F_{1, 48}$ = 10.17, MSE = 1.46, p = 0.003, η_p^2 = 0.18 (Figure 3.21A). There was no overall effect of genotype, $F_{1, 48}$ = 0.34, MSE = 1.13, p = 0.85, η_p^2 = 0.001, nor sex, $F_{1, 48}$ = 1.20, MSE = 1.13, p = 0.28, nor interaction between genotype * sex, $F_{1, 48}$ = 0.04, MSE = 1.13, p = 0.84, η_p^2 = 0.001.

There was also no significant interaction between flavour * genotype, $F_{1, 48}$ = 2.73, MSE = 1.46, p = 0.11, η_p^2 = 0.054, nor between flavour * sex, $F_{1, 48}$ = 0.035, MSE = 1.46, p = 0.85, η_p^2 = 0.001. There was a significant interaction between flavour * genotype * sex, $F_{1, 48}$ = 4.99, MSE = 1.46, p = 0.03, η_p^2 = 0.094. For this experiment, the most theoretically

interesting investigation was whether there is a difference in consumption between the two flavours, indicating formation of a reward-induced positive bias, in any of the sub groups. Fisher's LSD post-hoc analysis found consumption of CS-A^{++} was significantly greater than CS-B^{+} in male wild type rats (p <0.001), but not in male CACNA1$C^{+/-}$ rats (p = 0.69), female wild type rats (p = 0.088) or female CACNA1$C^{+/-}$ rats (p = 0.12).

Shapiro-Wilks test of normality found consumption data for CS-A^{++} was not normally distributed in male CACNA1$C^{+/-}$ rats, W(9) = 0.79, p = 0.015, nor male WT rats, W(19) = 0.76, p <0.001. Consumption data for CS-B^{+} was also not normally distributed in male WT rats, W(19) = 0.74, p <0.001. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA found no overall main effect of genotype on percentage preference for CS-A^{++} vs CS-B^{+}, $F_{1, 48}$ = 1.21, MSE = 0.056, p = 0.28, η_p^2 = 0.025, nor sex, $F_{1, 48}$ = 0.12, MSE = 0.056, p = 0.73, η_p^2 = 0.003. There was a significant interaction between genotype * sex, $F_{1, 48}$ = 5.58, MSE = 0.056, p = 0.022, η_p^2 = 0.10 (Figure 3.21B), with Fisher's LSD post-hoc analysis showing significantly lower preference for CS-A^{++} vs CS-B^{+} in male CACNA1$C^{+/-}$ rats compared to male WT rats (p = 0.013), with no difference in females nor between males and females.

Shapiro-Wilks test of normality found percentage preference for CS-A^{++} vs CS-B^{+} was not normally distributed in male WT rats only, W(19) = 0.85, p = 0.006. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA found only anecdotal evidence to support a lack of overall effect of genotype on preference for CS-A^{++} vs CS-B^{+}, BF_{01} = 1.71. Moderate evidence was found to support the lack of difference between males and females, BF_{01} = 3.17.

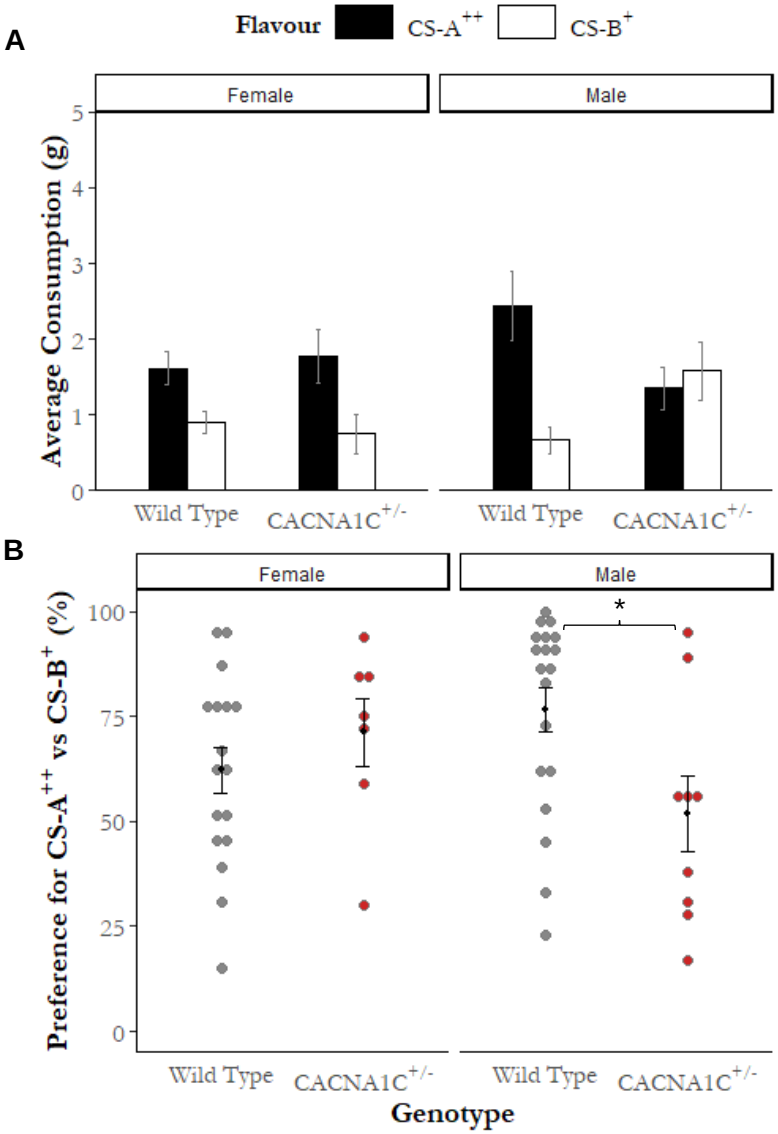


Figure 3.21. Graphs demonstrating CS-A^{++} vs CS-B^{+} choice test data for male and female wild type and CACNA1C$^{+/-}$ rats in Experiment 7.
No significant difference was found between genotypes or in overall consumptions of either flavour (A). Male CACNA1C$^{+/-}$ rats had significantly lower percentage preference for CS-A^{++} vs CS-B^{+} compared to male wild type, with no overall genotype/sex effect (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points. * = p<0.05.

Effects of CACNA1C heterozygous knockout on additional choice tests

Learning and memory of flavour-high reward associations

Overall, there was no preference for consumption of the flavour previously paired with a high value reward vs the previously unrewarded flavour, with no difference between the genotypes nor between males and females. However, Bayes analysis only provided anecdotal support for these lack of differences.

A repeated measures ANOVA found no overall difference in consumption of CS-A^{++} vs CS-C, $F_{1, 48} = 0.81$, MSE = 3.05, $p = 0.37$, $\eta_p^2 = 0.017$ (Figure 3.22A). There was no main effect of genotype, $F_{1, 48} = 0.001$, MSE = 1.24, $p = 0.97$, $\eta_p^2 < 0.001$, nor sex, $F_{1, 48} = 0.16$, MSE = 1.24, $p = 0.69$, $\eta_p^2 = 0.003$, on overall consumption. There was also no interaction between genotype * sex, $F_{1, 48} = 0.001$, MSE = 1.24, $p = 0.98$, $\eta_p^2 < 0.001$.

There was no interaction between genotype * flavour, $F_{1, 48} = 0.40$, MSE = 3.05, $p = 0.53$, $\eta_p^2 = 0.008$, nor between sex * flavour, $F_{1, 48} = 0.19$, MSE = 3.05, $p = 0.66$, $\eta_p^2 = 0.004$. There was a significant interaction between genotype * sex * flavour, $F_{1, 48} = 4.84$, MSE = 3.05, $p = 0.033$, $\eta_p^2 = 0.092$. For this experiment, the most theoretically interesting investigation was whether there is a difference in consumption between the two flavours in any of the sub groups. Fisher's LSD post-hoc analysis showed consumption of CS-A^{++} was significantly greater than CS-B^{+} in male wild type rats ($p = 0.035$), but not in male CACNA1C$^{+/-}$ rats ($p = 0.29$), female wild type rats ($p = 0.60$), or female CACNA1C$^{+/-}$ rats ($p = 0.25$).

Shapiro-Wilks test of normality found consumption data for CS-A^{++} was not normally distributed in female WT rats, W(17) = 0.89, $p = 0.04$, nor male CACNA1C$^{+/-}$, W(9) = 0.82, $p = 0.038$ or male WT rats, W(19) = 0.86, $p = 0.012$. Consumption data for CS-C was also not normally distributed in female WT rats, W(17) = 0.86, $p = 0.017$, nor male WT rats, W(19) = 0.68, $p < 0.001$. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA comparing percentage preference for CS-A^{++} vs CS-C (Figure 3.22B) found no main effect of genotype, $F_{1, 48} = 1.36$, MSE = 0.14, $p = 0.25$, $\eta_p^2 = 0.028$, nor of sex, $F_{1, 48} = 0.012$, MSE = 0.14, $p = 0.91$, $\eta_p^2 < 0.001$. There was a significant interaction between genotype * sex, $F_{1, 48} = 4.18$, MSE = 0.14, $p = 0.047$, $\eta_p^2 = 0.08$, with Fisher's LSD post-hoc analysis showing a significantly lower preference for CS-A^{++} vs CS-C in male CACNA1C$^{+/-}$ rats compared to male WT ($p = 0.02$), with no other differences.

Shapiro-Wilks test of normality found percentage preference for CS-A^{++} vs CS-B was not normally distributed in female WT rats, W(17) = 0.87, $p = 0.019$, male CACNA1C$^{+/-}$ rats,

W(9) = 0.81, p = 0.027, nor male WT rats, W(19) = 0.74, p <0.001. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA found only anecdotal evidence to support a lack of genotype effect on preference for CS-A^{++} vs CS-C, BF_{01} = 1.69, as well as the lack of main sex effect, BF_{01} = 2.56.

Learning and memory of flavour-low reward associations

Overall, there was no preference for consumption of the flavour previously paired with a low value reward vs the previously unrewarded flavour, with no difference between the genotypes nor between males and females, with moderate support from Bayes.

A repeated measures ANOVA found no overall difference in consumption of CS-B^+ vs CS-C, $F_{1, 48} = 0.68$, MSE = 2.97, $p = 0.41$, $\eta_p^2 = 0.014$ (Figure 3.23A). There was also no main effect of genotype, $F_{1, 48} = 0.062$, MSE = 1.30, $p = 0.80$, $\eta_p^2 = 0.001$, nor sex, $F_{1, 48} = 1.50$, MSE = 1.30, $p = 0.23$, $\eta_p^2 = 0.03$. There was no interaction between genotype * sex, $F_{1, 48} = 2.51$, MSE = 1.30, $p = 0.12$, $\eta_p^2 = 0.05$.

There was no interaction between genotype * flavour, $F_{1, 48} = 0.57$, MSE = 2.97, $p = 0.45$, $\eta_p^2 = 0.012$, nor between sex * flavour, $F_{1, 48} = 0.053$, MSE = 2.97, $p = 0.82$, $\eta_p^2 = 0.001$. There was also no interaction between genotype * sex * flavour, $F_{1, 48} = 1.66$, MSE = 2.97, $p = 0.20$, $\eta_p^2 = 0.033$.

Shapiro-Wilks test of normality found consumption data for CS-B^+ was not normally distributed in female WT rats, W(17) = 0.79, $p = 0.001$, male CACNA1$C^{+/-}$, W(9) = 0.83, $p = 0.043$ nor male WT rats, W(19) = 0.77, $p < 0.001$. Consumption data for CS-C was also not normally distributed in female WT rats, W(17) = 0.83, $p = 0.006$, nor male WT rats, W(19) = 0.79, $p < 0.001$. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA found no significant effect of genotype on preference for CS-B^+ vs CS-C (Figure 3.23B), $F_{1, 48} = 0.026$, MSE = 0.14, $p = 0.87$, $\eta_p^2 = 0.001$, nor of sex, $F_{1, 48} = 1.35$, MSE = 0.14, $p = 0.25$, $\eta_p^2 = 0.027$. There was also no interaction between genotype * sex, $F_{1, 48} = 2.29$, MSE = 0.14, $p = 0.14$, $\eta_p^2 = 0.046$.

Shapiro-Wilks test of normality found percentage preferences for CS-B^+ vs CS-C were not normally distributed in female WT rats, W(17) = 0.79, $p = 0.002$, nor male WT rats, W(19) = 0.86, $p = 0.009$. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA found moderate evidence to support the lack of main effect of genotype, $BF_{01} = 3.37$, and sex, $BF_{01} = 3.05$, on preference for CS-B^+ vs CS-C. There was also moderate evidence to support the lack of interaction between genotype * sex, $BF_{01} = 5.54$.

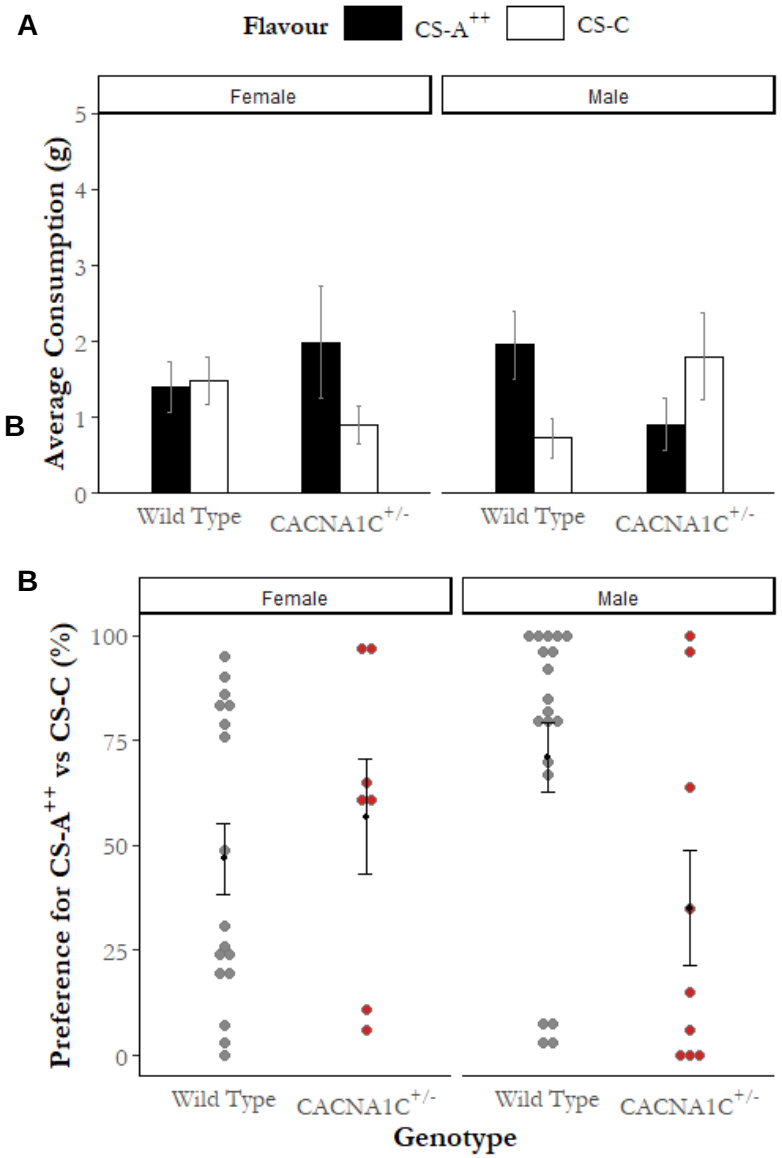


Figure 3.22. Graphs demonstrating CS-A^{++} vs CS-C choice test data for male and female wild type and CACNA1C heterozygous knockouts (CACNA1C$^{+/-}$) in Experiment 7.

No significant difference was found between genotypes or sex in overall consumption (A) nor percentage preference for flavours CS-A^{++} vs CS-C (B).

Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

Bars represent means ±SEM. Dots represent individual data points.

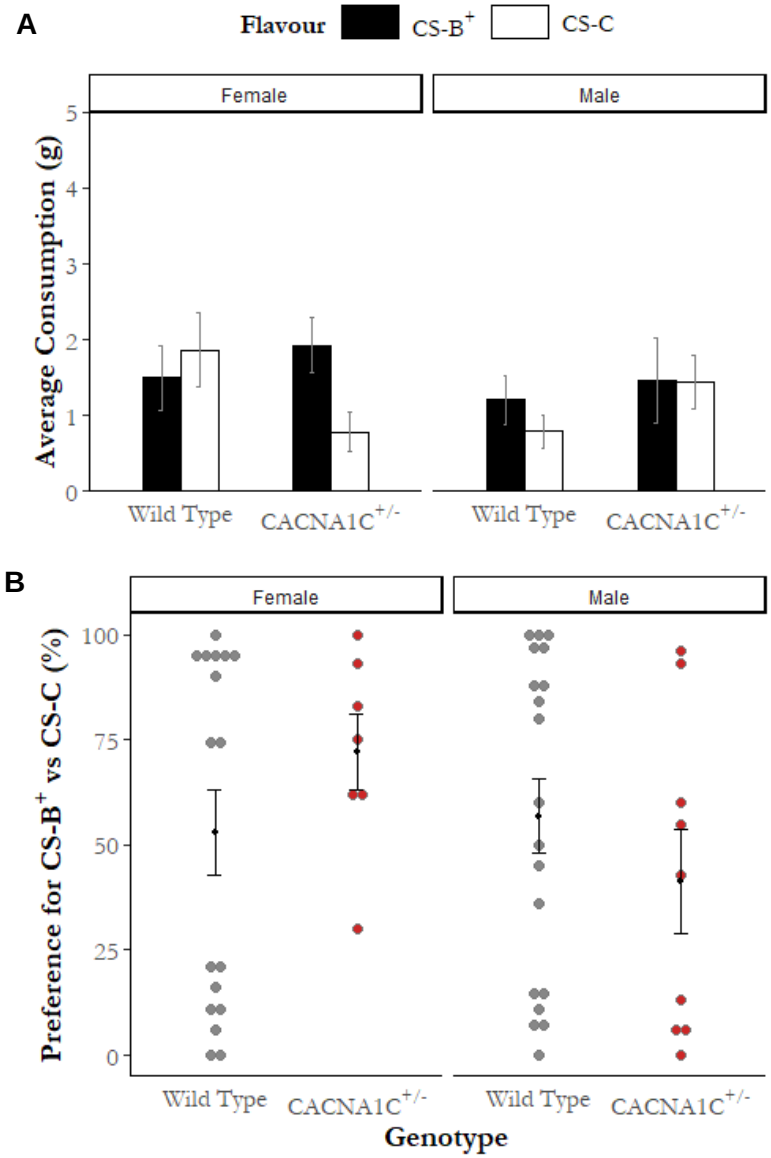


Figure 3.23. Graphs demonstrating CS-B$^+$ vs CS-C choice test data for male and female wild type and CACNA1C heterozygous knockouts (CACNA1C$^{+/-}$) in Experiment 7.

No significant difference was found between genotypes or sex in overall consumption (A) nor percentage preference for flavours CS-B$^+$ vs CS-C (B).

Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

Bars represent means ±SEM. Dots represent individual data points.

Avoidance of an unrewarded stimulus

Overall, there was no preference for the novel unrewarded flavour vs the familiar unrewarded flavour, indicating these rats did not learn to avoid the familiar flavour. There were no differences between genotypes nor sex on this avoidance, with moderate support from Bayes analysis.

A repeated measures ANOVA found no main effect of flavour on overall consumption of CS-D vs CS-C, $F_{1, 48}$ = 0.032, MSE = 3.33, p = 0.86, η_p^2 = 0.001, nor of genotype, $F_{1, 48}$ = 1.03, MSE = 1.11, p = 0.32, η_p^2 = 0.021. There was a significant main effect of sex, $F_{1, 48}$ = 4.86, MSE = 1.11, p = 0.032, η_p^2 = 0.092, where females consumed significantly more than males overall. There was no significant interaction between genotype * sex, $F_{1, 48}$ = 0.52, MSE = 1.11, p = 0.47, η_p^2 = 0.011 (Figure 3.24A).

There was no interaction between genotype * flavour, $F_{1, 48}$ = 0.94, MSE = 3.33, p = 0.34, η_p^2 = 0.019, nor between sex * flavour, $F_{1, 48}$ = 0.028, MSE = 3.33, p = 0.87, η_p^2 = 0.001. There was also no interaction between genotype * sex * flavour, $F_{1, 48}$ = 1.66, MSE = 3.33, p = 0.20, η_p^2 = 0.033.

Shapiro-Wilks test of normality found consumption data for CS-D was not normally distributed in female WT rats, W(17) = 0.85, p = 0.011, nor male WT rats, W(19) = 0.81, p = 0.002. Consumption data for CS-C was also not normally distributed in female CACNA1C$^{+/-}$ rats, W(7) = 0.73, p = 0.008, female WT rats, W(17) = 0.87, p = 0.024, nor male WT rats, W(19) = 0.73, p <0.001. However, a suitable alternative statistical test for this type of analysis was not available.

A univariate ANOVA found no significant main effect of genotype on preference for CS-D vs CS-C (Figure 3.24B), $F_{1, 48}$ = 0.15, MSE = 0.16, p = 0.70, η_p^2 = 0.003, nor of sex, $F_{1, 48}$ = 0.046, MSE = 0.16, p = 0.83, η_p^2 = 0.001. There was also no interaction between genotype * sex, $F_{1, 48}$ = 0.66, MSE = 0.16, p = 0.42, η_p^2 = 0.014.

Shapiro-Wilks test of normality found percentage preferences for CS-D vs CS-C were not normally distributed in female CACNA1C$^{+/-}$ rats, W(7) = 0.75, p = 0.012, female WT rats, W(17) = 0.77, p <0.001, male CACNA1C$^{+/-}$ rats, W(9) = 0.82, p = 0.033 or male WT rats, W(19) = 0.88, p = 0.022. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian univariate ANOVA found moderate evidence to support the lack of overall effect of genotype, BF_{01} = 3.24, and of sex, BF_{01} = 3.56. Strong evidence was found to support the lack of interaction between genotype * sex, BF_{01} = 10.53.

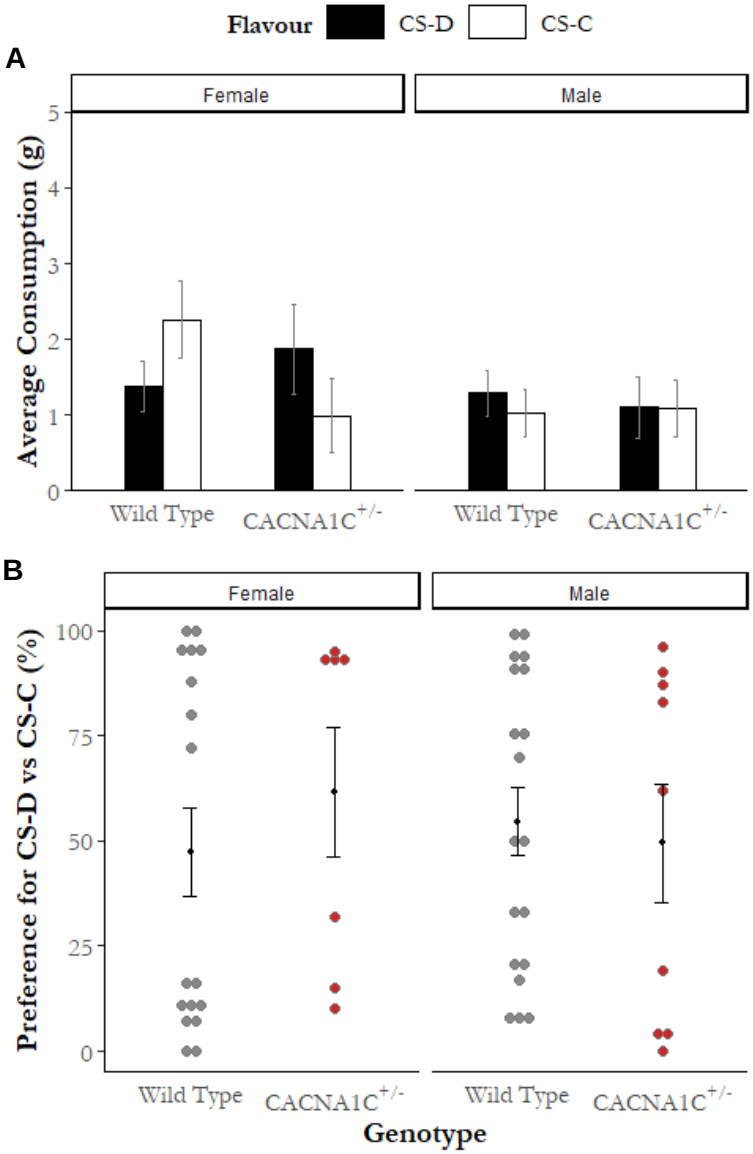


Figure 3.24. Graphs demonstrating CS-D vs CS-C choice test data for male and female wild type and CACNA1C heterozygous knockouts (CACNA1C$^{+/-}$) in Experiment 7.

No significant difference was found between genotypes in overall consumption, though consumption was greater for females over males (A). There was no difference in percentage preference for flavours CS-D vs CS-C (B).

Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

Bars represent means ±SEM. Dots represent individual data points.

3.6.3.3. Summary

Experiment 6 demonstrated a numerically reduced preference for the flavour previously paired with a higher value reward in female CACNA1C$^{+/-}$ rats compared with female controls, but this did not reach significance, and Bayes analysis suggested inconclusive evidence. Thus, to increase the sensitivity of the flavour m-ABT assay, Experiment 7 reverted back to the design used in Experiment 3 of 15% vs 5% sucrose as high vs low reward values, as well as investigating sex differences.

The findings from this experiment showed significantly reduced preference for the higher reward-paired flavour (CS-A^{++}) vs the lower reward-paired flavour (CS-B^{+}) during the main choice test in male CACNA1C$^{+/-}$ rats compared to male wild type only, with no difference again found in female rats. This suggests a potential deficit in forming reward-induced positive biases specific to males with reduced *CACNA1C* expression.

However, additional choice tests also found no overall preferences for CS-A^{++} or CS-B^{+} vs the unrewarded flavour, suggesting there was a lack of learning and recall of the reward-flavour associations. In addition, no group showed a preference for the novel unrewarded flavour (CS-D) vs the previously paired unrewarded flavour, with no differences between genotypes/males and females, indicating that these rats did not learn to avoid the unrewarded flavour given during pairing sessions.

3.7. Discussion of Chapter 3

In Chapter 3, the novel flavour m-ABT developed in Chapter 2 was applied to preclinical models of psychiatric disease to evaluate its validity as an assay for deficits in reward-induced biases like the digging m-ABT. Two pharmacological pro-depressants, CORT and IFN-α, which induce negative affective bias and in the case of chronic IFN-α reduce positive bias formation (Stuart et al., 2017), were evaluated in this novel assay to compare sensitivity, reliability and validity against the digging version. CACNA1C$^{+/-}$ rats were additionally tested in this assay to explore its use in other psychiatric disease models and determine whether this transgenic model shows deficits in reward-induced positive biases.

3.7.1. Effects of chronic CORT on forming reward-induced positive biases

The chronic CORT model was evaluated in the flavour m-ABT using 15% vs 5% sucrose in Experiment 3, and 16% vs 8% sucrose in Experiment 4, where preference for the higher valued reward-paired flavour (CS-A^{++}) compared to the lower valued flavour (CS-B^{+}) was used to indicate formation of a reward-induced positive bias.

In Experiment 3, CORT treated rats were tested alongside a group of IFN-α treated rats and a 'combined' control group (half given vehicle treatment to match CORT, and half given saline treatment to match IFN-α). Analysis of weight gain in these animals showed significantly lower percentage increase in body weight in chronic CORT treated rats compared to vehicle treated controls, which is expected based on previous studies (e.g. Lee et al., 2013; Sturm, Becker, Schroeder, Bilkei-Gorzo, & Zimmer, 2015). However, this control group was small, and this significance was reached only after removal of one CORT-treated rat identified as an outlier. Nevertheless, this indicates a positive control that chronic CORT treatment here appeared to work as expected.

Analysis of the preference for CS-A^{++} vs CS-B^{+} found no overall main effect of group, however after Bayes analysis indicated some evidence in support of a group effect, post-hoc analysis was examined which suggested that chronic CORT reduced positive bias, though this lack of firm evidence means results should be interpreted with caution. As this experiment was an initial pilot to evaluate this assay, there were several limitations in its design that may have contributed to these inconclusive findings.

One limitation was using a combined control group, although a direct comparison of the saline and vehicle control groups indicated no differences. There may still have been disparities due to the differing administration routes, especially since evidence has shown that i.p. injection via scruffing causes a negative affective bias when compared against a modified version without scruffing (Stuart & Robinson, 2015). Though the animals in this

experiment that received injections were also restrained without scruffing, the CORT/vehicle treatment groups had no injections or restraint. In addition, in the digging m-ABT, mean reward-induced positive biases are usually ~60% for control group and less than 55% for drug treatment groups (Stuart et al., 2017). However, in this experiment, preferences for CS-A^{++} were very high with a control group mean of 89.6% and CORT-treated group mean of 79.4%, which was likely due to using a 3:1 ratio of reward with 15% vs 5% sucrose, whereas the digging m-ABT uses a 2:1 pellet ratio. These high biases have the potential to produce 'ceiling effects', which may reduce its sensitivity in detecting deficits if all animals have a very strong preference for CS-A^{++}. Furthermore, reversal of any reduced positive biases with antidepressants may be more difficult to detect.

Therefore, Experiment 4 reduced the ratio between the high and low rewards to 2:1 by using 16% vs 8% sucrose to address this limitation, as well as providing a more robust experimental design with a greater sample size. In addition, recent evidence in the digging ABT have shown hormonal interventions can influence affective biases differently between males and females (Hinchcliffe et al., 2020), although some pharmacological interventions appear to be consistent across the sexes (Hinchcliffe et al., 2017). Thus, males and females were compared in Experiment 4.

Despite a more robust design to address limitations that may have reduced the ability to form conclusive interpretations in Experiment 3, this experiment found no deficit in forming positive biases following chronic CORT treatment, regardless of sex. This was unexpected since previous evidence in the digging ABT showed acute CORT treatment induced a negative affective bias (Stuart et al., 2017) and stress has been shown to impair reward learning and response biases (Bogdan et al., 2010; Bogdan & Pizzagalli, 2006; Bogdan et al., 2011). Although the mean preferences were lower than in Experiment 3 after reducing the ratio between rewards, these preferences were much more variable and in male control rats a preference for CS-A^{++} vs CS-B^{+} was not present. Thus, it is possible that reducing this ratio may have reduced the ability of the animals to generally form reward-induced positive biases.

However, CORT-treated animals in Experiment 4 did not show reduced weight gain compared to controls, and an ELISA measuring plasma CORT at the end of the experiment did not show a difference between the two groups. This might indicate issues with CORT intake/efficacy in this experiment, which could further explain this lack of difference.

Within the flavour m-ABT design, additional choice tests were also conducted to explore general cognitive processing and avoidance of the unrewarded stimulus, however, there are differences between the mechanics of these choice tests and the main CS-A^{++} vs CS-B^{+}

choice test that should be highlighted. Firstly, the main test was conducted on the first two days with no reward, and thus any tests conducted after this may be influenced by extinction of the stimulus-reward association. Furthermore, the main test was conducted with a counterbalance of flavour position over the two days to reduce side biases, whereas the additional choice tests were only conducted once with an internal counterbalance across animals on the day, thus side biases in the additional choice tests are less controlled.

In both Experiment 3 and Experiment 4, rats overall showed a preference for both previously reward-paired flavours vs the familiar unrewarded flavour, with no difference between control and CORT-treated rats. This indicates intact learning and memory recall of these reward-flavour associations that were not affected by CORT treatment.

Similarly, both Experiment 3 and Experiment 4 showed that rats overall preferred a novel unrewarded flavour to the familiar unrewarded flavour used throughout pairing sessions, regardless of CORT treatment nor sex. This indicates these rats demonstrated avoidance of the unrewarded flavour previously presented alongside the rewarded flavour, which also indicates these rats learned from the pairing sessions that the familiar unrewarded flavour contained no reward value. This avoidance could have been due to a negative contrast effect, however, this is normally considered when two different valued rewards are compared rather than two CS (see Flaherty & Rowan, 1985), and therefore this does not seem a likely explanation. In addition, there is no available data on avoidance of the unrewarded stimulus in the context of the digging ABT / m-ABT.

This learning and recall of the familiar unrewarded flavour was also not affected by CORT treatment. If treatment had affected this avoidance, and thus the learned value of the unrewarded flavour, then this could also cause the expected outcome of the reward-paired flavours to be altered and therefore could underpin any change in reward-induced biases. However, the demonstration that this avoidance occurred without an effect of treatment, whilst the formation of reward-induced positive bias was potentially reduced following CORT treatment in Experiment 3, indicates this deficit is not underpinned by altered avoidance of the unrewarded flavour. However, as mentioned, in Experiment 4 the reliability of CORT treatment was questioned so firm conclusions from the findings in this experiment cannot be made.

3.7.2. Effects of chronic IFN-α on forming reward-induced positive biases

The chronic IFN-α model was evaluated in the flavour m-ABT using 15% vs 5% sucrose in Experiment 3, and 16% vs 8% sucrose in Experiment 5. In Experiment 3, IFN-α treated rats were tested alongside the CORT-treated rats and the ‘combined’ control group described above.

As discussed, an overall group effect on preference for CS-A^{++} vs CS-B^{+} was not present in Experiment 3, but post-hoc analysis showed that the IFN-α treated group did appear to have a reduced positive bias compared to the combined control group. In contrast, no difference was found between control and IFN-α treated rats in Experiment 5, regardless of sex. Unlike with Experiment 4, a positive control for IFN-α treatment was found in these animals through a heightened immunological response in IFN-treated rats in several brain regions, most pronounced in males (see section 6.4.2.1). Although this was not a direct measurement of IFN-α levels and therefore cannot absolutely confirm that a lack of deficit was not due to poor treatment efficacy, this does indicate the IFN-treated group had an activated immune response which does not appear to influence reward-induced biases in the flavour m-ABT.

These findings were unexpected and could suggest variability in deficits caused by IFN-α treatment. Findings in other reward-related assays have been variable in this model (see section 4.1) and in humans long-term IFN-α treatment is associated with depression in 21 – 58% of patients (Raison, Borisov, et al., 2005; Raison, Broadwell, et al., 2005; Raison, Demetrashvili, Capuron, & Miller, 2005). Thus, not all patients develop depression, so it is possible that the same variability is seen in rats. Although environmental exposures in rodents would remain consistent, there may be innate differences that influence susceptibility to the development of “symptoms”.

However, chronic IFN-α treatment following this exact treatment dosage and regimen has been shown to reduce positive bias formation in the digging m-ABT (Stuart et al., 2017) as well as inducing negative biases in other decision-making tasks (e.g. Hales, Bartlett, Arban, Hengerer, & Robinson, 2021). In addition, IFN-α treatment in humans has been shown to reduce reward-induced response biases in probabilistic reward learning tasks (Harrison et al., 2016). Thus, this highlights potential flaws within the flavour m-ABT itself.

Firstly, the flavour m-ABT may not be sensitive enough to detect changes in reward-induced biases. The use of flavour-reward associations relies on measuring differences in overall consumption of flavours presented for the entire duration of the test, whereas in the digging m-ABT, once the animal makes a choice of substrate then the other substrate is blocked, which may reduce variability by limiting the animals’ ability to test both options. Data from the CS-A^{++} vs CS-B^{+} choice test in Experiment 3 appeared to be less variable than in Experiment 5, suggesting the 3:1 ratio of rewards may result in the formation of more stable biases, despite almost all groups showing a significant positive bias in Experiment 5 comparable to previously observed in the digging m-ABT.

Secondly, the digging m-ABT uses reward pellets containing a variety of dietary substances, whereas the flavour m-ABT uses pure sucrose. It is possible that more stable stimulus-reward associations are formed if the reward fulfils all dietary needs, rather than a reward based on sweet taste and high calorie content (see section 2.4.1).

Additional choice tests in Experiment 3 found that IFN-treated rats did show a preference for reward-paired flavours vs the unrewarded flavour, indicating intact learning and memory of the flavour-reward association, whereas the control group did not. However, there was no significant difference between the two groups. In contrast, all groups in Experiment 5 showed a preference for the reward-paired flavours vs unrewarded flavour, potentially suggesting that resolving the limitations of using a combined control group may have reduced variability in learning this flavour-reward association. However, this contrasts with findings from the control group used in Experiment 4, highlighting again variability across additional choice tests in this flavour m-ABT.

In Experiment 3, control rats appeared to avoid the familiar unrewarded flavour and preferred the novel unrewarded flavour, whilst IFN-treated rats did not, although no significant group difference was found. In Experiment 5, only female controls showed this preference for the novel flavour, with no overall group difference. Control females in this experiment were also the only group to not show a significant preference for CS-A^{++} vs CS-B^{+} in the main choice test, which may suggest that female rats learned the rewarding nature of flavours through avoidance of the unrewarded/negative flavour, so when given a choice without the unrewarded flavour present, they had no preference. However, given no overall differences were found between males and females in any choice test, and the variability of results seen across the two experiments, it is more probable that learning the value of CS-A^{++} and CS-B^{+} in Experiment 5 was more difficult due to the 2:1 ratio used, thus making results more variable.

3.7.3. Effects of heterozygous *CACNA1C* deletion on forming positive reward-induced biases

The CACNA1C$^{+/-}$ model was evaluated in the flavour m-ABT first using 16% vs 8% sucrose in females in Experiment 6 after initial limitations were identified following Experiment 3. However, after several inconclusive findings using this design in Experiments 4 – 6, a final investigation of this model in the flavour m-ABT was conducted using 15% vs 5% sucrose and comparison of males and females in Experiment 7.

Experiment 6 showed no difference between wild type and CACNA1C$^{+/-}$ rats in formation of positive biases, however only wild type rats showed a preference for CS-A^{++} vs CS-B^{+}, and Bayes analysis indicated inconclusive evidence here. In Experiment 7, male CACNA1C$^{+/-}$

rats had significantly reduced preference for CS-A^{++} vs CS-B^{+} compared to male wild type, with no significant difference in females. However, unlike in Experiment 6, in this experiment female CACNA1$C^{+/-}$ rats *did* show an overall preference for CS-A^{++}, although there was a much lower sample size in Experiment 7 which could have compromised the reliability of these results. Nevertheless, these findings suggest a deficit in forming reward-induced positive biases in male CACNA1$C^{+/-}$ rats, which corresponds with similar findings in human probabilistic reward learning tasks (Lancaster et al., 2014). In addition, other preclinical schizophrenia models have shown similar deficits in the digging m-ABT (Neill, Harte, Haddad, Lydall, & Dwyer, 2014). Thus, these findings highlight the potential for this CACNA1$C^{+/-}$ model of psychiatric disease, with a more powerful sample size, to be further explored in future designs of the flavour m-ABT or in the traditional digging m-ABT to confirm this deficit.

Additional choice tests in Experiment 6 showed no difference between the two genotypes, but individually that wild type rats showed intact learning about the higher valued flavour-reward association, but not lower valued flavour-reward association, whereas CACNA1$C^{+/-}$ rats showed the opposite pattern of results. In Experiment 7, male wild type rats showed intact learning about the higher valued flavour-reward association, with no preferences found in any other group. These inconsistent patterns of results in the additional choice tests reinforce previous inconsistencies here, further questioning the reliability of using additional choice tests without counterbalance for side biases and following two sessions of potential extinction of this flavour-reward association. However, in both experiments neither genotype showed a preference for the novel unrewarded flavour indicating these rats did not appear to learn through avoidance of the familiar unrewarded flavour during pairing sessions.

3.7.3. Conclusion

Chapter 3 evaluates the use of the flavour m-ABT in measuring deficits in reward-induced positive biases in preclinical models of psychiatric disease. Overall, this evidence indicates an overall lack of deficit in two pharmacological pro-depressant models of MDD, in contrast to other literature of reward learning deficits. Some suggestions of deficits were seen in these models; however, this was not replicated and so the flavour m-ABT task is not shown to be valid. In addition, a transgenic model of psychiatric disease indicated potential impairment in forming positive biases, but these findings were again inconsistent across replicating studies. Thus, these findings implicate limited sensitivity of the flavour m-ABT overall, particularly when using a 2:1 reward ratio design, highlighting further optimisation of the flavour m-ABT or alternative methods for assessing reward-induced biases are needed.

Chapter 4 – Analysis of consummatory anhedonia using lick cluster analysis in putative models of psychiatric disease

4.1. Introduction

Chapter 1 introduced the 'lick cluster analysis' (LCA) technique for measuring consummatory anhedonia in rodents and its advantages compared to the current gold standard sucrose preference test (SPT). As discussed previously, there is very limited current literature on applying this method to evaluate hedonic response in preclinical models of major depressive disorder (MDD) or schizophrenia, despite the growing concern over lack of progress in treating the negative symptoms of these disorders. Thus, the experiments in this chapter aimed to investigate the hedonic response in three preclinical models of psychiatric disease using this more sensitive assay of LCA.

Using the SPT, exogenous corticosterone (CORT) administration through subcutaneous injection or pellet implantation has induced a consistent phenotype of reduced sucrose preference in both mice (Chaves et al., 2019; Lopes et al., 2018; Sturm et al., 2015) and rats (Huang et al., 2011; Lou, Li, Wang, Xia, & Chen, 2018). CORT in drinking water is less commonly reported but has still demonstrated this same phenotype (e.g. Ding et al., 2018).

In contrast, evidence of anhedonia induced by interferon-alpha (IFN-α) administration has been much more variable. Sammut, Goodall, and Muscat (2001) used a less traditional 3-bottle sucrose test between 1%, 8% and 32% sucrose 20 minutes after acute administration of recombinant human recombinant IFN-α (hrIFN; 10, 100 or 10000IU/300g) or rat IFN-α (1, 10, 100IU/300g). They found that consumption of 1%, 8% and 32% sucrose was significantly lower following the highest dose of both hrIFN and rat IFN-α, with the greatest effect seen at the lowest sucrose concentration. Similarly in a follow-up study, rats administered with 10000IU/kg 20 minutes prior to sucrose testing for 33 days consumed significantly less of 1% sucrose compared to vehicle controls, whereas, consumption of 32% sucrose appeared to be elevated in the IFN-α treated group (Sammut, Bethus, Goodall, & Muscat, 2002). Although these studies demonstrate an apparent influence of IFN-α on sucrose consumption, particularly at a low concentration, the analysis of consumption alone may not be representative of anhedonia. Furthermore, the administration of IFN-α prior to testing in the latter study could mean the acute effects of IFN-α interfere with any chronic effect.

Interestingly, Fitzgibbon, Kerr, Henry, Finn, and Roche (2019) demonstrated that in male mice, 4 and 7 days of 8000IU/g daily hrIFN treatment significantly reduced preference for 1% sucrose vs water without impacting overall fluid consumption – where IFN-α was

administered at least 24 hours prior to behaviour testing. Similarly, Sprague-Dawley rats administered with 6000IU/kg hrIFN for 14 days showed a significantly reduced preference for 2% sucrose vs water, which was restored after treatment with an antidepressant (Bhatt et al., 2016), however, the timings of IFN-α administration were unclear in this study. Whereas, a study by Kosel, Bilkei-Gorzo, Zawatzky, Zimmer, and Schlaepfer (2011) chronically administered 600μg/mL rhIFN in mice twice over the course of 20 days and demonstrated no alteration to preference for 0.1% sucrose vs water at any time point. As mentioned in Chapter 3, Stuart et al. (2017) administered a much lower dose of 100IU/kg IFN-α than previously reported to avoid inducing sickness behaviours to Sprague-Dawley rats for 14 days, and demonstrated a lack of sucrose preference deficit in these rats despite showing a reduced reward-induced positive bias.

However, as argued in section 1.4.1, the SPT cannot appropriately measure hedonic response with absolute certainty that there are no motivational or cognitive influences on consumption. LCA provides a more sensitive method that may be able to dissociate hedonic response from these other reward-processing mechanisms.

Neither IFN-α nor CORT have been assessed via LCA, thus, the aim of Chapter 4 was, firstly, to apply the LCA technique to the investigation of anhedonia following chronic treatment with CORT or IFN-α, at a dose lower than that which produces sickness behaviours. Secondly, this chapter aimed to determine whether anhedonia may differ between the sexes, given the evidence of higher prevalence for depression in females (Albert, 2015), and whether the predisposed pro-depressant alters any sex differences.

In Experiment 8 and 10, the initial presence of a hedonic deficit in the CORT and IFN-α model, respectively, will be examined in males only, whereas Experiment 9 and 11 aimed to provide follow-up evidence for this deficit whilst evaluating any sex-specific hedonic changes.

4.2. General lick cluster analysis method

4.2.1. Subjects

For all experiments in Chapter 4, Sprague-Dawley rats supplied by Charles River UK were housed in cages of either two or three. All rats were handled and maintained on food restriction as described in section 3.2.1. See Appendix A for an outline of experimental trajectories. Sample sizes were determined as described in section 2.2.1.1 and husbandry details were as described for Experiment 1.

All experiments were conducted in compliance with the UK Animals (Scientific Procedures) Act 1986) and with local Cardiff University Ethics Review Committee approval.

4.2.2. Drug treatments

All drug treatment protocols in Chapter 4 were as described fully in section 3.2.4. Chronic CORT was administered at a dose of 50µg/mL in 1% ethanol in drinking water daily for 12 days, followed by dose tapering prior to any testing. Chronic IFN-α was administered as 100IU/kg daily i.p. injections for 14 days prior to any testing, plus at the end of each day thereafter.

4.2.3. Apparatus

The apparatus used in the experiments in Chapter 4 is the same as described in Chapter 2 (section 2.2.1.2). For LCA, a contact-sensitive lickometer recorded the timing of each lick to the nearest 0.01s via MED-PC software (Med Associates Inc., St Albans, VT, USA). The sucrose solutions used in all experiments for lick analysis contained commercial-grade cane sugar and distilled water only.

4.2.4. Pre-training

All animals received 10-minutes free access to two bottles of 8% sucrose in the experimental boxes, with the length of the drinking spouts reaching inside the box for easy detection on day one. The position of the spout was then retracted to line the tip of the spout with the edge of the box for the remainder of the experiment.

Once all rats had consumed more than 1ml from both bottles, they then received only one bottle of 8% sucrose on either the left- or right-hand side, counterbalanced, until a steady level of consumption was reached.

4.2.5. Testing

All animals receive access to either one bottle of 4% sucrose or 16% sucrose on a single testing day, followed by the alternate concentration the next day. This was repeated 3 times, giving 6 lick analysis-testing days. The order and spout positions were counterbalanced.

4.2.6. Data analysis

The weight of drinking bottles before and after each session was measured to calculate the volume of solution consumed by each animal. MED-PC data was used to calculate volume consumed per 1000 licks (vol/licks), the mean number of licks per bout (lick cluster size, LCS) in which a bout consists of a series of continuous licks separated by no more than a 0.5s pause – a criterion determined by Davis (1989), and inter-lick interval (ILI, the time between each lick).

After each testing session, data were screened for exclusion criteria based on results of the control variables, ILI's and vol/licks. If vol/licks were higher than 10ml/1000licks, or ILI's were over 180ms, all data from that animal on the given testing day was excluded. Values outside of these ranges indicate potential issues with recording of data or leaking of solutions. From the remaining data collected at the end of each experiment, the means for individual sucrose concentrations were calculated from each animal per test. These means were then used to calculate the overall control or treatment group means.

For all experiments in Chapter 4, an alpha level of 0.05 was used as the level of significance, and all analyses were conducted on SPSS software (v23, IBM). Where indicated, ANOVA's and independent samples t-tests used 'group' as the fixed factor whilst ANOVA's also applied 'sucrose concentration' as the within-subjects factor. 'Time' was added as a fixed factor to the analysis of different time points in Experiment 8, whilst 'sex' was a fixed factor added to the analysis of both males and females in Experiment 9 and 11. ANOVAs were compared using Fisher's Least Significant Difference (LSD) post-hoc analysis. Tests for normal distribution were conducted as described in section 2.2.1.5 and Bayesian analyses were conducted as described in section 2.2.1.6.

4.3. Anhedonia in the chronic corticosterone model

Deficits in hedonic response in the chronic CORT model were examined in the following experiments. Experiment 8 aimed to initially establish whether this model demonstrates a deficit in hedonic response to a low and/or high concentration of sucrose in only male rats. Experiment 9 further examined whether this model demonstrates a deficit in hedonic response to the same low and/or high concentration of sucrose in both male and female rats to confirm the findings of Experiment 8 and ensure deficits are consistent and can be generalised across the sexes. Based on previous findings in the SPT, chronic CORT treated rats are expected to demonstrate a reduction in LCS indicative of hedonic response, with no difference between males and females.

4.3.1. Experiment 8 - Establishing a deficit in hedonic response

4.3.1.1. Experimental design

48 naïve male Sprague-Dawley rats weighing 350-410g on *ad libitum* food at approx. 11 weeks of age were used for this experiment. Animals were split into two groups, receiving either the drug or vehicle (*n*=24 control, *n*=24 CORT) prior to food restriction.

For this experiment, pre-training began one day after the final CORT dose, with testing starting seven days after. Pre-training is described in 4.2.4, though in this experiment an adaptation of the SPT was incorporated into the testing schedule. For the test procedure, all animals received access to either one-bottle of 4% sucrose, one-bottle 16% sucrose, or a two-bottle SPT consisting of a choice between 1% sucrose and plain water, for 10 minutes per testing day. This was repeated 3 times to give a total of 9 days testing.

At the end of the test period, half this cohort were euthanized for histological and biochemical analyses (*n*=12 control, *n*=12 CORT-treated) approximately 18 days following their last CORT dose. The remaining animals were maintained as usual without re-exposure to CORT treatment until 47 days following the last test day, where they were given three short days of pre-training followed by the same 9 days of testing as previously described. This second testing group will hereby be referred to as ‘cohort two’. All animals in cohort two were then euthanized for histological and biochemical analyses approximately 60 days following their last CORT dose.

As described in section 4.2.6, data were screened for exclusion criteria after each testing session. According to these criteria, 12 data points were excluded from cohort one, and 27 data points excluded from cohort two. No animals were fully excluded from the experiment.

4.3.1.2. Results

Effects of chronic CORT treatment on weight gain

An independent samples t-test showed CORT-treated rats had significantly reduced percentage increase in weight gain compared to vehicle-treated controls, t(46) = -3.41, p = 0.001 (Figure 4.1). This demonstrates CORT treatment reduced weight gain and therefore worked as expected.

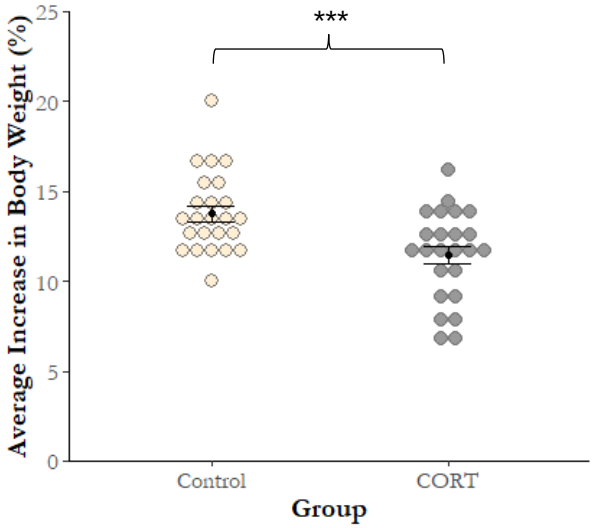


Figure 4.1. Percentage weight gain as confirmation of CORT treatment.
CORT treatment reduced the average percentage increase in weight (g) after 12 days compared to control rats.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points. *** = p<0.001.

Effects of chronic CORT treatment in an adaptation of the sucrose preference test

As discussed in section 4.3.1, the SPT has been used previously to demonstrate pro-depressant effects of chronic stress and CORT treatment through a reduced preference for sucrose when given the choice to drink either 1% sucrose or water (Stuart et al., 2017; Sturm et al., 2015). Thus, I added the SPT as a behavioural comparison to the lick analysis testing.

A repeated measures ANOVA indicated that all animals consumed significantly more of the sucrose compared to water, $F_{1,46}$ = 175.17, MSE = 1.24, p <0.001, with no interaction between sucrose concentrations * group, $F_{1,46}$ = 0.27, MSE = 1.24, p = 0.61, and no overall effect of group on consumption, $F_{1,46}$ = 1.3, MSE = 1.45, p = 0.26 (Figure 4.2A).

Shapiro-Wilks test of normality found that consumption of water was not normally distributed in the control group, W(24) = 0.89, *p* = 0.017, nor the CORT-treated group, W(24) = 0.89, *p* = 0.011. However, a suitable alternative statistical test for this type of analysis was not available.

Shapiro-Wilks test of normality also found that mean preference for 1% sucrose vs water was not normally distributed in control rats, W(24) = 0.88, *p* = 0.007, nor CORT-treated rats, W(24) = 0.77, *p* <0.001. Thus, Mann-Whitney U test was conducted to compare these two groups, which found no significant difference between the groups, U = 262, *p* = 0.59 (Figure 4.2B).

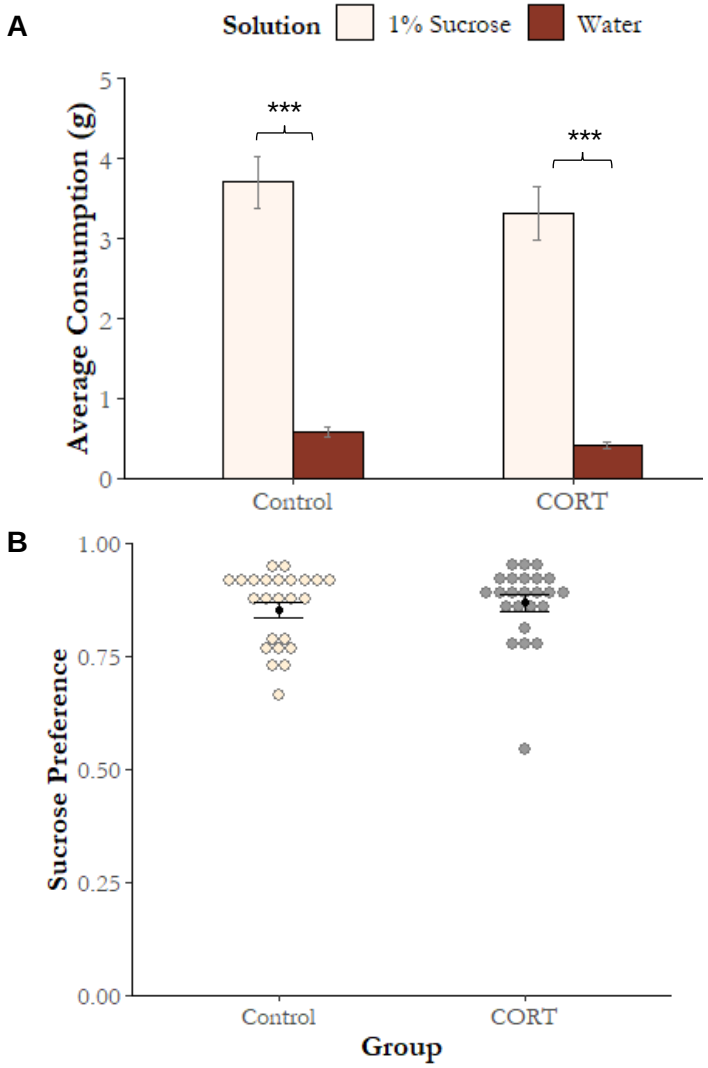


Figure 4.2. Effects of CORT treatment on an adaptation of the sucrose preference test (SPT).
All rats consumed significantly greater 1% sucrose compared to water, with no overall effect of CORT treatment on consumption (A), nor on overall sucrose preference, presented as percentage decimals (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points. *** = *p*<0.001.

A Bayesian independent samples t-test found anecdotal evidence to support this lack of overall difference in preference, BF_{01} = 2.93.

These findings demonstrate that CORT treatment had no effect on this adaptation of the SPT, though Bayes analysis deemed that this was inconclusive.

Effects of chronic CORT treatment on hedonic response to 4% and 16% sucrose

CORT treatment significantly reduced LCS compared to controls, indicating reduced hedonic response to reward, but Bayes analysis deemed the evidence to support this was inconclusive. This effect of CORT was independent of sucrose concentration, though this again was deemed inconclusive.

A repeated measures ANOVA revealed a significant effect of group, where CORT-treated rats had overall reduced LCS compared to controls, $F_{1,46}$ = 4.503, MSE = 1475.73, p = 0.039, η_p^2 = 0.089 (Figure 4.3A), indicating a reduced hedonic response to a sucrose reward. All animals had greater LCS for 16% compared to 4% sucrose, $F_{1,46}$ = 90.49, MSE = 535.95, p <0.001, η_p^2 = 0.66, suggesting a greater hedonic response for the sweeter solution. There was no overall interaction between sucrose concentration * group, $F_{1,46}$ = 2.19, MSE = 535.95, p = 0.15, η_p^2 = 0.046.

Shapiro-Wilks test of normality found that LCS for 4% sucrose was not normally distributed in the CORT-treated group, W(24) = 0.85, p = 0.002. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian repeated measures ANOVA found almost no evidence to support any effect of CORT treatment on LCS, BF_{10} = 1.08. Although Bayes analysis is mostly applied to non-significant results in this thesis, it was applied in this case to determine the strength of evidence for this CORT effect given contradictory evidence in further experiments and will be discussed in the chapter discussion. Anecdotal evidence was found to support the lack of interaction between group * sucrose concentration, BF_{01} = 1.86.

CORT treatment did not significantly influence overall consumption of sucrose, regardless of sucrose concentration.

There was no overall effect of group on sucrose consumption, $F_{1,46}$ = 0.04, MSE = 16.78, p = 0.84, η_p^2 = 0.001, but all rats consumed more 16% sucrose compared to 4% sucrose, $F_{1,46}$ = 76.32, MSE = 5.68, p <0.001, η_p^2 = 0.62 (Figure 4.3B). There was no interaction between sucrose concentration * group, $F_{1, 46}$ = 2.59, MSE = 5.68, p = 0.12, η_p^2 = 0.053.

A Bayesian repeated measures ANOVA found moderate evidence to support this lack of group effect on overall sucrose consumption, BF_{01} = 3.88. Only anecdotal evidence was found to support the lack of interaction between group * sucrose concentration on consumption, BF_{01} = 1.27.

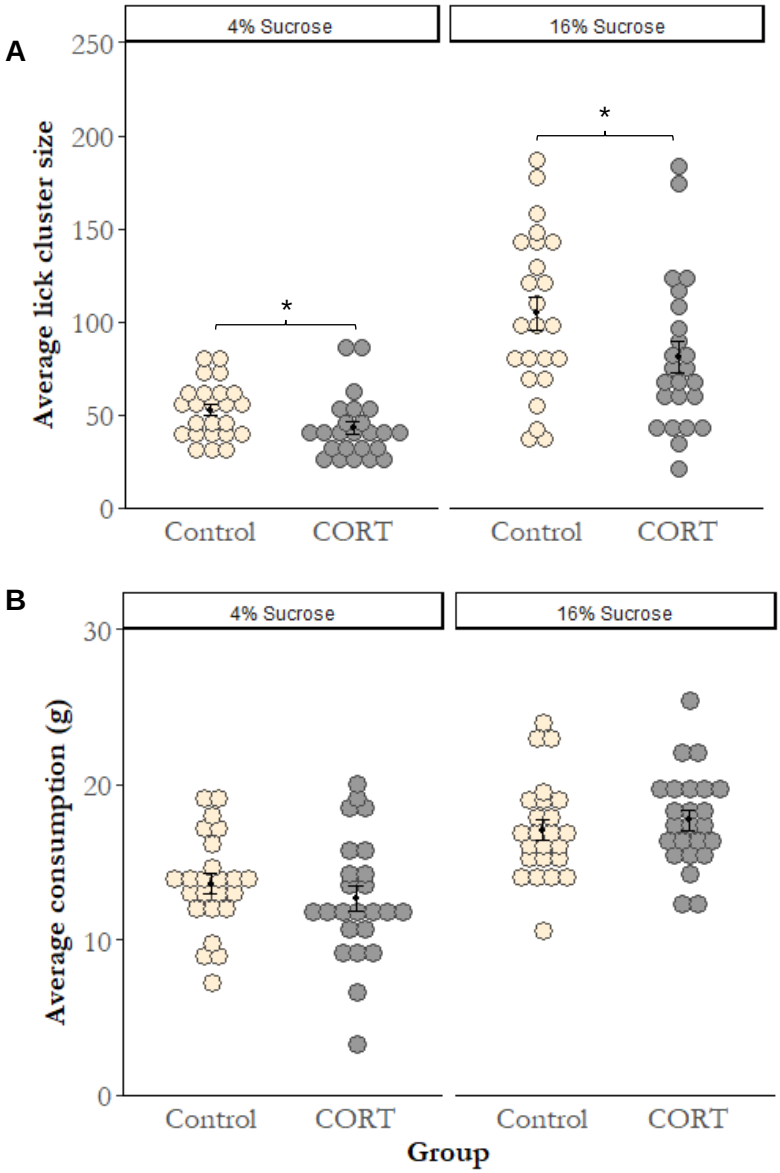


Figure 4.3. Effects of CORT treatment on hedonic response to sucrose and overall consumptions.
CORT-treated rats had significantly reduced overall lick cluster size compared to control rats, with no interaction between sucrose concentration * group (A). There were no group differences in overall average consumption of sucrose (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots indicate individual data points. * = p<0.05.

Repeated measures ANOVA was also conducted for each of the two control variables. Differences in both ILI's and vol/licks are expected between the two sucrose concentrations since a higher concentration will be more viscous, however, group differences could indicate motor deficits induced by CORT which may influence measurement of hedonic responses.

Results from these analyses showed ILI's and vol/licks were both influenced by sucrose concentration but CORT treatment had no effect on either control variable, regardless of sucrose concentration.

These showed that ILI's were significantly greater for 16% sucrose compared to 4% sucrose, $F_{1, 46} = 11.37$, MSE = 9.95, $p = 0.002$, $\eta_p^2 = 0.20$. There was no effect of group on ILI's, $F_{1,46} = 0.49$, MSE = 92.04, $p = 0.48$ (Figure 4.4A) and no overall interaction between sucrose concentration * group, $F_{1, 46} = 0.24$, MSE = 9.95, $p = 0.63$, $\eta_p^2 = 0.005$.

A Bayesian repeated measures ANOVA found only anecdotal evidence to support the lack of overall group effect on ILI's, $BF_{01} = 1.84$, as well as to support the lack of interaction between group * sucrose concentration on ILI's, $BF_{01} = 2.33$.

Further, vol/licks were greater for 4% sucrose compared to 16% sucrose, $F_{1, 46} = 4.67$, MSE = 0.299, $p = 0.036$, $\eta_p^2 = 0.092$. There was no effect of group on vol/licks, $F_{1, 46} = 1.39$, MSE = 0.91, $p = 0.24$, $\eta_p^2 = 0.029$ (Figure 4.4B), and no interaction between sucrose concentration * group, $F_{1, 46} = 0.11$, MSE = 0.299, $p = 0.75$, $\eta_p^2 = 0.002$. Shapiro-Wilks test of normality found that vol/licks for 4% sucrose was not normally distributed in the control group, W(24) = 0.89, $p = 0.019$. However, a suitable alternative statistical test for this type of analysis was not available.

Thus, these findings for ILI's and vol/licks show no indication that artefacts may have contributed to the observed hedonic impact of CORT on lick cluster size.

A Bayesian repeated measures ANOVA found only anecdotal evidence to support the lack of overall effect of group on vol/licks, $BF_{01} = 1.81$. Moderate evidence was found to support the lack of interaction between group * sucrose concentration, $BF_{01} = 3.65$.

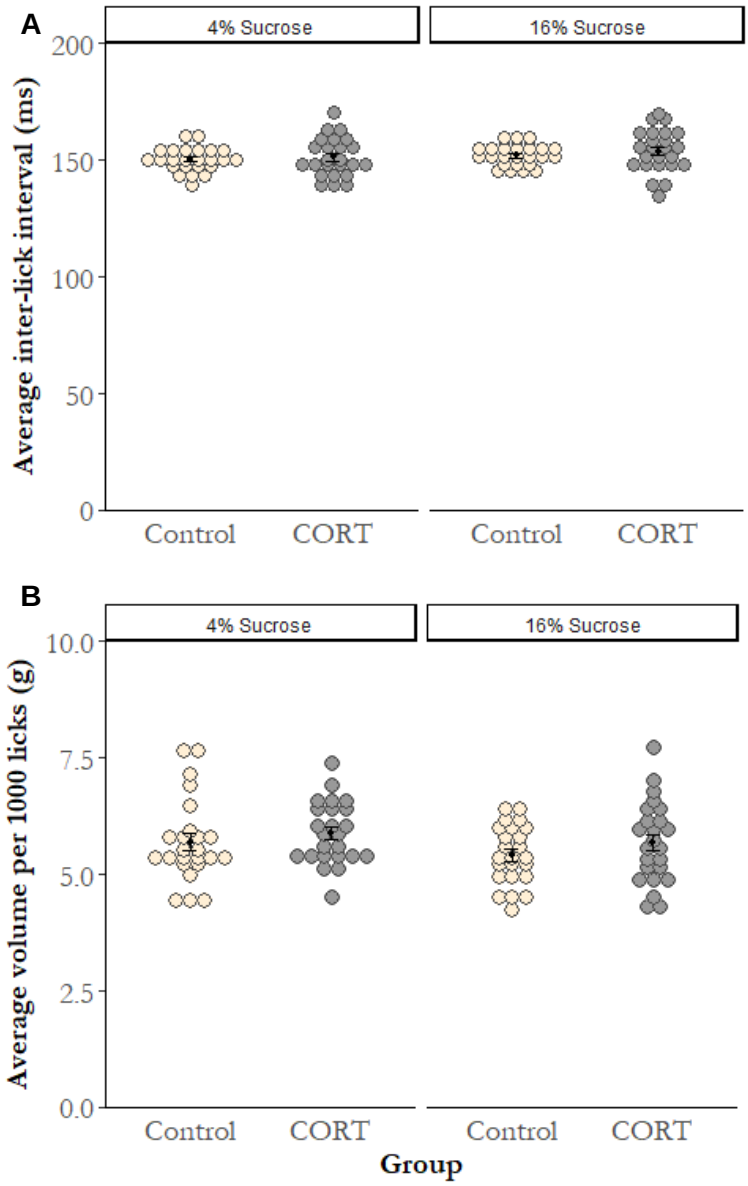


Figure 4.4. Effects of CORT treatment on control variables, inter-lick intervals (ILI's) and Vol/Licks (volume consumed per 1000 licks).
There was no effect of CORT treatment on either ILI's (A) or vol/licks (B) during lick cluster analysis. Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side. Bars represent means ±SEM. Dots indicate individual data points.

Delayed effects of chronic CORT treatment in an adaptation of the sucrose preference test

An additional investigation was made to determine whether the effects of CORT were long lasting. Half the original cohort were re-tested in the SPT and lick cluster analysis 7 weeks post-CORT treatment (*n*=12 per group).

A repeated measures ANOVA found there was no significant overall effect of group on consumption in the 1% sucrose vs water SPT, $F_{1, 22} = 0.25$, MSE = 2.61, $p = 0.63$, $\eta_p^2 = 0.011$ (Figure 4.5A). All rats consumed significantly greater 1% sucrose compared to water, $F_{1, 22} = 114.07$, MSE = 2.08, $p < 0.001$, $\eta_p^2 = 0.84$, with no interaction between sucrose concentration * group, $F_{1, 22} = 0.46$, MSE = 2.08, $p = 0.51$, $\eta_p^2 = 0.02$.

There was also no significant effect of time on consumption, $F_{1, 22} = 1.73$, MSE = 0.69, $p = 0.20$, $\eta_p^2 = 0.073$, nor between time * group, $F_{1, 22} = 0.035$, MSE = 0.69, $p = 0.85$, $\eta_p^2 = 0.002$. There was a significant interaction between sucrose concentration * time, $F_{1, 22} = 5.55$, MSE = 0.59, $p = 0.028$, $\eta_p^2 = 0.20$, in which consumption of 1% sucrose was numerically greater in the re-testing stage ($p = 0.073$) whilst consumption of water was greater in the original testing stage ($p = 0.11$), with neither finding reaching significance alone. There was no overall interaction between sucrose concentration * time * group, $F_{1, 22} = 0.51$, MSE = 0.59, $p = 0.49$, $\eta_p^2 = 0.022$.

Shapiro-Wilks test of normality found that consumption of 1% sucrose was not normally distributed in the control group at the late time point, W(12) = 0.82, $p = 0.015$. Consumption of water was also not normally distributed in the control group at the late time point, W(12) = 0.85, $p = 0.036$, nor the CORT-treated group at the late time point, W(12) = 0.48, $p < 0.001$. Consumption of water was also not normally distributed in the control group at the early time point, W(12) = 0.81, $p = 0.013$, nor the CORT-treated group at the early time point, W(12) = 0.81, $p = 0.013$. However, a suitable alternative statistical test for this type of analysis was not available.

A repeated measures ANOVA was also conducted on sucrose preference ratios in the SPT. There was no overall effect of group on preference, $F_{1, 22} = 0.45$, MSE = 0.005, $p = 0.51$, $\eta_p^2 = 0.02$. There was a significant effect of time, $F_{1, 22} = 10.03$, MSE = 0.004, $p = 0.004$, $\eta_p^2 = 0.31$, with preferences being higher overall in the re-testing stage compared to the original testing stage, and no interaction between time * group on sucrose preference, $F_{1, 22} = 1.18$, MSE = 0.004, $p = 0.29$, $\eta_p^2 = 0.051$ (Figure 4.5B).

Shapiro-Wilks test also found that mean preference for 1% sucrose vs water was not normally distributed in the control group at the early time point, W(12) = 0.73, $p = 0.002$, nor in the CORT-treated group at the late time point, W(12) = 0.72, $p = 0.001$. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian repeated measures ANOVA conducted on preference ratio data found only anecdotal evidence to support a lack of overall group effect on sucrose preference, BF_{01} = 2.56, as well as the lack of interaction between group * time, BF_{01} = 1.50.

These findings show conclusively that all rats had increased preference for sucrose vs water at the later time point compared to initial testing. This change in preference over time did not appear to be impacted by prior CORT treatment, though evidence here was not conclusive.

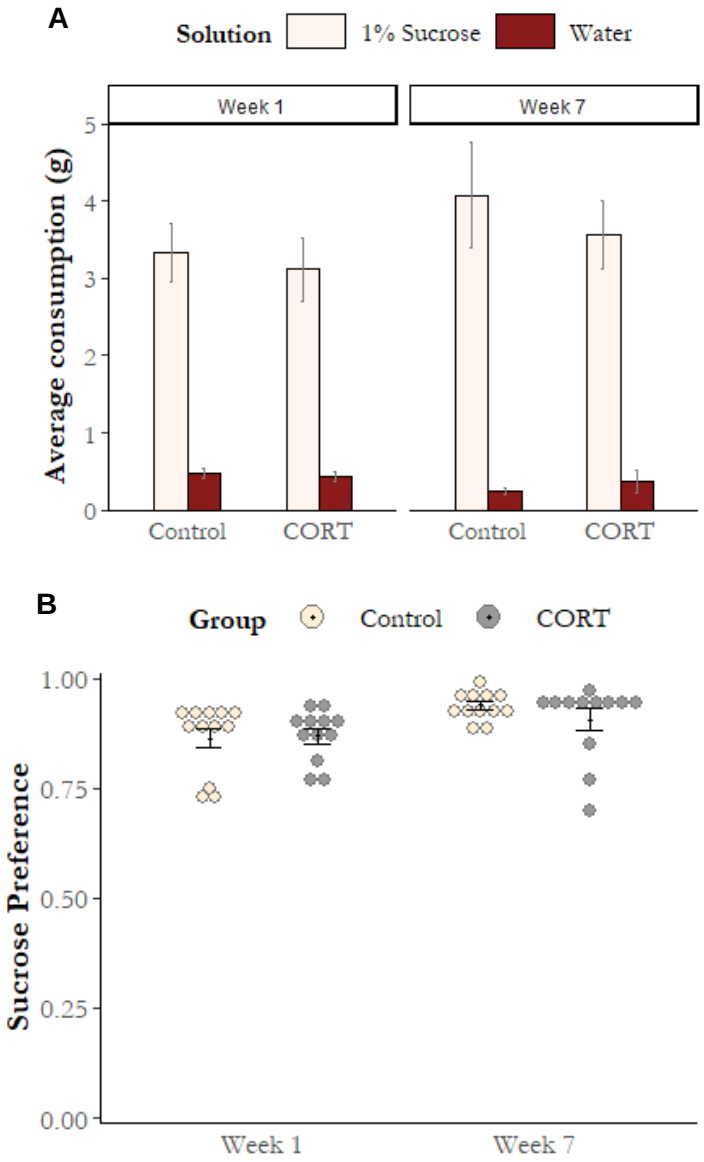


Figure 4.5. Comparison of immediate and delayed CORT effects on an adaptation of the sucrose preference test (SPT).
All rats consumed significantly greater 1% sucrose compared to water, with no overall effect of CORT treatment at either time point on consumption (A), nor on overall sucrose preference, presented as percentage decimals rounded to two significant figures (B). No interaction between group and time point was observed for either variable.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots indicate individual data points.

Delayed effects of chronic CORT treatment on hedonic response to 4% and 16% sucrose

At the later time point, there was no overall difference in LCS between control and CORT treated rats, with only anecdotal support from Bayes analysis. LCS also did not appear to change over time, regardless of sucrose concentration, which was strongly supported by Bayes.

A repeated measures ANOVA showed no overall effect of group on LCS, $F_{1, 22} = 2.46$, MSE = 1700.27, $p = 0.13$, $\eta_p^2 = 0.10$ (Figure 4.6A). All rats had significantly LCS size for 16% sucrose compared to 4%, $F_{1, 22} = 72.99$, MSE = 553.89, $p < 0.001$, $\eta_p^2 = 0.77$. There was no interaction between sucrose concentration * group on LCS, $F_{1, 22} = 2.55$, MSE = 553.89, $p = 0.13$, $\eta_p^2 = 0.10$.

There was no significant effect of time on LCS, $F_{1, 22} = 3.77$, MSE = 505.91, $p = 0.065$, $\eta_p^2 = 0.15$, and no interaction between time * group, $F_{1, 22} = 0.31$, MSE = 505.91, $p = 0.58$, $\eta_p^2 = 0.014$. Further, there was no interaction between sucrose concentration * time, $F_{1, 22} = 0.34$, MSE = 369.69, $p = 0.57$, $\eta_p^2 = 0.015$, nor between sucrose concentration * time * group, $F_{1, 22} = 0.19$, MSE = 369.69, $p = 0.66$, $\eta_p^2 = 0.009$.

Shapiro-Wilks test of normality found that LCS for 4% sucrose was not normally distributed in the CORT-treated group at the early time point, W(12) = 0.82, $p = 0.016$. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian repeated measures ANOVA found only anecdotal evidence to support the lack of overall effect of group on LCS, $BF_{01} = 1.58$, as well as to support the lack of overall effect of time on LCS, $BF_{01} = 2.01$. Anecdotal evidence was also provided to support the lack of interaction between time * group on LCS, $BF_{01} = 2.99$. Strong evidence was found to support the lack of three-way interaction between group * time * sucrose concentration, $BF_{01} = 10.82$.

Overall consumption was greater at the later time point compared to initial testing, which was independent of CORT treatment and/or sucrose concentration.

A repeated measures ANOVA showed no overall effect of group on sucrose consumption, $F_{1, 22} = 0.19$, MSE = 36.40, $p = 0.67$, $\eta_p^2 = 0.008$ (Figure 4.6B). All rats consumed significantly greater 16% sucrose compared to 4%, $F_{1, 22} = 52.94$, MSE = 6.19, $p < 0.001$, $\eta_p^2 = 0.71$ with no interaction between sucrose concentration * group on consumption, $F_{1, 22} = 0.90$, MSE = 6.19, $p = 0.35$, $\eta_p^2 = 0.039$.

There was a significant effect of time, $F_{1, 22} = 73.71$, MSE = 4.05, $p < 0.001$, $\eta_p^2 = 0.77$, where overall consumption was greater in the re-test stage. There was no interaction between time * group on consumption, $F_{1, 22} = 0.75$, MSE = 4.05, $p = 0.39$, $\eta_p^2 = 0.03$. There was a

significant interaction between sucrose concentration * time, $F_{1, 22} = 7.81$, MSE = 2.18, $p = 0.011$, $\eta_p^2 = 0.26$, where Fisher's LSD post-hoc comparisons revealed consumption was greater in the re-test stage for both 4% and 16% sucrose ($p < 0.001$). There was no interaction between sucrose concentration * time * group, $F_{1, 22} = 0.29$, MSE = 2.18, $p = 0.59$, $\eta_p^2 = 0.013$.

A Bayesian repeated measures ANOVA found anecdotal evidence to support the overall lack of group effect on consumption, $BF_{01} = 2.77$, and to support the lack of interaction between group * time, $BF_{01} = 2.58$. Moderate evidence was found to support the lack of interaction between group * time * sucrose concentration, $BF_{01} = 4.92$.

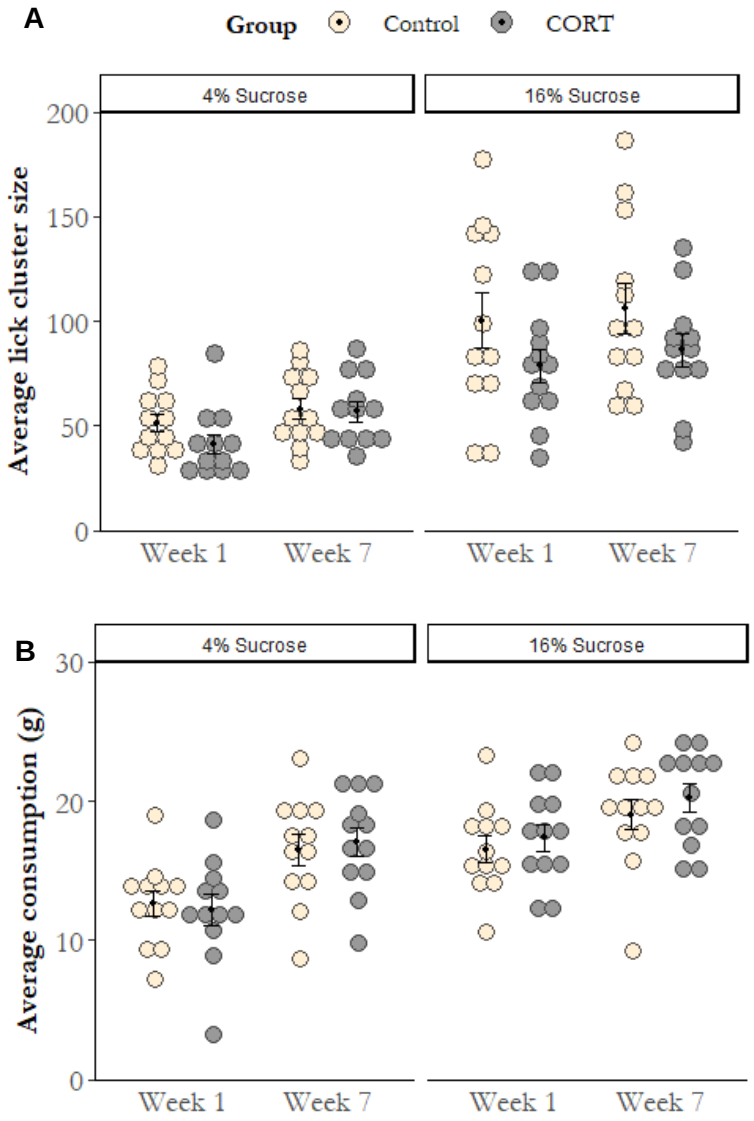


Figure 4.6. Comparison of immediate and delayed CORT treatment on hedonic response to sucrose and overall consumptions.
There was no significant difference between CORT-treated rats and control rats in lick cluster size (A) or overall consumption (B), nor any interactions between group and time point for either variable.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots indicate individual data points.

Analysis of control variables was also conducted via repeated measures ANOVA.

Both ILI's and vol/licks were greater at the later time point compared to initial testing, independently of CORT treatment and/or sucrose concentration.

There was no overall effect of group on ILI's, $F_{1, 22} = 0.46$, MSE = 215.82, $p = 0.51$, $\eta_p^2 = 0.02$, nor an overall effect of sucrose concentration, $F_{1, 22} = 3.34$, MSE = 10.16, $p = 0.081$, $\eta_p^2 = 0.13$ (Figure 4.7A). There was no interaction between sucrose concentration * group on ILI's, $F_{1, 22} = 0.12$, MSE = 10.16, $p = 0.73$, $\eta_p^2 = 0.006$. There was a significant effect of time on ILI's, $F_{1, 22} = 33.65$, MSE = 11.32, $p < 0.001$, $\eta_p^2 = 0.61$, with ILI's overall higher in the re-testing stage compared to the original testing. There was no interaction between time * group, $F_{1, 22} = 0.15$, MSE = 11.32, $p = 0.70$, $\eta_p^2 = 0.007$. Further, there were no interactions between sucrose concentration * time, $F_{1, 22} = 2.25$, MSE = 10.28, $p = 0.15$, $\eta_p^2 = 0.093$, nor between sucrose concentration * time * group, $F_{1, 22} = 2.94$, MSE = 10.28, $p = 0.10$, $\eta_p^2 = 0.12$.

A Bayesian repeated measures ANOVA found only anecdotal evidence to support the lack of group effect on ILI's, $BF_{01} = 1.64$. Moderate evidence was found to support the lack of interaction between time * group, $BF_{01} = 3.16$, and the lack of interaction between time * group * sucrose concentration, $BF_{01} = 7.63$.

Analysis of vol/licks also showed no overall effect of group, $F_{1, 22} = 0.39$, MSE = 1.34, $p = 0.54$, $\eta_p^2 = 0.018$ (Figure 4.7B). Vol/licks were overall significantly lower for 16% sucrose compared to 4% sucrose, $F_{1, 22} = 4.45$, MSE = 0.33, $p = 0.047$, $\eta_p^2 = 0.17$, with no interaction between sucrose concentration * group, $F_{1, 22} = 0.011$, MSE = 0.33, $p = 0.92$, $\eta_p^2 = 0.001$. There was a significant effect of time on vol/licks, $F_{1, 22} = 14.19$, MSE = 0.39, $p = 0.001$, $\eta_p^2 = 0.39$, where vol/licks were greater in the re-testing stage compared to the original testing, with no interaction between time * group, $F_{1, 22} = 0.001$, MSE = 0.39, $p = 0.97$, $\eta_p^2 < 0.001$. There was no overall interaction between sucrose concentration * time, $F_{1, 22} = 3.73$, MSE = 0.19, $p = 0.066$, $\eta_p^2 = 0.15$, nor between sucrose concentration * time * group, $F_{1, 22} = 4.04$, MSE = 0.19, $p = 0.057$, $\eta_p^2 = 0.16$.

Shapiro-Wilks test of normality found that vol/licks for 4% sucrose were not normally distributed in the CORT-treated group at the late time point, $W(12) = 0.85$, $p = 0.034$. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian repeated measures ANOVA found only anecdotal evidence to support the lack of group effect on vol/licks, $BF_{01} = 2.43$. Moderate evidence was found to support the lack of interaction between time * group, $BF_{01} = 4.03$, and the lack of interaction between time * group * sucrose concentration, $BF_{01} = 7.02$.

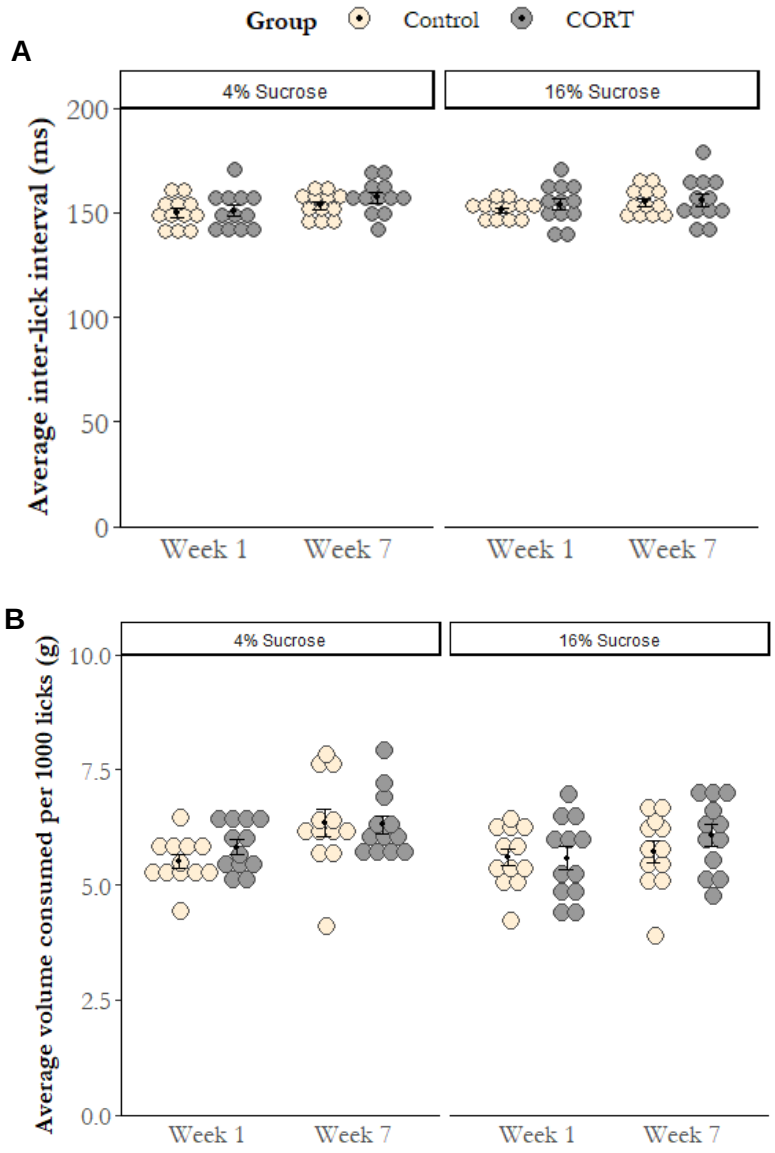


Figure 4.7. Comparison of immediate and delayed CORT treatment on control variables. There was no significant difference between CORT-treated rats and control rats on inter-lick intervals, ILI (A) or volume consumed per 1000 licks, vol/licks (B), nor any interactions between group and time point for either variable.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots indicate individual data points.

4.3.1.3. Summary

Experiment 8 aimed to establish a deficit in hedonic response to reward following chronic treatment with the pro-depressant, CORT, and to determine whether effects were long-lasting.

The findings from this experiment showed a reduction in hedonic response to two different concentrations of sucrose, high (16%) and low (4%), immediately following chronic treatment with CORT. This reduction in hedonic response was independent of any overall consummatory changes. To reduce external stressors, exogenous CORT was administered through drinking water, therefore a positive control was necessary to confirm incorporation of the drug into the system. Monitoring of rats' increase in body weight over time showed that CORT-treated animals gained less weight during drug administration compared to control animals.

Half of this original cohort were then re-tested 7 weeks post-CORT treatment to determine whether the effects of CORT remained after a long period and compared to their original test results. The findings from this re-test suggested no overall effect of CORT treatment on lick cluster size and no change over time, however, these findings were deemed inconclusive, likely due to reducing the sample size to a point of low power.

4.3.2. Experiment 9 – Re-examination of a CORT-induced deficit in hedonic response and comparing male/female differences

4.3.2.1. Experimental design

32 male and 32 female naïve Sprague-Dawley rats weighing 298g – 469g and 199g – 273g respectively on *ad libitum* food at approximately 11 weeks of age were used. Animals either received drug or vehicle treatment (*n*=16 control, *n*=16 CORT-treated, per sex) prior to food restriction.

For this experiment, pre-training began four days after the final CORT dose, following the description in section 4.2.4 and no sucrose preference test was conducted. Prior to LCA, the flavour modified affective bias test (m-ABT) was conducted (see section 3.4) immediately following pre-training. Then, all rats were tested in the general lick cluster method described in section 4.2.5, a total of 19 days following the final CORT dose.

As described in section 4.2.6, data were screened for exclusion criteria after each testing session. According to these criteria, 8 data points were excluded from this analysis. No animals were fully excluded from the experiment.

4.3.2.2. Results

Results from LCA only are presented here. For results on body weight and reward-induced bias in this same cohort, see section 3.4.3. In brief, there was no difference in percentage increase in body weight in the CORT-treated rats compared to control rats, which was unexpected and suggests CORT did not work as expected.

<u>Effects of chronic CORT treatment on hedonic response to 4% and 16% sucrose</u>

There was no overall difference in LCS between control and CORT treated rats in Experiment 9, regardless of sucrose concentration and/or sex, supported by Bayes analysis. Females had higher LCS compared to males when consuming 16% sucrose but not 4%.

A repeated measures ANOVA found no overall effect of group, $F_{1, 60} = 1.27$, MSE = 3234.94, $p = 0.26$, $\eta_p^2 = 0.021$, or sex, $F_{1, 60} = 3.65$, MSE = 3234.94, $p = 0.061$, $\eta_p^2 = 0.057$ on LCS (Figure 4.8A). There was no interaction between group * sex, $F_{1, 60} = 0.10$, MSE = 3234.94, $p = 0.75$, $\eta_p^2 = 0.002$.

Overall, there was a main effect of sucrose concentration with LCS greater for 16% sucrose compared to 4% sucrose, $F_{1, 60} = 60.96$, MSE = 1858.65, $p < 0.001$, $\eta_p^2 = 0.50$. There was a significant interaction between sucrose concentration * sex, $F_{1, 60} = 5.62$, MSE = 1858.65, $p = 0.021$, $\eta_p^2 = 0.086$. Fisher's LSD post-hoc analysis indicates that, overall, females had

greater LCS for 16% sucrose compared to males (p = 0.035) with no difference for 4% sucrose (p = 0.81). There was no interaction between sucrose concentration * group, $F_{1, 60}$ = 1.08, MSE = 1858.65, p = 0.30, η_p^2 = 0.018, nor between sucrose concentration * group * sex, $F_{1, 60}$ = 0.61, MSE = 1858.65, p = 0.44, η_p^2 = 0.01.

Shapiro-Wilks test of normality found that LCS for 16% sucrose was not normally distributed in female control rats only, W(16) = 0.75, p <0.001. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian repeated measures ANOVA found moderate evidence to support a lack of overall effect of group on LCS, BF_{01} = 3.04, and for the lack of interaction between group * sucrose concentration, BF_{01} = 3.10. Only anecdotal evidence was found to support the lack of difference between males and females in LCS, BF_{01} = 1.33. Moderate evidence supporting the lack of interaction between group * sex, BF_{01} = 4.14. There was also moderate evidence to support the lack of interaction between group * sex * sucrose concentration, BF_{01} = 7.21.

CORT treatment had no effect on overall consumption of sucrose, regardless of sucrose concentration and/or sex, with support from Bayes analysis. Males consumed overall more sucrose than females.

There was no overall effect of group on consumption, $F_{1, 60}$ = 0.002, MSE = 11.63, p = 0.96, η_p^2 <0.001 (Figure 4.8B). There was a significant effect of sex, $F_{1, 60}$ = 14.98, MSE = 11.63, p <0.001, η_p^2 = 0.20, with males consuming significant greater amounts than females, but no interaction between group * sex on consumption, $F_{1, 60}$ = 0.03, MSE = 11.63, p = 0.86, η_p^2 = 0.001.

All rats consumed significantly more of 16% sucrose than 4%, $F_{1, 60}$ = 43.17, MSE = 2.79, p <0.001, η_p^2 = 0.42. There was no interaction between sucrose concentration * group, $F_{1, 60}$ = 2.47, MSE = 2.79, p = 0.12, η_p^2 = 0.039, sucrose concentration * sex, $F_{1, 60}$ = 0.55, MSE = 2.79, p = 0.46, η_p^2 = 0.009, nor sucrose concentration * group * sex, $F_{1, 60}$ = 0.094, MSE = 2.79, p = 0.76, η_p^2 = 0.002.

A Bayesian repeated measures ANOVA found moderate evidence to support the lack of group effect on consumption, BF_{01} = 3.36, with only anecdotal evidence to support a lack of interaction between group * sex, BF_{01} = 2.84 and group * sucrose concentration, BF_{01} = 1.99. Strong evidence was found to support the lack of interaction between group * sex * sucrose concentration, BF_{01} = 11.71.

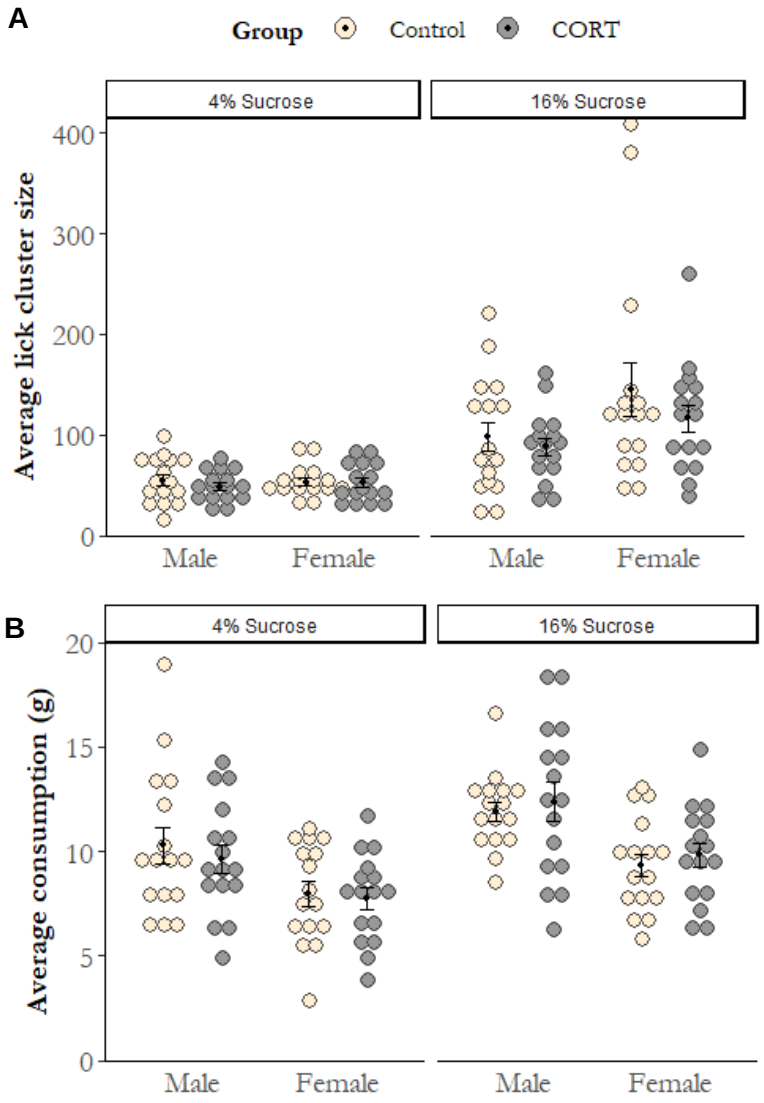


Figure 4.8. Effects of CORT treatment on hedonic response to sucrose and overall consumption in males and females.
There was no effect of CORT treatment on either lick cluster size, representing hedonic response (A), nor overall consumption (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

ILI's, but not vol/licks, were influenced by sucrose concentration. CORT treatment did not affect either control variable, with only anecdotal Bayesian support for this. Males had greater ILI's and vol/licks compared to females.

Repeated measures ANOVAs for control variables revealed no overall effect of group on ILI's, $F_{1, 60} = 2.85$, MSE = 110.98, $p = 0.096$, $\eta_p^2 = 0.045$. There was a significant effect of sex, $F_{1, 60} = 20.70$, MSE = 110.98, $p < 0.001$, $\eta_p^2 = 0.26$, in which males had greater ILI's than females, with no interaction between group * sex, $F_{1, 60} = 0.57$, MSE = 110.98, $p = 0.45$, $\eta_p^2 = 0.009$ (Figure 4.9A).

All rats had significantly greater ILI's for 16% sucrose compared to 4%, $F_{1, 60} = 56.94$, MSE = 9.98, $p < 0.001$, $\eta_p^2 = 0.49$, with no interaction between sucrose concentration * group, $F_{1, 60} = 1.74$, MSE = 9.98, $p = 0.19$, $\eta_p^2 = 0.028$; sucrose concentration * sex, $F_{1, 60} = 1.46$, MSE = 9.98, $p = 0.23$, $\eta_p^2 = 0.024$; nor between sucrose concentration * group * sex, $F_{1, 60} = 0.19$, MSE = 9.98, $p = 0.66$, $\eta_p^2 = 0.003$.

A Bayesian repeated measures ANOVA found only anecdotal evidence to support the lack of group effect on ILI's, $BF_{01} = 1.18$. Moderate evidence was found to support the lack of interaction between group * sex * sucrose concentration, $BF_{01} = 5.46$.

Furthermore, there was no overall effect of group on vol/licks, $F_{1, 60} = 0.54$, MSE = 0.99, $p = 0.47$, $\eta_p^2 = 0.009$. There was a significant effect of sex, $F_{1, 60} = 49.32$, MSE = 0.99, $p < 0.001$, $\eta_p^2 = 0.45$, with males having significantly greater vol/licks than females. There was no interaction between group * sex, $F_{1, 60} = 0.054$, MSE = 0.99, $p = 0.82$, $\eta_p^2 = 0.001$ (Figure 4.9B).

There was no effect of sucrose concentration on vol/licks, $F_{1, 60} = 1.53$, MSE = 0.11, $p = 0.22$, $\eta_p^2 = 0.025$, with no interactions between sucrose concentration * group, $F_{1, 60} = 0.48$, MSE = 0.11, $p = 0.49$, $\eta_p^2 = 0.008$, sucrose concentration * sex, $F_{1, 60} = 3.41$, MSE = 0.11, $p = 0.070$, $\eta_p^2 = 0.054$, nor between sucrose concentration * group * sex, $F_{1, 60} = 0.038$, MSE = 0.11, $p = 0.85$, $\eta_p^2 = 0.001$.

Shapiro-Wilks test of normality found that vol/licks for 4% sucrose was not normally distributed in the male CORT-treated group, $W(16) = 0.87$, $p = 0.025$. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian repeated measures ANOVA found anecdotal evidence to support the lack of overall effect of group on vol/licks, $BF_{01} = 1.84$, and the lack of effect of sucrose concentration, $BF_{01} = 2.71$. Strong evidence was found to support the lack of interaction between group * sex * sucrose concentration, $BF_{01} = 17.11$.

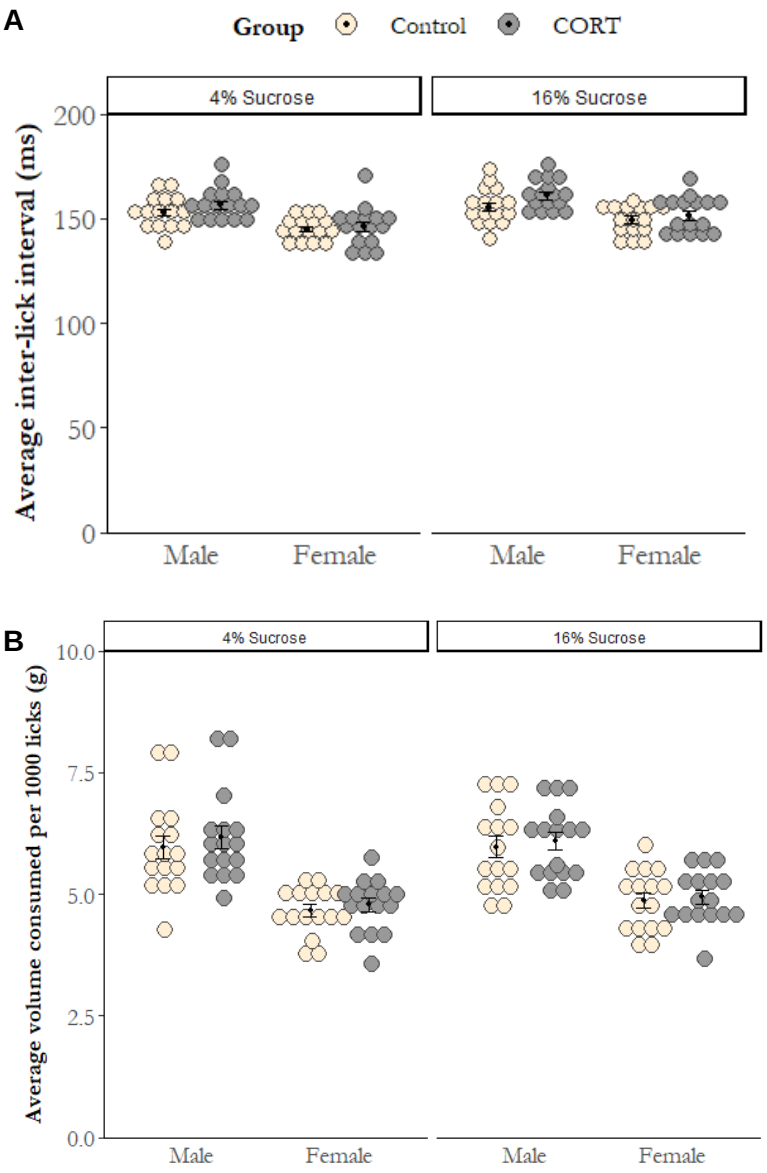


Figure 4.9. Effects of CORT treatment on control variables, inter-lick intervals (ILI's) and Vol/Licks (volume consumed per 1000 licks).
There was no effect of CORT treatment on either ILI's (A) or vol/licks (B) during lick cluster analysis. Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side. Bars represent means ±SEM. Dots represent individual data points.

4.3.2.3. Summary

Experiment 9 aimed to reproduce the findings from Experiment 8 by indicating the presence of a hedonic deficit in the chronic CORT rodent model. However, the findings of Experiment 9 indicated no change in hedonic response following chronic CORT treatment, along with the expected lack of changes in overall consumption and control variables.

As described in section 3.3.2, an ELISA demonstrated a lack of difference in circulating plasma CORT between the two groups in this experiment. In addition, there was no group difference in weight gain reported in this cohort. This lack of evidence for CORT working as expected might suggest issues with CORT intake and therefore a lack of behavioural effect here may instead be due to this drug administration issue.

This experiment does demonstrate some differences between male and female rats in their hedonic response to sucrose rewards. These results indicate that, although males consume significantly more sucrose overall, likely a result of gonadal hormone differences and/or body size, females appear to show increased hedonic response to a higher valued reward (16% sucrose) compared to males, but not in a lower valued reward (4% sucrose). Males did show greater ILI's and vol/licks compared to females. LCS and ILI's have been shown previously to shift in opposite directions as a result of artefacts from motor changes following PCP administration (Lydall et al., 2010), thus these findings suggest that females may have differences in motor function compared to males, rather than palatability to sucrose.

4.4. Anhedonia in the chronic interferon-alpha model

Deficits in hedonic response in the chronic IFN-α model were examined in the following experiments. Experiment 10 aimed to initially establish whether this model demonstrates a deficit in hedonic response to a low and/or high concentration of sucrose in only male rats. Experiment 11 further examined whether this model demonstrates a deficit in hedonic response to the same low and/or high concentration of sucrose in both male and female rats to confirm the findings of Experiment 10 and ensure any deficits are consistent and can be generalised across the sexes. Based on previous findings in the SPT, chronic IFN-α treated rats are not expected to demonstrate a change in LCS, indicative of hedonic response, and no difference between males and females.

4.4.1. Experiment 10 – Investigating IFN-α induced hedonic deficits.

4.4.1.1. Experimental design

48 male Sprague-Dawley rats weighing 303-384g on *ad libitum* food at approx. 9 weeks of age were used in this experiment. One rat was culled prior to testing due to complications from the injection, unrelated to any drug treatment. Animals either received IFN-α or saline treatment (*n*=23 control, *n*=24 IFN-treated) prior to food restriction.

Pre-training, testing and data collection were conducted exactly as described in section 4.2. For this experiment, pre-training was started during the injection period (after 5 days of treatment), and LCA testing began after 14 days of injections. According to the exclusion criteria, 17 data points were excluded from this experiment. No animals were completely removed.

4.4.1.2. Results

<u>Effects of chronic IFN-α treatment on hedonic response to 4% and 16% sucrose</u>

There was no difference in LCS between control and IFN-α treated rats, with moderate support from Bayes analysis, regardless of sucrose concentration.

A repeated measures ANOVA found no overall effect of group on LCS (Figure 4.10A), $F_{1, 45} = 0.54$, MSE = 1141.24, $p = 0.47$, $\eta_p^2 = 0.012$. There was a significant effect of sucrose concentration, whereby LCS were higher overall for 16% sucrose compared to 4%, $F_{1, 45} = 74.47$, MSE = 471.89, $p < 0.001$, $\eta_p^2 = 0.62$, however there was no interaction between sucrose concentration * group, $F_{1, 45} = 0.74$, MSE = 471.89, $p = 0.39$, $\eta_p^2 = 0.016$.

Shapiro-Wilks test of normality found that LCS for 16% sucrose was not normally distributed in control rats, W(23) = 0.88, $p = 0.009$, nor in IFN-treated rats, W(24) = 0.85, $p = 0.002$. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian repeated measures ANOVA revealed moderate support for this lack of group effect on LCS, BF_{01} = 3.27. Only anecdotal evidence was found to support the lack of interaction between group * sucrose concentration, BF_{01} = 2.36.

IFN-α treatment also had no effect on overall consumption, with only anecdotal evidence to support this.

There was also no overall effect of group on consumption (Figure 4.10B), $F_{1, 45}$ = 3.92, MSE = 10.90, p = 0.054, η_p^2 = 0.080. There was a significant effect of sucrose concentration on consumption, with all rats consuming more 16% compared to 4%, $F_{1, 45}$ = 229.75, MSE = 3.30, p <0.001, η_p^2 = 0.84, with no interaction between sucrose concentration * group, $F_{1, 45}$ = 2.37, MSE = 3.30, p = 0.13, η_p^2 = 0.050.

A Bayesian repeated measures ANOVA found only anecdotal evidence to support the lack of group effect on consumption, BF_{01} = 1.54, and the lack of interaction between group * sucrose concentration, BF_{01} = 1.70.

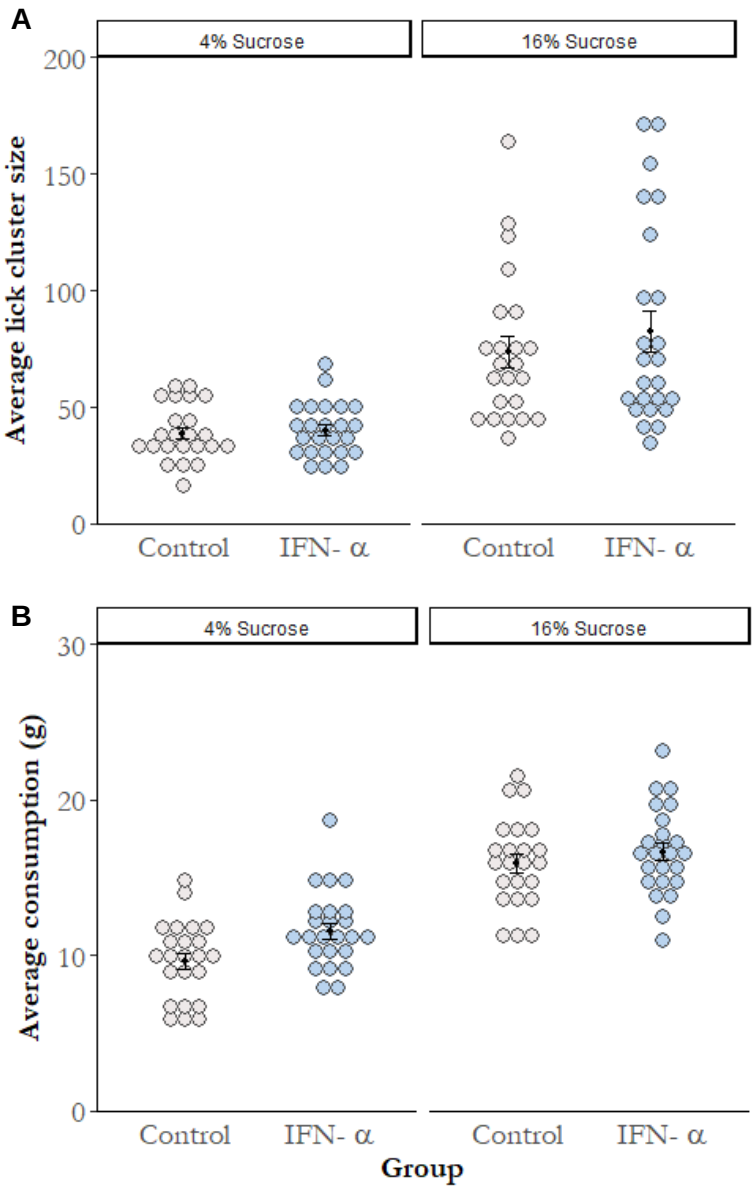


Figure 4.10. Effects of IFN-α treatment on hedonic response to sucrose and overall consumption.
There was no significant difference between control and IFN-treated rats in hedonic response, as measured by lick cluster size (A) nor overall consumption (B) of 4% or 16% sucrose.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

IFN-α treatment did not influence ILI's nor vol/licks, however only anecdotal evidence from Bayes analysis was found to support this.

Repeated measures ANOVAs for control variables revealed no overall effect of group on ILI (Figure 4.11A), $F_{1, 45} = 0.85$, MSE = 71.13, $p = 0.36$, $\eta_p^2 = 0.019$. There was a significant effect of sucrose concentration, $F_{1, 45} = 132.44$, MSE = 7.81, $p < 0.001$, $\eta_p^2 = 0.75$, where ILI's were higher for 16% vs 4% sucrose, with no interaction between sucrose concentration * group, $F_{1, 45} = 3.35$, MSE = 7.81, $p = 0.074$, $\eta_p^2 = 0.069$.

A Bayesian repeated measures ANOVA found only anecdotal evidence was found to support the overall lack of group effect, $BF_{01} = 2.08$, and lack of interaction between group * sucrose concentration, $BF_{01} = 1.51$, on ILI's.

Further, there was no overall effect of group on vol/licks (Figure 4.11B), $F_{1, 45} = 0.046$, MSE = 0.97, $p = 0.83$, $\eta_p^2 = 0.001$, nor an effect of sucrose concentration, $F_{1, 45} = 0.89$, MSE = 0.19, $p = 0.35$, $\eta_p^2 = 0.02$. There was no interaction between sucrose concentration * group, $F_{1, 45} = 0.17$, MSE = 0.19, $p = 0.68$, $\eta_p^2 = 0.004$.

Bayesian repeated measures ANOVA found anecdotal evidence to support the lack of group effect here, $BF_{01} = 2.46$. Strong evidence was found to support the lack of interaction between group * sucrose concentration, $BF_{01} = 11.56$.

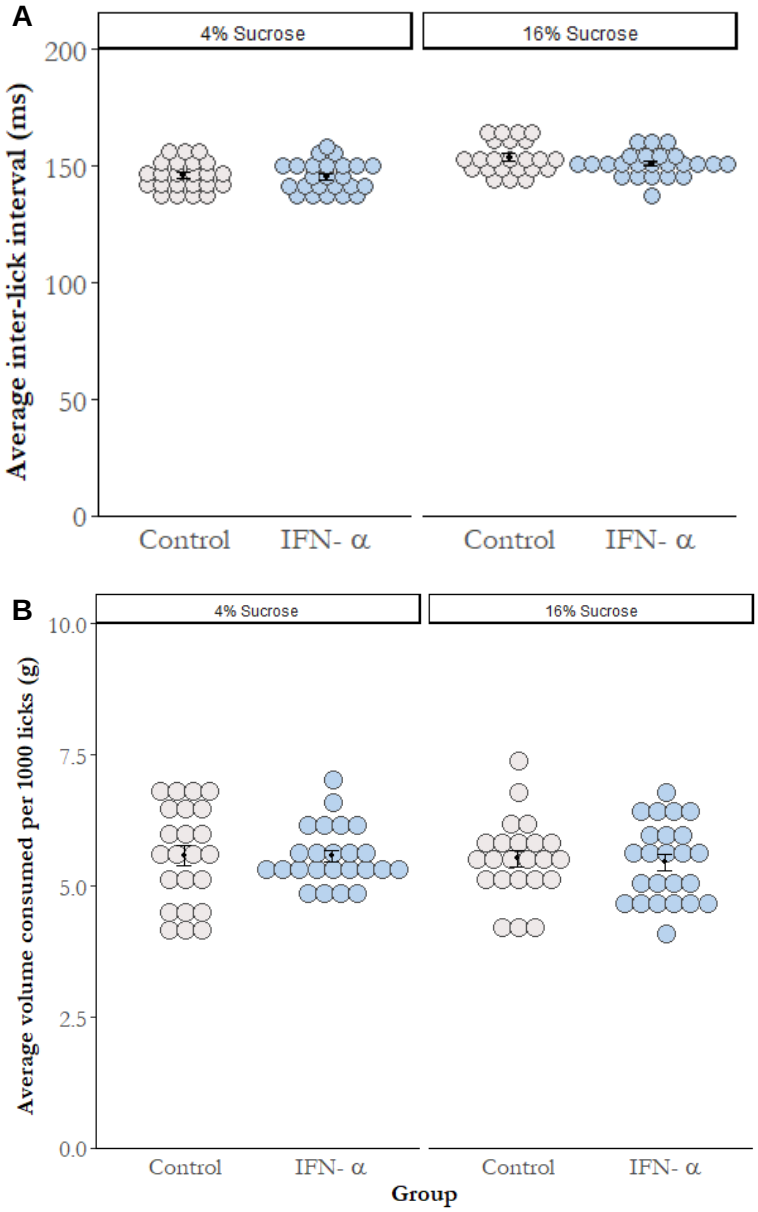


Figure 4.11. Effects of IFN-α treatment on control variables during lick cluster analysis.
There was no significant difference between control and IFN-treated rats in inter-lick intervals, ILI (A) nor volume consumed per 1000 licks, vol/licks (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

4.4.1.3. Summary

Experiment 10 aimed to determine whether chronic IFN-α treatment would induce a deficit in hedonic response to sucrose reward. Given previous research has indicated a lack of effect from this dose of IFN-α in alternative methods for assessing 'anhedonia', such as the SPT (Stuart et al., 2017), we hypothesised that chronic IFN-α treatment would also not induce a hedonic deficit in a more sensitive assay for anhedonia, LCA.

These findings demonstrate a lack of hedonic deficit following chronic treatment with IFN-α as LCS did not differ between control and treated rats. There were also no differences between the groups in overall consumption of sucrose, nor on control variables. Thus, these findings support previous research suggesting that although long-term IFN-α treatment is associated with the development of clinical depression, it does not appear to influence hedonic response to rewards in animal models.

4.4.2. Experiment 11 - Confirmation of a lack of hedonic deficit with IFN-α and male/female differences

4.4.2.1. Experimental design

32 male and 32 female naïve Sprague-Dawley rats weighing 310g – 460g and 221g – 304g respectively on *ad libitum* food at approximately 12 weeks of age were used. Animals received either drug or saline treatment (*n*=16 control, *n*=16 IFN-treated, per sex) prior to food restriction.

For this experiment, pre-training was started during the initial injection period (after 8 days of injections) and was conducted exactly as described in section 4.2.4. Prior to LCA, the flavour m-ABT was conducted (see section 3.5). Thus, LCA testing began after 23 days of injections. Testing and data collection procedures were conducted as described in section 4.2. According to exclusion criteria, 16 data points were excluded from this experiment. One animal from the control group met the exclusion criteria consistently during 4% sucrose testing sessions, so was removed from this analysis.

4.4.2.2. Results

There was no difference in LCS between control and IFN-α treated rats, regardless of sucrose concentration or sex, thus indicating IFN-α treatment had no effect on hedonic response, with only anecdotal evidence to support this following Bayes analysis. LCS was overall greater in females than males.

A repeated measures ANOVA revealed no overall effect of group on LCS (Figure 4.12A), $F_{1, 59} = 1.19$, MSE = 5346.75, $p = 0.28$, $\eta_p^2 = 0.02$. There was a significant effect of sex, $F_{1, 59} = 7.38$, MSE = 5346.75, $p = 0.009$, $\eta_p^2 = 0.11$, with LCS greater in females than males, and no interaction between group * sex, $F_{1, 59} = 0.74$, MSE = 5346.75, $p = 0.39$, $\eta_p^2 = 0.012$.

There was a significant effect of sucrose concentration, $F_{1, 59} = 73.17$, MSE = 2481.69, $p < 0.001$, $\eta_p^2 = 0.55$, with LCS greater for 16% sucrose vs 4%. There was no interaction between sucrose concentration * group, $F_{1, 59} = 1.19$, MSE = 2481.69, $p = 0.28$, $\eta_p^2 = 0.02$, but there was a significant interaction between sucrose concentration * sex, $F_{1, 59} = 6.38$, MSE = 2481.69, $p = 0.014$, $\eta_p^2 = 0.098$, with LCS greater for females when consuming 16% sucrose ($p = 0.009$) but not 4% sucrose ($p = 0.056$). There was no significant interaction between sucrose concentration * group * sex, $F_{1, 59} = 0.899$, MSE = 2481.69, $p = 0.35$, $\eta_p^2 = 0.015$.

Shapiro-Wilks test of normality found that LCS for 4% sucrose was not normally distributed in female control rats, $W(16) = 0.65$, $p < 0.001$, nor female IFN-treated rats, $W(16) = 0.80$, $p = 0.003$. LCS for 16% sucrose was also not normally distributed in female control rats,

W(16) = 0.70, p <0.001, female IFN-treated rats, W(16) = 0.69, p <0.001, nor male IFN-treated rats, W(16) = 0.79, p = 0.002. However, a suitable alternative statistical test for this type of analysis was not available.

A Bayesian repeated measures ANOVA found anecdotal support for a lack of group effect on LCS, BF_{01} = 2.93, and a lack of interaction between group * sucrose concentration, BF_{01} = 2.59 or group * sex, BF_{01} = 2.58 on LCS. Moderate evidence was found to support the lack of interaction between group * sex * sucrose concentration, BF_{01} = 3.43.

There was no difference between controls and IFN-α treated rats in overall sucrose consumption, regardless of sucrose concentration and/or sex, with moderate support from Bayes analysis.

There was also no overall effect of group on consumption (Figure 4.12B), $F_{1, 59}$ = 0.49, MSE = 9.21, p = 0.48, η_p^2 = 0.008, nor an effect of sex, $F_{1, 59}$ = 0.19, MSE = 9.21, p = 0.66, η_p^2 = 0.003. There was no interaction between group * sex, $F_{1, 59}$ = 0.22, MSE = 9.21, p = 0.64, η_p^2 = 0.004.

There was a significant effect of sucrose concentration on overall consumption, $F_{1, 59}$ = 31.61, MSE = 4.18, p <0.001, η_p^2 = 0.35, where consumption was highest for 16% sucrose compared to 4%. There was no interaction between sucrose concentration * group, $F_{1, 59}$ = 0.39, MSE = 4.18, p = 0.54, η_p^2 = 0.006, between sucrose concentration * sex, $F_{1, 59}$ = 2.37, MSE = 4.18, p = 0.13, η_p^2 = 0.039, nor between sucrose concentration * group * sex, $F_{1, 59}$ = 0.024, MSE = 4.18, p = 0.88, η_p^2 <0.001.

A Bayesian repeated measures ANOVA found moderate evidence to support a lack of group effect on consumption, BF_{01} = 3.44, and a lack of sex effect, BF_{01} = 3.86. There was also moderate evidence to support a lack of interaction between group * sucrose concentration, BF_{01} = 6.09, and group * sex, BF_{01} = 9.11. Very strong evidence was found to support the lack of interaction between group * sex * sucrose concentration, BF_{01} = 45.19.

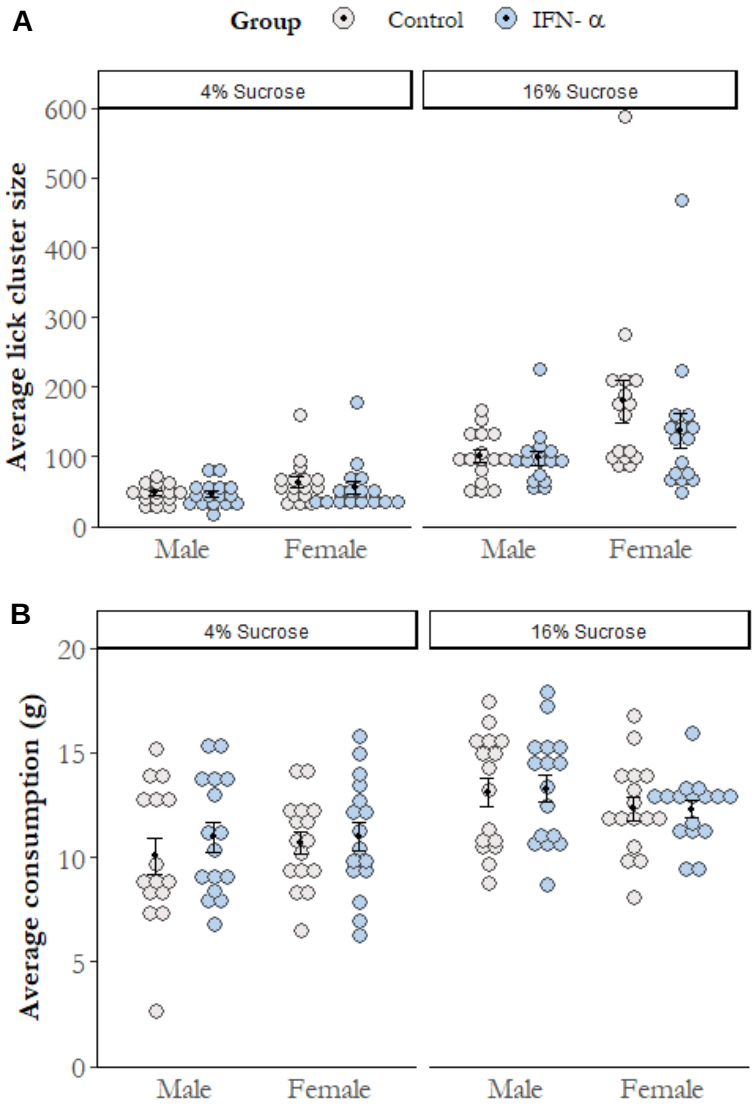


Figure 4.12. Effects of IFN-α treatment and/or sex on hedonic response to sucrose and overall consumption.
There was no significant difference between control and IFN-treated rats in hedonic response, as measured by lick cluster size (A) nor overall consumption (B) of 4% or 16% sucrose.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

IFN-α treatment did not influence ILIs or vol/licks, regardless of sucrose concentration and/or sex, however, Bayes analysis found little evidence to support this. For both control variables, males showed higher values than females.

Repeated measures ANOVAs for control variables revealed no significant effect of group on ILI's (Figure 4.13A), $F_{1, 59} = 3.77$, MSE = 78.64, $p = 0.057$, $\eta_p^2 = 0.06$. There was a significant effect of sex, $F_{1, 59} = 9.69$, MSE = 78.64, $p = 0.003$, $\eta_p^2 = 0.14$, with higher ILI's for males compared to females. There was no significant interaction between group * sex, $F_{1, 59} = 1.44$, MSE = 78.64, $p = 0.24$, $\eta_p^2 = 0.024$.

There was a significant effect of sucrose concentration on ILI's, $F_{1, 59} = 24.01$, MSE = 9.59, $p < 0.001$, $\eta_p^2 = 0.29$, with ILIs higher for 16% sucrose compared to 4%. There was no significant interaction between sucrose concentration * group, $F_{1, 59} = 0.058$, MSE = 9.59, $p = 0.81$, $\eta_p^2 = 0.001$, nor between sucrose concentration * sex, $F_{1, 59} = 2.73$, MSE = 9.59, $p = 0.10$, $\eta_p^2 = 0.044$, nor between sucrose concentration * group * sex, $F_{1, 59} = 0.12$, MSE = 9.59, $p = 0.74$, $\eta_p^2 = 0.002$.

Shapiro-Wilks test for normality found that ILI's for 16% sucrose were not normally distributed in female IFN-treated rats, W(16) = 0.88, $p = 0.034$, nor male IFN-treated rats, W(16) = 0.78, $p = 0.001$.

A Bayesian repeated measures ANOVA found almost no evidence to support a lack of group effect on ILI's, $BF_{01} = 0.91$, as well as a lack of interaction between group * sex, $BF_{01} = 0.93$. Anecdotal evidence was found to support the lack of interaction between group * sucrose concentration on ILI's, $BF_{01} = 2.27$. Moderate evidence supported the lack of interaction between group * sex * sucrose concentration, $BF_{01} = 5.53$.

Further, there was no overall effect of group on vol/licks (Figure 4.13B), $F_{1, 59} = 1.84$, MSE = 1.46, $p = 0.18$, $\eta_p^2 = 0.03$. There was a significant effect of sex, $F_{1, 59} = 12.91$, MSE = 1.46, $p = 0.001$, $\eta_p^2 = 0.18$, where males have higher vol/licks than females, and no interaction between group * sex, $F_{1, 59} = 1.14$, MSE = 1.46, $p = 0.29$, $\eta_p^2 = 0.019$.

There was no significant effect of sucrose concentration on vol/licks, $F_{1, 59,} = 3.34$, MSE = 0.16, $p = 0.073$, $\eta_p^2 = 0.054$. There was no interaction between sucrose concentration * group, $F_{1\ 59} = 1.87$, MSE = 0.16, $p = 0.18$, $\eta_p^2 = 0.031$, nor between sucrose concentration * sex, $F_{1, 59} = 2.90$, MSE = 0.16, $p = 0.094$, $\eta_p^2 = 0.047$. There was no interaction between sucrose concentration * group * sex on vol/licks, $F_{1, 59} = 0.56$, MSE = 0.16, $p = 0.46$, $\eta_p^2 = 0.009$.

A Bayesian repeated measures ANOVA found anecdotal evidence to support a lack of group effect, $BF_{01} = 1.43$, as well as a lack of interaction between group * sucrose

concentration, BF_{01} = 2.18, and group * sex, BF_{01} = 1.16. Moderate evidence supported the lack of interaction between group * sex * sucrose concentration, BF_{01} = 4.18.

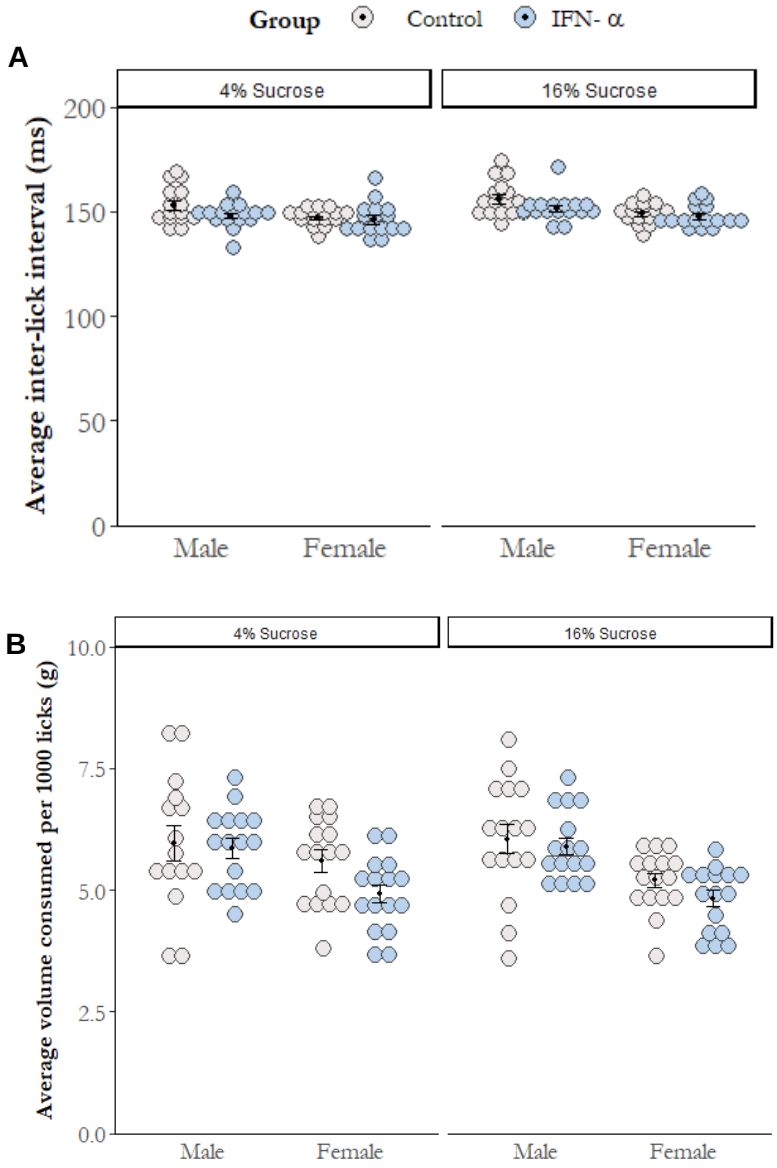


Figure 4.13. Effects of IFN-α treatment on control variables during lick cluster analysis. There was no significant difference between control and IFN-treated rats in inter-lick intervals, ILI (A) nor volume consumed per 1000 licks, vol/licks (B).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points rounded to the nearest whole number.

4.4.3.3. Summary

Experiment 11 aimed to validate the findings of Experiment 10 and confirmed that chronic IFN-α treatment does not appear to induce a deficit in hedonic response to rewards. I also investigated whether this lack of deficit was conserved across both male and female rats. These findings indicate that chronic IFN-α treatment does not influence hedonic responses to reward in either males or females.

In addition, these findings provide more support to Experiment 9, demonstrating that females have higher lick cluster size for 16% sucrose compared to males. Males also showed greater ILI's compared to females, again confirming findings from Experiment 9 that indicate this difference in LCS is likely due to motor differences rather than an underlying difference in palatability.

To confirm that the lack of hedonic response deficit in IFN-α rats was not a result of ineffective IFN-α treatment, histological analysis was performed to confirm a heightened immunological response in IFN-treated rats, which suggests IFN-α treatment did work as expected (see section 6.4.2).

4.5. Discussion of Chapter 4

This chapter aimed to evaluate the use of LCA as a technique for measuring anhedonia in preclinical models of psychiatric disease and determine whether pro-depressant treatment with CORT or IFN-α influence hedonic response to reward.

4.5.1. Effects of chronic CORT on hedonic processing of rewards

Experiments 8 and 9 evaluated the chronic CORT model using LCA, however, the findings from these experiments were contradictory.

Firstly, in Experiment 8, rats treated with chronic CORT showed a clear reduction in weight gain as expected, and reduced LCS indicating a hedonic deficit, with no change in overall consumption. These animals also received an adaptation of the SPT that was planned as a positive control since previous literature indicates a sucrose preference deficit (Ding et al., 2018; Li et al., 2016; Sturm et al., 2015); however, no difference was found between the two groups. The preference ratios here were higher than have been reported previously with this method, which potentially arose as a function of testing under food restriction rather than water restriction, along with the use of this LCA apparatus compared to home cages described in the standard SPT protocol (Eagle et al., 2016). Thus, remaining experiments in this chapter focused on the LCA technique only, using physiological or biochemical changes as the positive control.

In addition to demonstrating this hedonic deficit following chronic CORT treatment, the LCA method provides in-depth control measures to indicate whether these findings may have been due to disturbances in motor function (Ahmed et al., 2017). Since ILI's and vol/licks did not differ between the groups, this indicates CORT did not appear to influence motor function and thus there were no interferences with the interpretation of this change in LCS.

In the second part of experiment 8, half this cohort were maintained to examine whether the hedonic deficit was conserved over time. However, there was no significant difference, as measured by LCS, between control and CORT-treated rats at either the immediate or delayed time point, and no change over time. Bayesian analysis deemed these findings inconclusive, likely due to the reduced sample size in this cohort to a point of low power. Thus, the implications of long-term effects from CORT treatment on hedonic response to reward remains unclear.

In contrast to experiment 8, chronic CORT-treated rats in experiment 9 did not show any reduction in weight gain and showed a lack of hedonic deficit. Since a change in weight was expected, this positive control for CORT treatment was not present, so a further investigation of circulating CORT hormone levels was conducted via ELISA which also

failed to provide a positive control for CORT treatment (see section 3.4.3). Given these results, it can be suggested that chronic CORT administration did not work as expected in experiment 9 and therefore the behavioural results obtained do not accurately reflect the effects of this treatment.

This method of chronic CORT administration via drinking water was chosen to reduce any external stressors (i.e., injections) interacting with the impact of this exogenous stress hormone on behaviour. Since the drinking water method appears to be unreliable and produces inconsistencies, a further experiment to confirm the effects of chronic CORT on hedonic response to reward should administer chronic injections of exogenous CORT to better control the dose rats receive. I was unable to complete this confirmatory experiment due to time constraints; however, this was completed by other researchers in this laboratory. In this experiment, CORT (25mg/kg or 40mg/kg) was administered to male Sprague Dawley rats for 10 days via subcutaneous injection, followed by 3 days washout period before pre-training and testing in the 'lick cluster analysis' technique as described in this chapter. The treatment period of 10 days initially aimed to be 12 days to match with previous literature, but CORT-treated rats began losing weight to a point where treatment had to be halted at 10 days for safety concerns. Findings from this experiment showed no significant difference between controls and CORT treated rats in sucrose consumption, LCS or control variables. The difference in weight between control and CORT-treated rats indicates this drug was incorporated as expected in these animals, however, such a strong metabolic change could potentially interfere with results from a consummatory task such as this.

Nevertheless, this finding indicates that chronic CORT treatment does not influence hedonic processing of rewards. Returning to my initial analysis of Experiment 8, Bayes was applied to determine the strength of evidence supporting the group effect here, and this showed almost no evidence to support this. Thus, this supports that chronic CORT treatment does not appear to influence hedonic processing of rewards. Prior studies that use alternative techniques such as the SPT had suggested that chronic CORT treatment induced a deficit here, but this may represent other aspects of reward processing and by using the more sensitive LCA, I have been able to measure hedonic response more specifically.

4.5.2. Effects of chronic IFN-α on hedonic processing of rewards

Findings from Experiment 10 and 11 investigating the effect of chronic IFN-α showed a clear lack of deficit in hedonic response using the LCA technique, supporting previous research evaluating the dose of IFN-α used in this experiment in the SPT (Stuart et al., 2017). Furthermore, chronic IFN-α did not influence overall consumption, nor control variables. As discussed in section 3.6.2, an activated immune response was identified in the cohort of

animals used in Experiment 11, suggesting this immune response does not appear to be responsible for changes in hedonic response.

As discussed in the introduction of this chapter, several studies have found varying effects of IFN-α treatment on sucrose consumption depending on drug dose, type (e.g., human or rat/mouse), and behavioural method. These studies tend to show changes in sucrose consumption following higher doses of IFN-α than used here, and demonstrate the greatest reduction in sucrose preference with 1% sucrose (e.g. Sammut et al., 2002; Sammut et al., 2001).

Taken with the findings in this chapter, this could indicate that although the immune response becomes activated following 100IU/kg recombinant rat IFN-α, this dose was not high enough to induce a hedonic deficit. Higher doses of IFN-α have been associated with sickness behaviours (e.g. De La Garza et al., 2005; Sammut et al., 2001), whereas this dose was chosen in a previous study as it did not induce these sickness behaviours (Stuart et al., 2017). Thus, it could be suggested that the presence of a hedonic deficit is reliant upon sickness behaviours, rather than the general immune activating properties of IFN-α. Furthermore, the indication that these higher doses of IFN-α reduced preference of 1% sucrose could in fact indicate a deficit in "reward sensitivity", i.e., an inability to distinguish between the low concentration of sucrose and water in a state of food and water deprivation, rather than reducing hedonic experience.

Further investigation should determine whether higher doses of IFN-α treatment can induce hedonic deficits, as measured by LCA, and monitor sickness behaviours to correlate these with any deficits observed, thus being able to clarify whether any hedonic deficits are indeed a result of sickness behaviours, rather than immune activation alone. Measuring overall consumption along with LCS could help to answer the question of whether hedonic deficits resulting from sickness behaviours are indeed a reflection of reduced enjoyment of a reward, or whether this reflects solely a change in general appetite.

4.5.4. Conclusion

In summary, findings from Chapter 4 show that chronic CORT treatment does not appear to impair hedonic response to reward, with no influence on overall consumption or motor function, although these findings were variable depending on treatment administration route. In addition, consistent evidence from these experiments indicates that chronic IFN-α treatment does not impair hedonic response. These findings indicate discrepancies between hedonic response to reward measured by the more sensitive LCA assay and the traditionally used SPT in these two depression-related preclinical rodent models. Thus, this

highlights the need for more sensitive assays of reward processing deficits to reliably model psychiatric disease and in the development of novel treatments.

Chapter 5 – Effort-related decision making and motivational deficits in putative models of psychiatric disease

5.1. Introduction

Motivation for reward is the third main mechanism of reward processing discussed in Chapter 1 and will be the focus of this chapter. As described in section 1.4.2, progressive ratio (PR) tasks measure changes in the number of lever presses (representing 'effort') an animal is willing to make to obtain a reward. However, by measuring lever presses alone this task is subject to influence from motor impairments or deficits in other psychological processes such as habit formation or impulse control. Instead, the effort-related choice paradigm (EfR) measures this willingness to produce more effort for a high value reward given alongside the choice to consume free chow, which has a lower reward value requiring no effort, therefore allowing general consumption deficits to be considered. EfR is also directly translational to the human effort expenditure for reward task (EEfRT) task described in section 1.2.5 (Treadway et al., 2009) which has demonstrated reduced effort for a higher reward in MDD patients.

Previous studies using PR have demonstrated reduced lever presses following chronic corticosterone (CORT) treatment in both mice (Dieterich et al., 2019) and rats (Olausson et al., 2013) suggesting this stress hormone can reduce motivation to produce effort for a reward. However, in addition to potential influence from other deficits, PR may not accurately dissociate motivational aspects of reward processing from hedonic (Salamone, Correa, Mingote, & Weber, 2003). Therefore, in Experiment 12, I investigate the effects of chronic CORT treatment on effort-related choice in the EfR task to elucidate the influence of overactivation of the HPA axis on motivation for reward. Given the traditional use of EfR has been in the investigation of acute drug effects (see section 1.4.2) with limited reports using chronic manipulations, the acute effects of CORT will also be examined in Experiment 13.

In contrast, to my knowledge the effects of interferon-alpha (IFN-α) have not been evaluated using any motivational operant task. Previous literature has shown reduced lever presses and increased chow intake following acute treatment with other pro-inflammatory cytokines IL-1β and IL-6 (Nunes et al., 2014; Yohn, Arif, et al., 2016), suggesting a similar effect could be seen following IFN-α treatment. In patients, IFN-α treatment has been associated with reduced motivation as measured in self-report assessments (e.g. Capuron et al., 2012). Thus, to elucidate the impact of IFN-α on deficits in this aspect of reward processing, the effects of chronic and acute IFN-α treatment in the EfR will be investigated in Experiment 12 and 13, respectively.

Similarly, to my knowledge the effects of the *CACNA1C* risk mutation have not been explored in any motivational task, in humans or preclinical models. Schizophrenia has commonly been characterised by the presence of motivational deficits (Andreasen, 1995) and the *CACNA1C* risk variant is often expressed in multiple neuronal circuits related to motivation (Dedic et al., 2018). To compare the effects of this genetic risk factor in all three mechanisms of reward processing, the effects of heterozygous *CACNA1C* knockout (CACNA1C$^{+/-}$) on effort-related choice and motivational deficits was also explored in Experiment 14.

Thus, Chapter 5 aims to assess the use of the EfR to measure motivational decision-making deficits in these three models of psychiatric disease and exploring the neurobiological mechanisms by which motivational deficits occur.

5.2. General ‘effort-related choice test’ methods

5.2.1. Subjects

For experiments in Chapter 5, Sprague-Dawley rats supplied by Charles River or Envigo were housed in cages of two or three. CACNA1C heterozygous knockouts were sourced from a breeding stock held at Charles River UK. All rats were handled and maintained on food restriction prior to training as described in section 3.2.1 and husbandry details were as described for Experiment 1.

All experiments were conducted in compliance with the UK Animals (Scientific Procedures Act 1986) and with local Cardiff University Ethics Review Committee approval.

5.2.2. Apparatus

Eight identical operant boxes (Med Associates, VT) measuring 30x24x21cm and placed within a sound-attenuating shell with a ventilation fan were used for experiments in Chapter 5. Each box contained aluminium sidewalls with a clear acrylic front, back and top, and 19 steel rods (4.8mm diameter, 16mm apart) comprised the flooring, sat above a stainless-steel tray.

Reward pellets (45mg, TestDiet) were delivered to a recessed food well in the centre of the left wall, which automatically recorded the presence of the rat entering the well as “nose pokes”. Pellets used initially in experiment 12 were 5TUT (#1811251), but these were changed to 5TUL (#1811155) after initial training and for the entirety of Experiment 13. Two retractable levers were located 3cm to the left and right of the food well.

MED-PC software was used to insert levers and deliver reward pellets, while also recording food-well entries (nose pokes) and lever presses at one-minute intervals. For all sessions, only the left-hand lever was protracted during lever press sessions.

5.2.3. Experimental procedure

5.2.3.1. Operant lever press training

All rats were initially trained to press on a fixed ratio (FR) schedule in the following protocol: Firstly, rats received one session of magazine training where reward pellets were delivered on a variable-time (VT) 60s schedule with both levers retracted for the entire session. They then received two sessions of continuous reinforcement (CRF), where one lever press resulted in one reward pellet delivered.

After all rats had reached 50 reinforced trials (RFT) on the final CRF session, they were moved to FR2 schedule where two lever presses were required for one reward pellet delivery, and this increased exponentially until FR8, with no maximum number of RFTs.

All sessions lasted for a maximum of 30 minutes, held on separate days between 9am – 12pm.

5.2.3.2. Chow vs reward pellet preference testing

Once all rats were producing a stable number of lever presses at the final FR8 schedule for at least three consecutive days, they were shifted to the chow vs reward pellet choice testing. During choice test sessions, a clear glass bowl containing approximately 50g chow was placed in the back corner of the operant box opposite the lever and food well at the beginning of the session. The lever was extended, and rats could then choose whether to consume free chow or press the lever set to an FR8 schedule to receive reward pellets, for a maximum of 30 minutes.

5.2.4. Data analysis

Glass bowls containing chow were weighed before and after each session, including any spilled chow, to determine the amount of chow consumed. Chow consumption, number of lever presses, and percentage preference for reward pellets vs chow (calculated from the weight consumed of each) were analysed as the primary outcome measures.

For all experiments in Chapter 5, an alpha level of 0.05 was used as the level of significance and all analyses of choice test data were conducted using SPSS software (v23, IBM). Where indicated, repeated measures ANOVAs were used to compare the within-subjects factor ‘time’ or ‘dose’ with fixed factor of ‘group’ and/or ‘sex’. For Experiment 14, ‘genotype’ and ‘sex’ were compared in a univariate ANOVA. ANOVAs were compared using Fisher’s Least Significant Difference (LSD) post-hoc analysis. Tests for normal distribution were conducted as described in section 2.2.1.5 and Bayesian analyses were conducted as described in section 2.2.1.6.

5.3. Experiment 12 – Assessing effort-related choice following chronic CORT or IFN-α treatment

Experiment 12 aimed to pilot the use of the effort-related choice task and to determine whether chronic CORT and chronic IFN-α models showed a deficit in motivation for reward using the effort-related choice task. As this was an initial pilot experiment, only male rats were used. Based on previous findings in other motivation-related tasks, chronic CORT and chronic IFN-α were expected to reduce the number of lever presses produced to gain the higher valued reward and increase consumption of the lower valued free chow, demonstrating a reduced motivation for reward.

5.3.1. Experimental design

Chronic CORT and IFN-α treatment protocols are described fully in section 3.2.4. Chronic CORT was administered at a dose of 50µg/mL in 1% ethanol in drinking water daily for 12 days, followed by 6 days dose-tapering. Chronic IFN-α was administered via i.p. injections of 100IU/kg IFN-α daily for 14 days prior to testing, and at the end of every test day thereafter.

Experiment 12 used 4 treatment groups (vehicle control, *n*=8; CORT-treated, *n*=15; saline control, *n*=8; IFN-treated, *n*=16). One CORT-treated rat was removed from analysis after being identified as an outlier with greater weight gain compared to all other rats (see 3.3.2). All rats received lever press training in operant boxes and baseline choice tests prior to drug treatment, and then underwent an initial flavour modified affective bias test (m-ABT) experiment before re-entering the final stages of this experiment (see section 3.3 and Appendix A). The sample size used in this experiment was determined for the flavour m-ABT, and therefore as described in section 2.2.1.1.

For this cohort, all rats were trained up to a schedule of FR8 and then moved to FR8 vs chow choice test sessions, however, the experiment was halted after two choice sessions due to an unexpected lack of lever presses. To determine the reason behind this lack of response, an assessment of preference for three types of reward pellet vs chow took place. Rats were individually presented with two bowls in a separate home cage for 20 minutes, one containing chow and the other filled with either the 5TUT standard sucrose pellets used in the initial stage of the experiment, 5TUT chocolate flavour pellets, or 5TUL reward pellets. Both bowls were weighed before and after to determine the amount consumed, and the percentage preference for the reward pellets was calculated. A one-sample t-test was conducted from individual preference testing, and results are presented in the next section. In summary, rats showed a significant preference for the 5TUL reward pellets vs chow, but

did not prefer either of the 5TUT pellets, thus the remainder of the experiment used 5TUL reward pellets.

After this, all rats underwent one additional FR8 lever press training session with the new pellets before proceeding to complete four days of FR8 vs chow choice test baseline sessions. Following chronic drug treatment and the flavour m-ABT, all rats then received two days of 'reminder' lever press sessions, the first consisting of 5 minutes CRF, 5 minutes FR4 and 20 minutes FR8, with the final session following FR8 schedule only. They then received four additional choice test sessions with FR8 vs chow.

The mean number of lever presses and chow intake at baseline and post-treatment were calculated from the final 3 days of choice tests for statistical analysis.

5.3.2. Results

Results on differences in weight gain following treatment can be found in section 3.3.2. In brief, chronic CORT-treated rats had significantly reduced weight gain compared to vehicle-treated controls, with no difference between IFN-α and saline groups.

Pellet preference testing

As discussed above, a sudden lack of lever pressing during FR8 vs chow choice testing indicated a potential problem with the reward pellets chosen in this study. Thus, preference tests were conducted between different pellets vs chow, comparing the total amount consumed in 20 minutes.

A one-sample t-test using 50% as the test value showed there was no significant preference for the 5TUT sucrose pellets compared to normal home cage chow, $t(47) = -1.59$, $p = 0.12$, indicating the lack of lever presses was likely due to the reward itself. Switching to chocolate flavoured 5TUT pellets, rats actually showed a significant preference for the chow, $t(47) = -14.54$, $p < 0.001$. However, A one-sample t-test found that rats did show a significant preference for the 5TUL reward pellets compared to chow, $t(47) = 11.51$, $p < 0.001$.

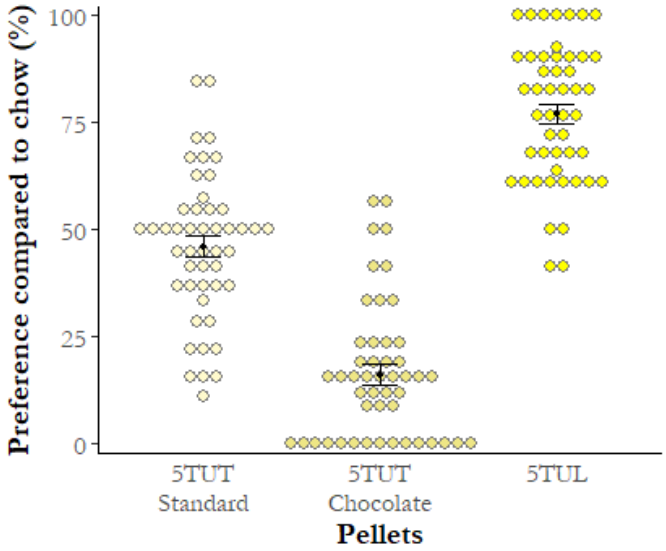


Figure 5.1. Percentage preference consumption for various types of reward pellet compared to chow.
One-sample t-tests showed no overall significant preference when assessing 5TUT standard pellets vs chow; a significant preference for chow compared to 5TUT chocolate pellets; and a significant preference for 5TUL pellets vs chow. Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

Comparison of control groups on lever presses and chow consumption

As in Experiment 3, the vehicle and saline control groups alone contained a small sample size, and it was planned prior to beginning the experiment that these two would be combined for analysis. However, an initial analysis of whether there were group differences between these two had to be established first.

The findings from this analysis demonstrated no difference between lever presses post-treatment vs pre-treatment for either saline or vehicle-treated rats. Overall, chow consumption was lower, and thus preference for pellets vs chow was greater, post-treatment compared to pre-treatment, with no difference between the two groups (see Table 5.1).

A repeated measures ANOVA found no overall significant difference between the mean number of lever presses during baseline choice tests compared to post-treatment tests, $F_{1, 14} = 0.24$, MSE = 25616.54, $p = 0.63$, $n_p^2 = 0.017$, with no interaction between group * time, $F_{1, 14} = 0.047$, MSE = 25616.52, $p = 0.83$, $n_p^2 = 0.003$. There was no group effect on overall lever presses, $F_{1, 14} = 90731.49$, $p = 0.99$, $n_p^2 < 0.001$. Shapiro-Wilks test for normality found that lever presses post-treatment in the vehicle-treated group were not normally distributed, W(8) = 0.80, $p = 0.03$. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian repeated measures ANOVA provided anecdotal evidence to support this lack of difference between the two groups in overall lever presses, BF_{01} = 1.97, as well as to support the lack of overall difference between pre- and post-treatment lever presses, BF_{01} = 2.70. Moderate evidence was found to support the lack of interaction between group * time, BF_{01} = 3.65.

Analysis of chow consumption found that overall chow consumption was significantly greater during baseline choice tests compared to post-treatment tests, $F_{1, 14}$ = 68.32, MSE = 0.95, p =<0.001, n_p^2 = 0.83, with no interaction between group * time, $F_{1, 14}$ = 0.44, MSE = 0.95, p = 0.52, n_p^2 = 0.031. There was also no group effect on overall chow consumption, $F_{1, 14}$ = 0.077, MSE = 5.44, p = 0.79, n_p^2 = 0.005. Shapiro-Wilks test for normality found that chow consumption pre-treatment in the saline-treated group was not normally distributed, W(8) = 0.79, p = 0.02. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian repeated measures ANOVA provided anecdotal evidence to support the lack of group difference in overall chow consumption, BF_{01} = 2.34. Anecdotal evidence was found to support the lack of interaction between group * time, BF_{01} = 1.95.

Finally, percentage preference for the reward pellets were analysed. A repeated measures ANOVA found that overall preference for pellets was lower during baseline choice tests compared to post-treatment tests, $F_{1, 14}$ = 43.35, MSE = 0.01, p <0.001, n_p^2 = 0.76, with no interaction between group * time, $F_{1, 14}$ = 0.98, MSE = 0.01, p = 0.34, n_p^2 = 0.065. There was no group effect on overall preference for pellets, $F_{1, 14}$ <0.001, MSE = 0.07, p = 0.99, n_p^2 <0.001. Shapiro-Wilks test for normality found that this preference post-treatment in the vehicle-treated group was not normally distributed, W(8) = 0.82, p = 0.044. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian repeated measures ANOVA provided anecdotal evidence to support the lack of overall group difference in preference for pellets vs chow, BF_{01} = 2.27. Anecdotal evidence was found to support the lack of interaction between group * time, BF_{01} = 1.64.

Table 5.1. A summary of results from vehicle and saline groups in the effort-related choice task of Experiment 12.

	Pre-treatment			Post-treatment		
Group	**Chow consumption (g)**	**Mean lever presses**	**Preference for pellets (%)**	**Chow consumption (g)**	**Mean lever presses**	**Preference for pellets (%)**
Saline	6.5 (±0.55)	489 (±88.7)	28.5 (±0.05)	3.4 (±0.77)	505 (±81.9)	55.8 (±0.09)
Vehicle	6.0 (±0.60)	478 (±97.7)	32.2 (±0.06)	3.4 (±0.58)	518 (±70.5)	52.4 (±0.07)

Comparison of combined control group and treatment groups

Given the evidence above suggests there were no significant differences between the saline and vehicle control groups, these two groups were combined and analysed with both CORT and IFN-treated rats in mixed ANOVAs.

It was found that lever presses did not differ pre- and post-treatment, with no difference between the three groups and moderate evidence to support this from Bayes analysis. Consumption of chow was significantly lower post-treatment vs pre-treatment with no overall group difference, with moderate Bayes support. Preference for pellets vs chow was significantly greater post-treatment vs pre-treatment for both control and CORT-treated rats, but not for IFN-α treated rats. However, there was no overall group difference in this preference, moderately supported by Bayes.

A repeated measures ANOVA found no overall difference between lever presses during pre- vs post-treatment, $F_{1, 44} = 0.17$, MSE = 39522.89, $p = 0.68$, $n_p^2 = 0.004$, with no interaction between group * time, $F_{2, 44} = 0.86$, MSE = 39522.89, $p = 0.43$, $n_p^2 = 0.038$ (Figure 5.2A). There was no significant main effect of group, $F_{2, 44} = 0.19$, MSE = 193364.43, $p = 0.83$, $n_p^2 = 0.009$. Shapiro-Wilks test for normality found that lever presses in the IFN-treated group were not normally distributed pre-treatment, W(16) = 0.85, $p = 0.016$ nor post-treatment, W(16) = 0.87, $p = 0.03$. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian repeated measures ANOVA found moderate evidence to support this lack of group effect on overall lever presses, $BF_{01} = 3.36$, as well as to support the lack of overall difference between pre- and post-treatment lever presses, $BF_{01} = 4.17$. Strong evidence was also found to support the lack of interaction between group * time, $BF_{01} = 19.25$.

Analysis of chow consumption found overall lower consumption post-treatment compared to pre-treatment, $F_{1, 44} = 59.48$, MSE = 1.37, $p < 0.001$, $n_p^2 = 0.58$. There was a significant interaction between group * time, $F_{2, 44} = 5.59$, MSE = 1.37, $p = 0.007$, $n_p^2 = 0.20$

(Figure 5.2B), where Fisher's LSD post-hoc analysis revealed no between-group differences but reduced chow consumption post-treatment for control (p <0.001), CORT-treated (p <0.001) and IFN-treated rats (p = 0.036). There was no main effect of group on chow consumption, $F_{2, 44}$ = 0.20, MSE = 7.52, p = 0.82, n_p^2 = 0.009. Shapiro-Wilks test for normality found that chow consumption pre-treatment was not normally distributed in the control group, W(16) = 0.88, p = 0.037. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian repeated measures ANOVA found moderate evidence to support the lack of group effect on overall chow consumption, BF_{01} = 5.04.

Furthermore, a repeated measures ANOVA found overall percentage preference for reward pellets vs chow was significantly greater post-treatment compared to pre-treatment, $F_{1, 44}$ = 34.53, MSE = 0.014, p <0.001, n_p^2 = 0.44. There was a significant interaction between group * time, $F_{2, 44}$ = 3.93, MSE = 0.014, p = 0.027, n_p^2 = 0.15 (Figure 5.2C), with Fisher's LSD post-hoc analysis revealing no between-group differences, but greater preference post-treatment vs pre-treatment in control (p <0.001) and CORT-treated rats (p = 0.009), but not IFN-treated rats (p = 0.076). There was no main effect of group, $F_{2, 44}$ = 0.071, MSE = 0.086, p = 0.93, n_p^2 = 0.003.

A Bayesian repeated measures ANOVA found moderate evidence to support the overall lack of group effect on preference for pellets vs chow, BF_{01} = 4.49.

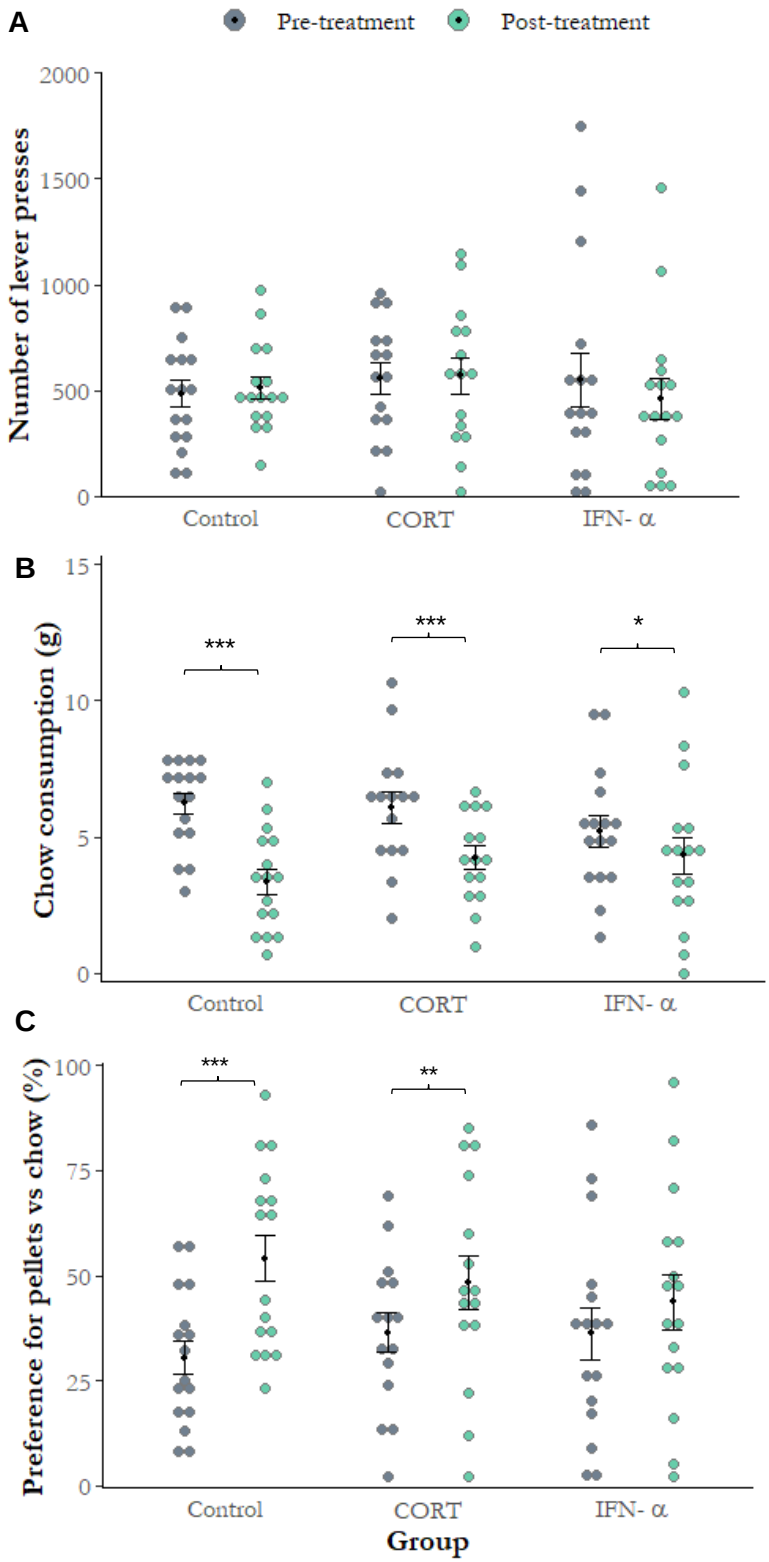


Figure 5.2. Graphs demonstrating lever presses, chow consumption and preference for pellets vs chow in the effort-related choice task of Experiment 12. No significant group differences were found in either lever presses (A), chow consumption (B) or preference for pellets vs chow (C) between pre-treatment and post-treatment time points. However, all rats showed a decrease in chow consumption post-treatment and thus an increase in preference for pellets.
Bars represent mean ±SEM. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.
Dots indicate individual data points.

5.3.3. Summary

Experiment 12 was an initial pilot experiment aiming to assess whether motivational deficits in the chronic CORT or IFN-α model could be measured using the effort-related choice task.

Firstly, an unexpected lack of lever presses was found at the beginning of this experiment when using standard 5TUT sucrose pellets as the reward. A comparison of three different types of reward pellets commonly used in the behavioural laboratory was conducted, in which rats were able to choose freely between one type of pellet or chow. This showed that rats only preferred the new 5TUL reward pellets compared to chow, whilst there was no preference between standard 5TUT pellets vs chow, and rats preferred chow over 5TUT chocolate flavoured sucrose pellets. Given these findings, the pellets used were changed to 5TUL for the remainder of this experiment.

Individual vehicle and saline control groups had small sample sizes and prior to the experiment it was planned to combine these two groups for analysis. An initial comparison of these two control groups found no significant group differences and so they were combined, however Bayesian analyses did not provide firm conclusive evidence to support this lack of differences and so results should be interpreted with caution.

The findings comparing this combined control group with the CORT-treated and IFN-treated groups demonstrate that there appeared to be no effect of either treatment on overall lever presses, chow consumption, or preference for pellets vs chow. However, all groups showed a significant reduction in chow consumption post-treatment compared to pre-treatment. Since there was no difference in lever presses after treatment, this change in chow consumption resulted in a significant increase post-treatment vs pre-treatment in the animals preference for producing effort to gain reward pellets vs consuming free chow.

An interaction between group * time showed this increase in preference for pellets post-treatment was most prominent in control rats, lesser so in CORT-treated rats, and non-significant in IFN-treated. This potentially indicates an effect of treatment such that the preference for the effortful reward option reduces following chronic CORT or IFN-α treatment but using unfavourable pellets at the beginning could have interfered with providing conclusive evidence here. There were no direct between-group differences found so this interpretation should be taken cautiously.

From these findings, it could be suggested that the change in chow consumption for all animals may reflect the problems with using 5TUT pellets for training at the start. Given that rats did not prefer the 5TUT pellets in general and they originally learned the lever-pellet association with 5TUT, this association may not yet have been replaced with the new 5TUL pellets and thus their preference for chow was greater.

5.4. Experiment 13 – Effects of acute CORT or IFN-α on effort-related choice.

After Experiment 12 showed inconclusive findings possibly due to issues during training in the operant task, Experiment 13 again aimed to pilot the use of the effort-related choice task but utilised an acute treatment design to match the previous literature using this task and to fit with the time requirements of this PhD. It also aimed to determine whether CORT and IFN-α models showed a deficit in motivation for reward. Based on previous findings in other motivation-related tasks, acute CORT and acute IFN-α were expected to reduce the number of lever presses produced to gain the higher valued reward and increase consumption of the lower valued free chow, demonstrating a reduced motivation for reward, however the dose required to induce this deficit was not predicted. Haloperidol was used as a positive control for the assay itself, which was expected to reduce lever presses and increase chow consumption in a dose-dependent manner. For all drugs in this experiment, deficits induced were expected to be consistent across males and females.

5.4.1. Experimental design

In Experiment 13, 16 male and 16 female Sprague-Dawley rats were used in a within-subjects design where all animals received each drug dose and appropriate vehicle, in which the order of doses was randomised using a Latin square design. The sample sizes used in this experiment and design followed were determined from previous research (Griesius, Mellor, & Robinson, 2020).

Once all rats had been trained to respond stably on the FR8 schedule, they received seven days of FR8 vs free chow preference testing without drug treatment to achieve stable responses across both lever presses and chow consumption.

The remainder of the experiment followed a schedule over the course of 2 weeks in which baseline (non-drug or vehicle) sessions were conducted on Monday and Thursday, while drug/vehicle sessions were conducted on Tuesday and Friday. No animal was run on Wednesday, Saturday, or Sunday. Each 2-week treatment course was separated by one full week of baseline FR8 vs chow sessions (see Appendix A for experimental trajectory).

Three drugs were assessed in this experiment, all of which were administered in a final volume of 1mL/kg (see Table 5.1 for the list of treatments and doses).

Table 5.1. Treatments used in Experiment 13.
mg/kg, milligrams per kilogram of weight; *min*, minute; *DMSO*, dimethyl sulfoxide; *i.p,* intraperitoneal

Sequence	Treatment	Administration route	Dose	Vehicle	Time before task (min)
1	Haloperidol	i.p.	0.01, 0.03, 0.1 (mg/kg)	1% DMSO, 2% Cremaphor, 97% Saline	50
2	CORT	subcutaneous	3, 10, 30 (mg/kg)	10% DMSO, 20% Cremaphor, 70% Saline	30
3	IFN-α	i.p.	10, 100, 1000 (IU/kg)	100% Saline	30

All rats were weighed prior to drug treatment. The percentage increase in body weight between each treatment was calculated for each rat, and this was analysed to compare the effects of each drug treatment dose on weight gain 2-3 days post-treatment.

5.4.2. Results

Acute effects of haloperidol

Weight gain

A repeated measures ANOVA found no overall effect of dose on percentage weight gain, $F_{3, 90} = 0.55$, MSE <0.001, $p = 0.65$, $\eta_p^2 = 0.018$, with no interaction between dose * sex, $F_{3, 90} = 0.80$, MSE <0.001, $p = 0.49$, $\eta_p^2 = 0.026$ (Figure 5.3). There was a significant overall effect of sex, $F_{1, 30} = 37.96$, MSE <0.001, p <0.001, $\eta_p^2 = 0.56$, where males had greater percentage weight gain than females. Shapiro-Wilks test for normality found that the percentage weight gain for females after 0.03mg/kg Haloperidol was not normally distributed, W(16) = 0.86, $p = 0.021$. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian repeated measures ANOVA found strong evidence to support this lack of effect of dose on percentage weight gain, $BF_{01} = 11.10$. Strong evidence was also found to support the lack of interaction between dose * sex, $BF_{01} = 16.46$.

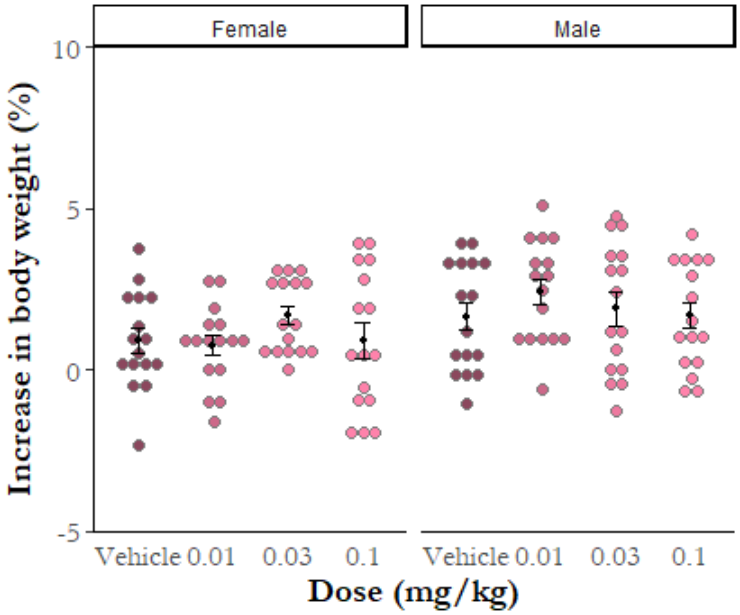


Figure 5.3. Percentage increase in body weight following acute haloperidol treatment. There was no significant change in weight gain following acute haloperidol treatment.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent means ±SEM. Dots represent individual data points.

Effort-related choice

Findings from the effort-related choice task demonstrated that the highest dose of haloperidol (0.1mg/kg) significantly increased chow consumption and reduced lever presses and preference for pellets. There was no significant overall difference following

acute treatment with 0.03mg/kg and 0.01mg/kg haloperidol, nor an overall difference between males and females.

A repeated measures ANOVA found a significant overall effect of dose on lever presses, $F_{3, 90} = 33.06$, MSE = 46111.20, $p < 0.001$, $\eta_p^2 = 0.52$ (Figure 5.4A). Fisher's LSD post-hoc analysis showed a dose-dependent decrease in lever presses, with a significant reduction following 0.1mg/kg treatment ($p < 0.001$), a non-significant reduction following 0.03mg/kg treatment ($p = 0.063$), and no significant change following 0.01mg/kg ($p = 0.72$) compared to vehicle. There was no interaction between dose * sex, $F_{3, 90} = 0.98$, MSE = 46111.20, $p = 0.41$, $\eta_p^2 = 0.032$, and no overall effect of sex on lever presses, $F_{1, 30} = 0.18$, MSE = 169895.58, $p = 0.68$, $\eta_p^2 = 0.006$.

Shapiro-Wilks test for normality found that lever presses after 0.1mg/kg was not normally distributed for females W(16) = 0.67, $p < 0.001$, nor males, W(16) = 0.75, $p < 0.001$. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian repeated measures ANOVA found moderate evidence to support the lack of effect of sex, $BF_{01} = 3.57$, as well as the lack of interaction between dose * sex, $BF_{01} = 5.79$.

There was also a significant overall effect of haloperidol dose on chow consumption, $F_{3, 90} = 8.61$, MSE = 1.27, $p < 0.001$, $\eta_p^2 = 0.22$ (Figure 5.4B), where the highest dose significantly increased chow consumption compared to vehicle ($p < 0.001$), with no significant change after treatment with 0.03mg/kg ($p = 0.36$) or 0.01mg/kg ($p = 0.31$). There was no significant interaction between dose * sex, $F_{3, 90} = 0.63$, MSE = 1.27, $p = 0.59$, $\eta_p^2 = 0.021$. There was a significant overall effect of sex on chow consumption, $F_{1, 30} = 24.62$, MSE = 3.84, $p < 0.001$, $\eta_p^2 = 0.45$, with males consuming more than females.

Shapiro-Wilks test for normality found that chow consumption in females was not normally distributed after treatment with vehicle, W(16) = 0.76, $p < 0.001$, 0.01mg/kg Haloperidol, W(16) = 0.83, $p = 0.008$, nor 0.03mg/kg Haloperidol, W(16) = 0.87, $p = 0.029$. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian repeated measures ANOVA found anecdotal evidence to support the lack of interaction between dose * sex, $BF_{01} = 2.04$.

Analysis of the preference ratio for reward pellets vs chow with a repeated measures ANOVA found a significant effect of haloperidol dose, $F_{3, 90} = 35.11$, MSE = 0.036, $p < 0.001$, $\eta_p^2 = 0.54$ (Figure 5.4C). Fisher's LSD post-hoc analysis found that the preference for reward pellets was significantly reduced following treatment with 0.1mg/kg ($p < 0.001$), with a non-significant reduction following 0.03mg/kg ($p = 0.066$) and no significant change following 0.01mg/kg ($p = 0.32$). There was also a significant overall effect of sex on

preference, $F_{1, 30} = 5.85$, MSE = 0.098, $p = 0.022$, $\eta_p^2 = 0.16$, with females having a greater preference compared to males overall.

There was a significant interaction between dose * sex, $F_{3, 90} = 3.09$, MSE = 0.036, $p = 0.031$, $\eta_p^2 = 0.093$, where females had significantly greater preference for reward pellets compared to males following vehicle ($p = 0.002$) and 0.01mg/kg treatment ($p = 0.004$), but not following 0.03mg/kg ($p = 0.14$) or 0.1mg/kg ($p = 0.77$). Comparing only female rats found a significant reduction in preference following both 0.1mg/kg ($p < 0.001$) and 0.03mg/kg ($p = 0.026$) treatment compared to vehicle, with no change following 0.01mg/kg ($p = 0.47$). Comparing male rats only found a significant reduction following 0.1mg/kg compared to vehicle ($p = 0.001$), with no significant change following 0.03mg/kg ($p = 0.73$) nor 0.01mg/kg ($p = 0.49$).

Shapiro-Wilks test for normality found that percentage preference in females was not normally distributed after treatment with vehicle, W(16) = 0.86, $p = 0.022$, nor 0.1mg/kg Haloperidol, W(16) = 0.77, $p < 0.001$. However, no suitable alternative statistical test was available for this type of analysis.

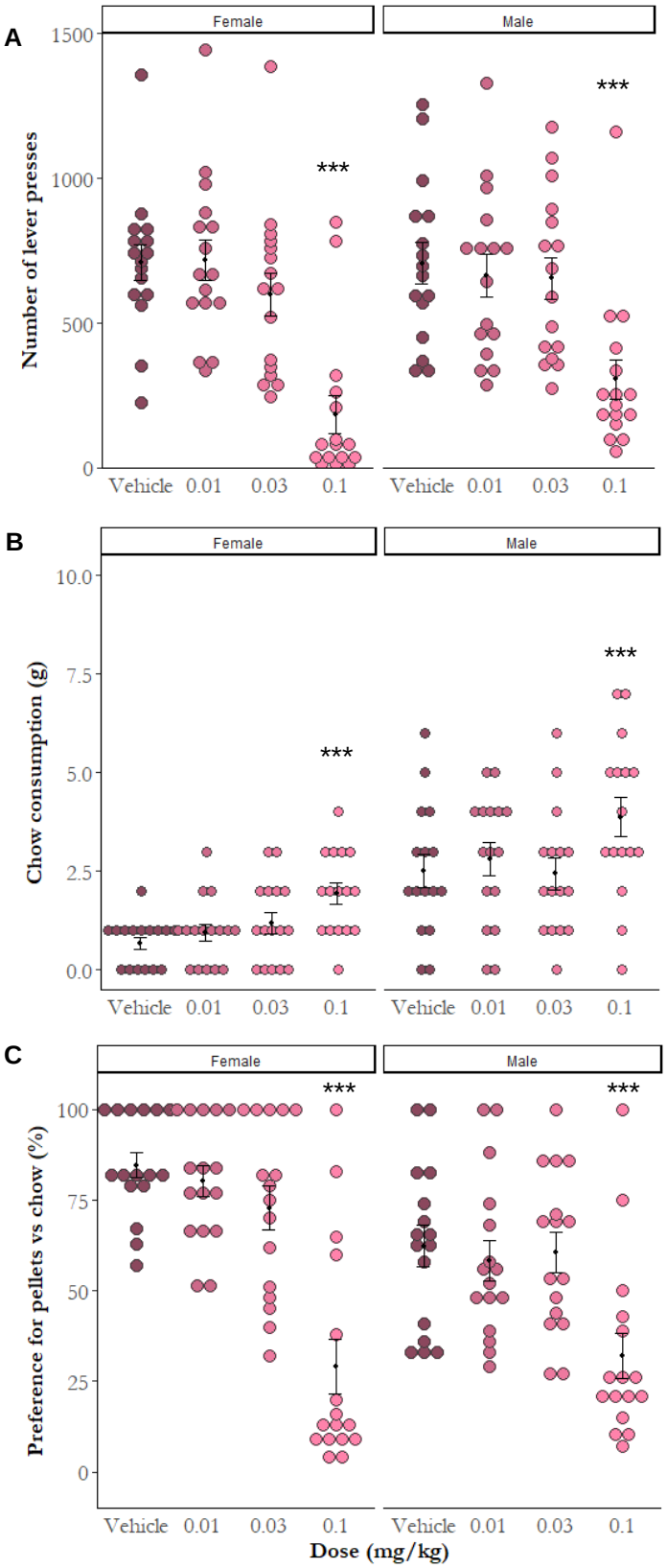


Figure 5.4. Effects of acute haloperidol treatment on effort-related choice in Experiment 13. Haloperidol significantly reduced lever presses (A) and percentage preference for pellets (C), and increased chow consumption (B) in a dose-dependent manner in both males and females. Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side. Bars indicate mean ±SEM. Dots represent individual data points. *** = p <0.001 compared to vehicle.

Acute effects of CORT

Weight gain

A repeated measures ANOVA found a significant overall effect of dose on percentage weight gain, $F_{3, 90} = 79.97$, MSE = 2.73, $p < 0.001$, $\eta_p^2 = 0.73$. Doses of 3mg/kg ($p = 0.022$), 10mg/kg ($p < 0.001$) and 30mg/kg ($p < 0.001$) all resulted in significantly reduced weight gain compared to vehicle with the highest doses resulting in weight loss (Figure 5.5). There was also a significant overall effect of sex on weight gain, $F_{1, 30} = 20.24$, MSE = 1.47, $p < 0.001$, $\eta_p^2 = 0.40$, with females having overall significantly greater weight gain compared to males.

There was a significant interaction between dose * sex, $F_{3, 90} = 12.43$, MSE = 2.73, $p < 0.001$, $\eta_p^2 = 0.29$, where females had significantly greater weight gain compared to males following 30mg/kg CORT only ($p < 0.001$). Both males and females individually showed a significant reduction in weight gain following 10mg/kg and 30mg/kg CORT compared to vehicle ($p < 0.001$), but only females showed this reduction following 3mg/kg ($p = 0.036$) with males showing a non-significant reduction ($p = 0.23$) compared to vehicle.

Shapiro-Wilks test for normality found that the percentage weight gain for females after treatment with vehicle was not normally distributed, W(16) = 0.81, $p = 0.003$. However, no suitable alternative statistical test was available for this type of analysis.

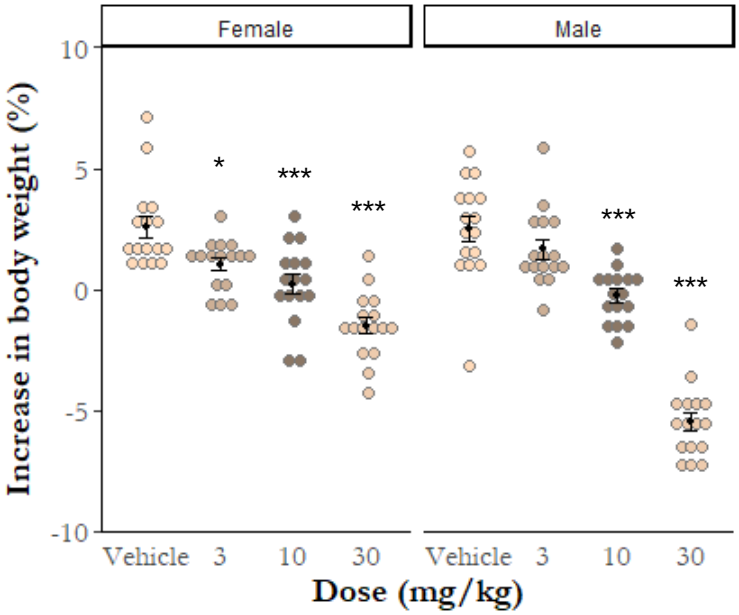


Figure 5.5. Percentage increase in body weight following acute CORT treatment. There was a significant dose-dependent decrease in weight gain following CORT treatment, with those receiving the highest dose losing weight. Bars indicate mean ±SEM. Dots represent individual data points rounded to the nearest whole number. * = p <0.05, *** = p <0.001 compared to vehicle.

Effort-related choice

Findings from the effort-related choice task showed no difference between control and CORT treated rats in lever presses, regardless of dose and/or sex. Chow consumption was significantly greater in males overall than females, but it was also shown that 10mg/kg CORT resulted in an increase in chow consumption compared to vehicle in male rats only, with no difference found with other doses nor in females. There were no differences between control and CORT treated rats in preference for pellets vs chow, regardless of sex and/or dose.

A repeated measures ANOVA found no overall effect of CORT dose on lever presses, $F_{3, 90} = 0.45$, MSE = 23314.88, $p = 0.72$, $\eta_p^2 = 0.015$, with no significant interaction between dose * sex, $F_{3, 90} = 0.68$, MSE = 23314.88, $p = 0.57$, $\eta_p^2 = 0.022$ (Figure 5.6A). There was also no significant overall effect of sex on lever presses, $F_{1, 30} = 0.66$, MSE = 218621.82, $p = 0.42$, $\eta_p^2 = 0.022$.

A Bayesian repeated measures ANOVA found strong evidence to support the lack of overall effect of dose on lever presses, $BF_{01} = 14.01$, with only anecdotal evidence to support the lack of difference between males and females, $BF_{01} = 1.80$. Moderate evidence was found to support the lack of interaction between dose * sex on lever presses, $BF_{01} = 3.69$.

Analysis of chow consumption found no significant overall effect of CORT dose, $F_{3, 90} = 2.69$, MSE = 0.58, $p = 0.051$, $\eta_p^2 = 0.082$. There was a significant overall effect of sex on chow consumption, $F_{1, 30} = 4.39$, MSE = 2.71, $p = 0.045$, $\eta_p^2 = 0.13$, where males generally consumed more than females.

There was also a significant interaction between dose * sex, $F_{3, 90} = 3.73$, MSE = 0.58, $p = 0.014$, $\eta_p^2 = 0.11$ (Figure 5.6B). Fisher's LSD post-hoc analysis of male rats found a significant increase in chow consumption after 10mg/kg CORT administration compared to vehicle ($p < 0.001$), with no significant change following 3mg/kg ($p = 0.31$) or 30mg/kg ($p = 0.10$). In female rats, there was no significant change in chow consumption following 3mg/kg ($p = 0.21$), 10mg/kg ($p = 0.77$), or 30mg/kg ($p = 0.27$), compared to vehicle.

Shapiro-Wilks test for normality found that chow consumption for females was not normally distributed after treatment with vehicle, W(16) = 0.75, $p < 0.001$, 3mg/kg CORT, W(16) = 0.83, $p = 0.006$, 10mg/kg CORT, W(16) = 0.73, $p < 0.001$, nor 30mg/kg CORT, W(16) = 0.79, $p = 0.002$. Chow consumption in males was also not normally distributed after treatment with vehicle, W(16) = 0.83, $p = 0.006$, 3mg/kg CORT, W(16) = 0.77, $p = 0.001$, nor 30mg/kg CORT, W(16) = 0.82, $p = 0.004$. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian repeated measures ANOVA found only anecdotal evidence to support the lack of overall dose effect on chow consumption, BF_{01} = 1.43.

When comparing preference ratios for reward pellets vs chow, a repeated measures ANOVA showed no significant effect of CORT dose, $F_{3, 90}$ = 1.27, MSE = 0.019, p = 0.29, η_p^2 = 0.04, with no significant interaction between dose * sex, $F_{3, 90}$ = 2.57, MSE = 0.019, p = 0.059, η_p^2 = 0.079 (Figure 5.6C). There was also no significant effect of sex, $F_{1, 30}$ = 3.85, MSE = 0.082, p = 0.059, η_p^2 = 0.11.

Shapiro-Wilks test for normality found that the percentage preference in females was not normally distributed after treatment with vehicle, W(16) = 0.82, p = 0.004, 3mg/kg CORT, W(16) = 0.88, p = 0.034, 10mg/kg CORT, W(16) = 0.77, p <0.001, nor 30mg/kg CORT, W(16) = 0.82, p = 0.006. In males, preferences were not normally distributed after treatment with vehicle, W(16) = 0.88, p = 0.043, 3mg/kg CORT, W(16) = 0.87, p = 0.027, nor 30mg/kg CORT, W(16) = 0.83, p = 0.007. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian repeated measures ANOVA found moderate evidence to support the overall lack of effect of CORT dose on preference ratios, BF_{01} = 5.89. Almost no evidence was found to support the lack of interaction between dose * sex on preference ratios, BF_{01} = 0.971.

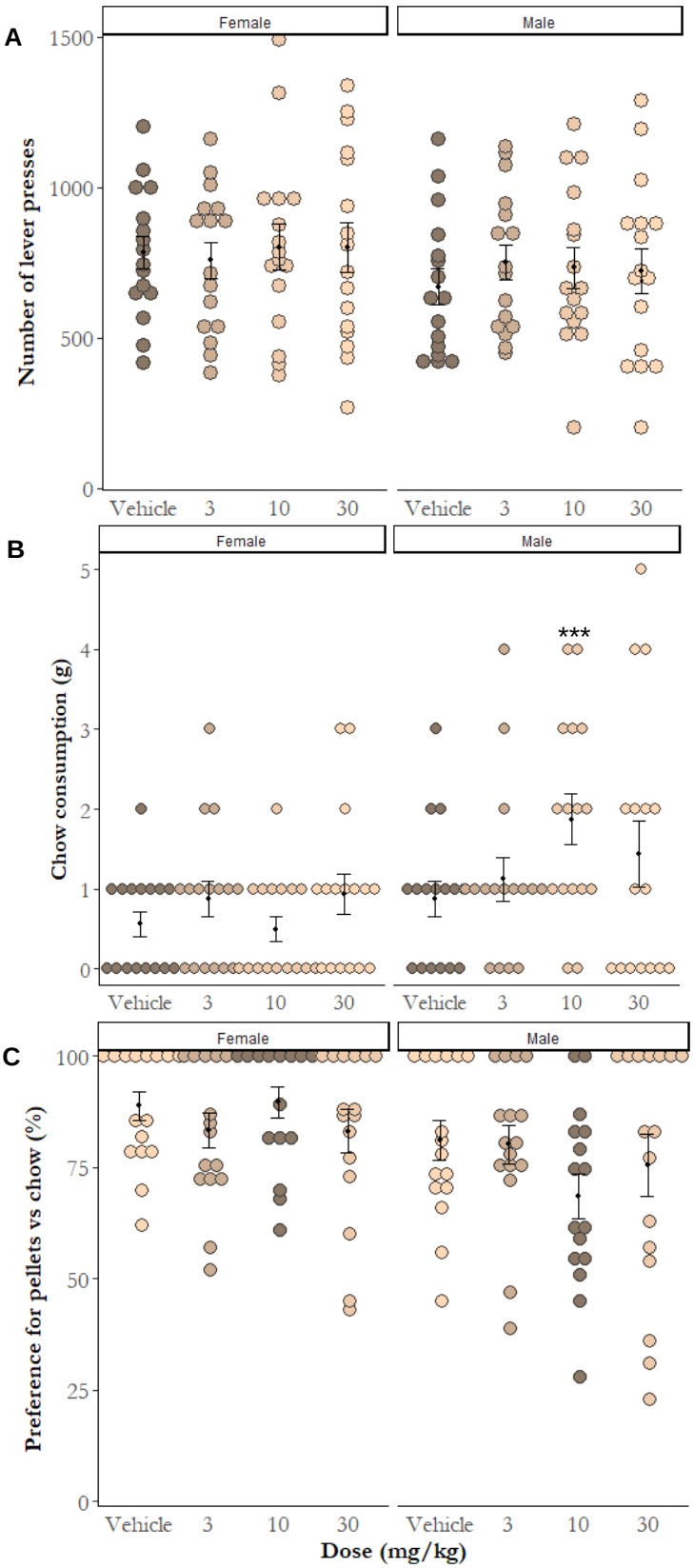


Figure 5.6. Effects of acute CORT treatment on effort-related choice in Experiment 13. CORT had no significant effect on lever presses (A). In male rats, 10mg/kg CORT significantly increased chow consumption compared to vehicle, with no other significant effects on chow consumption (B). CORT had no significant effect on preference for pellets vs chow (C).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars indicate mean ±SEM. Dots represent individual data points. *** = p <0.001 compared to vehicle.

Acute effects of IFN-α

Weight gain

Due to the required timescale for culling these animals, not all weights were analysed in the days post-IFN-α treatment. For this reason, only the comparison between the available weights post-vehicle and post-1000IU/kg IFN-α treatment were statistically analysed, but all available data are presented graphically here (Figure 5.7).

A repeated measures ANOVA found there was no significant effect of IFN-α treatment on percentage increase in body weight, $F_{1, 14} = 0.11$, MSE = 2.19, $p = 0.75$, $\eta_p^2 = 0.008$, with no interaction between dose * sex, $F_{1, 14} = 0.007$, MSE = 2.19, $p = 0.94$, $\eta_p^2 < 0.001$. There was no significant overall effect of sex on body weight, $F_{1, 14} = 0.94$, MSE = 0.39, $p = 0.35$, $\eta_p^2 = 0.063$.

A Bayesian repeated measures ANOVA found anecdotal evidence to support the lack of effect of IFN-α on body weight, $BF_{01} = 2.79$, as well as to support the overall lack of difference between males and females, $BF_{01} = 2.43$. Moderate evidence was found to support the lack of interaction between dose * sex, $BF_{01} = 3.89$.

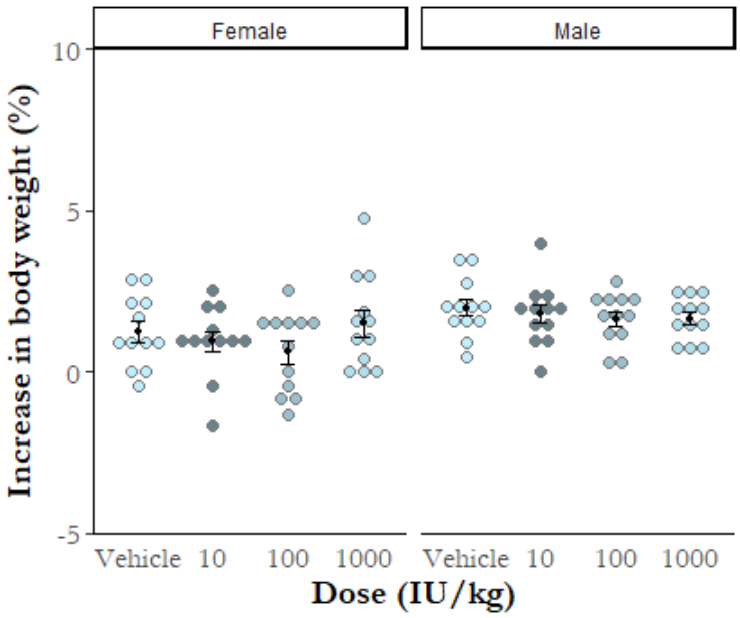


Figure 5.7. Percentage increase in body weight following acute IFN-α treatment. There was no significant change in weight gain following acute IFN-α treatment.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars indicate mean ±SEM. Dots represent individual data points.

Effort-related choice

Findings from the effort-related choice task showed no difference between control and IFN-α treated rats on lever presses, with only anecdotal evidence to support this, regardless of sex and/or dose. There was also no effect of IFN-α treatment on chow consumption nor preference for pellets vs chow, with moderate evidence to support this.

A repeated measures ANOVA showed no significant effect of IFN-α dose on lever presses, $F_{3, 90} = 2.43$, MSE = 25209.92, $p = 0.071$, $\eta_p^2 = 0.075$, with no significant interaction between dose * sex, $F_{3, 90} = 0.66$, MSE = 25209.92, $p = 0.58$, $\eta_p^2 = 0.021$ (Figure 5.8A). There was no significant effect of sex, $F_{1, 30} = 1.17$, MSE = 222532.51, $p = 0.29$, $\eta_p^2 = 0.037$.

A Bayesian repeated measures ANOVA found only anecdotal evidence to support the lack of overall effect of IFN-α dose on lever presses, BF_{01}= 1.51, and the lack of overall sex effect, $BF_{01} = 1.55$. There was moderate evidence to support the lack of interaction between dose * sex, $BF_{01} = 8.32$.

When looking at chow consumption, a repeated measures ANOVA found no significant effect of dose, $F_{3, 90} = 0.99$, MSE = 0.46, $p = 0.39$, $\eta_p^2 = 0.032$, with no significant interaction between dose * sex, $F_{3, 90} = 0.45$, MSE = 0.46, $p = 0.72$, $\eta_p^2 = 0.015$ (Figure 5.8B). There was a significant effect of sex, $F_{1, 30} = 12.42$, MSE = 2.91, $p = 0.001$, $\eta_p^2 = 0.29$, with males consuming more than females.

Shapiro-Wilks test for normality found that chow consumption in females was not normally distributed after treatment with vehicle, W(16) = 0.73, p <0.001, 10IU/kg IFN-α, W(16) = 0.75, p <0.001, 100IU/kg IFN-α, W(16) = 0.73, p <0.001, nor 1000IU/kg IFN-α, W(16) = 0.77, $p = 0.001$. Chow consumption in males was also not normally distributed after treatment with vehicle, W(16) = 0.85, $p = 0.012$, nor 100IU/kg IFN-α, W(16) = 0.89, $p = 0.047$. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian repeated measures ANOVA found moderate evidence to support the lack of overall effect of IFN-α dose on chow consumption, $BF_{01} = 7.69$. Strong evidence was found to support the lack of interaction between dose * sex, $BF_{01} = 22$.

Furthermore, there was no significant effect of IFN-α dose on preference ratio for pellets vs chow, $F_{3, 90} = 1.69$, MSE = 0.013, $p = 0.17$, $\eta_p^2 = 0.054$, with no significant interaction between dose * sex, $F_{3, 90} = 0.53$, MSE = 0.013, $p = 0.66$, $\eta_p^2 = 0.017$ (Figure 5.8C). There was a significant overall effect of sex, $F_{1, 30} = 11.10$, MSE = 0.081, $p = 0.002$, $\eta_p^2 = 0.27$, where females had a greater preference overall than males.

Shapiro-Wilks test for normality found that percentage preference in females was not normally distributed after treatment with vehicle, $W(16) = 0.76$, $p < 0.001$, 10IU/kg IFN-α, $W(16) = 0.76$, $p < 0.001$, 100IU/kg IFN-α, $W(16) = 0.77$, $p = 0.001$, nor 1000IU/kg IFN-α, $W(16) = 0.81$, $p = 0.004$. Percentage preference in males was also not normally distributed after treatment with 100IU/kg IFN-α, $W(16) = 0.88$, $p = 0.036$. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian repeated measures ANOVA provided moderate evidence to support the lack of overall effect of IFN-α dose on preference ratios, $BF_{01} = 3.25$. Moderate evidence was found to support the lack of interaction between dose * sex, $BF_{01} = 9.35$.

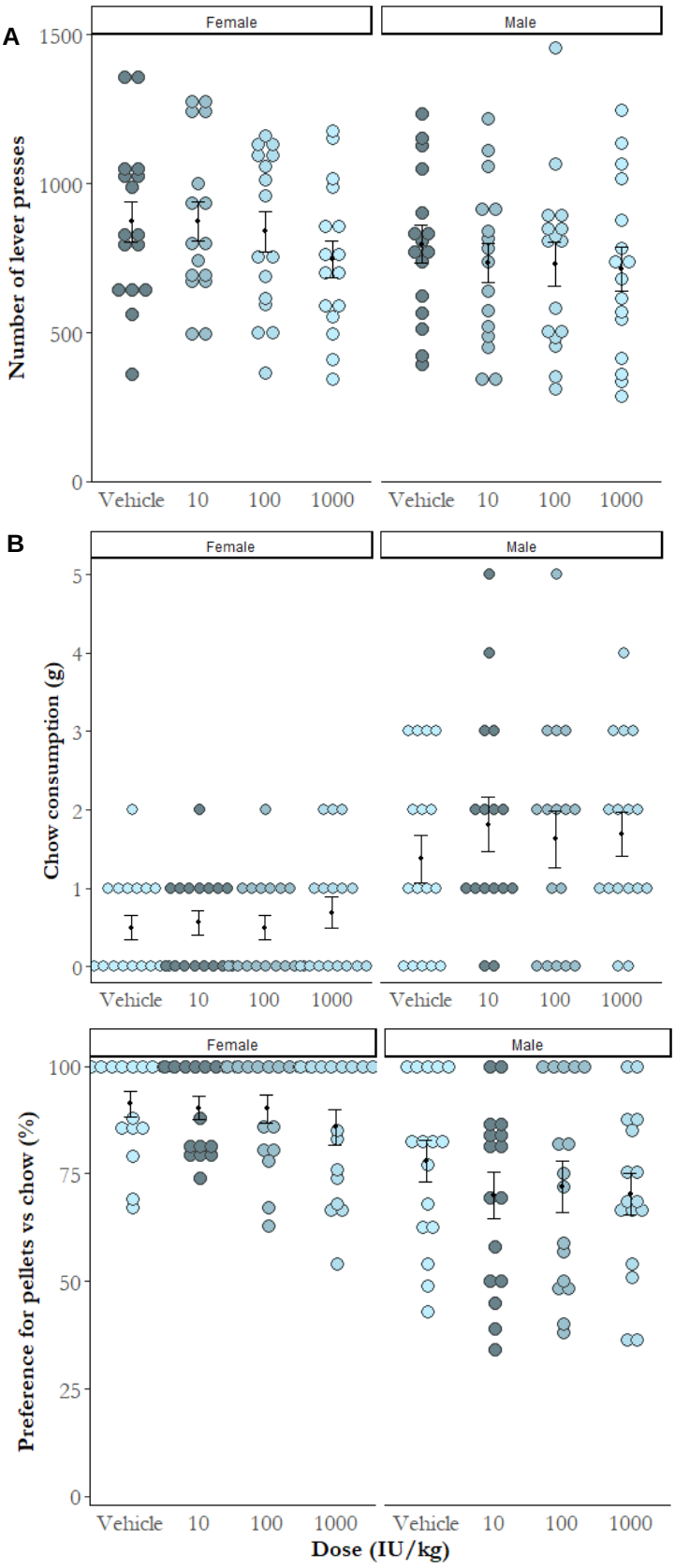


Figure 5.8. Effects of acute IFN-α treatment on effort-related choice in Experiment 13.
IFN-α had no significant effect on lever presses (A), chow consumption (B), or preference for reward pellets vs chow (C).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side. Bars indicate mean ±SEM. Dots represent individual data points.

5.4.3. Summary

Experiment 13 aimed to investigate motivational deficits following acute CORT and IFN-α treatment, with haloperidol used as a positive control.

Firstly, haloperidol induced a clear reduction in lever presses during the FR8 vs chow choice tests. Alongside this, there was an increase in chow consumption during this choice test, indicating that the reduction in lever presses was not due to haloperidol reducing overall hunger, but rather a reduced willingness to produce effort for a reward gain, which matches to previous findings (Salamone et al., 1991) and therefore serves as a positive control for this assay.

CORT treatment had a dose-dependent effect on weight gain, indicating CORT had been incorporated into the system as expected. Findings from the effort-related choice test showed that acute CORT treatment did not influence lever presses, but chow consumption was increased specifically in male rats in a dose-dependent manner. These results suggest that willingness to produce effort for a reward is not impaired by acute CORT treatment, but general hunger may be increased. It is possible that since females consumed much less chow than males in general, a CORT-induced change in this consumption may be too small to be detected in this task.

IFN-α treatment did not appear to influence either lever presses or chow consumption, although Bayes analysis deemed the lack of effect on lever presses to be inconclusive as a numerical trend appeared to show a possible dose-dependent reduction.

5.5. Experiment 14 – Evaluating the heterozygous *CACNA1C* deletion model in effort-related choice

Experiment 14 aimed to examine whether the CACNA1C$^{+/-}$ model showed a deficit in motivation for reward, and whether this deficit was consistent across males and females. Based on the association between the disorders related to *CACNA1C* variants and motivational deficits, CACNA1C$^{+/-}$ rats were expected to have reduced lever presses for the higher valued reward and increased consumption of the lower valued free chow, indicating reduced motivation for reward.

5.5.1. Experimental design

CACNA1C$^{+/-}$ rats described in section 1.3.3. were bred from an outsourced colony at Charles River UK. Automated genotyping of these rats was completed by TransnetYX, Memphis, USA.

For Experiment 14, 39 male rats (WT *n* = 30, CACNA1C$^{+/-}$ *n* = 9) weighing 190g – 400g and 31 female rats (WT *n* = 24, CACNA1C$^{+/-}$ *n* = 7) weighing 150g – 257g on *ad libitum* food varying from approx. 7 – 13 weeks of age were used. Note, there was a low sample size of CACNA1C$^{+/-}$ mutants available from this cohort due to an error made by the technician during genotyping (see section 3.6.3).

Once all rats had been trained to respond stably on the FR8 schedule, they received nine days of FR8 vs free chow preference testing followed by 2 additional FR8 only (no chow) sessions. The mean number of lever presses and chow intake during FR8 vs chow choice tests were calculated from all nine days for statistical analysis. The mean number of lever presses during FR8 only sessions (without chow present) were also calculated from the final 4 training days plus the 2 additional days post-choice tests.

5.5.2. Results

Effects of genotype and/or sex on FR8 lever presses without chow present

During training sessions, there was no difference between wild type and CACNA1C$^{+/-}$ rats on lever presses, with only anecdotal evidence to support this. Lever presses in these genotypes was also not influenced by sex, however, Bayes analysis indicated the evidence for this favoured the alternate hypothesis. Thus, post-hoc analysis was conducted and demonstrated greater lever presses in male CACNA1C$^{+/-}$ rats compared to male wild type rats.

A univariate ANOVA found no significant main effect of genotype on lever presses (Figure 5.9), $F_{1, 64} = 3.96$, MSE = 44394.35, $p = 0.051$, $n_p^2 = 0.058$. There was a significant main effect of sex, $F_{1, 64} = 1.26$, MSE = 44394.35, $p < 0.001$, $n_p^2 = 0.45$, with males overall responding with greater lever presses compared to females. There was no interaction between genotype * sex, $F_{1, 64} = 1.62$, MSE = 44394.35, $p = 0.27$, $n_p^2 = 0.019$.

A Bayesian univariate ANOVA found only anecdotal evidence to support the overall lack of genotype effect on lever presses, $BF_{01} = 1.52$. Anecdotal evidence was found in the direction of support for a significant interaction between genotype * sex, $BF_{10} = 1.32$. Considering this result from Bayes analysis, post-hoc Fisher's LSD analysis was conducted on this interaction. This showed significantly greater lever presses in male CACNA1C$^{+/-}$ rats compared to male wild type rats ($p = 0.026$).

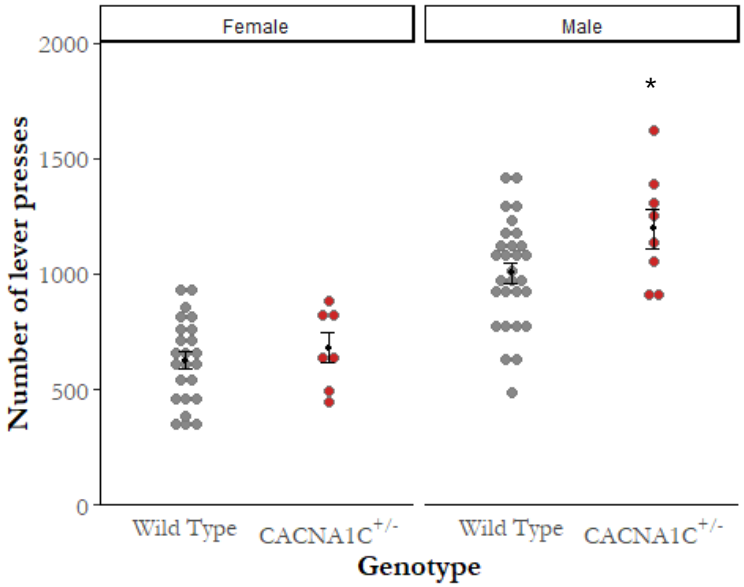


Figure 5.9. Average lever presses during FR8 only (without chow) training sessions in Experiment 14.
No overall significant differences between genotypes were found in lever presses, nor an interaction between genotype and sex, however males made greater lever presses overall than females.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent mean ±SEM. Dots indicate individual data points. * = p <0.05 compared to wild type.

Effects of genotype and/or sex on effort-related choice

During the effort-related choice task, there was no overall difference in lever presses between CACNA1C$^{+/-}$ and wild type rats, regardless of sex. However, again Bayes analysis found only anecdotal evidence to support the lack of overall difference and found evidence in support of the alternate hypothesis for a lack of sex influencing a genotype difference. Thus, post-hoc analysis showed that male CACNA1C$^{+/-}$ rats made significantly greater lever presses compared to wild type rats. There was no difference between the genotypes in chow consumption nor preference for pellets vs chow.

A univariate ANOVA comparing the mean lever presses across choice test days found no significant main effect of genotype (Figure 5.10A), $F_{1, 64}$ = 2.49, MSE = 62607.4, p = 0.12, n_p^2 = 0.038. There was a significant main effect of sex, $F_{1, 64}$ = 22.76, MSE = 62607.4, p <0.001, n_p^2 = 0.26, with males making a greater number of lever presses overall compared to females. There was no significant interaction between genotype * sex, $F_{1, 64}$ = 1.95, MSE = 62607.4, p = 0.17, n_p^2 = 0.03.

A Bayesian univariate ANOVA found only anecdotal evidence for a lack of overall genotype effect on lever presses, BF_{01} = 1.55. Anecdotal evidence was also found in the direction of support for a significant interaction between genotype * sex, BF_{10} = 1.21. Considering this result from Bayes analysis, post-hoc Fisher's LSD analysis was conducted on this interaction. This showed male CACNA1C$^{+/-}$ rats made significantly greater lever presses than wild type males (p = 0.032).

There was also no significant main effect of genotype on mean chow consumption during choice tests (Figure 5.10B), $F_{1, 64}$ = 0.008, MSE = 0.60, p = 0.93, n_p^2 <0.001. There was a significant main effect of sex, $F_{1, 64}$ = 35.64, MSE = 0.60, p <0.001, n_p^2 = 0.36, with males consuming more chow than females, with no interaction between genotype * sex, $F_{1, 64}$ = 0.65, MSE = 0.60, p = 0.42, n_p^2 = 0.01.

Shapiro-Wilks test for normality found that chow consumption was not normally distributed in female CACNA1C$^{+/-}$ rats, W(7) = 0.75, p = 0.014, female WT rats, W(24) = 0.83, p <0.001, nor male CACNA1C$^{+/-}$ rats, W(9) = 0.79, p = 0.017. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian univariate ANOVA found moderate evidence to support the lack of overall genotype effect on chow consumption, BF_{01} = 3.44, with only anecdotal evidence to support the lack of interaction between genotype * sex, BF_{01} = 1.99.

No significant main effect of genotype was found in a univariate ANOVA comparing mean preference for pellets vs chow during choice tests (Figure 5.10C), $F_{1, 64}$ = 0.051, MSE = 0.023, p = 0.82, n_p^2 = 0.001. There was a significant main effect of sex, $F_{1, 64}$ = 8.67, MSE = 0.023, p = 0.005, n_p^2 = 0.12, where overall females had a greater preference for pellets vs chow than males. There was no significant interaction between genotype * sex, $F_{1, 64}$ = 1.46, MSE = 0.023, p = 0.23, n_p^2 = 0.022.

Shapiro-Wilks test for normality found that percentage preference was not normally distributed in female CACNA1C$^{+/-}$ rats, W(7) = 0.76, p = 0.017, female WT rats, W(24) = 0.79, p <0.001, nor male CACNA1C$^{+/-}$ rats, W(9) = 0.77, p = 0.008. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian univariate ANOVA found moderate evidence to support the lack of overall genotype effect on preference for pellets vs chow, BF_{01} = 3.32, with only anecdotal evidence to support the lack of interaction, BF_{01} = 1.42.

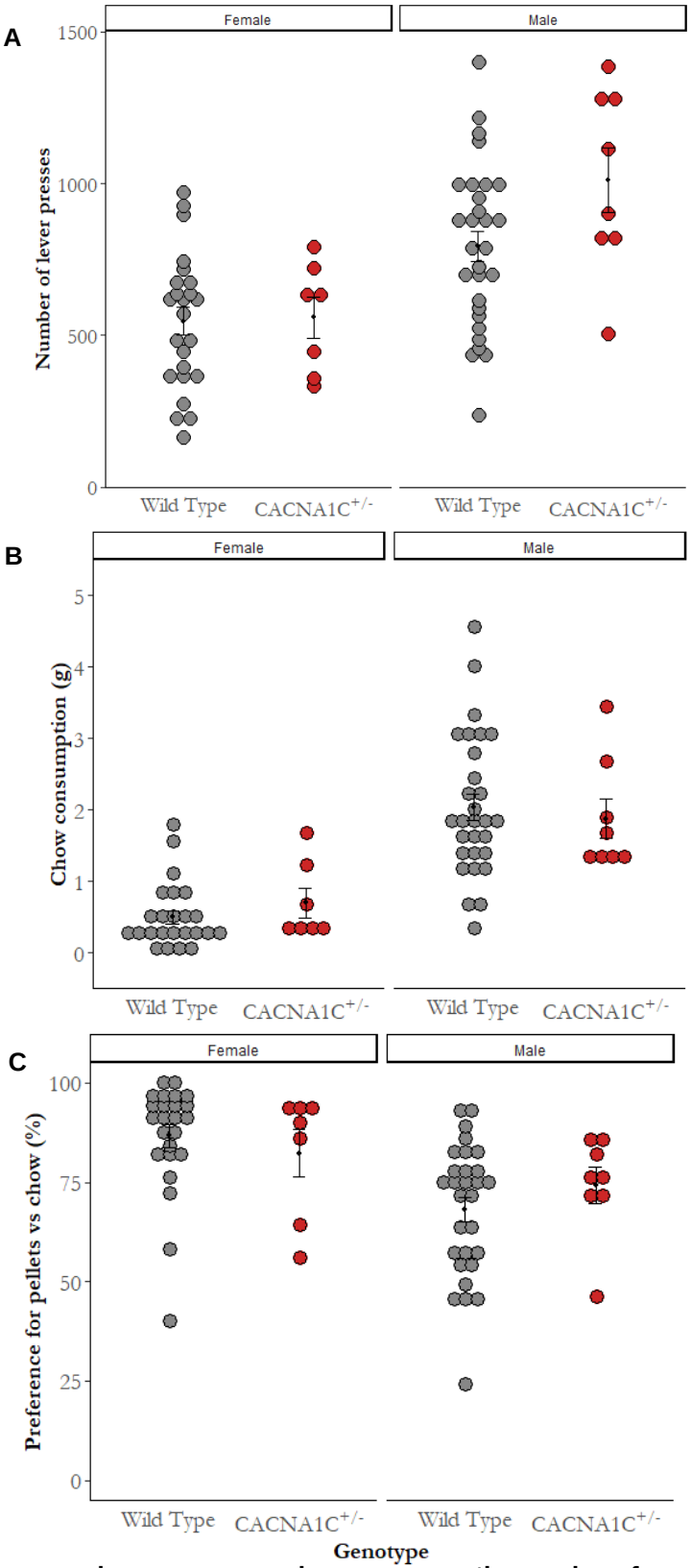


Figure 5.10. Average lever presses, chow consumption and preference for pellets vs chow in the effort-related choice task of Experiment 14.
There were no significant effects of genotype or interaction between genotype and sex on lever presses (A), chow consumption (B) or preference for pellets vs chow (C). Males made greater lever presses, had increased chow intake, and lower preference compared to females.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Bars represent mean ±SEM. Dots indicate individual data points.

5.5.3. Summary

Experiment 14 explored the effects of heterozygous *CACNA1C* deletion on effort-related decision making in the EfR task.

This experiment found no overall effects of genotype, regardless of sex, on lever presses on an FR8 schedule without the presence of chow. However, Bayes analysis indicated limited support in direction of an interaction between genotype and sex. Looking further into this interaction, and at the numerical values of the results, showed greater lever presses for male CACNA1C$^{+/-}$ rats than wild type.

Examining the choice test data showed a similar pattern of results where male CACNA1C$^{+/-}$ rats had numerically higher lever presses for the reward pellets compared to male wild type rats, yet this was also not significant in the overall analysis. There was no overall effect of genotype on chow consumption or calculated percentage preference for pellets vs chow.

The lack of overall significant findings means these results must be interpreted with caution, and a greater sample size would likely resolve this uncertainty, but these findings potentially indicate an elevated willingness to produce effort for high value reward in these transgenic models, with no effect on general consumption.

However, looking at wild type rats where the sample size was much greater, there was a lot of variability between individual animals in both lever presses and the amount of chow consumed, despite the mean across several test days being calculated. This possibly suggests the responding behaviour of individual animals is too varied to make reliable conclusions from a between-subjects design such as this.

5.6. Discussion of Chapter 5

In Chapter 5, the effort-related choice task was applied to the three preclinical models of psychiatric disease assessed in previous chapters of this thesis to elucidate the influence of risk factors stress, immune response, and genetic variation on motivation for reward.

5.6.1. Effects of CORT on motivation for reward

The chronic CORT model was initially examined in Experiment 12 and compared against a 'combined control' group where half were given vehicle treatment to match the CORT regimen, and the other half were given saline to match the IFN-α regimen. Findings from this experiment did not show a clear effect of chronic CORT treatment on lever press responses for the higher valued reward pellets or chow consumption, nor overall preference for the pellets. However, all groups (including control) reduced chow consumption post-treatment and thus had a greater preference for pellets vs chow compared to pre-treatment. This difference pre vs post-treatment was lower in chronic CORT treated rats than controls, which could indicate a deficit in these CORT-treated rats, though no between-group differences were identified. Given the issues identified with the initial reward pellets used for operant training pre-treatment, it is possible that this interfered with the animals learning the lever press-reward association and thus influenced their overall consumption and potentially the ability to detect changes in lever presses.

Since I conducted this experiment, Dieterich et al. (2020) published a study in which mice given 35µg/mL CORT in drinking water for 4 weeks were assessed in the EfR, and showed these mice had reduced lever presses for the reward pellets with no change in general chow consumption. However, in this experiment treatment was continued throughout the behaviour schedule for a total of 15 weeks, and responses were only analysed as a between-subjects design post-treatment, thus the analysis and dosing regimen was different to that followed for Experiment 12. Nevertheless, this study indicates that the EfR task should be sensitive enough to detect differences in effort-related choice following chronic CORT administration. Along with evidence of reduced responding in PR tasks, and the potential indication of a deficit in this experiment, this indicates further exploration of the chronic CORT model, possibly following the subcutaneous injection method for more reliable drug efficacy (see Chapter 4), may reveal similar deficits, which could allow a full characterisation of the reward processing deficits in this model.

In addition to chronic CORT, the effects of acute CORT administration were also examined in Experiment 13 using only the 5TUL pellets identified as most preferred. Surprisingly, acute CORT did not appear to influence lever presses, but elevated chow consumption in males in a dose-dependent manner. This finding suggests that willingness to work for a

higher valued reward was not impaired by acute CORT, but general appetite may be increased. This could further explain the potential deficit seen in Experiment 12, with chronic CORT-treated rats showing less of a reduction in chow consumption post-treatment vs pre-treatment than the control rats, but also supports no change in lever presses. In addition, these rats in Experiment 13 showed a dose-dependent reduction in percentage weight gain following drug administration that could increase hunger, and subsequent consumption of the easy access chow. This follows previous literature in PR tasks briefly mentioned in section 1.4.2 suggesting that stress may enhance general appetite rather than motivation (Koob, 2008).

5.6.2. Effects of IFN-α on motivation for reward

Chronic IFN-α treatment was also examined in Experiment 12, which showed no difference between control and treated rats on lever presses, chow consumption, or preference for pellets vs chow. However, as above, chow consumption reduced post-treatment for all groups possibly due to interference from the use of less rewarding pellets during training. Compared to controls, this reduction in chow consumption was much smaller in chronic IFN-α treated rats, and this group did not show the increase in preference for pellets vs chow seen in the other groups. This could indicate that chronic IFN-α treatment caused a deficit in the willingness to produce effort for reward, but the lack of between-group differences and interference from the unfavourable pellets during training could underlie the lack of firm conclusions here.

The acute effects of IFN-α were assessed in Experiment 13, which also found no overall effect of treatment on lever presses or chow consumption. However, Bayes analysis deemed the lack of effect on lever presses to be inconclusive, and further examination of the numerical values shows a potential dose-dependent reduction, but this cannot be accurately inferred without statistical significance.

As discussed in section 4.6.2, other literature using the IFN-α model tend to administer a higher dose that can induce sickness behaviours, whereas the doses used in this experiment were likely too low to do so. Draper et al. (2018) found that sickness behaviours (plus elevation of IL-6 and TNF-α) following LPS treatment in humans was associated with reduced ‘acceptance’ of high effort choices in a self-report task, suggesting perhaps deficits in motivation may be dependent upon the presence of sickness behaviours. However, the evidence in this area is limited. A future study examining higher doses of acute IFN-α in the EfR task, along with monitoring of sickness behaviours, may reveal whether the lack of effect in this experiment was due to this low dose used or indeed there is no deficit induced by IFN-α.

5.6.3. Effects of *CACNA1C* risk variant on motivation for reward

The effects of heterozygous deletion of the *CACNA1C* gene were examined in the EfR task in Experiment 14. This experiment found no overall effect of this genetic mutation on lever presses, chow consumption or preference for pellets. Further exploration of the interaction between genotype and sex revealed a significant increase in lever presses for male CACNA1C$^{+/-}$ rats compared to male wild type rats, however, this was despite a non-significant overall interaction result and therefore should be interpreted cautiously. The sample size of transgenic rats for this experiment was very low, thus a future study with a more powerful sample may be able to reveal whether this difference was indeed present.

Elevated lever presses in the EfR task can indicate greater motivation for reward or general appetite, however, since there was stronger evidence to suggest no effect on the consumption of chow then the former interpretation would be more likely. Whilst this change in behaviour may not fit with the hypothesis that the *CACNA1C* mutation would result in impaired motivation for reward, it instead suggests increased motivation for reward. However, as mentioned in section 5.1, lever presses alone could be indicative of other processes such as habit formation or impulse control issues. Given the limited characterisation of this model, or the *CACNA1C* risk variant itself, this finding still provides important implications for the role of *CACNA1C* reward processing and provides a basis for future exploration. However, from visualisation of these data from wild type rats, the lever presses made by individual animals can be very variable and therefore a between-subjects design such as this may be limited in its sensitivity to detect deficits.

5.6.4. Conclusion

Chapter 5 provides findings from experiments that apply the EfR task to measure motivation for reward in three preclinical models of psychiatric disease. These findings suggest CORT may influence general appetite rather than motivational responses, whilst IFN-α at low doses has no influence on motivation or general appetite. The transgenic CACNA1C$^{+/-}$ model showed indication of enhanced motivation for reward, but this evidence was limited and only during training sessions without chow present. Future research would expand upon this initial suggestion of a CACNA1C influence on motivation, and the impact of IFN-α at higher doses.

Chapter 6 – Neurogenic and biochemical changes in putative models of psychiatric disease

6.1. Introduction

Chapter 6 aimed to identify changes in various biological markers in the chronic corticosterone (CORT) and chronic interferon-alpha (IFN-α) models of depression, and potentially relate these with their behavioural characterisation in Chapters 3, 4 and 5.

As discussed in section 1.2, there are several hypotheses for the pathophysiology of psychiatric disease. One such hypothesis is that a reduction in neurotrophic factors, leading to neuronal atrophy and impaired neurogenesis, could play a role in the development of major depressive disorder (MDD) (Duman & Li, 2012). Whilst much evidence has implicated a role of neurogenesis in cognitive deficits (e.g. Apple, Fonseca, & Kokovay, 2017), some evidence has also suggested that deficits in neurogenesis can lead to impaired hedonic response to reward (e.g. Snyder, Soumier, Brewer, Pickel, & Cameron, 2011) and motivation for low value rewards (e.g. Karlsson, Wang, Sonti, & Cameron, 2018). Thus, markers for neurogenic changes were examined in these two models of MDD to determine whether any behavioural changes associated with these models could also be related to diminished neurogenesis.

Previous research has demonstrated a reduction in the number and density of Doublecortin (DCX)-positive neurons, a marker of immature neurons, in both the dorsal and ventral hippocampus following chronic CORT treatment (e.g. Diniz et al., 2013; e.g. Haas, Wang, Saffar, Mooney-Leber, & Brummelte, 2020; Jiang, Xu, Zou, Yang, & Wang, 2013). Similarly, a reduction in the expression of Ki67-positive neurons, a marker of cells in the proliferative stage of neurogenesis, has been demonstrated in chronic CORT treated rodents (e.g. Agasse et al., 2020; Brummelte & Galea, 2010). Thus, these previous findings have shown reduced expression of the neurogenic markers, DCX and Ki67, in the chronic CORT model, which indicates a potential deficit in the formation of new neurons in this model of MDD. However, most of these studies use subcutaneous implants of CORT-releasing pellets or injections. Limited literature has reported neurogenic deficits following CORT in drinking water, with those reporting this showing variable results. Thus, to determine whether the specific dose and administration route of CORT used in these animals also influenced changes in neurogenesis, it was important to replicate this.

In mice chronically treated with IFN-α, DCX and Ki67-positive neurons have been shown to be reduced in the hippocampus (Zheng et al., 2014), varying depending upon dosage. *In vitro*, direct application of IFN-α over 10 days to human hippocampal progenitor cells

significantly reduced the number of DCX-positive cells expressed (Borsini et al., 2018), and recently Kaneko, Nakamura, and Sawamoto (2020) found reduced DCX expression in the hippocampus of marmosets following 4 weeks of IFN-α treatment. Another study demonstrated a reduction in an alternative marker of cell proliferation, BrdU, in the hippocampus of chronic IFN-α treated rats (Kaneko et al., 2006).

In contrast, Borsini et al. (2019) administered serum extracted from hepatitis C patients to human hippocampal progenitor cells *in vitro* and found the number of DCX-positive neurons increased after patients received 4 weeks of IFN-α treatment compared to baseline, but that this increase was smaller in those who later developed depression, with no change in Ki67. Thus, literature reporting changes in neurogenic markers following IFN-α treatment has been limited in rodent models, and shows variable findings in human hippocampal cell cultures, but this evidence generally implicates reduced neurogenic markers could be associated with IFN-α induced depression.

Another hypothesis for the pathophysiology of MDD postulates that symptoms may arise from dysregulation of the glutamatergic neurotransmitter system. There are several subunits of NMDA receptors, in which NR1, NR2A and NR2B are considered the predominant subunits of NMDA receptors in neurons (Rauner & Köhr, 2011), and are recognised as the major subunits involved in inducing long-term potentiation (LTP) in the hippocampus, a process underpinning synaptic plasticity and learning and memory (Paoletti, Bellone, & Zhou, 2013). Genetic studies have identified hypermethylation of NR2A in the prefrontal cortex (PFC) in MDD patients, a process resulting in repressed gene expression (Kaut et al., 2015), as well as polymorphisms in the NR2B gene associated with treatment resistant MDD (Zhang et al., 2014).

In addition, AMPA receptors are involved in NMDAR-dependent synaptic plasticity, whereby NMDA receptor activation allows the influx of Ca^{2+} into the neurons, which in turn upregulated AMPA receptor expression in the synaptic membrane – an essential process in memory formation (Henley & Wilkinson, 2013). GluA1, encoded by the *GRIA1* gene, is the best characterised subunit and is suggested to act independently in both LTP and long-term depression (LTD), which conversely reduces synaptic plasticity (Lee, Takamiya, He, Song, & Huganir, 2010). Genome-wide association studies have identified a significant association with a single nucleotide polymorphism located upstream of *GRIA1* and schizophrenia (Ripke et al., 2013), whilst evidence shows transgenic mice lacking GluA1 have reduced hedonic response to reward measured by lick cluster analysis (LCA), demonstrating potential involvement of the glutamatergic system in hedonic processing (Austen et al., 2017; Strickland et al., 2021).

Thus, the expression of NR1, NR2A, NR2B, and GluA1, were examined in these two models of MDD to investigate which, if any, reward processing deficits may result from alterations in glutamatergic receptors. Furthermore, the synaptic scaffolding protein PSD-95 may control AMPA receptor regulation during synaptic plasticity (Ehrlich & Malinow, 2004), and stabilise NMDA receptors (Won, Incontro, Nicoll, & Roche, 2016). Thus, any changes in NMDA or AMPA receptor subunits could result from overall PSD-95 changes, so the expression of this protein was also analysed.

Previous studies have found no change in these NMDA receptor subunits in the frontal cortex or dorsal hippocampus following chronic CORT treatment (e.g. Buret & van den Buuse, 2014), whereas other studies demonstrate an increase in hippocampal NR1 and NR2A expression 1-2 hours following acute CORT exposure (e.g. Tse, Bagot, Hutter, Wong, & Wong, 2011). In contrast, Cohen et al. (2011) found reduced NR1 and PSD-95 expression in the whole brain of mice chronically treated with CORT compared to controls, whilst Gourley et al. (2009) found reduced NR2B expression in the medial PFC (mPFC) with no change in GluA1 expression following chronic CORT treatment in rats. In contrast, Li, Xie, et al. (2017) found GluA1 expression in the hippocampus was significantly upregulated following chronic CORT treatment in rats. Thus, evidence of chronic CORT effects on these major glutamatergic receptor subunits has been variable and may change with dosage or brain regions analysed.

The literature on glutamatergic receptor changes in the chronic IFN-α model is sparse, with no studies to my knowledge that measure changes in the expression of NR1, NR2A, or NR2B in this model. Although one study appears to show elevated PSD-95 expression, with no significant change in GluA1, in the hippocampus of mice chronically treated with IFN-α (Sanchez-Mendoza et al., 2020). Therefore, I aimed to characterise changes in the expression of these proteins in the hippocampus and PFC of the chronic CORT and IFN-α models used throughout this thesis to match biological changes more accurately with potential behavioural deficits.

In addition, interactions between the stress and inflammation pathways have been suggested previously, such that one may influence the other to contribute to depression development (Maydych, 2019). Whilst several pro-inflammatory cytokines exist, there are three major cytokines commonly associated with MDD and that are elevated following exposure to stress (Hodes, Kana, Menard, Merad, & Russo, 2015). Polymorphisms in the IL-1β and TNF-α genes have been directly associated with MDD (Lezheiko et al., 2019), whilst a polymorphism in IL-6 was associated with the development of IFN-α induced depressive symptoms (Shadrina, Bondarenko, & Slominsky, 2018). To investigate the stress-inflammation interactions in the models used throughout this thesis, and the

association between cytokine expression and reward-related deficits, analysis of blood plasma was undertaken to measure circulating CORT levels in the chronic IFN-α model, and levels of pro-inflammatory cytokines IL-1β, TNF-α and IL-6 in both models.

Chronic mild stress (CMS) has been shown to elevate plasma TNF-α and IL-1β in rats and mice (e.g. Grippo, Francis, Beltz, Felder, & Johnson, 2005; Liu et al., 2014; Liu et al., 2015). To my knowledge, there is no literature investigating the direct effects of chronic CORT on these cytokines in plasma, however, TNF-α and IL-1β mRNA expression in the hippocampus was shown to be unaffected by chronic CORT, whereas hippocampal IL-6 mRNA expression was reduced (Kelly, Miller, Bowyer, & O'Callaghan, 2012). In contrast, Donner, Montoya, Lukkes, and Lowry (2012) found that chronic CORT treatment had no effect on plasma IL-6 concentration.

Rats treated with chronic IFN-α showed elevated serum CORT, IL-1β, and IL-6 (Callaghan et al., 2018), however, acute IFN-α treatment in mice increased plasma CORT but only elevated TNF-α and IL-6 following exposure to an additional stressor (Anisman, Poulter, Gandhi, Merali, & Hayley, 2007).

Previous literature has demonstrated inconsistent or limited findings on the effects of chronic CORT or IFN-α treatment on various markers of neurogenesis, glutamatergic neurotransmission, and immunological response, which may result from variability in drug dosage and treatment protocols. To understand the neurobiological underpinnings leading to the development of reward-related deficits in psychiatric disease, it is important to identify relationships between changes in these markers and these deficits as measured through sensitive behavioural assays.

In addition, microglia are activated in response to elevated cytokines, including IFN-α (e.g. Colton, Yao, Keri, & Gilbert, 1992; Wachholz et al., 2016). Mice treated with IFN-α have shown altered microglia morphology in the mPFC and hippocampus (Aw, Zhang, & Carroll, 2020) as well as general increased expression of microglia in the mPFC and dorsal and ventral hippocampus (Li, Huang, et al., 2017). To provide a positive control for IFN-α treatment, the expression of microglia in these same brain regions was analysed in the chronic IFN-α model here.

Thus, in Chapter 6, chronic CORT and IFN-α treated rats previously characterised behaviourally will be examined for changes in the expression of neurogenic markers in the dorsal and ventral hippocampus, glutamatergic receptor subunits in the whole hippocampus and PFC, cytokines and CORT in plasma. Chronic CORT-treated rats previously showing a hedonic deficit with LCA, although with potentially inconclusive evidence (section 4.3.1) were examined for changes in the expression of neurogenic markers and glutamatergic

receptor subunits, whilst those previously not demonstrating a hedonic deficit nor deficit in reward-induced positive bias in the flavour modified affective bias test (m-ABT, sections 3.4 and 4.3.2) had plasma cytokines measured. Chronic IFN-α treated rats shown not to have a hedonic deficit in LCA (section 4.4.1) were examined for changed in expression of glutamatergic receptor subunits, whilst the cohort demonstrating both a lack of hedonic deficit and lack of reward-induced positive bias in the flavour m-ABT (sections 3.5 and 4.4.2) were analysed for microglial activation in the amygdala, dorsal and ventral hippocampus, neurogenic markers, and plasma cytokines / CORT production.

6.2. General methods

6.2.1. Immunohistochemistry

6.2.1.1. Sample preparation

All rats were terminally anaesthetised with sodium pentobarbital (Euthatal, Merial, UK) and brains were removed from the skull. The left hemisphere was fixed in 4% paraformaldehyde (PFA) for 24 hours before transferring to 25% sucrose in 0.1M phosphate-buffered saline (PBS) for at least 24 hours prior to slicing. The right hemisphere was used for protein extraction and Western blotting (see 6.2.2).

6.2.1.2. Procedure

Post-fixed brains were cut coronal at 40µm thickness into a 1:5 series using a freezing microtome, and stored in cryo-protectant at -20˚C. For each stain, a single series was placed into one 15mm Netwell insert (Corning, Sigma-Aldrich) within a 12-well plate containing phosphate buffered saline (PBS). See Appendix C for optimisation protocols and immunohistochemistry solution details.

Sections were washed three times in PBS before quenching for 10 minutes in 3% H_2O_2, 10% methanol and distilled water. Sections were washed three more times in PBS-T (PBS with 0.2% Triton X-100) and then blocked in 3% normal serum in PBS-T for 1 hour. The sections were then incubated overnight at room temperature (RT) in the primary antibody solution containing PBS-T and 1% serum (see Table 6.1 for antibody concentrations).

Table 6.1. List of primary antibodies used for immunohistochemistry.

Primary Antibody	Target	Concentration	Species Raised in	Blocking Serum	Manufacturer
Ki-67	Proliferating cells	1:1000	Rabbit	Goat	Abcam, Ab15580
Doublecortin (DCX)	Immature neurons	1:1000	Guinea Pig	Goat	Sigma-Aldrich, AB2253
Iba1	Microglia	1:1000	Rabbit	Goat	Abcam, Ab178846

The following day, sections were washed in PBS-T three times before incubation in a biotinylated secondary antibody in 1% normal serum and PBS-T for 3 hours (see Table 6.2 for antibody details). Sections were then washed in PBS-T three times and incubated for a further 2 hours in an ABC kit (Vector Laboratories Ltd, Cambridgeshire, UK) containing 1% normal serum.

Table 6.2. List of secondary antibodies used for immunohistochemistry.

Secondary Antibody	Concentration	Manufacturer
Biotinylated Goat Anti-Rabbit IgG	1:1000	Vector Laboratories, BA1000.
Goat Anti-Guinea pig IgG H&L (Biotin) Pre-adsorbed	1:1000	Abcam, ab7138.

Sections were washed three times in PBS-T followed by two washes in TRIS Non-Saline (TNS) before leaving to develop in 3,3'-diaminobenzidine (DAB, Vector Laboratories Ltd) with Nickel. To stop the reaction, sections were washed in PBS, and then mounted on to subbed 1% gelatinised slides (Thermo Scientific, Menzel Gläser). Slides air-dried for 48 hours, or 24 hours in an incubator set to 37°C, before dehydration in increasing concentrations of industrial methylated spirit (IMS, 70%, 90% and 100%) followed by clearing in 100% xylene. Finally, slides were cover-slipped using distyrene plasticizer and xylene (DPX, Thermo Scientific, Raymond Lamb, Leicestershire, UK) and allowed to dry before analysis.

6.2.1.3. Cell counts and analysis

DCX and Ki67 in the hippocampus

DCX and Ki67 were analysed in the chronic CORT/control group behaviourally characterised in Experiment 8 (section 4.3.1), as well as the chronic IFN-α/control group characterised in both Experiment 5 (section 3.5) and 11 (section 4.4.2). See Appendix A for experimental trajectories.

Images were first taken at 1.6x magnification using CellSens Dimension software (Olympus, US) on a brightfield microscope (Leica DMRB). Each section was assigned a region based on their position anteroposterior (AP) to Bregma, matching to those defined by the Paxinos and Watson rat brain atlas (Paxinos & Watson, 2007). Sections between -1.8mm to -5.2mm below Bregma were considered as dorsal hippocampus, while those between -5.2mm to -6.7mm below Bregma were considered as ventral, as described by Kott, Mooney-Leber, Shoubah, and Brummelte (2016).

Once each section was assigned a region level, samples were matched to the four most common shared regions in the dorsal hippocampus (outlined in the relevant results sections), which would be used for further analysis. Damaged or missing sections were excluded from analysis. If enough shared regions were also available in the ventral hippocampus, these would also be analysed separately to dorsal sections.

Using ImageJ software (Schneider, Rasband, & Eliceiri, 2012), the measurement scale was first calibrated to the 500µm marker on one image and then the area of the granular cell layer of the dentate gyrus (GrDG) was calculated for each of the four sections per sample by drawing around the region of interest. Images were then taken of the GrDG in the selected sections at 20x magnification and stitched together to form one large 20x magnified image. ImageJ was used to count immuno-positive cells manually, then for each section, the total number of immuno-positive cells counted were divided by the GrDG area to give

the number of cells per mm^2. The mean number of cells per mm^2 per region were then calculate first for each sample, then group means were calculated.

Iba1 stained sections

Iba1 was analysed in the chronic IFN-α/control group behaviourally characterised in both Experiment 5 (section 3.5) and 11 (section 4.4.2).

As above, images were first taken at 1.6x magnification using CellSens Dimension software (Olympus, US) on a brightfield microscope. Each section was assigned a region based on their position anteroposterior (AP) to Bregma, matching to those defined by the Paxinos and Watson rat brain atlas (Paxinos & Watson, 2007).

For each sample, three images per section were then taken at 20x magnification of various regions depending on the area of interest. Following the methodology used by Li, Huang, et al. (2017), images were taken of sections that fall between -1.92mm to -2.4mm from Bregma for amygdala analysis; between -3.6mm to -4.08mm for dorsal hippocampus analysis; and between -5.28mm to -6.00mm for ventral hippocampus analysis.

Using ImageJ (Schneider et al., 2012), the measurement scale was first calibrated to the 100µm marker on one image and then the area of interest was calculated for each section by drawing around the region of interest. Immuno-positive cells were manually counted and for each section, the total number of immuno-positive cells were divided by the area to give the density of cells per mm^2. The mean number of cells per mm^2 per region were then calculate first for each sample, then group means were calculated.

6.2.1.4. Data analysis

Where indicated, an independent samples t-test was used in SPSS (v23, IBM) to compare 'group' when only one between-subjects variable was used. A univariate ANOVA was also used to compare 'group' and 'sex' where applicable. Bonferroni corrections for multiple comparisons were applied after initial analysis for any significant results for DCX and Ki67 since these analytes can be grouped into neurogenic markers in the hippocampus. As Iba1 is a marker of microglia rather than neuronal and was analysed in a different manner to the other markers, it was not classed as the same group for a correction to be applied.

Tests for normal distribution were conducted as described in section 2.2.1.5 and Bayesian analyses were also conducted, where indicated, as described in section 2.2.1.6.

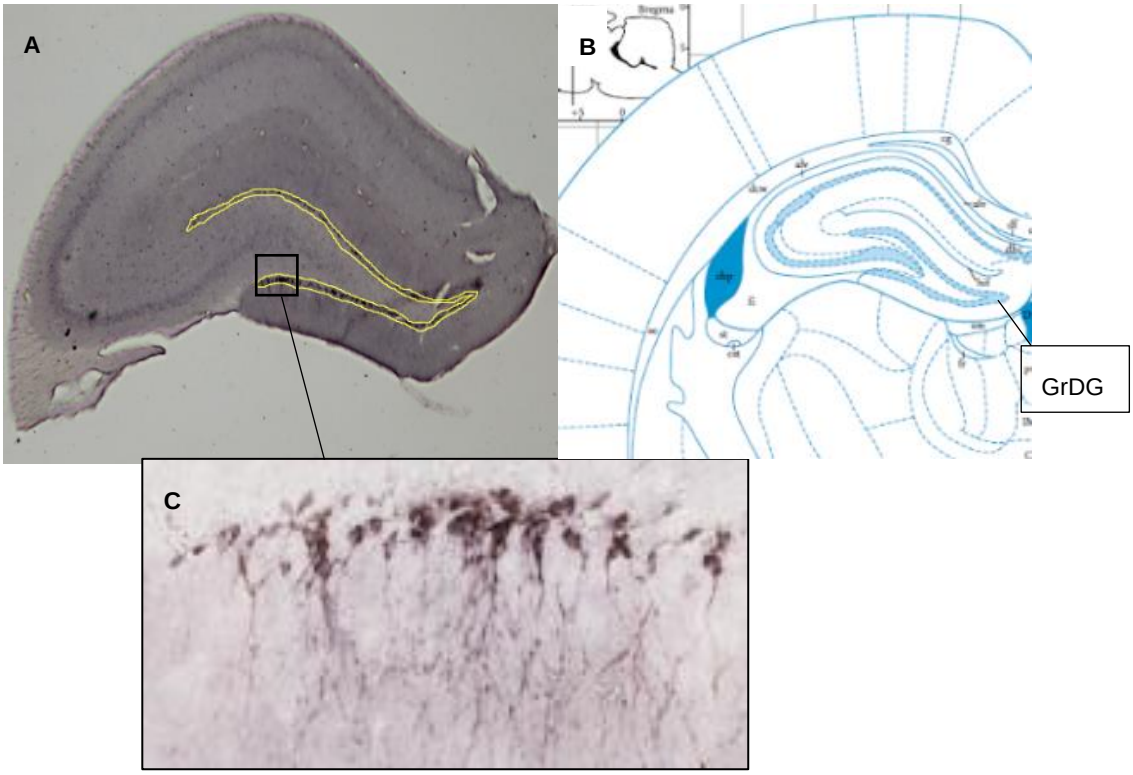


Figure 6.1. Example dorsal section of the hippocampus stained for doublecortin (DCX) illustrating how the region of interest, granular cell layer of the dentate gyrus (GrDG), was outlined at 1.6x magnification (A) to calculate area, using the Paxinos and Watson atlas (B) as a reference. DCX^+ neurons were then counted at 20x magnification (C).

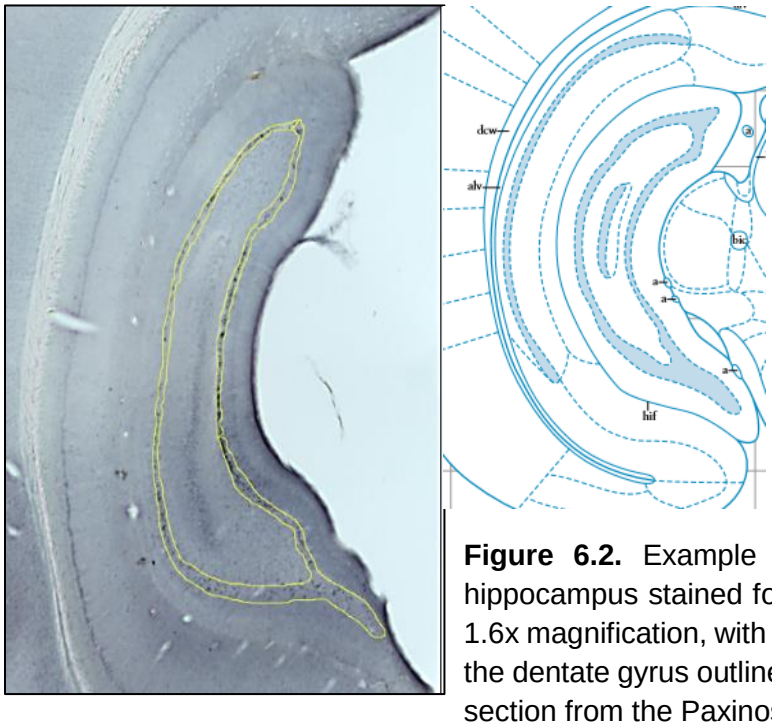


Figure 6.2. Example ventral section of the hippocampus stained for doublecortin (DCX) at 1.6x magnification, with the granular cell layer of the dentate gyrus outlined (A) and the reference section from the Paxinos and Watson atlas (B).

6.2.2. Western blotting

6.2.2.1. Tissue preparation

All rats were terminally anaesthetised with sodium pentobarbital (Euthatal, Merial, UK) and their brains were removed from the skull. The right hemisphere was dissected similarly to the protocol described by Chiu, Lau, Lau, and Chang (2007), with some adaptations: Firstly, the olfactory bulb and cerebellum were removed and the two hemispheres were separated using a scalpel. To extract the hippocampus using forceps, the selected hemisphere was placed on its lateral side, so the diencephalon and mesencephalon could be gently removed from the cerebral cortex. The hippocampus could be identified beneath, as it is a paler, grey colour compared to surrounding tissue. Forceps were hooked underneath the ventral portion of the hippocampus to roll it slowly free from the cortex. Finally, the frontal cortex, defined as the remaining area anterior to the corpus callosum, was dissected from the tissue. Hippocampal and frontal cortex samples were snap frozen in liquid nitrogen and stored at -80˚C.

6.2.2.2. Synaptosomal protein extraction

The proteins of interest consisted of receptor subunits, which lay within the synaptosome of the neurons, therefore a synaptosomal protein extraction was conducted. Dissected samples were first homogenised in Syn-PER reagent (Thermo Scientific, UK) with 1% protease inhibitor cocktail (539134, Merck-Millipore, UK) and 2% phosphatase inhibitor cocktail (524629, Merck-Millipore, UK) at 10µl/mg of wet tissue weight. Homogenised samples were centrifuged at 1200 x *g* for 10 minutes at 4˚C. The resulting homogenate was then separated and centrifuged at 15000 x *g* for 25 minutes at 4˚C. The cytosolic supernatant was carefully transferred to a separate Eppendorf, and the remaining synaptosomal pellet was re-suspended in 1.5µl/mg Syn-PER reagent to be used throughout the remainder of the protocol.

6.2.2.3. Bicinchonic Acid (BCA) protein assay

To determine the protein concentration of the synaptosomal fraction, a BCA assay kit (ThermoScientific, UK) was used. Serial dilutions of the bovine serum albumin (BSA) standards were prepared ranging from 0.008–2mg/ml. On a 96 well plate, 25µl of these standards and a negative blank control (distilled H_2O, dH_2O) were loaded along with 1µl of the samples and sample blank controls (Syn-PER). All standards, samples and blanks were loaded in duplicate. 200µl of BCA Working Reagent (50 parts Reagent A:1 part Reagent B), was added to all wells. The plate was mixed for 15 seconds on a plate shaker followed by incubation at 37˚C for 30 minutes. Using a spectrophotometer, each plate was read at

an absorbance of 540nm, and the protein concentrations of each sample was calculated from the standard curve generated by the BSA standards using Microsoft Excel.

6.2.2.4. Western blot assay

All Western blot assays were conducted in the chronic CORT/control group behaviourally characterised in Experiment 8 (section 4.3.1), as well as the chronic IFN-α/control group characterised in Experiment 10 (section 4.4.1). See Appendix A for experimental trajectories.

Extracted samples were diluted to 3:1 with 3x sample buffer, heated at 90°C for 40 minutes then frozen at -20°C for storage. Prior to first use, samples were thawed and heated at 70°C for ten minutes before loading, and for any subsequent use, they were heated at 70°C for five minutes.

10% polyacrylamide gels were prepared fresh and loaded with 8µL molecular weight marker (Precision Plus Protein Standards marker, Bio-rad Laboratories, Hercules, California, USA), 10µg of each sample and 10µL 1x Sample Buffer. For each experiment, one control sample was used as a standard and loaded into each gel at 10µg. Samples were separated via sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) in 1x tank buffer. Separated proteins were electrophoretically transferred from gels to 0.45µm Nitrocellulose membranes (GE Healthcare, Amersham, UK) before washing once quickly in Tris-buffered saline with Tween 20 (TBS-T).

Membranes were then blocked for 1 hour in 5% milk blotto at RT, before being cut horizontally at approximately 60kDa to separate sections into a 'top' and 'bottom' membrane. The bottom section was incubated in conjugated β-actin (Sigma-Aldrich, A3854; 1:15000) whilst the top section was incubated in the chosen primary antibody solution (see table 6.3) with 1% milk blotto in TBS-T on a roller either overnight at 4°C or for 1.5 hours at RT.

Table 6.3. List of primary antibodies used in Western blot analyses.

Primary antibody	Molecular weight	Concentration	Manufacturer
NMDAR1	120kDa	1:500	BD Biosciences, 556308
NR2A	170kDa	1:1000	Sigma-Aldrich, 07-632
NR2B	166 – 180 kDa	1:1000	Abcam, Ab65783
GluA1	100kDa	1:2000	Abcam, Ab31232
PSD95	80kDa	1:1000	Abcam, Ab18258

Next, 'top' membranes were washed in TBS-T three times for 5 minutes and incubated in 1% milk blotto containing the matching secondary antibody conjugated to horseradish peroxidase (HRP, see table 6.4) for 1 hour at RT. All membranes were then washed as above, and immuno-positive bands were visualised using enhanced chemiluminescence

with the ECL substrate (Thermo Scientific, UK) and exposed to high performance chemiluminescent X-ray film (GE Healthcare, Amersham, UK) images were taken on a GBox Chemi-XX6 imaging system (Syngene, UK). For β-Actin staining of the 'bottom' membrane, after incubation with the primary antibody the membrane went directly to washes followed by ECL visualisation and imaging.

Table 6.4. List of secondary antibodies used in Western blot analyses.

Secondary Antibody	Concentration	Manufacturer
Anti-Mouse, HRP-linked	1:1000	Vector Labs, PI2000
Anti-Rabbit IgG, HRP-linked	1:2000	Cell Signalling Technology, 7074S

6.2.2.5. Data analysis

Western blot images were inverted and analysed using ImageJ software. Plots of each lane were generated, and the area of these plots were used to determine the density of luminescence from each sample. This density was first normalised to the matching β-Actin level for each sample, then each sample within a gel was normalised to the 'standard' sample for that gel.

Normalised optical densities for individual proteins were compared between the fixed factor 'group' using independent samples t-tests in SPSS (v23, IBM). Where indicated, a univariate ANOVA was used to compare 'group' and 'time' as fixed factors separately for individual proteins. Bonferroni corrections for multiple comparisons were applied after initial analysis for any significant results to appropriate groups of markers based on their receptor-type and brain region analysed, for example NMDAR-related receptors (NMDAR1, NR2A, NR2B) within the hippocampus or AMPAR-related receptors (GluA1) in the frontal cortex.

Tests for normal distribution were conducted as described in section 2.2.1.5 and Bayesian analyses were also conducted, where indicated, as described in section 2.2.1.6.

6.2.3. Enzyme-linked immunosorbent assays (ELISA)

6.2.3.1. Sample preparation

All rats were terminally anaesthetised with sodium pentobarbital (Euthatal, Merial, UK) before undergoing decapitation and trunk blood was collected in 4ml heparin-coated tubes (BD367883, Fisher Scientific, UK). Collected blood immediately underwent centrifugation at 2600 x *g* for 10 minutes at RT, and then the supernatant plasma was collected and stored at -20°C until analysis, following manufacturer instructions.

6.2.3.2. Procedure and analysis

All ELISAs were performed according to manufacturer's instructions (see Table 6.5), but protocols are described in brief below. One strip from each kit was used as an optimisation strip to determine any required sample dilutions.

<u>IL-1β, IL-6 and TNF-α</u>

Plasma cytokines IL-1β, IL-6, and TNF-α were analysed in the chronic CORT/control group behaviourally characterised in both Experiment 4 (section 3.4) and 9 (section 4.3.2), as well as the chronic IFN-α/control group characterised in both Experiment 5 (section 3.5) and 11 (section 4.4.2). See Appendix A for experimental trajectories.

For the analysis of circulating cytokines, the following sandwich ELISA kits were purchased from R&D Systems, Abingdon, UK: Rat IL-1beta Quantikine® ELISA kit (RLB00); Rat IL-6 Quantikine® ELISA kit (R6000B); and Rat TNF-alpha Quantikine® ELISA kit (RTA00).

An initial optimisation strip showed neat plasma was the most appropriate sample dilution state for all kits. First, 50µL assay diluent was added to each well of a provided 96-well microplate pre-coated with a polyclonal antibody specific to the cytokine target. Then, 50µL of each standard, internal control or neat sample was added separately to wells, in duplicate, covered with adhesive strip and incubated for 2 hours at RT.

Following incubation, each well was aspirated and filled with 400µL wash buffer for five washes. After the final wash, the plate was inverted and blotted against clean paper towel to remove any remaining buffer. 100µL of polyclonal antibody against the target cytokine conjugated to HRP was then added to each well, covered and incubated for a further 2 hours at RT.

Then, washes were repeated as before and 100µL Substrate Solution was added to each well, the microplate was covered with aluminium foil to protect from light and incubated for 30 minutes at RT. After incubation, 100µL of Stop Solution was added to each well and

gently mixed on a microplate shaker before the optical density of the wells were analysed using a microplate reader set at a wavelength of 450nm.

Standard curves were calculated from the standards of known concentration provided within individual kits using Prism (v8, GraphPad). The concentration of unknown samples was then interpolated from the standard curve.

Corticosterone

Plasma CORT was analysed in the chronic CORT/control group behaviourally characterised in both Experiment 4 (section 3.4) and 9 (section 4.3.2), as well as the chronic IFN-α/control group characterised in both Experiment 5 (section 3.5) and 11 (section 4.4.2). See Appendix A for experimental trajectories.

Plasma CORT levels were analysed using a competitive ELISA kit (Abcam, ab108821). An initial optimisation strip showed a dilution of 1:50 fit within the standard curve most accurately, so all samples were diluted 1:50 with Diluent S.

First, 25µL of each standard or diluted sample was added to a 96-well microplate in duplicate, followed by 25µL Biotinylated Corticosterone Protein to each well, which were gently mixed on a microplate shaker. This was covered and incubated for 2 hours at RT.

Following incubation, each well was aspirated and filled with 200µL Wash Buffer for five washes, before blotting on paper towel. 50µL of SP Conjugate was then added to each well and mixed, then incubated for 30 minutes, followed by five more washes. Finally, 50 µL of Chromagen Substrate was added to each well and incubated for 30 minutes before the addition of 50µL Stop Solution to each well. Immediately, the absorbance of each well was measured using a microplate reader set at a wavelength of 450nm.

Standard curves were calculated from the standards of known concentration provided within individual kits using Prism (v8, GraphPad). The concentration of unknown samples was then interpolated from the standard curve.

Table 6.5. Summary of ELISA kits used, including sample dilutions required as per optimisation and reference ranges for the internal control provided.

Target	Manufacturer	Sample dilution	Internal control
IL-1β	R&D Systems, RLB00	Neat	227 – 328pg/mL
IL-6	R&D Systems, R6000B	Neat	135 – 226pg/mL
TNF-α	R&D Systems, RTA00	Neat	107 – 178pg/mL
corticosterone	Abcam, ab108821	1:50	N/A

6.2.3.3. Data analysis

SPSS (v23, IBM) was used to compare circulating cytokines/hormones individually, with 'group' and 'sex' as fixed factors. Bonferroni correction was applied following initial analysis for any significant results in the analysis of cytokines.

Tests for normal distribution were conducted as described in section 2.2.1.5 and Bayesian analyses were also conducted, where indicated, described in section 2.2.1.6.

6.3. Chronic corticosterone model

The analyses conducted in section 6.3 aimed to establish whether chronic CORT treatment affected neurogenesis or the expression of glutamatergic receptors or plasma cytokines. Based on previous findings following chronic CORT treatment, it was expected that chronic CORT would reduce the expression of neurogenic markers, glutamatergic receptor subunits, and elevate plasma cytokine levels.

6.3.1. Experimental design

Two separate cohorts were used for analysis of neurobiological changes in the chronic CORT model. Drug treatment protocols are described in section 3.2.4. In brief, CORT was administered at a dose of 50µg/mL dissolved in 1% ethanol in drinking water for 12 days, followed by dose tapering.

Changes in neurogenesis and glutamatergic neurotransmission were analysed in the cohort of animals used in Experiment 8 (see 4.3.1), which were split into two time points following CORT treatment. For the analysis of neurogenic markers, only the group sacrificed 18 days post-CORT treatment were included due to a lack of viable tissue in the delayed group, therefore two groups were compared here. For the analysis of glutamatergic signalling, both time points were included and so four groups were compared. The ‘early’ groups refer to those sacrificed 18 days post-CORT treatment, and the ‘delayed’ group refers to those sacrificed 60 days post-CORT treatment.

Changes in circulating cytokines or CORT measured via ELISA were analysed in the cohort from both Experiment 4 (see 3.4) and 9 (see 4.3.3), consisting of males and females. Blood samples were extracted 25-27 days after the final CORT dose (see Appendix A for experimental trajectories).

For all analyses, sample sizes were dependent on extracted sample volume and viability during the assay. Exact sample sizes are reported for individual proteins/markers in the results section. In the analysis of circulating cytokines, some levels were below the threshold for detection and thus allocated a value of ‘0’. The internal control provided for cytokine kits was checked to ensure its value was within the expected reference range as quality control for the assay.

6.3.2. Results

For results on plasma CORT levels in the chronic CORT model, see section 3.4.2. In brief, there was no difference in plasma CORT between control or CORT-treated rats, and no main effect or interaction with sex.

6.3.2.1. Changes in neurogenesis in the chronic CORT model

Effects of chronic CORT treatment on cell proliferation and immature neurons in the hippocampus

For Ki67 analyses (control n = 11, CORT n = 12), Bregma regions -3.00, -3.6, -4.44, -4.8 were analysed and the mean was calculated for each rat. For DCX analyses (control n = 6, CORT n = 8), regions used were -3.36, -3.6, -4.44, and -4.68.

An independent samples t-test found no significant difference in the mean number of total $Ki67^+$ cells, $t(21) = 0.35$, $p = 0.73$, area of GrDG, $t(21) = 0.66$, $p = 0.52$, nor in the mean number of cells per mm^2, $t(21) = 0.036$, $p = 0.97$ (Figure 6.3A).

Similarly there were no significant differences in the number of DCX^+ cells, $t(12) = 2.005$, $p = 0.068$, area of GrDG, $t(12) = 1.18$, $p = 0.26$, nor DCX^+ cells per mm^2, $t(12) = 0.49$, $p = 0.64$ (Figure 6.3B).

Bayesian independent-samples t-tests found only anecdotal evidence to support the lack of difference between the two groups in total $Ki67^+$ cells, $BF_{01} = 2.53$, area of the GrDG, $BF_{01} = 2.09$, and number of $Ki67^+$ cells per mm^2, $BF_{01} = 2.64$.

Similarly, only anecdotal evidence was found to support the lack of difference between the groups in area of the GrDG, $BF_{01} = 1.49$, and DCX^+ cells per mm^2, $BF_{01} = 2.06$. A small amount of evidence was found to support the direction of a significant difference between total number of DCX^+ cells, but this was again only anecdotal, $BF_{10} = 1.48$.

These findings indicate that, in this cohort, there was no change in neurogenic markers of cell proliferation and immature neurons in chronic CORT-treated rats compared to controls, although Bayesian analysis indicates that this is inconclusive.

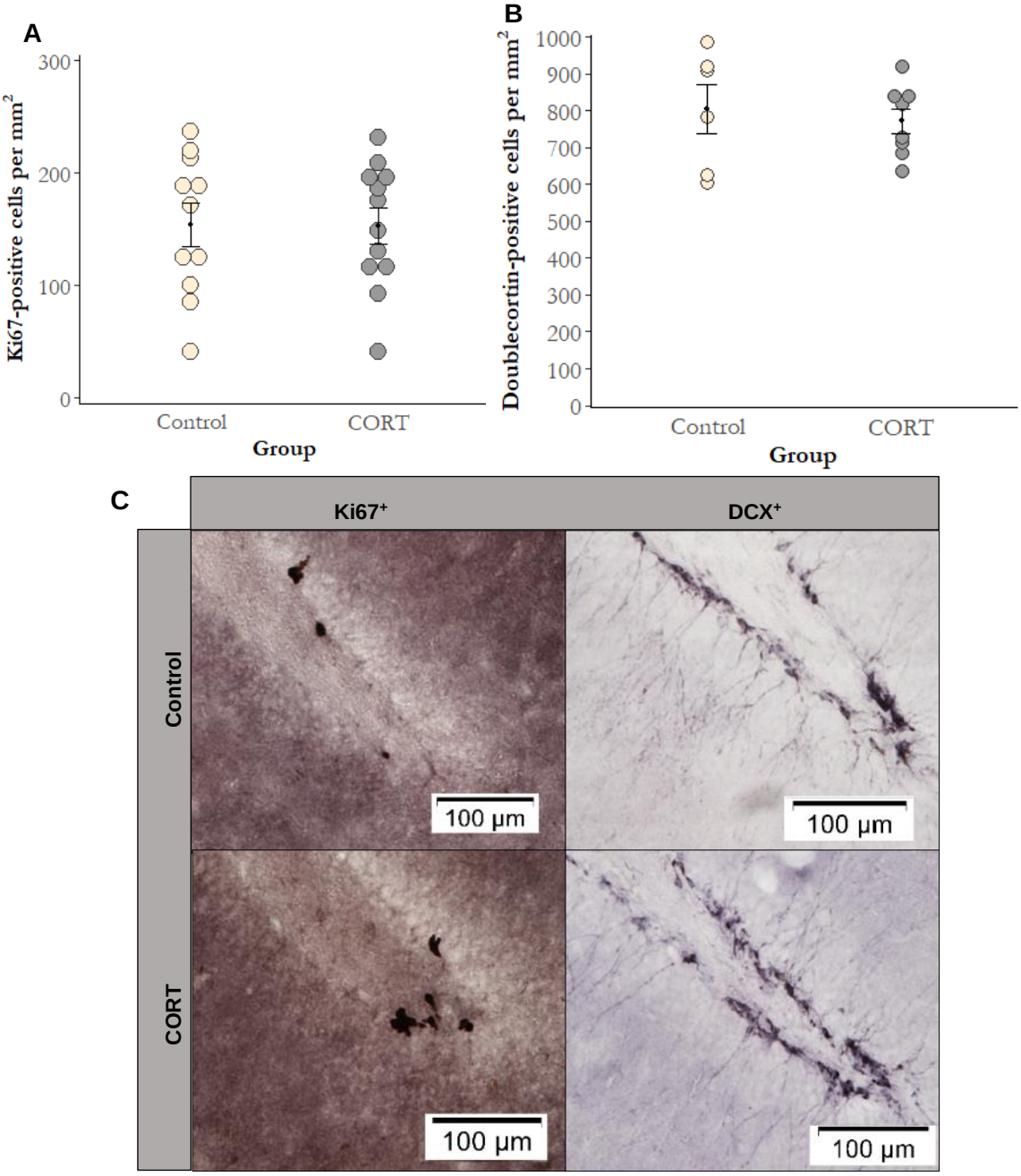


Figure 6.3. Effects of chronic CORT treatment on neurogenic markers. The number of Ki67$^+$ (A) cells per mm^2 and DCX$^+$ cells per mm^2 (B) in the dorsal hippocampus did not differ significantly between control and CORT-treated rats. Representative images of Ki67$^+$ and DCX$^+$ cells in the dorsal hippocampus are shown in panel C.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Dots indicate individual data points, with mean ±SEM.

6.3.2.2. Changes in glutamatergic neurotransmission in the chronic CORT model

Western blot analysis of glutamatergic/synaptic protein expression in the hippocampus

A univariate ANOVA comparing hippocampal NMDAR1 expression between rats taken early post-CORT treatment (vehicle *n*=11, CORT *n*=11) and delayed post-CORT treatment (vehicle *n*=12, CORT *n*=9) found no overall difference between control and CORT-treated animals (Figure 6.5A), $F_{1, 39} = 1.32$, MSE = 5.79, $p = 0.26$, $\eta_p^2 = 0.03$, nor between time points, $F_{1, 39} = 0.84$, MSE = 5.79, $p = 0.37$, $\eta_p^2 = 0.021$. There was also no interaction between group * time, $F_{1, 39} = 0.14$, MSE = 5.79, $p = 0.71$, $\eta_p^2 = 0.004$.

Shapiro-Wilks test for normality found that NMDAR1 expression was not normally distributed in the control group at the delayed time point, W(12) = 0.60, $p < 0.001$, nor in the CORT group at the delayed time point, W(9) = 0.77, $p = 0.01$. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian univariate ANOVAs found only anecdotal evidence to support the lack of overall effect of group, $BF_{01} = 2.03$, and time, $BF_{01} = 2.46$. Moderate evidence was found to support a lack of interaction between group * time, $BF_{01} = 6.37$.

A univariate ANOVA comparing GluA1 expression (early cohort: vehicle *n*=11, CORT *n*=11; delayed cohort: vehicle *n*=11, CORT *n*=9) showed no overall difference between groups (Figure 6.5B), $F_{1, 38} = 0.25$, MSE = 4.59, $p = 0.62$, $\eta_p^2 = 0.007$, nor between time points, $F_{1, 38} = 1.26$, MSE = 4.59, $p = 0.27$, $\eta_p^2 = 0.032$. A significant interaction was found between group * time, $F_{1, 38} = 5.01$, MSE = 4.59, $p = 0.031$, $\eta_p^2 = 0.12$. Post-hoc analysis of this interaction with Fisher's LSD comparison showed that there was a significant increase in GluA1 expression at the later time point compared to the earlier time point ($p = 0.019$), with no difference in the CORT-treated group ($p = 0.45$). There were no significant differences between vehicle and CORT-treated rats in either the early post-CORT treatment cohort ($p = 0.21$) or in the delayed post-CORT treatment cohort ($p = 0.067$).

Shapiro-Wilks test for normality found that GluA1 expression was not normally distributed in the control group at the early time point, W(11) = 0.71, $p < 0.001$. However, no suitable alternative statistical test was available for this type of analysis.

A univariate ANOVA found moderate evidence to support the lack of overall group effect, $BF_{01} = 3.07$, whilst only anecdotal evidence was found to support a lack of effect of time, $BF_{01} = 1.83$.

Analysis of NR2A expression (early cohort: vehicle *n*=11, CORT *n*=11; delayed cohort: vehicle *n*=10, CORT *n*=9) showed no overall difference between groups (Figure 6.5C), $F_{1, 37} = 0.01$, MSE = 0.49, $p = 0.92$, $\eta_p^2 < 0.001$, nor between time points, $F_{1, 37} = 0.69$,

MSE = 0.49, p = 0.41, η_p^2 = 0.02. There was no interaction between group * time, $F_{1, 37}$ = 0.26, MSE = 0.49, p = 0.61, η_p^2 = 0.007.

A Bayesian univariate ANOVA found only anecdotal evidence to support a lack of effect of time, BF_{01} = 2.47. Moderate evidence was found to support the lack of overall group effect, BF_{01} = 3.23, and lack of interaction between group * time, BF_{01} = 9.14.

NR2B expression (early cohort: vehicle n=11, CORT n=11; delayed cohort: vehicle n=12, CORT n=9) also did not differ overall between groups (Figure 6.5D), $F_{1, 39}$ = 0.17, MSE = 1.99, p = 0.68, η_p^2 = 0.004, nor between time points, $F_{1, 39}$ = 0.06, MSE = 1.99, p = 0.81, η_p^2 = 0.002. There was no interaction between group * time, $F_{1, 39}$ = 0.15, MSE = 1.99, p = 0.15, η_p^2 = 0.051.

Shapiro-Wilks test for normality found that NR2B expression was not normally distributed in the control group at the early time point, W(11) = 0.77, p = 0.004, the CORT group at the early time point, W(11) = 0.85, p = 0.036, nor the CORT group at the delayed time point, W(9) = 0.82, p = 0.036. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian univariate ANOVA found moderate evidence to support a lack of group effect, BF_{01} = 3.13, time, BF_{01} = 3.15, and interaction between group * time, BF_{01} = 5.05.

Finally, analysis of PSD-95 (early cohort: vehicle n=11, CORT n=11; delayed cohort: vehicle n=12, CORT n=9) showed no overall difference between the groups (Figure 6.5E), $F_{1, 39}$ = 0.22, MSE = 0.49, p = 0.64, η_p^2 = 0.006, nor between time points, $F_{1, 39}$ = 0.35, MSE = 0.49, p = 0.56, η_p^2 = 0.009. There was also no interaction between group * time, $F_{1, 39}$ = 2.07, MSE = 0.49, p = 0.16, η_p^2 = 0.05.

Only anecdotal evidence was found in a univariate ANOVA to support the lack of effect of time, BF_{01} = 2.69. Moderate evidence was found to support the overall lack of group effect, BF_{01} = 3.04, and lack of interaction between group * time, BF_{01} = 4.49.

These findings indicate no differences between control and CORT-treated rats in general expression of these receptor subunits in the hippocampus, and that this lack of difference did not change over time (see Figure 6.4 for Western blot images). However, this evidence of a lack of group effect was inconclusive for NMDAR1 expression. Evidence that GluA1 expression was not influenced by CORT-treatment over time was also inconclusive.

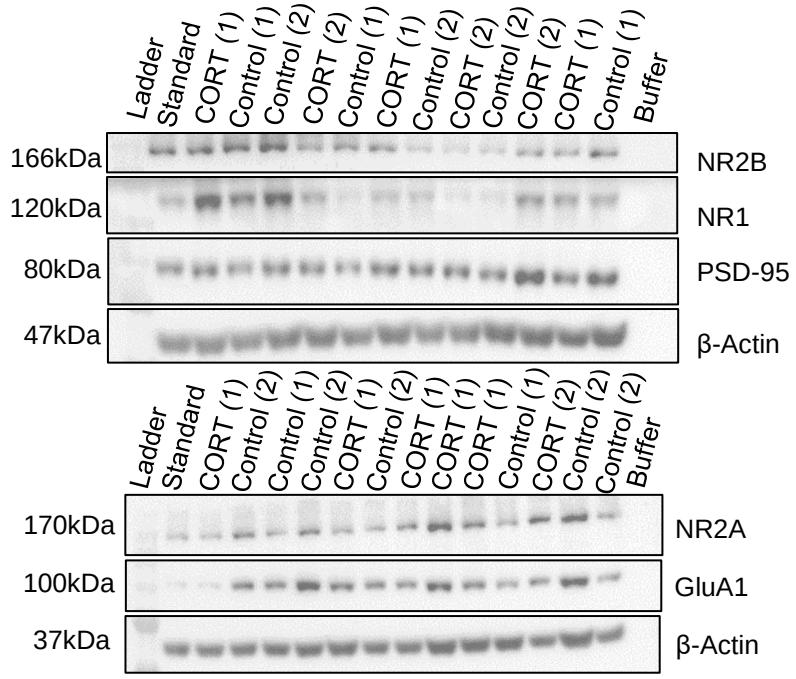


Figure 6.4. Western blot images of glutamatergic/synaptic receptor protein expression in the hippocampus. Images generated from two Western blot membranes demonstrating expression and molecular weights of NR1, NR2B, PSD-95 (A), and GluA1 and NR2A (B) against the reference protein β-Actin in control or CORT-treated rats analysed in cohort one, i.e. 'early' time point (1) or cohort two, i.e., 'delayed' time point (2).

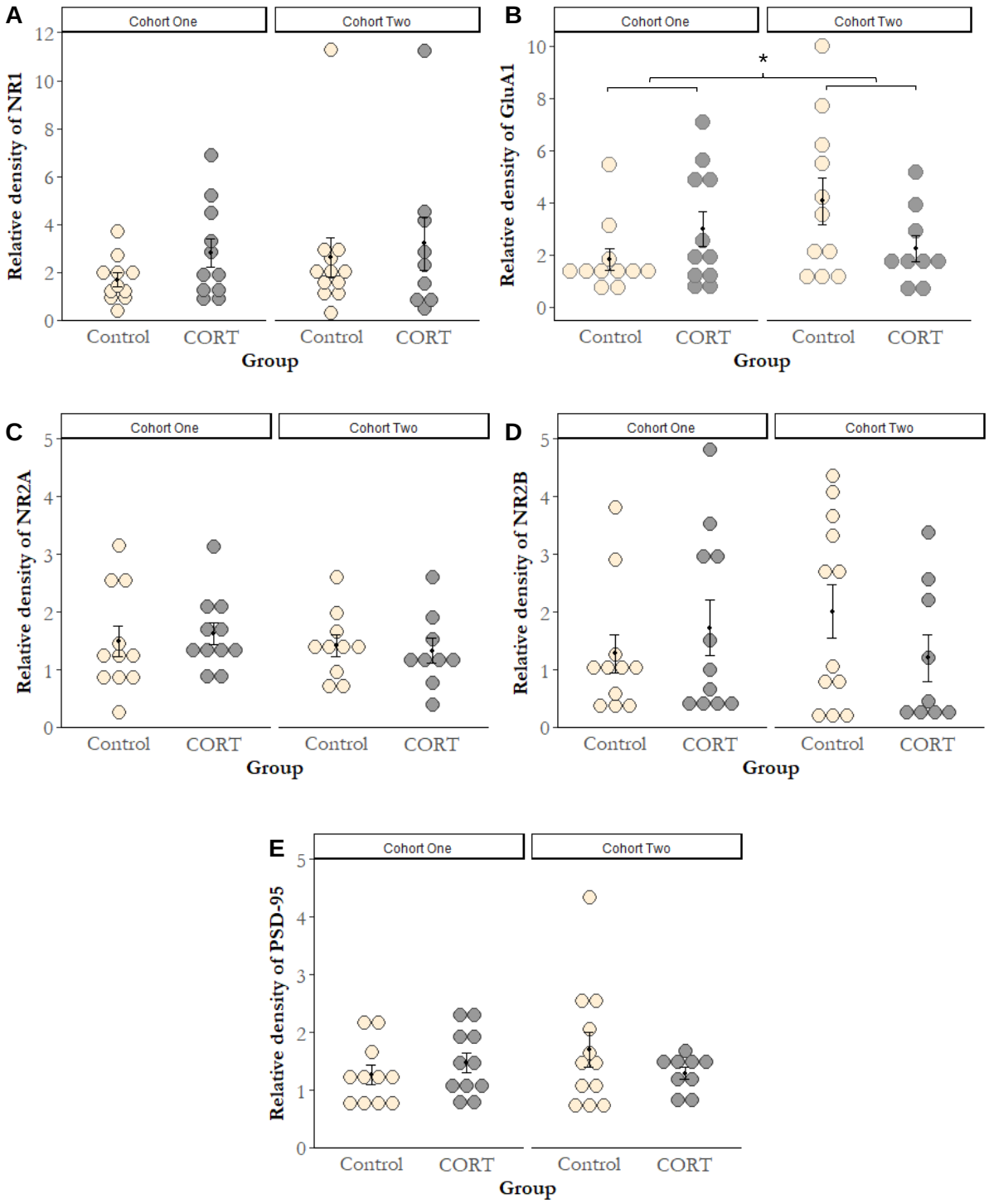


Figure 6.5. Quantified expression of glutamatergic/synaptic receptor proteins in the hippocampus. Analysis of NR1 (A), GluA1 (B), NR2A (C), NR2B (D), and PSD-95 (E) expression in the hippocampus found no difference between control and CORT-treated rats, regardless of time point for tissue extraction. Cohort two groups overall had greater expression of GluA1 compared to cohort one.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Dots indicate individual data points, with mean ±SEM. 'Cohort One' = 'early' time point; 'Cohort Two' = 'delayed'. * = p <0.05.

Western blot analysis of glutamatergic/synaptic protein expression in the frontal cortex

A univariate ANOVA comparing NMDAR1 expression in the frontal cortex between groups taken early post-CORT treatment (vehicle *n*=11, CORT *n*=12) and delayed post-CORT treatment (vehicle *n*=12, CORT *n*=9) found no overall significant difference between the groups (Figure 6.7A), $F_{1, 40} = 3.98$, MSE = 0.84, $p = 0.053$, $\eta_p^2 = 0.09$. There was also no significant difference between the time points, $F_{1, 40} = 0.002$, MSE = 0.84, $p = 0.96$, $\eta_p^2 < 0.001$, nor interaction between group * time, $F_{1, 40} = 1.21$, MSE = 0.84, $p = 0.28$, $\eta_p^2 = 0.029$.

Shapiro-Wilks test for normality found that NMDAR1 expression was not normally distributed in the control group at the early time point, W(11) = 0.77, $p = 0.004$, the CORT group at the early time point, W(12) = 0.84, $p = 0.027$, the control group at the delayed time point, W(12) = 0.83, $p = 0.021$, nor in the CORT group at the delayed time point, W(9) = 0.74, $p = 0.005$. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian univariate ANOVA found moderate evidence to support a lack of effect of time, $BF_{01} = 3.31$, and lack of interaction between group * time, $BF_{01} = 3.34$.

Analysis of GluA1 expression (early cohort: vehicle *n*=11, CORT *n*=12; delayed cohort: vehicle *n*=12, CORT *n*=9) found no significant difference between groups (Figure 6.7B), $F_{1, 40} = 0.70$, MSE = 13.40, $p = 0.41$, $\eta_p^2 = 0.02$, nor between time points, $F_{1, 40} = 0.056$, MSE = 13.40, $p = 0.82$, $\eta_p^2 = 0.001$. There was also no significant interaction between group * time, $F_{1, 40} = 0.61$, MSE = 13.40, $p = 0.44$, $\eta_p^2 = 0.015$.

Shapiro-Wilks test for normality found that GluA1 expression was not normally distributed in the control group at the early time point, W(11) = 0.74, $p = 0.002$, the CORT group at the early time point, W(12) = 0.75, $p = 0.003$, the control group at the delayed time point, W(12) = 0.78, $p = 0.005$, nor in the CORT group at the delayed time point, W(9) = 0.72, $p = 0.003$. However, no suitable alternative statistical test was available for this type of analysis.

Only anecdotal evidence was found in a Bayesian univariate ANOVA to support the lack of overall group effect, $BF_{01} = 2.53$. Moderate evidence was found to support the lack of effect of time, $BF_{01} = 3.19$, and lack of interaction between group * time, $BF_{01} = 7.73$.

NR2A expression (early cohort: vehicle *n*=11, CORT *n*=12; delayed cohort: vehicle *n*=12, CORT *n*=9) was similarly overall unchanged between the groups (Figure 6.7C), $F_{1, 40} = 0.03$, MSE = 0.34, $p = 0.86$, $\eta_p^2 = 0.001$, and between time points, $F_{1, 40} = 0.14$, MSE = 0.34, $p = 0.71$, $\eta_p^2 = 0.003$. There was also no interaction between group * time, $F_{1, 40} = 0.11$, MSE = 0.34, $p = 0.75$, $\eta_p^2 = 0.003$.

Moderate evidence was found in a Bayesian univariate ANOVA to support the lack of overall group effect, BF_{01} = 3.31, and effect of time, BF_{01}= 3.14, as well as strong evidence for a lack of interaction between group * time, BF_{01}= 11.38.

Expression of NR2B (early cohort: vehicle *n*=11, CORT *n*=12; delayed cohort: vehicle *n*=12, CORT *n*=9) was not significantly different between groups (Figure 6.7D), $F_{1, 40}$ = 2.77, MSE = 5.99, *p* = 0.10, η_p^2 = 0.065, nor between time points, $F_{1, 40}$ = 1.97, MSE = 5.99, *p* = 0.17, η_p^2 = 0.047. There was no interaction between group * time, $F_{1, 40}$ = 0.33, MSE = 5.99, *p* = 0.57, η_p^2 = 0.008.

A Bayesian univariate ANOVA found only anecdotal evidence to support a lack of overall group effect, BF_{01} = 1.36, and lack of effect of time, BF_{01} = 1.87. Moderate evidence was found to support the lack of interaction between group * time, BF_{01} = 3.52.

Finally, expression of PSD-95 (early cohort: vehicle *n*=11, CORT *n*=12; delayed cohort: vehicle *n*=12, CORT *n*=9) was overall unchanged between the groups (Figure 6.7E), $F_{1, 40}$ = 3.42, MSE = 1.95, *p* = 0.072, η_p^2 = 0.079. Similarly, there was no difference between time points overall, $F_{1, 40}$ = 0.006, MSE = 1.95, *p* = 0.94, η_p^2 <0.001, and no interaction between group * time, $F_{1, 40}$ = 0.93, MSE = 1.95, *p* = 0.34, η_p^2 = 0.023.

Shapiro-Wilks test for normality found that NMDAR1 expression was not normally distributed in the control group at the delayed time point, W(12) = 0.86, *p* = 0.042. However, no suitable alternative statistical test was available for this type of analysis.

Almost no evidence was found in a Bayesian univariate ANOVA in the direction of support for a group effect, BF_{10} = 1.12. Moderate evidence was found to support the lack of effect of time, BF_{01} = 3.33, and lack of interaction between group * time, BF_{01} = 4.

These findings indicate there was generally no difference between control and CORT-treated rats in the expression of these receptor subunits in the frontal cortex and that this lack of difference did not change over time (see Figure 6.6 for Western blot images). However, evidence was only conclusive for NR2A expression, with all other changes lacking substantial evidence to conclude this lack of group effect.

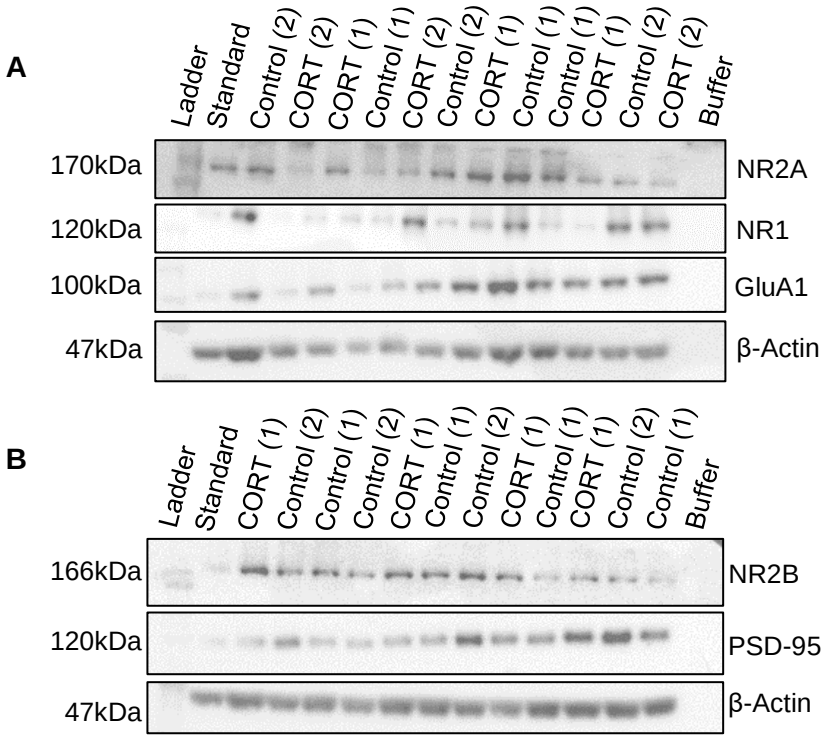


Figure 6.6. Western blot images of glutamatergic/synaptic receptor protein expression in the frontal cortex. Images generated from two Western blot membranes demonstrating expression and molecular weights of NR1, NR2B, PSD-95 (A), and GluA1 and NR2A (B) against the reference protein β-Actin in control or CORT-treated rats analysed in cohort one, i.e. 'early' time point (1) or cohort two, i.e., 'delayed' time point (2).

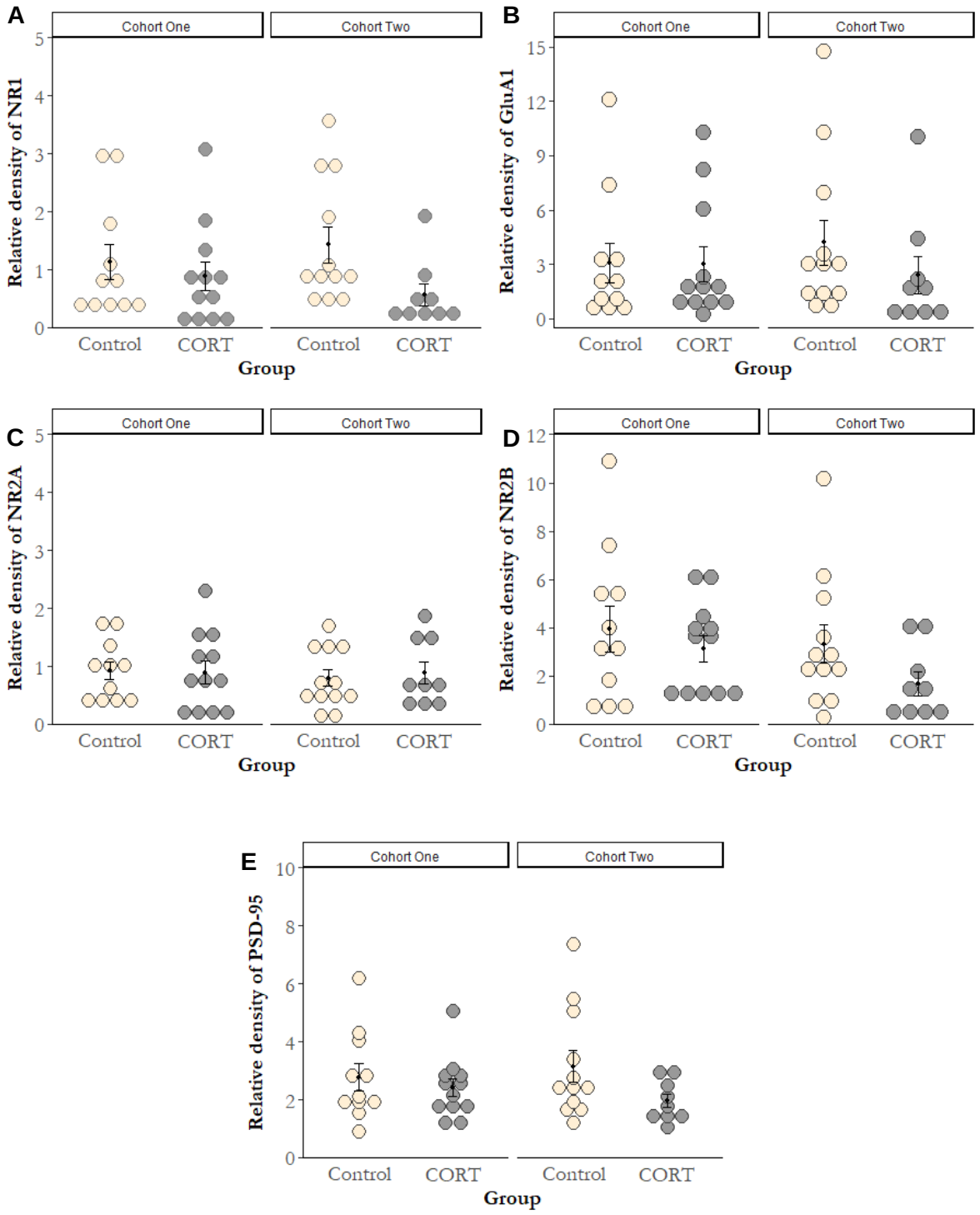


Figure 6.7. Quantified expression of glutamatergic/synaptic receptor proteins in the frontal cortex. Analysis of NR1 (A), GluA1 (B), NR2A (C), NR2B (D), and PSD-95 (E) expression in the frontal cortex found no difference between control and CORT-treated rats, regardless of time point for tissue extraction.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Dots indicate individual data points, with mean ±SEM. 'Cohort One' = 'early' time point; 'Cohort Two' = 'delayed'.

6.3.2.3. Changes in circulating cytokines in the chronic CORT model

Plasma IL-1β levels in the chronic CORT model

For assay quality control, an internal control was run in the ELISA with the samples. The internal control provided had a predicted range between 227-328pg/mL. The actual concentration for this control was 278.04pg/mL for the first ELISA plate and 328.12pg/mL for the second plate[2]. One animal was identified as an outlier, with a plasma IL-1β concentration of 218.46pg/mL[3].

A univariate ANOVA comparing vehicle controls (male *n*=16, female *n*=16) and CORT-treated rats (male *n*=15, female *n*=16) found no overall difference between the two groups in plasma IL-1β concentration, $F_{1, 59} = 0.98$, MSE = 47.86, $p = 0.33$, $n_p^2 = 0.016$, nor between males and females, $F_{1, 59} = 0.32$, MSE = 47.86, $p = 0.57$, $n_p^2 = 0.005$. There was no significant interaction between group * sex, $F_{1, 59} = 3.42$, MSE = 47.86, $p = 0.069$, $n_p^2 = 0.055$ (Figure 6.8).

A Bayesian univariate ANOVA found only anecdotal evidence to support a lack of overall group effect on IL-1β concentration, $BF_{01} = 2.68$, with moderate evidence to support a lack of difference between males and females, $BF_{01} = 3.48$, and lack of interaction between group * sex, $BF_{01} = 3.09$.

These findings demonstrate no overall differences in IL-1β concentration between control and CORT-treated rats, nor between males and females. However, many values were

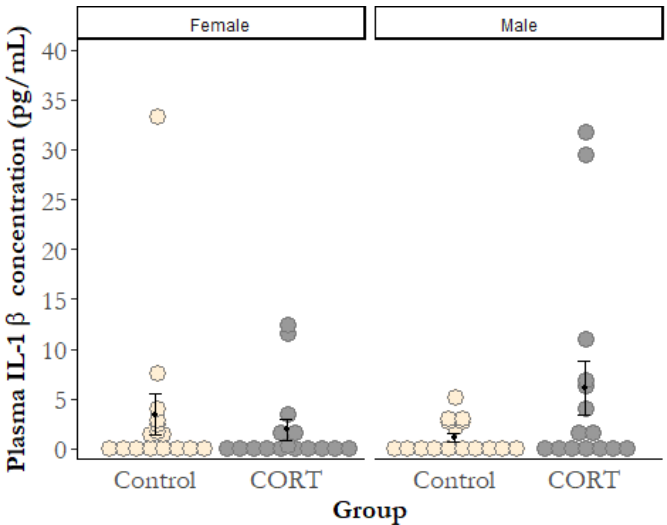


Figure 6.8. Concentration of plasma IL-1β measured via ELISA. There was no difference in IL-1β concentration between control and CORT-treated rats, nor between males and females. Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side. Dots indicate individual data points, with mean ±SEM.

[2] Although the internal control value was slightly higher than the expected range, removal of the data from this plate did not material affect the results from analysis.
[3] Removal of this animal did not materially impact the results from analysis.

below the threshold of detection or ~0pg/mL, and this lack of group effect was deemed inconclusive from Bayes analysis.

These data for plasma IL-1β were not normally distributed as identified via Shapiro-Wilk test for either controls, W(19) = 0.84, p = 0.005, or CORT-treated rats, W(20) = 0.64, p <0.001. Thus, a Kruskal-Wallis non-parametric test was conducted on these data. With males and females combined, there was no significant difference in plasma IL-1β concentration between control and CORT-treated rats, H(1) = 0.057, p = 0.81. Separately, there was no difference between male control and CORT-treated rats, H(1) = 1.68, p = 0.19, nor female control and CORT-treated rats, H(1) = 0.85, p = 0.36.

Plasma IL-6 levels in the chronic CORT model

The internal control provided had a predicted range between 135-226pg/mL. The actual concentration determined for this control was 246.98pg/mL, indicating all values reported here may be higher than their true value and therefore caution should be taken when interpreting these results.

Analysis of plasma IL-6 concentrations with a univariate ANOVA found no overall difference between vehicle-treated controls (male n=9, female n=10) and CORT-treated rats (male n=10, female n=10), $F_{1, 35}$ = 0.36, MSE = 5096.92, p = 0.56, η_p^2 = 0.01. There was also no difference overall between males and females, $F_{1, 35}$ = 2.10, MSE = 5096.92, p = 0.16, η_p^2 = 0.057, and no interaction between group * sex, $F_{1, 35}$ = 1.89, MSE = 5096.92, p = 0.18, η_p^2 = 0.051 (Figure 6.9).

A Bayesian univariate ANOVA found only anecdotal evidence to support the overall lack of effect of group, BF_{01}= 2.81, and sex, BF_{01} = 1.38, as well as the lack of interaction between group * sex, BF_{01} = 2.75 on plasma IL-6 concentration.

These findings demonstrate no overall differences in IL-6 concentration between control and CORT-treated rats, nor between males and females. However, several values were below the threshold of detection and Bayes analysis deemed these findings inconclusive.

These data for plasma IL-6 were not normally distributed as identified via Shapiro-Wilk test for either controls, W(19) = 0.73, p < 0.001, or CORT-treated rats, W(20) = 0.55, p <0.001. Thus, a Kruskal-Wallis non-parametric test was conducted on these data. With males and females combined, there was no significant difference in plasma IL-6 concentration between control and CORT-treated rats, H(1) = 0.84, p = 0.36. Separately, there was no difference between male control and CORT-treated rats, H(1) <0.001, p = 1.00, nor female control and CORT-treated rats, H(1) = 2.02, p = 0.16.

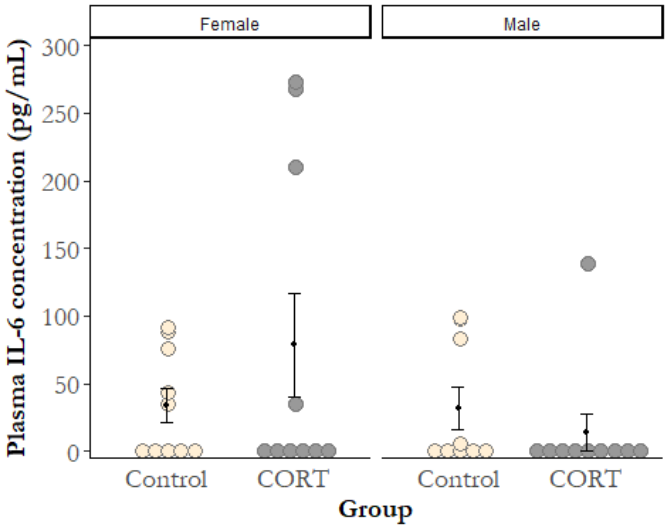


Figure 6.9. Concentration of plasma IL-6 measured via ELISA. There was no difference in IL-6 concentration between control and CORT-treated rats, nor between males and females.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Dots indicate individual data points, with mean ±SEM.

Plasma TNF-α levels in the chronic CORT model

The internal control for TNF-α had a predicted range between 107-178pg/mL. The actual concentration determined for this control was 151.92pg/mL.

A univariate ANOVA found no overall difference between vehicle controls (male *n*=9, female *n*=10) and CORT-treated rats (male *n*=10, female *n*=10) in plasma TNF-α concentration, $F_{1, 35} = 0.36$, MSE = 0.30, $p = 0.55$, $\eta_p^2 = 0.01$. Female rats overall had a significantly higher plasma concentration of TNF-α compared to males, $F_{1, 35} = 10.36$, MSE = 0.30, $p = 0.003$, $\eta_p^2 = 0.23$, with no interaction between group * sex, $F_{1, 35} = 0.66$, MSE = 0.30, $p = 0.42$, $\eta_p^2 = 0.02$ (Figure 6.10). With Bonferroni correction for three comparisons, this significant effect of sex remains significant.

A Bayesian univariate ANOVA found only anecdotal evidence to support a lack of group effect, $BF_{01} = 2.74$, and lack of interaction between group * sex, $BF_{01} = 1.95$.

These findings demonstrate no overall differences in TNF-α concentration between control and CORT-treated rats, however, this lack of group effect was deemed inconclusive from Bayes analysis. Evidence did demonstrate that, overall, female rats had greater plasma TNF-α concentration compared to males, which was strongly supported by Bayes analysis.

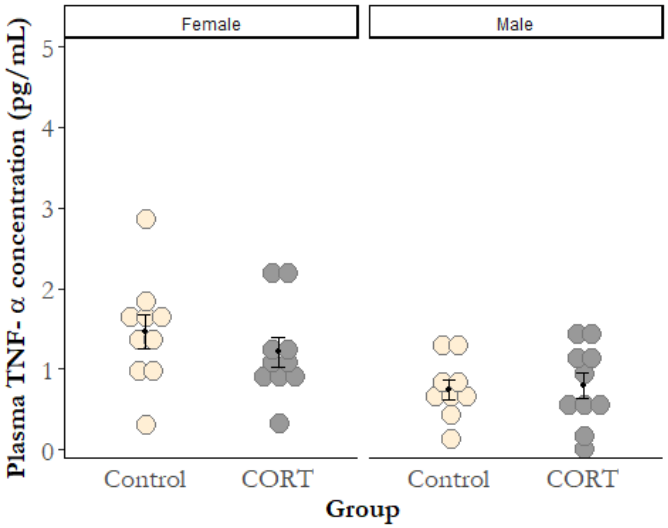


Figure 6.10. Concentration of plasma TNF-α measured via ELISA.
There was no difference in TNF-α concentration between control and CORT-treated rats, however female rats had overall higher concentration compared to males (p <0.01).
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Dots indicate individual data points, with mean ±SEM.

6.3.2.4. Identifying correlations between behaviour and neurobiological markers

Since this thesis aims to elucidate the neurobiological mechanisms underpinning different aspects of reward processing, Pearson's correlations within individual groups were conducted between neurobiological markers and lick cluster size (reported in section 4.3) or preference for CS-A^{++} vs CS-B^{+} as an indication of positive affective bias (reported in section 3.3). Only significant correlations between behaviour and neurobiological markers are reported here. It should be noted that interpretation of these correlations must be done cautiously considering the many comparisons undertaken, many non-significant correlations and the likelihood of significant correlations becoming non-significant if a multiple-comparison correction had been applied.

Experiment 8 cohort – early post-CORT treatment

Control rats showed a significant negative correlation between NR2B expression in the frontal cortex and lick cluster size for 4% sucrose, R = -0.63, p = 0.038 (Figure 6.11A). There was a trend toward a negative correlation between NR2B expression in the frontal cortex and lick cluster size for 16% sucrose, but this did not reach significance, R = -0.56, p = 0.071.

Control rats also showed a significant negative correlation between NR2A expression in the frontal cortex and lick cluster size for 16% sucrose, R = -0.62, p = 0.042 (Figure 6.11B).

In contrast, CORT-treated rats had no significant correlations between any neurobiological markers and lick cluster size.

Experiment 8 cohort – delayed post-CORT treatment

There were no significant correlations between lick cluster size and any neurobiological markers in control or CORT-treated rats.

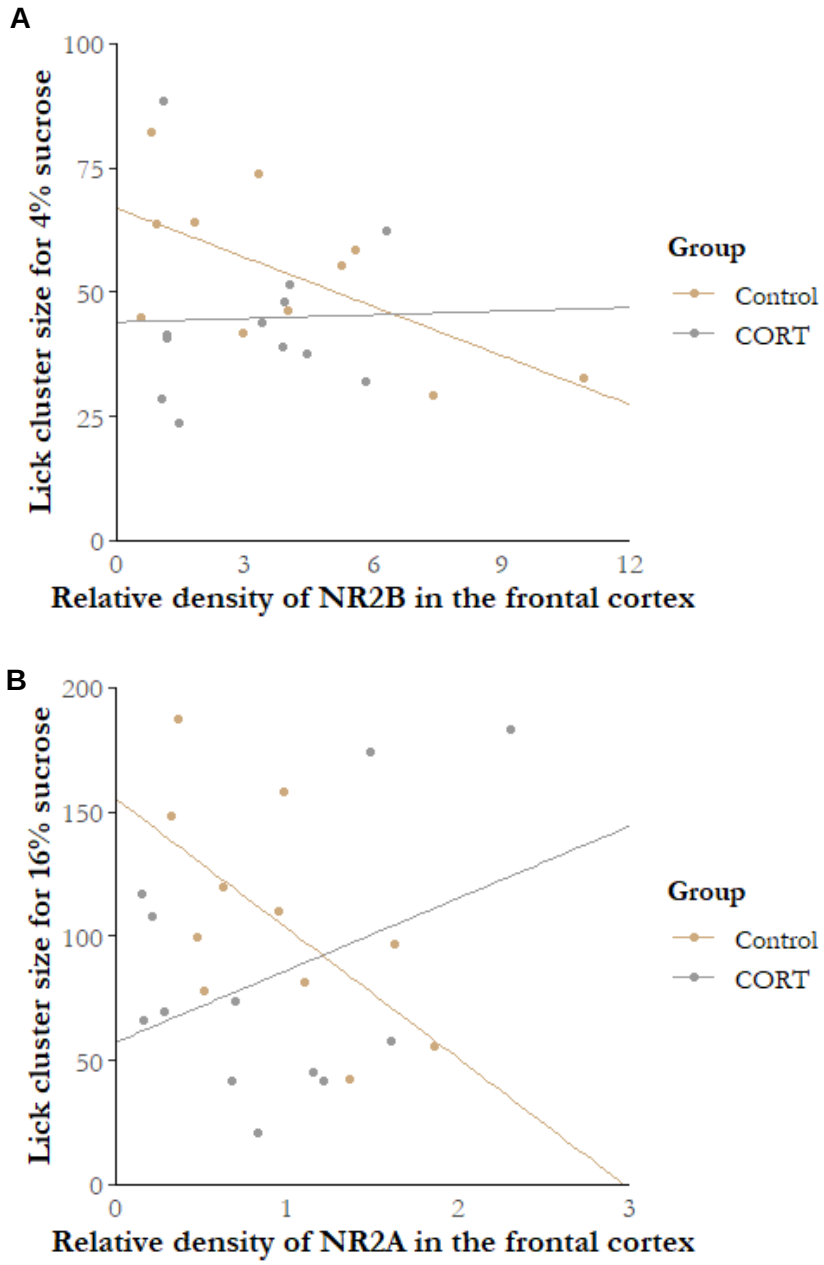


Figure 6.11. Correlations between lick cluster size and relative density of NMDA receptor subunits in the Experiment 8 cohort examined early post-treatment.
In control rats, a significant negative correlation was found between lick cluster size for 4% sucrose and relative density of NR2B in the frontal cortex (A) and between lick cluster size for 16% sucrose and relative density of NR2A in the frontal cortex (B). No significant correlations within the CORT-treated group were found.
Dots indicate individual data points with the mean ±SEM.

Experiment 9 cohort

Male control rats showed a significant negative correlation between lick cluster size during consumption of 4% sucrose and plasma TNF-α concentration, R = -0.72, p = 0.03 (Figure 6.12), whilst this correlation was not significant in male CORT-treated rats, nor female control or CORT-treated. No other significant correlations were found with circulating plasma proteins.

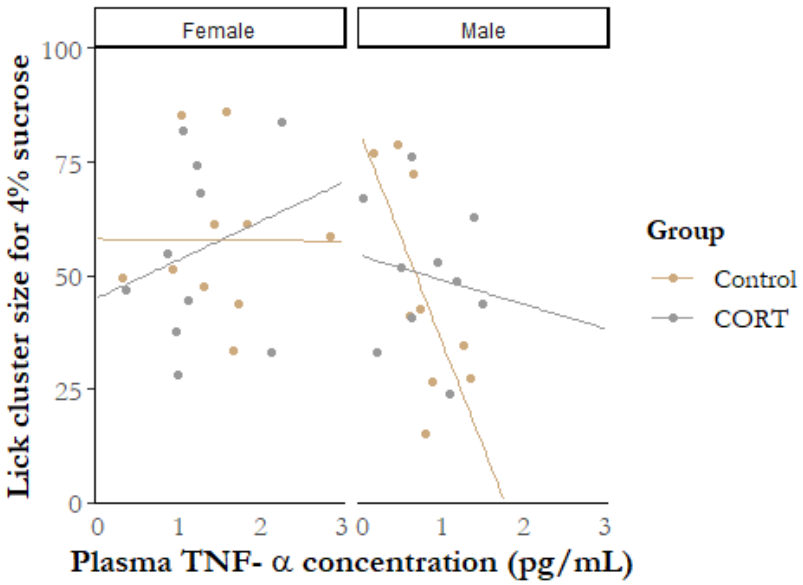


Figure 6.12. Correlation between plasma TNF-α concentration and lick cluster size for 4% sucrose. A significant negative correlation between lick cluster size for 4% sucrose and plasma TNF-α concentration was found within male control rats only (p <0.05).
Dots indicate individual data points with the mean ±SEM.

Together these findings demonstrate that CORT-treated rats did not show any correlations between neurogenic markers measured here with affective biases or hedonic response to reward. However, potential relationships were found within male control rats of Experiment 8 and 9, demonstrating an association between reduced lick cluster size when consuming 4% sucrose with elevated plasma TNF-α concentration and NR2B expression in the frontal cortex. Reduced lick cluster size when consuming 16% sucrose was also associated with elevated NR2A expression in the frontal cortex. However, these interpretations should be taken with caution and are stated simply to demonstrate potential avenues for future exploration.

6.3.3. Summary

Section 6.3 of this thesis aimed to characterise the neuronal and biological changes associated with chronic CORT exposure, with the aim of highlighting a relationship between these changes and associated reward-related deficits. Here, I present findings from the immunohistochemical analysis of neurogenic markers, Western blot analysis of glutamatergic receptor subunits, and immunoassays of circulating cytokines.

The findings from these analyses demonstrated no significant changes in neurogenic markers, glutamatergic receptor subunits or circulating cytokines in chronic CORT-treated rats compared to control rats. However, much of this evidence was not substantial enough to be conclusive from Bayesian analysis. Given the potential issues with chronic CORT treatment for one cohort of animals here as discussed in Chapter 3 (see section 3.4), the findings gathered from ELISA analysis of circulating cytokines may not accurately reflect any changes resulting from chronic CORT treatment. Similarly, although behavioural and physiological changes were present in the other cohort of animals examined here, this model may not be reliable enough to provide conclusive evidence of neurobiological changes.

Despite potential flaws of using this model, some correlations were identified between behaviours characterised in previous chapters of this thesis and neurobiological markers here. Although no correlations were identified in CORT-treated rats, in male control rats a greater lick cluster size for 4% sucrose was associated with reduced NR2B expression in the frontal cortex and reduced plasma TNF-α, whereas greater lick cluster size for 16% sucrose was associated with reduced NR2A in the frontal cortex.

6.4. Chronic interferon-alpha model

The analyses conducted in section 6.4 aimed to establish whether chronic IFN-α treatment affected neurogenesis, the expression of glutamatergic receptors, as well as circulating plasma CORT or cytokines. Based on previous findings following IFN-α treatment and hypothesised effects on synaptic plasticity, it was expected that chronic IFN-α would reduce the expression of neurogenic markers, glutamatergic receptor subunits, and elevate plasma cytokine levels.

6.4.1. Experimental design

Two separate cohorts were used for analysis of neurobiological changes in the chronic IFN-α model.

Changes in glutamatergic neurotransmission were analysed in the cohort of animals used in Experiment 10 (see 4.4.2), comparing saline vs IFN-treated male rats. Tissue samples were extracted after a total of 21-23 IFN-α injection days in this cohort.

Changes in neurogenic markers, microglial activation and circulating proteins measured via ELISA were analysed in the cohort of animals used in both Experiment 5 and 11 (see 3.5 and 4.4.3, respectively), consisting of males and females. Tissue and blood samples were extracted after a total of 29-30 IFN-α injection days in this cohort.

For all analyses, sample sizes were dependent on extracted sample volume and viability during the assay. Exact sample sizes are reported for individual proteins/markers in the results section. In the analysis of circulating cytokines, some levels were below the threshold for detection and were allocated a value of ‘0’. For IL-6, all samples were below the detectable threshold and so statistical analyses could not be performed. The internal control provided for cytokine kits was checked to ensure its value was within the expected reference range as quality control for the assay.

6.4.2. Results

6.4.2.1. Neuro-inflammatory changes in the chronic IFN-α model

Amygdala

A univariate ANOVA showed no significant effect of IFN-α treatment on the mean number of Iba1$^+$ cells per mm^2 in the amygdala, $F_{1, 28}$ = 1.36, MSE = 103.05, p = 0.19, η_p^2 = 0.06 (Figure 6.13). There was a significantly greater number of Iba1$^+$ cells per mm^2 in males compared to females, $F_{1, 28}$ = 6.03, MSE = 103.05, p = 0.021, η_p^2 = 0.18, with no interaction between group * sex, $F_{1, 28}$ = 1.36, MSE = 103.05, p = 0.25, η_p^2 = 0.046.

Bayesian univariate ANOVAs found only anecdotal evidence to support the lack of overall group effect, BF_{01} = 1.67, with almost no evidence to support a lack of interaction between group * sex, BF_{01} = 1.25.

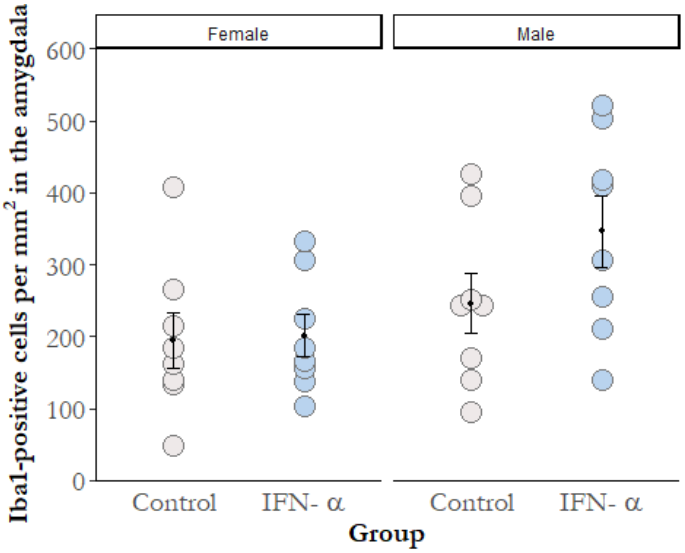


Figure 6.13. Markers of microglial activation in the amygdala.
The average number of Iba1$^+$ cells per mm^2 showed no differences between the two groups. Dots indicate individual data points, with the mean ±SEM.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

Dorsal hippocampus

IFN-α treated rats had a significantly greater number of Iba1$^+$ cells per mm^2 in the dorsal hippocampus compared to controls, $F_{1, 28}$ = 7.092, MSE = 11648.23, p = 0.013, η_p^2 = 0.20 (Figure 6.14). There were also more Iba1$^+$ cells per mm^2 dorsal hippocampus in males compared to females, $F_{1, 28}$ = 6.28, MSE = 11648.23, p = 0.018, η_p^2 = 0.18, with no interaction between group * sex, $F_{1, 28}$ = 0.79, MSE = 11648.23, p = 0.38, η_p^2 = 0.028.

Shapiro-Wilks test for normality found that expression of Iba1$^+$ cells per mm^2 in the dorsal hippocampus was not normally distributed in female IFN-α treated rats, W(8) = 0.81, p = 0.036. However, no suitable alternative statistical test was available for this type of analysis.

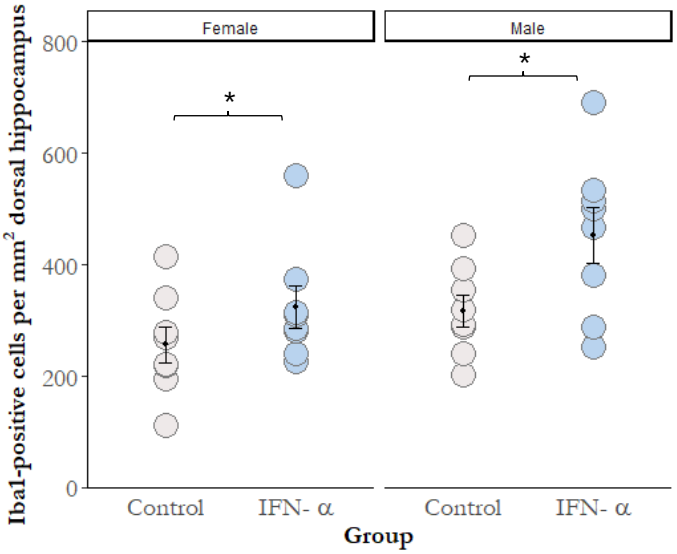


Figure 6.14. Markers of microglial activation in the dorsal hippocampus.
The average number of Iba1$^+$ cells per mm^2 showed overall significantly more Iba1$^+$ cells per mm^2 in IFN-α treated rats compared to controls.
Dots indicate individual data points with the mean ±SEM. * = p<0.05.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

Ventral hippocampus

There was no significant effect of IFN-α treatment on Iba1$^+$ cells per mm^2 in the ventral hippocampus, $F_{1, 26} = 1.96$, MSE = 19668.11, $p = 0.17$, $\eta_p^2 = 0.07$ (Figure 6.15). There was also no difference between males and females, $F_{1, 26} = 3.49$, MSE = 19668.11, $p = 0.073$, $\eta_p^2 = 0.12$, nor interaction between group * sex, $F_{1, 26} = 0.68$, MSE = 19668.11, $p = 0.42$, $\eta_p^2 = 0.025$.

Shapiro-Wilks test for normality found that expression of Iba1$^+$ cells per mm^2 in the ventral hippocampus was not normally distributed in male control, W(7) = 0.71, $p = 0.005$. However, no suitable alternative statistical test was available for this type of analysis.

Bayesian univariate ANOVAs found only anecdotal evidence to support a lack of group effect on the number of Iba1$^+$ cells per mm^2, $BF_{01} = 1.54$ and lack of interaction between group * sex, $BF_{01} = 2.04$.

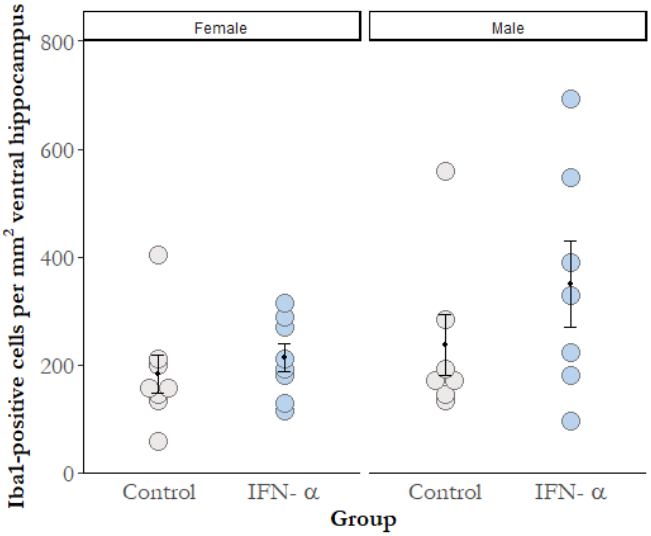


Figure 6.15. Markers of microglial activation in the ventral hippocampus.
The average number of Iba1$^+$ cells per mm^2 showed no significant difference between the two groups.
Dots indicate individual data points with the mean ±SEM. * = $p<0.05$.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

Together, these findings demonstrate significantly number of microglial cells in the dorsal hippocampus of IFN-α treated rats compared to controls, with overall greater microglial expression in males compared to females in the amygdala and dorsal hippocampus (see Figure 6.16 for images used in analysis).

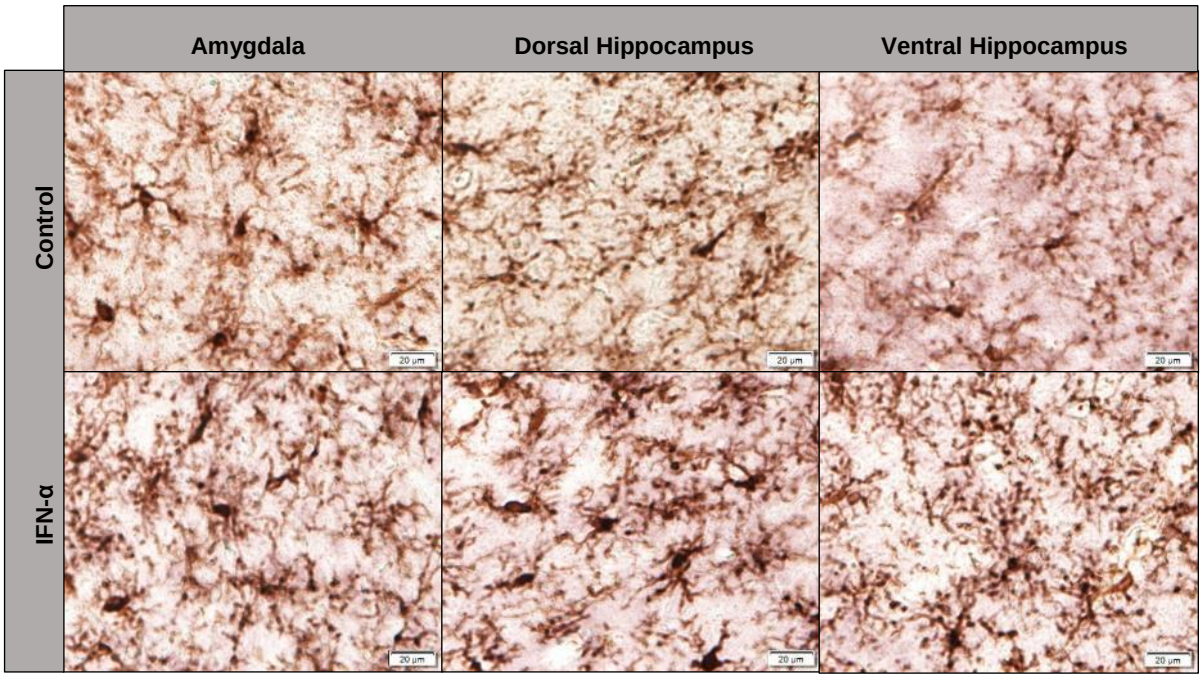


Figure 6.16. Representative images of Iba1 staining in control and IFN-α treated rats. Images were taken of the centre of the amygdala, dorsal hippocampus, and ventral hippocampus at x20 magnification in both control and IFN-α rats, to be analysed via cell counting.

6.4.2.1. Neurogenic changes in the chronic IFN-α model

Effects of chronic IFN-α treatment on cell proliferation and immature neurons in the dorsal hippocampus

From the analysis of doublecortin- and Ki67- positive cells in the dorsal hippocampus outlined below, it was found that IFN-α treated rats had significantly greater expression of immature neurons compared to control rats with no overall group effect on cell proliferation, however this latter evidence was deemed inconclusive. A significant interaction indicated that male IFN-α treated rats did show greater cell proliferation compared to male control rats, with no overall difference between males and females in either DCX or Ki67 expression, however, again evidence for this was deemed inconclusive. It should be noted that these results should be interpreted cautiously due to some poor staining such as in the DCX-stained samples, as seen in Figure 6.17, which may have arisen due to potential issues with a different batch of antibodies used.

Doublecortin

A univariate ANOVA showed a significantly greater number of total DCX^+ cells in the dorsal hippocampus of IFN-treated rats compared to controls, $F_{1, 33} = 5.12$, MSE = 454.43, $p = 0.03$, $\eta_p^2 = 0.13$, with no overall difference between males and females, $F_{1, 33} = 2.66$, MSE = 454.43, $p = 0.11$, $\eta_p^2 = 0.074$. There was no interaction between group * sex, $F_{1, 33} = 3.21$, MSE = 454.43, $p = 0.083$, $\eta_p^2 = 0.089$.

IFN-α treated rats also had significantly smaller mean GrDG area in the dorsal hippocampus compared to controls, $F_{1, 33} = 6.68$, MSE <0.001, $p = 0.014$, $\eta_p^2 = 0.17$, with overall males having significantly larger area compared to females, $F_{1, 33} = 25.75$, MSE <0.001, p <0.001, $\eta_p^2 = 0.44$. There was no interaction between group * sex, $F_{1, 33} = 0.003$, MSE <0.001, p = 0.96, η_p^2 <0.001.

Adjusting for differences in area, results showed that IFN-α treated rats had significantly greater DCX^+ cells per mm^2 in the dorsal hippocampus compared to controls, $F_{1, 33} = 14.66$, MSE = 24810.16, $p = 0.001$, $\eta_p^2 = 0.31$ (Figure 6.17A). There was no significant difference between males and females, $F_{1, 33} = 0.62$, MSE = 24810.16, $p = 0.44$, $\eta_p^2 = 0.02$, nor an interaction between group * sex, $F_{1, 33} = 2.91$, MSE = 24810.16, $p = 0.097$, $\eta_p^2 = 0.081$. Using Bonferroni correction for two neurogenic markers analysed in this cohort, the effect of group here remains significant.

A Bayesian univariate ANOVA found only anecdotal evidence to support the lack of overall effect of sex on DCX^+ cells per mm^2, $BF_{01} = 2.51$.

Ki-67

In the dorsal hippocampus, a univariate ANOVA found no overall significant effect of IFN-α treatment on mean number of Ki-67 cells, $F_{1, 28} = 0.12$, MSE = 20.21, $p = 0.74$, $\eta_p^2 = 0.004$. Male rats had significantly more Ki-67 cells compared to female rats, $F_{1, 28} = 9.01$, MSE = 20.21, $p = 0.006$, $\eta_p^2 = 0.24$, with a significant interaction between group * sex, $F_{1, 28} = 13.09$, MSE = 20.21, $p = 0.001$, $\eta_p^2 = 0.32$. Fisher's LSD post-hoc analysis revealed that male IFN-α treated rats had significantly greater Ki-67$^+$ cells compared to male control rats ($p = 0.009$), whereas female IFN-α treated rats had significantly less Ki-67$^+$ cells compared to female controls ($p = 0.028$).

Analysis of GrDG areas found no significant effect of IFN-α treatment, $F_{1, 28} = 3.89$, MSE <0.001, $p = 0.058$, $\eta_p^2 = 0.12$. Males had a significantly larger mean GrDG area compared to females, $F_{1, 28} = 9.36$, MSE <0.001, $p = 0.005$, $\eta_p^2 = 0.25$, with no interaction between group * sex, $F_{1, 28} = 1.27$, MSE <0.001, $p = 0.27$, $\eta_p^2 = 0.043$.

Taking the area of the GrDG into account, there was no significant overall effect of IFN-α treatment on the number of Ki-67 cells per mm^2, $F_{1, 28} = 3.82$, MSE = 1932.18, $p = 0.061$, $\eta_p^2 = 0.12$, nor sex, $F_{1, 28} = 0.92$, MSE = 1932.18, $p = 0.35$, $\eta_p^2 = 0.032$ (Figure 6.17B). There was a significant interaction between group * sex, $F_{1, 28} = 13.36$, MSE = 1932.18, $p = 0.001$, $\eta_p^2 = 0.32$, where Fisher's LSD post-hoc analysis showed male IFN-α treated rats had significantly more Ki-67 cells per mm^2 than male control rats ($p = 0.003$), with no significant difference in females ($p = 0.067$). Using Bonferroni correction for two neurogenic markers analysed in this cohort, this interaction remains significant.

A Bayesian univariate ANOVA revealed only anecdotal evidence to support the lack of overall group effect on number of Ki67$^+$ cells per mm^2, $BF_{01} = 2.35$. Almost no evidence was found to support the lack of difference between males and females, $BF_{01} = 1.07$.

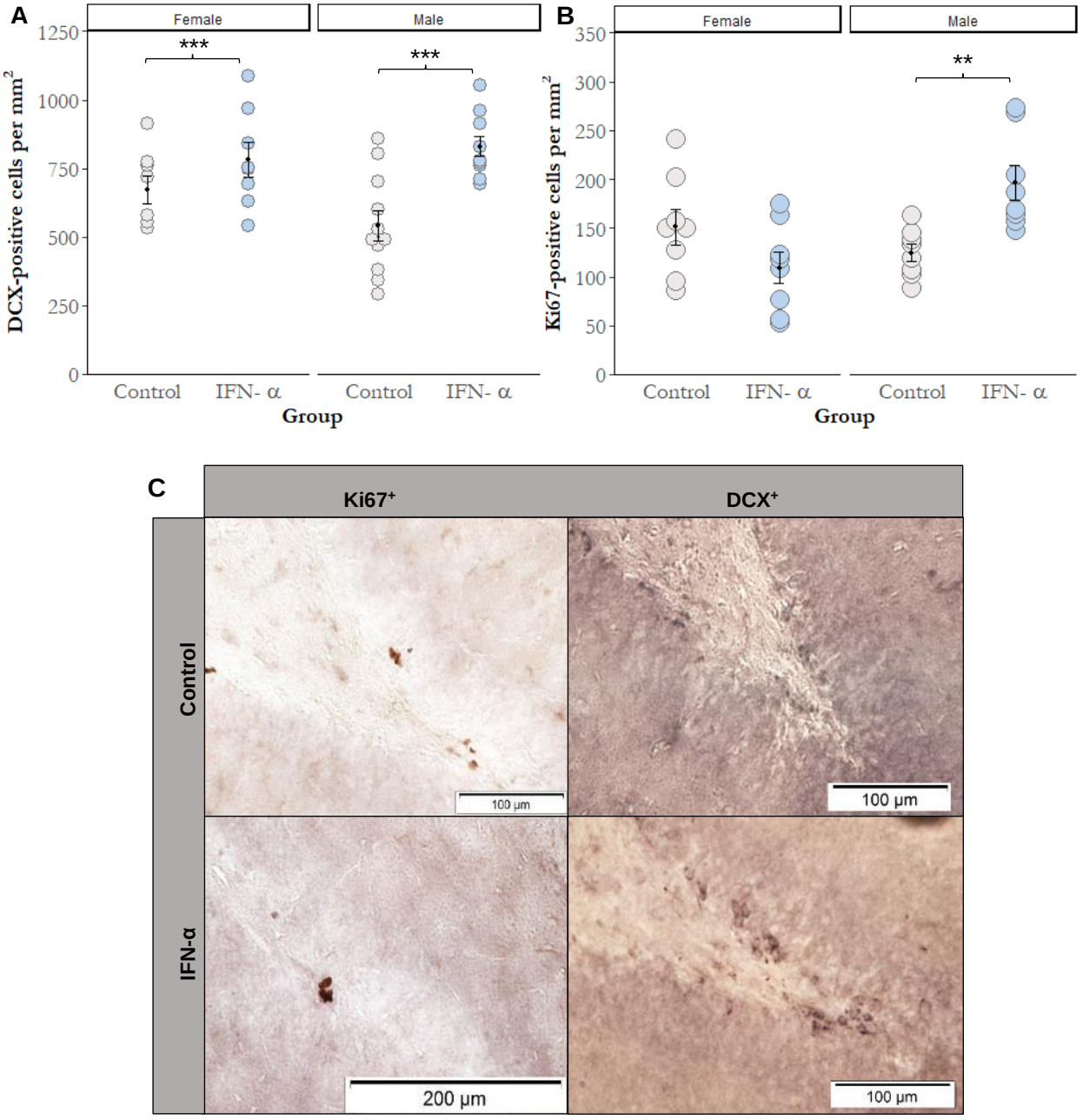


Figure 6.17. Average number of neurogenic marker cells per mm² in the dorsal hippocampus.
(A) The number of Ki-67⁺ cells per mm² was significantly greater in male IFN-α rats compared to male controls, with no difference in females. (B) Number of DCX⁺ cells per mm² was significantly greater in IFN-α rats overall compared to controls. (C) shows representative images of Ki67⁺ and DCX⁺ cells in the dorsal hippocampus.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.
Dots indicate individual data points, with mean ±SEM.
** = p<0.01, *** = p ≤0.001.

Effects of chronic IFN-α treatment on cell proliferation and immature neurons in the ventral hippocampus

From the analysis of doublecortin-positive cells in the ventral hippocampus outlined below, no difference in the expression of immature neurons was found between IFN-α treated rats and controls, nor between males and females. In contrast, Ki67-positive cells, used as markers of cell proliferation, were significantly lower in IFN-α treated rats compared to controls with no difference between males and females.

Doublecortin

A univariate ANOVA showed there was no significant effect of treatment on mean number of DCX^+ cells in the GrDG of the ventral hippocampus, $F_{1, 20} = 0.73$, MSE = 1353.22, $p = 0.40$, $\eta_p^2 = 0.035$, nor of sex, $F_{1, 20} = 0.57$, MSE = 1353.22, $p = 0.46$, $\eta_p^2 = 0.028$. There was no interaction between group * sex, $F_{1, 20} = 1.38$, MSE = 1353.22, $p = 0.25$, $\eta_p^2 = 0.065$.

There was no significant effect of treatment on mean area of the GrDG, $F_{1, 20} = 2.67$, MSE = 0.006, $p = 0.12$, $\eta_p^2 = 0.12$. There was no effect of sex, $F_{1, 20} = 0.05$, MSE = 0.006, $p = 0.83$, $\eta_p^2 = 0.003$, nor an interaction between group * sex, $F_{1, 20} = 0.45$, MSE = 0.006, $p = 0.51$, $\eta_p^2 = 0.022$.

Analysis of the mean number of DCX^+ cells per mm^2 in the ventral hippocampus found no significant effect of treatment, $F_{1, 20} = 0.94$, MSE = 5825.11, $p = 0.35$, $\eta_p^2 = 0.045$ (Figure 6.18A). Males had significantly greater DCX^+ cells per mm^2 compared to females, $F_{1, 20} = 5.40$, MSE = 5825.11, $p = 0.031$, $\eta_p^2 = 0.21$, with no interaction between group * sex, $F_{1, 20} = 0.77$, MSE = 5825.11, $p = 0.39$, $\eta_p^2 = 0.037$. Using Bonferroni correction for two neurogenic markers analysed in this cohort, this significant effect of sex becomes no longer significant.

Shapiro-Wilks test for normality found that expression of DCX^+ cells per mm^2 in the ventral hippocampus was not normally distributed in female control rats, $W(6) = 0.73$, $p = 0.011$. However, no suitable alternative statistical test was available for this type of analysis.

A Bayesian univariate ANOVA found only anecdotal evidence to support a lack of overall group effect on the number of DCX^+ cells per mm^2 in the ventral hippocampus, $BF_{01} = 2.01$, as well as the lack of interaction between group * sex, $BF_{01} = 1.49$.

Ki-67

A univariate ANOVA found significantly less $Ki\text{-}67^+$ cells in the ventral hippocampus in IFN-α treated rats compared to control rats, $F_{1, 21} = 9.46$, MSE = 94.19, $p = 0.006$, $\eta_p^2 = 0.31$. There was no significant effect of sex, $F_{1, 21} = 1.61$, MSE = 94.19, $p = 0.22$, $\eta_p^2 = 0.071$, with no interaction between group * sex, $F_{1, 21} = 0.55$, MSE = 94.19, $p = 0.47$, $\eta_p^2 = 0.026$.

There was no significant effect of group, $F_{1, 21} = 0.23$, MSE = 0.004, $p = 0.63$, $\eta_p^2 = 0.011$, nor sex, $F_{1, 21} = 0.81$, MSE = 0.004, $p = 0.38$, $\eta_p^2 = 0.037$, on area of the GrDG. There was no interaction between group * sex, $F_{1, 21} = 2.44$, MSE = 0.004, $p = 0.13$, $\eta_p^2 = 0.10$.

Accounting for area, an overall significant reduction was found in number of $Ki67^+$ cells per mm^2 in IFN-α treated rats compared to control rats, $F_{1, 21} = 5.41$, MSE = 1165.5, $p = 0.03$, $\eta_p^2 = 0.21$ (Figure 6.18B). There was no overall effect of sex here, $F_{1, 21} = 1.73$, MSE = 1165.5, $p = 0.20$, $\eta_p^2 = 0.076$, nor an interaction between group * sex, $F_{1, 21} = 1.79$, MSE = 1165.5, $p = 0.19$. Using Bonferroni correction for two neurogenic markers analysed here, this significant effect of group becomes no longer significant.

A Bayesian univariate ANOVA found only anecdotal evidence to support a lack of group effect on $Ki67^+$ cells per mm^2, $BF_{01} = 1.39$, with almost no evidence to support the lack of interaction between group * sex, $BF_{01} = 1.04$.

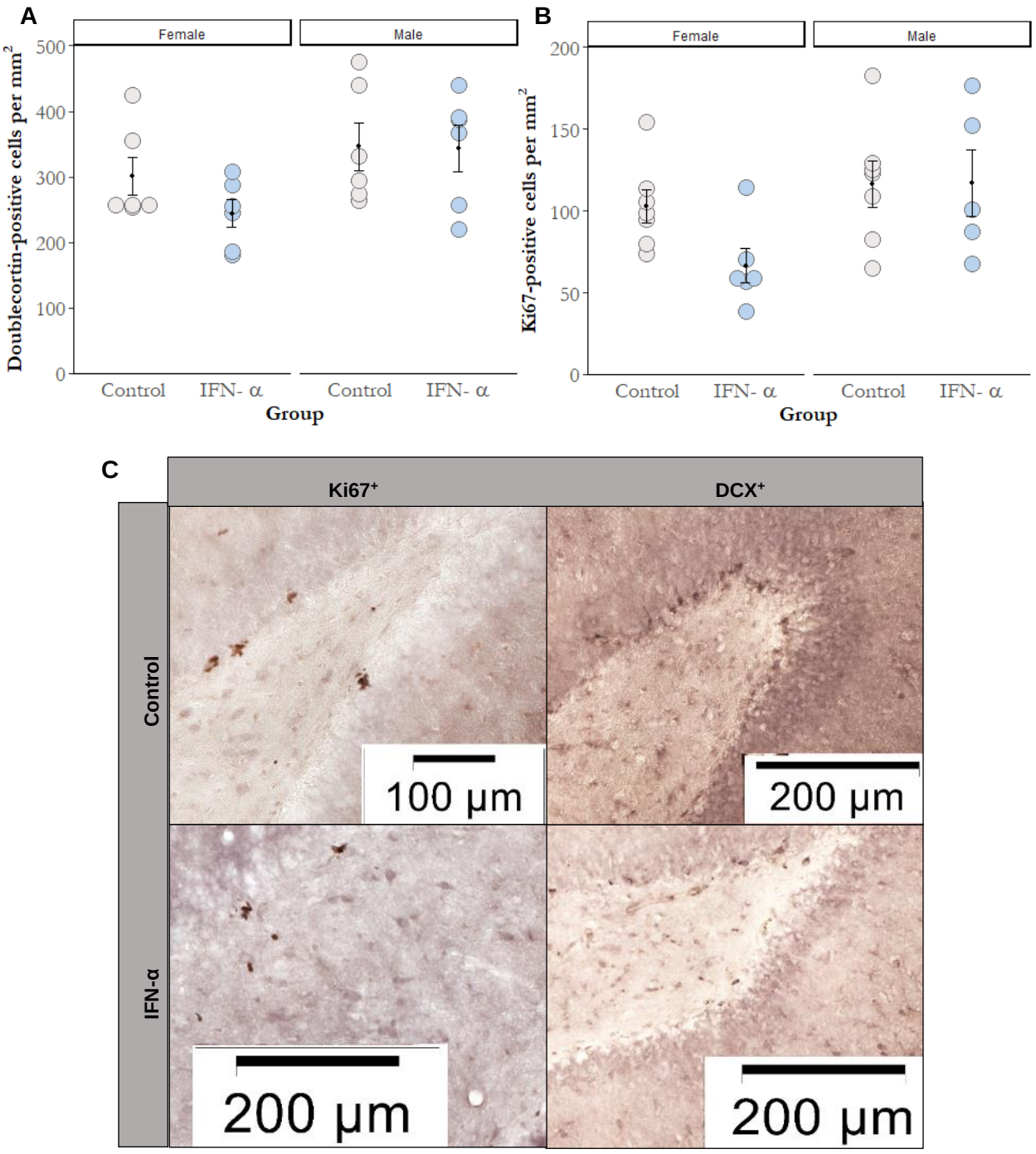


Figure 6.18. Average number of neurogenic marker cells per mm² in the ventral hippocampus.

(A) The number of Ki-67⁺ cells per mm² was significantly lower overall in IFN-α rats compared to controls, with no difference across males and females. (B) There was no significant difference in number of DCX⁺ cells per mm². (C) Example images of Ki67⁺ and DCX⁺ cells in the ventral hippocampus. Dots indicate individual data points with mean ±SEM.

Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

Dots indicate individual data points, with mean ±SEM.

6.4.2.2. Changes in glutamatergic neurotransmission in the chronic IFN-α model

Western blot analysis of glutamatergic/synaptic protein expression in the hippocampus

Independent samples t-tests were conducted comparing receptor protein expression in the hippocampus of control vs IFN-α treated rats, followed by Bayesian independent samples t-tests for each protein analysed.

No significant difference was found in expression of NR1 (Figure 6.20A), t(22) = -1.87, p = 0.075, however, Bayes found anecdotal evidence in support of the alternate hypothesis, BF_{10} = 1.28.

There was no difference in NR2A expression, NR2A (Figure 6.20B), t(22) = 0.91, p = 0.38, supported only by anecdotal evidence, BF_{01} = 1.99.

A significant reduction in NR2B expression was found in IFN-α treated rats compared to controls, t(22) = 2.64, p = 0.015 (Figure 6.20C). Using Bonferroni's correction for three NMDAR-related receptors in this analysis, this difference remains significant.

No difference was found in GluA1 (Figure 6.20D), t(22) = -0.79, p = 0.44, supported by anecdotal evidence, BF_{01} = 2.15.

In addition, there was no difference in PSD-95 expression (Figure 6.20E), t(22) = -1.37, p = 0.18, supported by anecdotal evidence, BF_{01} = 2.15.

These findings demonstrate no apparent differences between control and IFN-α treated rats in the expression of NMDAR1, NR2A, GluA1 and PSD95 in the hippocampus, however this evidence was deemed inconclusive. IFN-α treated rats did demonstrate lower expression of the NR2B subunit compared to controls (see Figure 6.19 for all Western blot images).

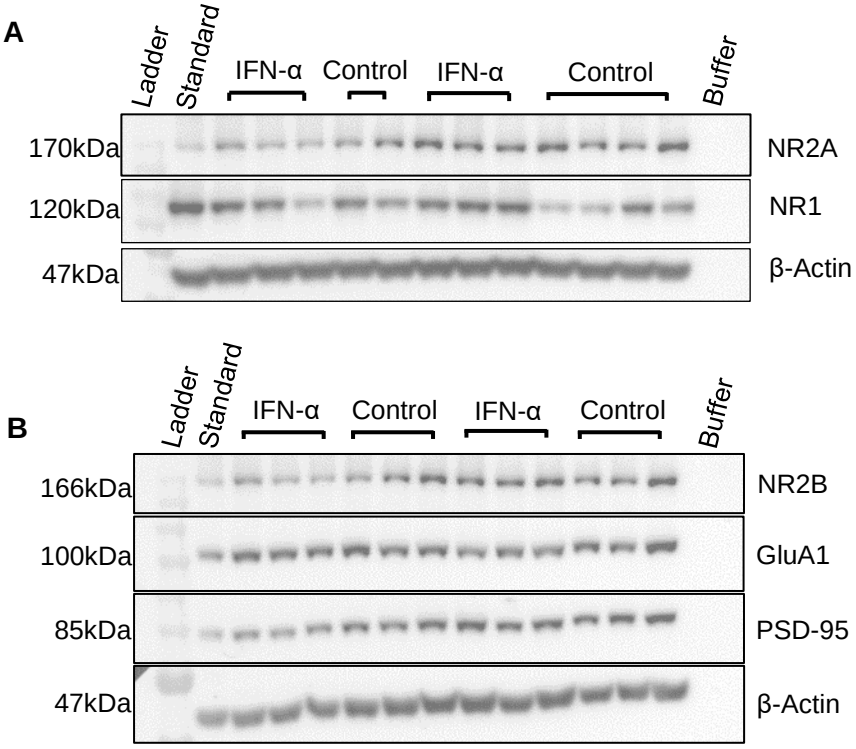


Figure 6.19. Western blot images of glutamatergic/synaptic receptor protein expression in the hippocampus.
Images generated from two Western blot membranes demonstrating expression and molecular weights of NR1, and NR2A (A), and NR2B, GluA1, and PSD-95 (B) against the reference protein β-Actin in control or IFN-α treated rats from Experiment 10.

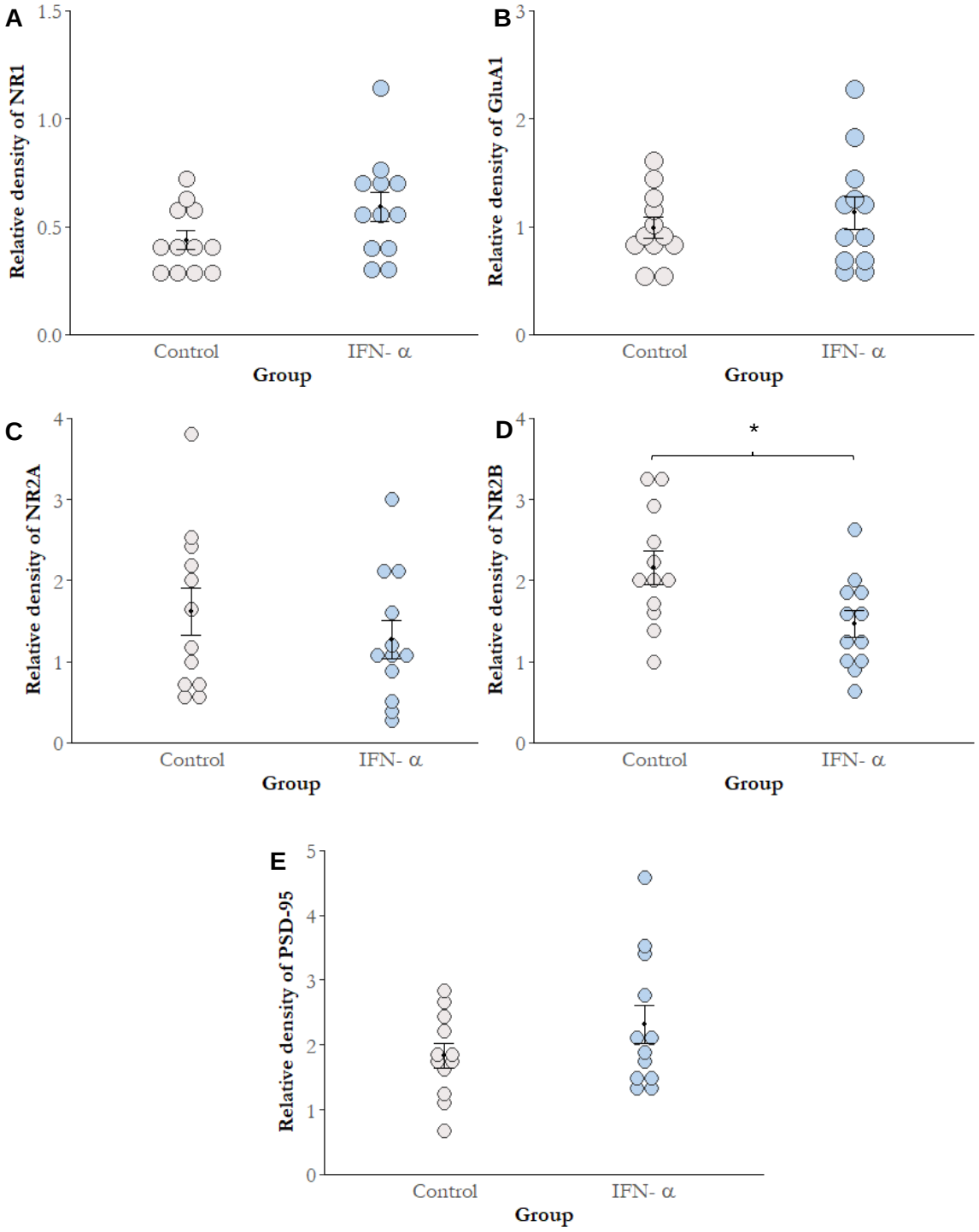


Figure 6.20. Quantified expression of glutamatergic/synaptic receptor proteins in the hippocampus.
Analysis of NR1 (A), GluA1 (B), NR2A (C), and PSD-95 (E) expression in the hippocampus found no difference between control and IFN-α treated rats. Expression of NR2B (D) was significantly reduced in IFN-α treated rats compared to controls.
Dots indicate individual data points, with mean ±SEM. * = p <0.05.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

Western blot analysis of glutamatergic/synaptic protein expression in the frontal cortex

Independent samples t-tests were conducted comparing receptor protein expression in the frontal cortex of control vs IFN-α treated rats, followed by Bayesian independent samples t-tests for each protein analysed.

No difference was found in NR1 expression (Figure 6.22A), $t(22) = 0.81$, $p = 0.43$, supported with only anecdotal evidence, $BF_{01} = 2.09$.

There was also no difference in NR2A (Figure 6.22B), $t(22) = 1.45$, $p = 0.16$, supported by anecdotal evidence, $BF_{01} = 1.26$.

NR2B expression did not differ between the two groups (Figure 6.22C), $t(22) = 0.72$, $p = 0.48$, with also only anecdotal evidence to support this, $BF_{01} = 2.21$.

No significant difference was found for GluA1 expression (Figure 6.22D), $t(22) = 1.89$, $p = 0.071$, however, Bayes analysis found anecdotal evidence in support of a significant group effect, $BF_{10} = 1.30$.

There was no significant difference in PSD-95 expression (Figure 6.22E), $t(22) = 0.34$, $p = 0.74$, supported by anecdotal evidence, $BF_{01} = 2.57$.

These findings show inconclusive evidence indicating no differences between control and IFN-α treated rats in the expression of these receptor subunits in the frontal cortex (see Figure 6.21 for Western blot images).

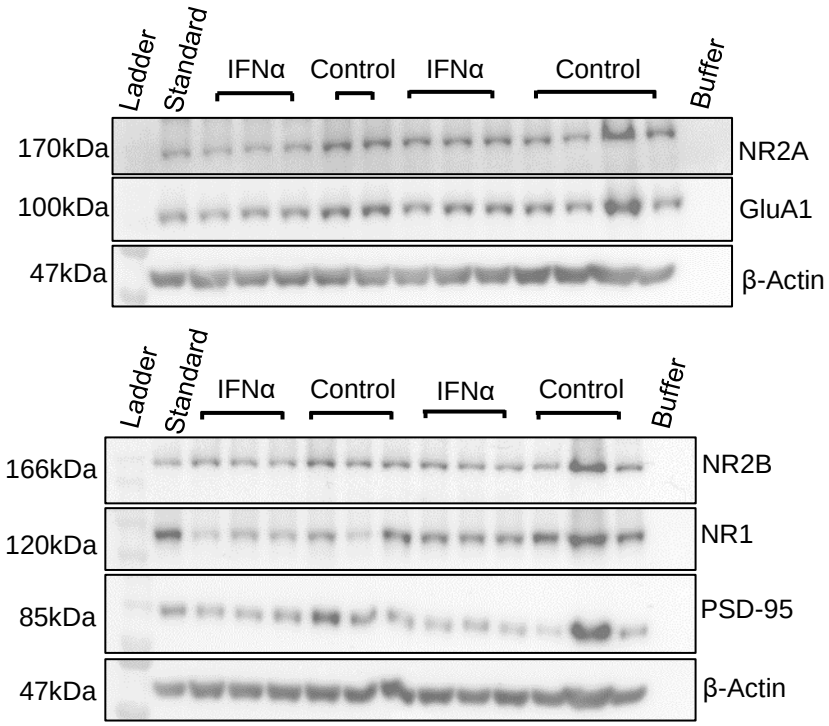


Figure 6.21. Western blot images of glutamatergic/synaptic receptor protein expression in the frontal cortex.

Images generated from two Western blot membranes demonstrating expression and molecular weights of NR2A and GluA1 (A), and NR2B, NR1, and PSD-95 (B) against the reference protein β-Actin in control or IFN-α treated rats from Experiment 10.

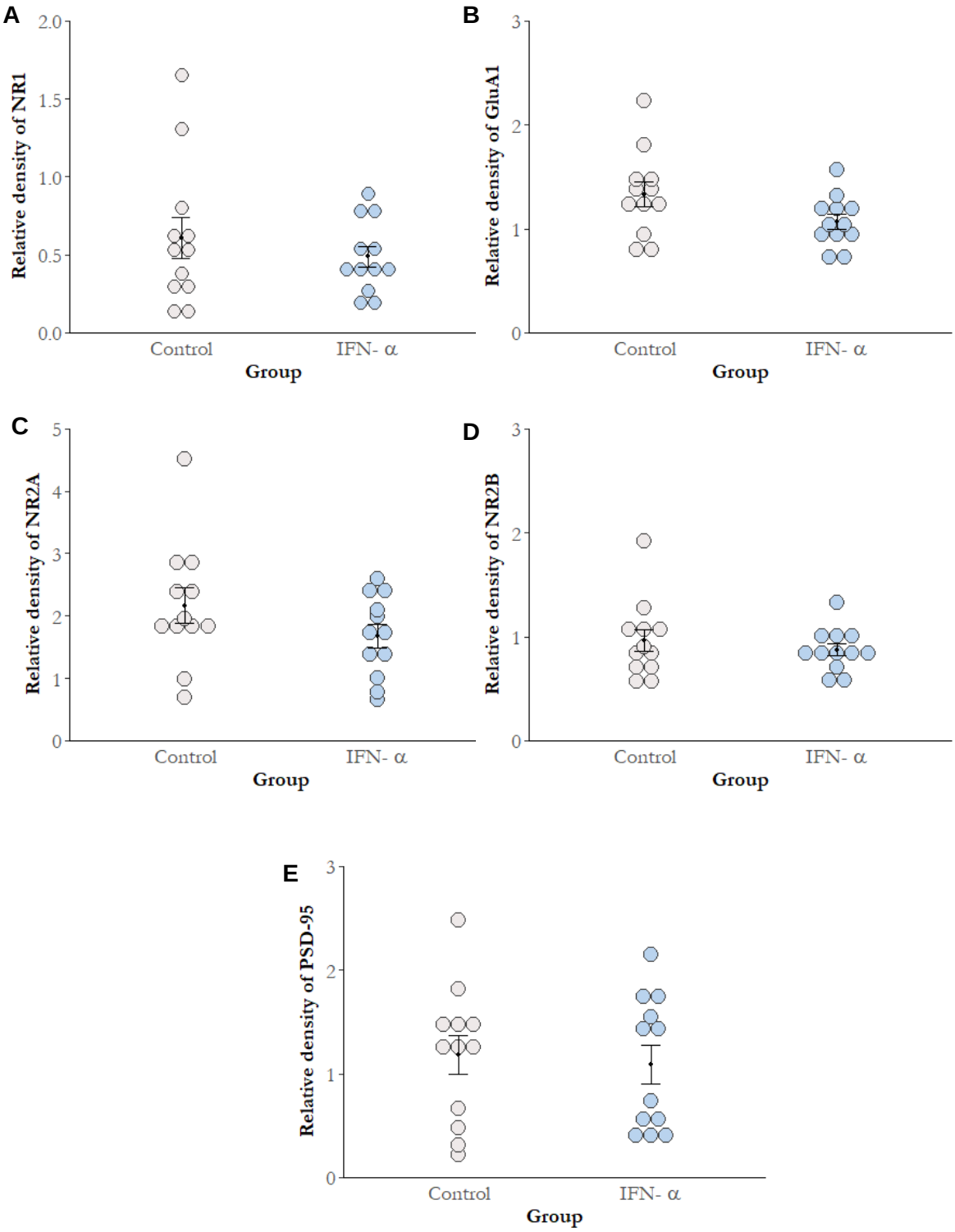


Figure 6.22. Quantified expression of glutamatergic/synaptic receptor proteins in the frontal cortex.
Analysis of NR1 (A), GluA1 (B), NR2A (C), NR2B (D) and PSD-95 (E) expression in the frontal cortex found no difference between control and IFN-α treated rats.
Dots indicate individual data points, with mean ±SEM.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

6.4.2.3. Changes in circulating cytokines and corticosterone in the chronic IFN-α model

Plasma IL-1β levels in the chronic IFN-α model

For assay quality control, an internal control was run in the ELISA with the samples. The internal control provided had a predicted concentration range of 227-328pg/mL. The actual value for this control was 294pg/mL.

A univariate ANOVA showed no overall significant difference between saline controls (male *n*=16, female *n*=16) and IFN-treated rats (male *n*=16, female *n*=15) in plasma IL-1β concentration, $F_{1, 59} = 2.004$, MSE = 772.98, $p = 0.16$, $\eta_p^2 = 0.033$. There was also no difference between males and females, $F_{1, 59} = 1.21$, MSE = 772.98, $p = 0.28$, $\eta_p^2 = 0.02$, and no significant interaction between group * sex, $F_{1, 59} = 2.52$, MSE = 772.98, $p = 0.12$, $\eta_p^2 = 0.041$ (Figure 6.23).

A Bayesian univariate ANOVA found only anecdotal evidence to support a lack of group effect on plasma IL-1β, $BF_{01} = 1.63$, as well as to support the lack of difference between males and females, $BF_{01} = 2.39$ and lack of interaction between group * sex, $BF_{01} = 2.59$.

These findings demonstrate no overall differences in plasma IL-1β concentration between control and IFN-α treated rats, nor between males and females. However, many values were below the threshold of detection or ~0pg/mL, and Bayesian analysis deemed this inconclusive.

These data for plasma IL-1β were not normally distributed as identified via Shapiro-Wilk test for either controls, W(18) = 0.87, $p = 0.018$, or IFN-α treated rats, W(16) = 0.57, $p < 0.001$. Thus, a Kruskal-Wallis non-parametric test was conducted on these data. With males and females combined, there was no significant difference in plasma IL-1β concentration between control and IFN-α treated rats, H(1) = 0.28, $p = 0.60$. Separately, there was no difference between male control and IFN-α treated rats, H(1) = 0.023, $p = 0.88$, nor female control and IFN-α treated rats, H(1) = 1.32, $p = 0.25$.

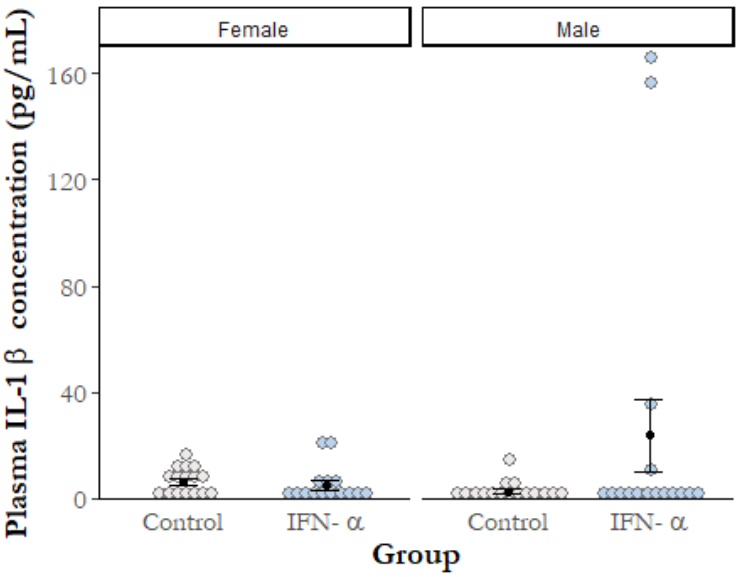


Figure 6.23. Concentration of plasma IL-1β.
There was no difference in IL-1β concentration between control and IFN-treated rats, nor between males and females.
Dots indicate individual data points with the mean ±SEM.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

Plasma TNF-α levels in the chronic IFN-α model

The internal control provided had a predicted concentration range of 107-178pg/mL. The actual value of this control was 143.95pg/mL.

A univariate ANOVA comparing saline control (male *n*=9, female *n*=9) and IFN-treated (male *n*=9, female *n*=8) found no overall significant difference between the two groups in plasma TNF-α concentration, $F_{1, 31} = 3.24$, MSE = 0.64, $p = 0.082$, $\eta_p^2 = 0.095$, nor between males and females, $F_{1, 31} = 0.029$, MSE = 0.64, $p = 0.87$, $\eta_p^2 = 0.001$. There was no interaction between group * sex, $F_{1, 31} = 0.11$, MSE = 0.64, $p = 0.74$, $\eta_p^2 = 0.004$ (Figure 6.24).

A Bayesian univariate ANOVA found almost no evidence in the direction of support for a group effect on plasma TNF-α, $BF_{10} = 1.19$. Moderate evidence was found to support a lack of difference between males and females, $BF_{01} = 3.01$, and lack of interaction between group * sex, $BF_{01} = 4.41$.

These findings demonstrate no overall differences in plasma TNF-α concentration between control and IFN-α treated rats, nor between males and females. However, many values were again below the threshold of detection and Bayesian analysis deemed these findings inconclusive.

These data for plasma TNF-α were not normally distributed as identified via Shapiro-Wilk test for either controls, W(18) = 0.56, $p < 0.001$, or IFN-α treated rats, W(16) = 0.34, $p < 0.001$. Thus, a Kruskal-Wallis non-parametric test was conducted on these data. With males and females combined, there was no significant difference in plasma TNF-α concentration between control and IFN-α treated rats, H(1) = 2.76, $p = 0.097$. Separately, there was no difference between male control and IFN-α treated rats, H(1) <0.001, $p = 1.00$. However, there was a significant difference between female control and IFN-α treated rats, H(1) = 4.28, $p = 0.039$, where female controls had greater plasma TNF-α concentration compared to female IFN-α treated rats.

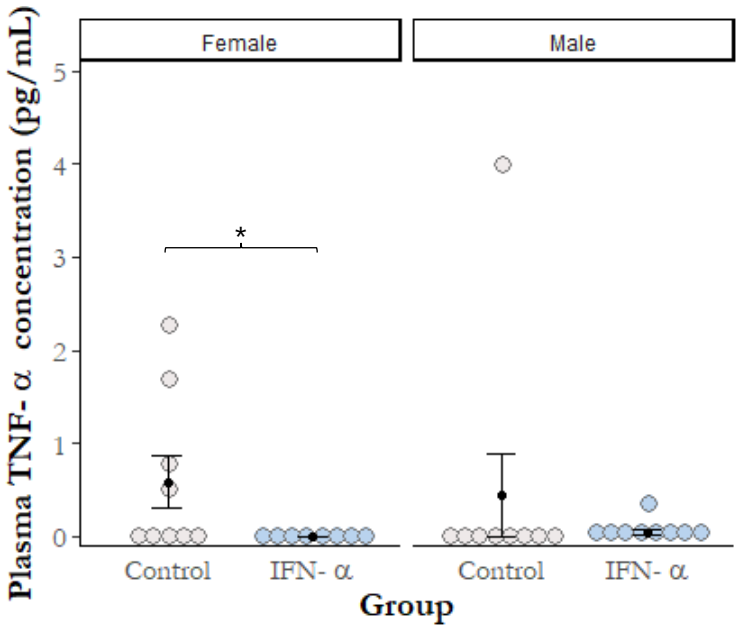


Figure 6.24. Concentration of plasma TNF-α.
There was no overall difference in TNF-α concentration between control and IFN-treated rats, nor between males and females. Female controls had greater plasma TNF-α concentration than female IFN-α treated rats when compared via Kruskal-Wallis non-parametric test.
Dots indicate individual data points with the mean ±SEM. * = $p<0.05$.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

Plasma CORT levels in the chronic IFN-α model

Analysis of plasma CORT levels in a univariate ANOVA showed there was no overall significant difference between saline controls (male n=15, female n=15) and IFN-treated rats (male n=16, female n=16), $F_{1, 58} = 0.035$, MSE = 2065.36, $p = 0.85$, $\eta_p^2 = 0.001$. There was also no overall difference between males and females, $F_{1, 58} = 2.27$, MSE = 2065.36, $p = 0.14$, $\eta_p^2 = 0.038$. There was a significant interaction between group * sex, $F_{1, 58} = 4.56$, MSE = 2065.36, $p = 0.037$, $\eta_p^2 = 0.073$ (Figure 6.25). Fisher's LSD post-hoc analysis

indicated that female IFN-treated rats had significantly higher plasma concentration of CORT compared to male IFN-treated rats (p = 0.011).

A Bayesian univariate ANOVA found moderate evidence to support the lack of overall group effect on plasma CORT, BF_{01} = 3.81. Anecdotal evidence was found to support the lack of difference between males and females, BF_{01}= 1.43.

These findings demonstrate no overall differences in plasma CORT concentration between control and IFN-α treated rats. Female IFN-treated rats showed significantly greater plasma CORT levels compared to male IFN-treated rats, with no overall difference between males and females. However, Bayesian analysis deemed these null findings inconclusive.

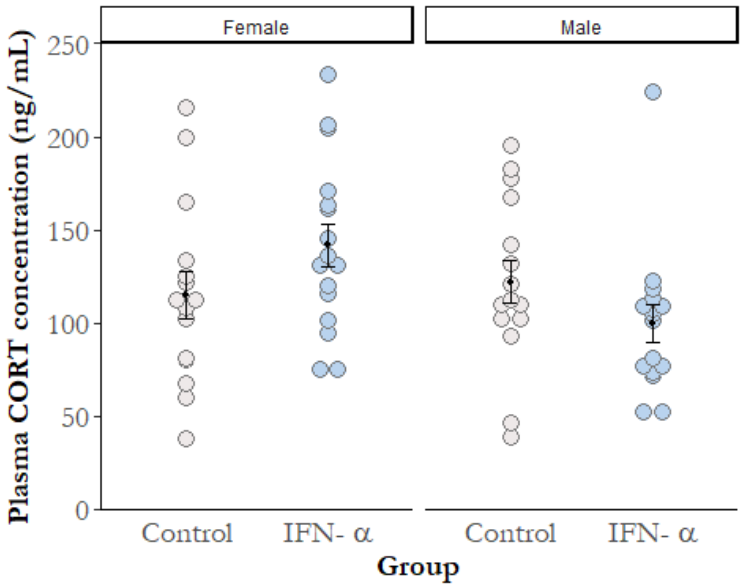


Figure 6.25. Concentration of plasma corticosterone. There was no overall difference in CORT concentration between control and IFN-treated rats. Female IFN-treated rats had significantly greater plasma CORT than male IFN-treated rats.
Dots indicate individual data points with the mean ±SEM.
Note, in some cases the difference on the observed values between animals is smaller than the resolution of the figure. Where this occurs, individual data points are represented side by side.

6.4.2.4. Identifying correlations between behaviour and neurobiological markers

Pearson's correlations within individual groups were conducted between neurobiological markers and lick cluster size (reported in section 4.4) and/or preference for CS-A^{++} vs CS-B^{+} as an indication of positive affective bias (reported in section 3.4). Only significant correlations between behaviour and neurobiological markers are reported here. It should be noted that interpretation of these correlations must be done cautiously considering the many comparisons undertaken, many non-significant correlations and the likelihood of significant correlations becoming non-significant if a multiple-comparison correction had been applied.

Experiment 10 cohort

Control rats showed a significant positive correlation between lick cluster size for 16% sucrose and NR2A expression in the frontal cortex, R = 0.61, p = 0.036 (Figure 6.26A). IFN-treated rats showed a significant negative correlation between lick cluster size for 4% sucrose and NR2B expression in the hippocampus, R = -0.58, p = 0.05 (Figure 6.26B) as well as with NR1 expression in the frontal cortex, R = -0.64, p = 0.025 (Figure 6.26C).

Experiment 11 cohort

Female control rats showed a significant positive correlation between preference for CS-A^{++} vs CS-B^{+} in the flavour m-ABT and the number of Ki67-positive neurons in the ventral hippocampus, R = 0.77, p = 0.042 (Figure 6.27A). There was also a significant negative correlation between this preference for CS-A^{++} vs CS-B^{+} and plasma CORT concentration, R = -0.57, p = 0.027 (Figure 6.27B).

In male control rats, there was a significant negative correlation between preference for CS-A^{++} vs CS-B^{+} in the flavour m-ABT and the number of Iba1-positive cells in both the amygdala, R = -0.74, p = 0.036 (Figure 6.27C), and the ventral hippocampus, R = -0.77, p = 0.044 (Figure 6.27D). A non-significant trend was found between this preference for CS-A^{++} and Iba1-positive cells in the dorsal hippocampus, R = -0.68, p = 0.064. A significant positive correlation was also found between lick cluster size for 16% sucrose and the number of DCX-positive cells in the ventral hippocampus, R = 0.84, p = 0.037 (Figure 6.27E).

Female IFN-treated rats showed no significant correlations between behaviour and any neurobiological markers.

Male IFN-treated rats showed a significant positive correlation between lick cluster size for 16% sucrose and plasma IL-1β concentration, R = 0.71, p = 0.002 (Figure 6.27F). No other significant correlations were found.

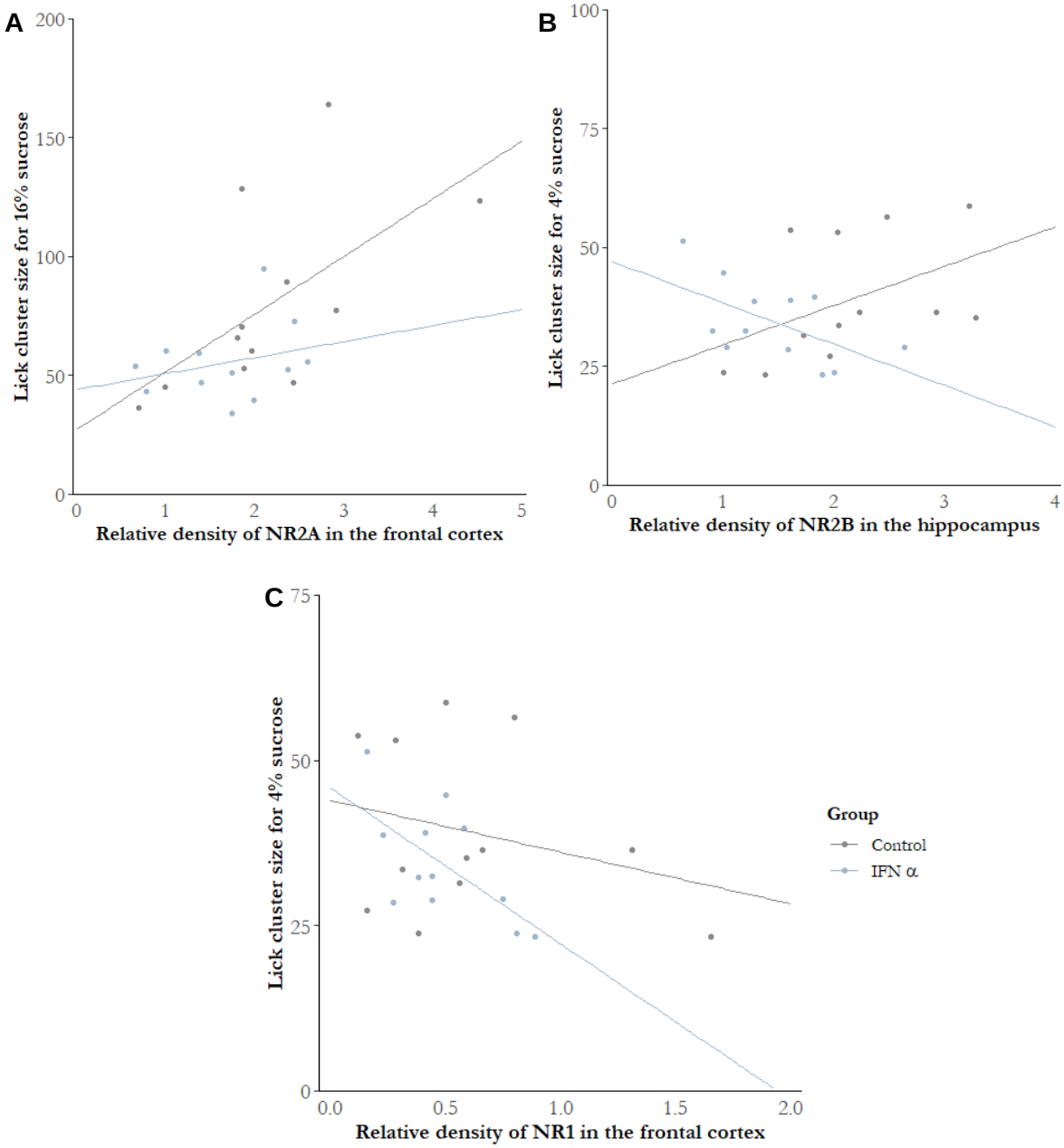


Figure 6.26. Correlations between lick cluster size and relative density of NMDA receptor subunits in the Experiment 10 cohort.
In control rats, a significant positive correlation was found between lick cluster size for 16% sucrose and relative density of NR2A in the frontal cortex (A). In IFN-treated rats, a significant negative correlation was found between lick cluster size for 4% sucrose and relative density of NR2B in the hippocampus (B) and relative density of NR1 in the frontal cortex (C). Dots indicate individual data points with the mean ±SEM.

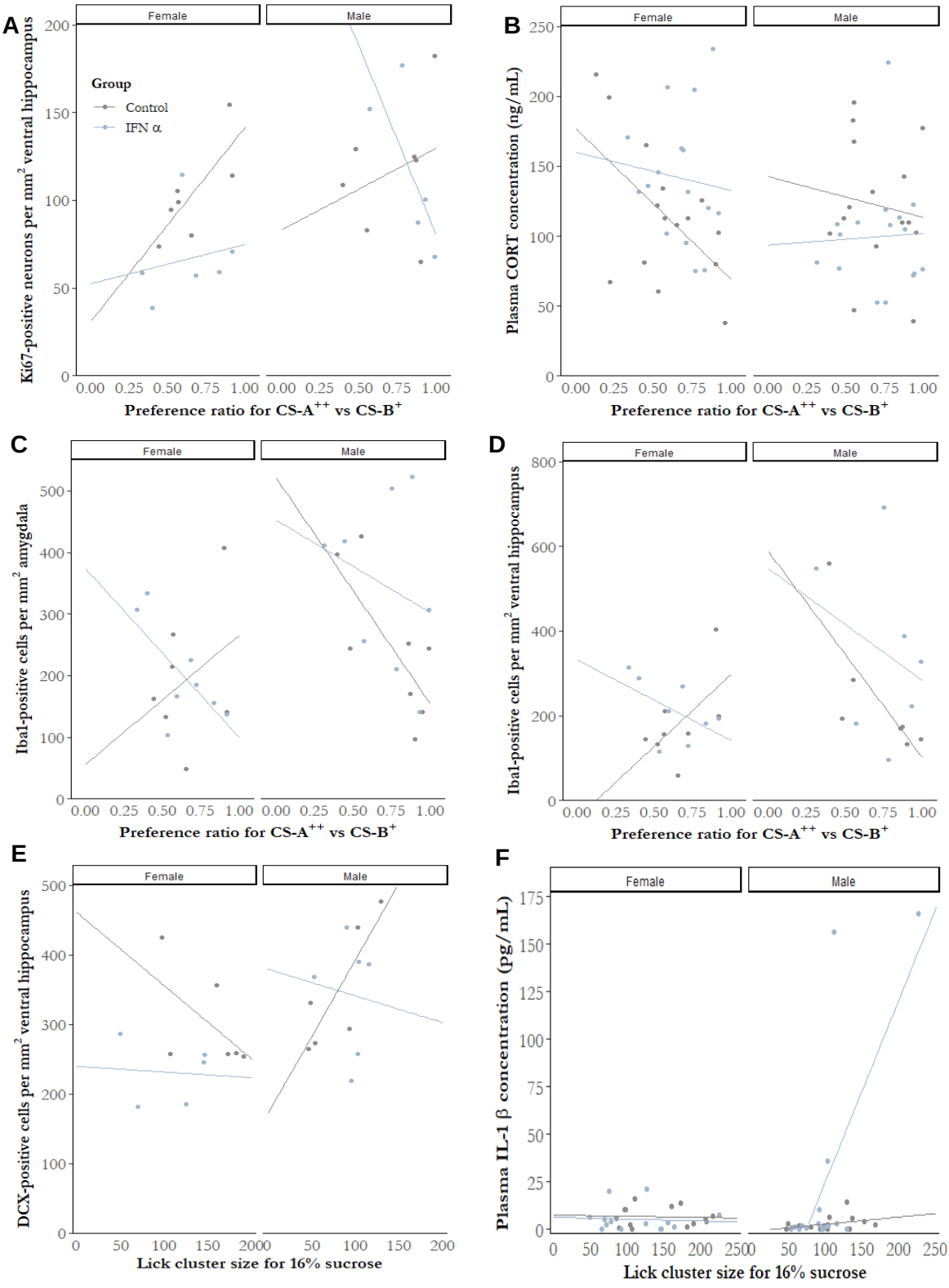


Figure 6.27. Correlations in the Experiment 11 cohort.
In female control rats, positive affective bias was positively correlated with proliferating cells in the ventral hippocampus (A) and negatively correlated with plasma CORT concentration (B). In male controls, positive affective bias was negatively correlated with the number of microglial cells in the amygdala (C) and ventral hippocampus (D). Lick cluster size for 16% sucrose was positively correlated with number of immature neurons in the ventral hippocampus (E). In male IFN-treated rats, lick cluster size for 16% sucrose was positively correlated with plasma IL-1β (F). No correlations were found in female IFN-treated rats. Dots indicate individual data points with the mean ±SEM.

Together these findings demonstrate potential associations between behaviour and some neurobiological markers in control vs IFN-α treated rats. However, these interpretations should be taken with caution and are stated simply to demonstrate potential avenues for future exploration.

Male control rats in Experiment 10 appeared to show an association between greater lick cluster size for 16% sucrose and increased NR2A expression in the frontal cortex. Of note, this was the opposite relationship to be found in control rats in Experiment 8.

In Experiment 11, male control rats showed an association between reduced positive affective bias and elevated microglia expression in the amygdala and ventral hippocampus. In addition, male controls showed an association between greater lick cluster size for 16% and increased immature neuron expression in the ventral hippocampus, which again was not a relationship consistent with the results from male control rats in Experiment 8.

In contrast, male IFN-treated rats did not show any of these same correlations but did appear to show an association between greater lick cluster size for 16% sucrose and elevated plasma IL-1β concentration. Female control rats demonstrated an association between greater positive affective bias and increased cell proliferation in the ventral hippocampus, as well as reduced plasma CORT.

6.4.3. Summary

Section 6.4 of this thesis aimed to characterise the neuronal and biological changes associated with chronic IFN-α treatment, with the aim of highlighting a relationship between these changes and associated reward-related deficits. Here, I present findings from the immunohistochemical analysis of neurogenic markers, Western blot analysis of glutamatergic receptor subunits, and immunoassays of circulating cytokines.

These findings demonstrate overall greater expression of microglia per mm^2 in male rats compared to females in the amygdala and dorsal hippocampus. There was no overall group effect on microglia expression in the amygdala or ventral hippocampus, but significantly more microglia per mm^2 of dorsal hippocampus were found in IFN-treated rats compared to controls. This finding suggests greater microglia activation in IFN-α treated rats compared to controls, which indicates that IFN-α treatment did appear to activate the immune response.

Analysis of neurogenic markers demonstrated that IFN-α treated rats had significantly greater expression immature neurons in the dorsal hippocampus compared to controls. The number of proliferating cells per mm^2 of dorsal hippocampus was elevated only in male IFN-

treated rats compared to male controls, with no overall group difference. There was no effect of IFN-α treatment on expression of neurogenic markers in the ventral hippocampus.

There were no overall differences between the two groups in expression of NR1, NR2A, GluA1 or PSD95 in either the hippocampus or frontal cortex. IFN-α treated rats had greater expression of NR2B in the hippocampus, but not frontal cortex, compared to controls. In addition, there were no differences between the groups in plasma IL-1β or CORT concentrations. After non-parametric analysis, TNF-α concentration in female controls was found to be greater compared to female IFN-α treated rats, with no difference in males.

However, much of this evidence was again deemed inconclusive by Bayes analysis, suggesting a greater sample size may be required in future to determine conclusive evidence of the effects of this low dose of IFN-α treatment on various neurobiological markers.

Correlations of these markers with behaviours characterised in previous chapters of this thesis demonstrated that in female controls, a greater preference for a higher rewarded stimulus compared to a lower rewarded stimulus – indicative of reward-induced positive bias - was associated with a greater number of proliferating cells in their ventral hippocampus, as well as reduced plasma CORT concentrations. In male controls, a greater positive bias was associated with a reduced number of microglial cells in the amygdala and ventral hippocampus. In addition, a greater lick cluster size for 16% sucrose was associated with a greater number of immature neurons in the ventral hippocampus. Whereas, in male IFN-treated rats, a greater lick cluster size for 16% sucrose was associated with elevated plasma IL-1β concentration. As previously, these interpretations of correlations should be taken with caution and are highlighted to demonstrate potential avenues for future exploration only.

6.5. Discussion of Chapter 6

This chapter aimed to characterise changes in various biological markers relating to the pathophysiology of psychiatric disease in two models of MDD, the chronic CORT and chronic IFN-α models. Identifying these changes could elucidate the biological pathways affected by these risk factors of stress and inflammation, and by combining these findings with behavioural characterisation of these models could allow the detection of neurobiological changes that lead to the development of reward-related deficits.

6.5.1. Effects of chronic CORT treatment on markers of neurogenesis, glutamatergic receptors and circulating cytokines

Chronic CORT and vehicle treated rats from Experiment 8 and 9 were analysed for changes in markers of neurogenesis, glutamatergic receptor subunits, and circulating cytokines. The general findings from these analyses demonstrated no significant changes in chronic CORT-treated rats compared to vehicle-treated rats in any of these markers.

Whilst previous research on the effects of chronic CORT on these markers has in some cases been variable, a substantial amount of evidence had demonstrated that chronic CORT treatment reduces the number of DCX-positive neurons in the dorsal hippocampus, as discussed in section 6.1. Published studies demonstrating this effect mostly used subcutaneous implants of CORT-releasing pellets over 21 days (e.g. Diniz et al., 2013), or subcutaneous injection of 40mg/kg CORT daily for between 14-21 days (e.g. Haas et al., 2020; Jiang et al., 2013; Yau et al., 2016). In contrast, several studies administering 35µg/ml CORT via drinking water to mice for between 4-12 weeks did not demonstrated this same reduction in DCX-positive neurons in the hippocampus (e.g. Agasse et al., 2020; Mekiri, Gardier, David, & Guilloux, 2017; Mendez-David et al., 2020). However, Kott et al. (2016) did find that female rats that received 200µg/mL CORT dissolved in their drinking water daily for 20 days had reduced DCX-positive neurons in the ventral, but not dorsal, hippocampus.

Thus, although evidence mostly suggested that chronic CORT treatment in rats would reduce the expression of DCX-positive neurons, these findings have been variable depending upon the route of administration and dose. It is possible that administration of CORT in the drinking water is not a reliable method for inducing neurogenic deficits associated with stress. In addition, for many of the studies using alternative methods of administration, rats were anaesthetised with their brains perfused within 48 hours of the final CORT dose. Administration of CORT in drinking water requires a washout period, and in the case of my experiments 8 and 9 behavioural assessments were undertaken following

this washout, so there was a time delay from the end of CORT administration and collection of tissue, which may have influenced the findings here.

Previous evidence has suggested that the number of Ki67-positive cells in the hippocampus reduces following chronic CORT treatment via either subcutaneous pellet implants or injections in rats (e.g. Brummelte & Galea, 2010; Huang & Herbert, 2005; Pinnock & Herbert, 2008), as well as following CORT administration in drinking water in mice (e.g. Agasse et al., 2020). Although measurement of Ki67-positive cells following chronic CORT administration via drinking water has been sparsely reported, this one example does suggest it is possible to detect disruption to neurogenesis in this model, thus the lack of difference detected in this chapter could again be due to the time delay or variability with this method of administration.

Similarly, evidence of changes in glutamatergic receptor expression in the hippocampus and/or frontal cortex has been reported previously following chronic CORT treatment via subcutaneous pellet implants (e.g. Cohen et al., 2011), intraperitoneal injection (e.g. Li, Xie, et al., 2017) and drinking water (Gourley et al., 2009). However, these studies have variable findings for different subunits, and other studies have also reported no change following chronic CORT treatment via drinking water (e.g. Buret & van den Buuse, 2014), thus the lack of change reported in this thesis is not entirely unexpected.

In addition, no other studies to my knowledge have measured plasma concentrations of IL-1β or TNF-α in the chronic CORT model however, as discussed in the introduction of this chapter, other models of chronic stress have shown to elevate both these cytokines whilst chronic CORT was not expected to change IL-6 concentration. Unexpectedly, this cohort of animals showed no change in plasma CORT concentration (see section 3.4.3) contrary to previous literature that has demonstrated elevated serum or plasma CORT following chronic CORT injections (e.g. Karatsoreos et al., 2010; Lee, Sur, Shim, Lee, & Hahm, 2015). Although Xu et al. (2011) showed no overall change in serum CORT after 8 weeks of low dose (20µg/mL) CORT in drinking water, whilst Karatsoreos et al. (2010) only found elevated plasma CORT levels during the “light” phase in rats that received a higher dose of 100µg/mL. This could suggest that the reason plasma CORT was not elevated was due to the time of day for blood collection rather than CORT treatment not working, however, body weight was also unchanged in these animals to support this theory that CORT was not incorporated appropriately. Therefore, measurement of cytokines was unreliable here.

6.5.2. Effects of chronic IFN-α treatment on markers of neurogenesis, glutamatergic receptors and circulating hormones/cytokines

Results from this chapter indicated that IFN-treated rats from Experiment 10 had increased expression of NR2B in the hippocampus compared to controls, with no other changes in glutamatergic or postsynaptic receptor subunits. NR2B is thought to be implicated in LTP and thus is important for synaptic plasticity (see Loftis & Janowsky, 2003). Previous research has indicated NR2B in the hippocampus plays a role in spatial working memory (von Engelhardt et al., 2008) and overexpression of NR2B in mice was associated with increased performance in learning and memory tasks such as novel object recognition or fear conditioning (Tang et al., 1999). In addition, evidence has shown decreased NR2B expression in the frontal cortex linked to reduced depression-like symptoms in mice (Miller et al., 2014), whilst other rodent models of depression also display elevated NR2B expression (e.g. Peng et al., 2016). Together with previous clinical development of an NR2B antagonist for treating depression ("NR2B antagonist pursued for treatment-resistant depression," 2009) and evidence of NR2B antagonism reducing depression symptoms in rodent models and MDD patients (e.g. Jiménez-Sánchez, Campa, Auberson, & Adell, 2014; Preskorn et al., 2008), this evidence suggests elevated NR2B expression could contribute to development of depression-like symptoms.

IFN-treated rats from Experiment 11 showed elevated expression of microglia in the dorsal hippocampus. This elevation in microglia demonstrates an immune response to IFN-α treatment that corresponds with previous literature (e.g. Aw et al., 2020; Wachholz et al., 2016) and indicates that IFN-α treatment in these animals worked as expected.

Analysis of neurogenic markers showed increased expression of immature neurons in the dorsal hippocampus of IFN-treated rats compared to controls, with elevated proliferating cells also found only in male IFN-treated rats. It should be noted that results from these analyses should be treated with caution due to poor staining in some samples, as seen in Figure 6.17, due to potential issues with antibodies.

This finding is not consistent with previous literature discussed in the introduction of this chapter, which indicate reduced neurogenic markers following chronic treatment with IFN-α. However, several differences between the cohort of animals here and those used in previous studies are of note; firstly, the dose of IFN-α administered in this cohort was very low compared to other studies (100IU/kg compared to range from 5000IU/kg to 1000000IU/kg). It has been suggested that an alternate type of microglial activation enhances neurogenesis, whereas classic microglial activation can reduce this (e.g. Kohman & Rhodes, 2013). Whilst I did not conduct detailed analysis of the morphology of these

microglia as the measurement of Iba1 staining was conducted to simply act as a positive control for IFN-α treatment, it is possible that a lower dose of IFN-α may activate microglia in this alternate way whereas a higher dose as used in previous literature could cause the classical activation resulting in reduced neurogenesis.

In addition, Zheng et al. (2014) reported no change in Ki67 or DCX expression after 2 weeks of daily IFN-α treatment, but a reduction after 4 weeks, whilst Kaneko et al. (2020) also demonstrated reduced DCX expression in marmosets after 4 weeks. In this cohort, rats received 2 weeks of IFN-α treatment before starting behavioural assessment (see Appendix A for timelines) with continuous treatments giving a total of 29-30 days of IFN-α administration. Thus, the number of treatments received does not explain this variability, and Kaneko et al. (2006) found a reduction in cell proliferation after only 7 consecutive days of treatment.

However, one major difference was that in this cohort behaviour tests were conducted prior to analysis of brain tissue, whereas in these previous studies only brain tissue analysis was conducted after drug treatments. Therefore, it is possible that IFN-α alone may reduce neurogenesis, but when exposed to a task involving learning and memory such as the flavour m-ABT described in Chapter 3, this effect is reversed. Adult neurogenesis has been suggested to play an essential role in the ability to learn and form memories, in addition to some links with regulating emotional memory and stress response (Cameron & Glover, 2015). However, neurogenesis has been shown to be enhanced with learning (e.g. Gould, Tanapat, Rydel, & Hastings, 2000; Shors, Townsend, Zhao, Kozorovitskiy, & Gould, 2002). These theories suggest that the formation of new neurons may be a consequence of learning and could be essential in storing learned information as memory. Thus, a learning task such as the flavour m-ABT would be expected to increase neurogenesis overall. This finding that IFN-α treatment appeared to elevate this further could indicate that activation of the immune system increases the dynamic nature of the dentate gyrus to allow rapid formation of new neurons in response to learning.

Furthermore, results from this chapter demonstrated no significant difference between IFN-treated rats and controls in circulating CORT or IL-1β levels. TNF-α concentration appeared to be reduced in female IFN-treated rats compared to female controls, with no change in males and only following non-parametric analysis. In contrast, Callaghan et al. (2018) reported elevated serum CORT, IL-1β and IL-6 after chronic treatment with 170000IU/kg human IFN-α in rats, whilst Anisman et al. (2007) showed acute administration of murine IFN-α ranging from 25000 – 200000IU/kg in mice resulted in elevated plasma CORT with no change in TNF-α or IL-6. These previous findings suggest a change in CORT and potential changes in cytokines would be expected following chronic IFN-α treatment.

Thus, the differences found in this cohort is again likely due differences in the IFN-α dose administered, with a low dose of 100IU/kg potentially not activating the HPA axis to elevate CORT levels and having a lesser effect on other pro-inflammatory cytokines. Comparison with reports of baseline CORT levels in Sprague-Dawley rats indicate the values reported here fall within the expected normal range (Bekhbat, Glasper, Rowson, Kelly, & Neigh, 2018). With regards to both the CORT cohort discussed above and this IFN-α cohort, the cytokine levels reported appear to be relatively low and in several cases were below the detectable threshold, but these values are similar to those reported in the plasma of control rats elsewhere (e.g. Anisman et al., 2007; Ruff & DeVore, 2014). Serum levels as reported elsewhere appear to be generally higher than these values and therefore extracting serum may better maintain the integrity of cytokines for future analysis (Abramova et al., 2013; Callaghan et al., 2018; Mukhamedshina et al., 2017).

6.5.3. Correlations between behaviour and neurobiological markers

Correlations between the behaviours measured in previous chapters of this thesis and these biological markers were explored in this chapter. Some relationships were identified, particularly in male control groups, however these were not preserved across the different control groups of the CORT and IFN-α experiments. In addition, there were limited sample sizes for many markers that increased the difficulty of providing conclusive interpretation from these results. Given the number of correlations conducted, it is possible that any significant findings here were due to chance, which could explain the lack of similar results found between control groups.

Nevertheless, the correlations reported in the CORT experiments indicate potential relationships between hedonic response and NMDA receptors, as well as plasma TNF-α. Whilst the IFN-α experiments indicate potential relationships between affective bias and neurogenesis, neuroinflammation, and circulating CORT, whereas hedonic response was associated with neurogenesis and plasma IL-1β. These findings suggest there may be some relationships between these neurobiological markers and reward processing, and further investigation is required to provide firm conclusions regarding these relationships.

6.5.4. Summary

In conclusion, the findings from Chapter 6 are variable against previous literature which could be due to several factors including limited reliability of the CORT administration method, varying doses used in other literature, and exposure to behaviour prior to analysis of tissue. These findings can be used to characterise the effects of these specific drug doses on biological markers which will be discussed with regards to behavioural changes in the general discussion of this thesis.

Chapter 7 – General Discussion

7.1. Thesis overview

Deficits in reward processing are characteristic of psychiatric disorders such as MDD and schizophrenia, but the heterogeneity of these conditions and lack of effective treatments highlights a clinical need to dissociate these reward-related deficits and improve understanding of their neurobiological underpinnings.

The overall aim of this thesis was to develop and apply rodent behavioural assays to separate three main mechanisms of reward processing: cognition, motivation, and hedonic response; and to elucidate their underlying neurobiological mechanisms. The aims and key findings for each experimental chapter are summarised in Table 7.1.

In Chapter 2, novel flavour preference-based assays for affective bias and reward-induced bias were developed, where the flavour modified-affective bias test (m-ABT) appeared to be capable of detecting a reward-induced positive bias. This flavour m-ABT was then applied to preclinical models of psychiatric disease in Chapter 3, where some evidence suggested deficits were present, but these findings were inconsistent across study replications. Chapter 4 evaluated the effects of these preclinical models on hedonic response to reward and demonstrated evidence to support a lack of deficit following chronic corticosterone (CORT) treatment, contradictory to prior evidence in traditional assays for consummatory anhedonia, as well as an expected lack of hedonic deficit following chronic interferon-alpha (IFN-α) treatment.

Similarly, motivation for reward evaluated in Chapter 5 appeared to be unaffected by acute CORT and IFN-α treatment, with some suggestion that chronic treatment may impair this, but evidence was limited and influenced by issues during training. Some preliminary evidence indicated increased general motivation or habitual responding in the heterozygous *CACNA1C* model (CACNA1C$^{+/-}$) model, though only in male rats and with low sample power. Chapter 6 assessed neurobiological changes following chronic CORT and IFN-α treatment, aiming to associate differences in these changes with any discrepancies in behavioural deficits. Findings in this chapter highlighted potential changes in neurogenic markers and glutamatergic markers in the IFN-α model, but not in the CORT model, however, these findings were variable and require further assessment.

Overall, the results of these experiments raise some issues with the validity of the novel flavour m-ABT and the methods used to induce a depressive state in these preclinical models, but have also identified potential failings using traditional methods for consummatory anhedonia and highlighted possible avenues for future exploration.

Table 7.1. A summary of experimental aims, key findings, and future directions for each chapter of this thesis.

Aims	Key findings	Future directions
Chapter 2 Develop and evaluate a novel flavour preference-based assay for affective bias (Experiment 1) and reward-induced positive bias (Experiment 2)	• The flavour ABT did not show changes in affective bias following acute pro- or anti-depressant treatments. • The flavour m-ABT detected reward-induced positive bias in healthy rats, whereas the digging m-ABT could not detect this in these same animals.	• Re-examine and optimise the flavour m-ABT design, e.g., including sucrose reward during choice tests for positive feedback. • Evaluate alternative automated methods for detecting biases in reward learning or affective bias, e.g., lever-based, or texture-based operant task.
Chapter 3 Apply the novel flavour m-ABT to investigate the effects of chronic CORT (Experiment 3 and 4), chronic IFN-α (Experiment 3 and 5), and heterozygous *CACNA1C* deletion (Experiment 6 and 7) on reward-induced positive bias	• An initial experiment showed a potential deficit in reward-induced positive bias in the chronic CORT and IFN-α models, however, follow-up experiments did not replicate this. • The CACNA1C model initially showed no deficit in positive bias, but a follow-up experiment with altered sucrose concentrations did find a deficit.	

Aims	Key findings	Future directions
Chapter 4 Examine hedonic deficits following chronic CORT (Experiment 8 and 9), and chronic IFN-α treatment (Experiment 10 and 11)	• A deficit was found initially in the chronic CORT model, but this was not replicated in follow-ups. • A consistent lack of hedonic deficit was shown in the chronic IFN-α model, matching with previous evidence.	• Application of this assay to other models of psychiatric disease, such as following exposure to environmental stress, and assessment of treatments alone or as symptom reversal.
Chapter 5 Investigate motivation for reward following CORT and IFN-α treatment (Experiment 12 and 13), and heterozygous *CACNA1C* deletion (Experiment 14)	• All groups showed elevated preference for pellets vs chow post-treatment but this was reduced in the chronic CORT and IFN-α groups compared to controls. Evidence of a direct deficit in motivation for reward was limited. • Acute CORT treatment increased chow consumption, with no impact on lever presses. Acute IFN-α had no effect on either variable. • The CACNA1C model had elevated lever presses without chow present, but no change with the presence of chow.	• Dose-response study with acute IFN-α at higher doses and monitoring sickness behaviours. • Replication of CACNA1C study with greater sample size.
Chapter 6 Examine neurogenic and biochemical changes in the chronic CORT and IFN-α models	• No changes were found in plasma CORT, IL-1β, or IL-6 following chronic CORT or IFN-α treatment. • Chronic CORT did not alter expression of DCX, Ki67, NR1, NR2A, NR2B, GluA1, nor PSD-95. • Chronic IFN-α elevated expression of DCX, Ki67, NR2B, and microglial activation in the hippocampus. TNF-α was reduced in female IFN-α rats only.	• Examine neurobiological changes in CACNA1C model. • Investigate changes in these markers immediately following chronic IFN-α treatment, without exposure to behavioural assessments first.

7.2. Dissociating cognitive, motivational, and hedonic aspects of reward processing

7.2.1. Separating reward-related deficits in preclinical models of psychiatric disease

Together, findings from Chapter 3, and 4 suggest there may be a potential reward learning deficit in the chronic CORT and IFN-α models without the presence of hedonic deficits, which would support previous evidence in the IFN-α model where a deficit was present in the digging m-ABT but not in the SPT (Stuart et al., 2017). These findings would suggest that hedonic and cognitive processing of rewards can be dissociated from cognitive processing.

Chapter 5 did not directly demonstrate an effect of chronic or acute CORT or IFN-α treatment on motivational deficits, but issues from the chronic effort-related choice (EfR) study (Experiment 12) resulted in an increase in preference for pellets vs chow in all groups during the post-treatment time point compared to pre-treatment. Both CORT and IFN-α treatment groups had lower increases in this preference than the control group, which could indicate a deficit was present post-treatment, however, this was not clearly evidenced and so firm conclusions here cannot be made. In addition, given the variability of results from the flavour m-ABT, the reliability of evidence for reward learning deficits measured in this assay is unclear, but the digging m-ABT evidence previously gathered in these models can support this conclusion for IFN-α treatment (Stuart et al., 2017). Repetition of the chronic study in EfR that addresses the issues identified in my experiment and exploring chronic CORT in the digging m-ABT could provide more conclusive evidence of the ability to separate these three mechanisms.

In addition, previous evidence has demonstrated a hedonic deficit in the CACNA1C$^{+/-}$ model (Gasalla Canto et al., 2019) whilst my findings suggest this model also appears to show a deficit in reward-induced positive bias, without a deficit in motivation. The evidence of positive bias deficit was only present in male rats and together with the motivation for reward data it was limited by the low sample size of this experiment, but this may indicate that motivation for reward can be dissociated from hedonic and cognitive processing. Thus, along with the chronic pro-depressant experiments, these findings would support the hypothesis that these aspects of reward processing are mediated by separate mechanisms and should be treated as isolated processes when evaluating preclinical models or treatments.

In all three of these preclinical models, there was a potential suggestion of reward learning deficit as well as no clear deficit in motivation. Whilst it can be implied that hedonic processing is separate from motivational and cognitive given the variation in this aspect

between different models, the lack of motivational deficit means it cannot be confirmed that the presence of such a deficit would not concurrently impair other processing mechanisms. Thus, it would be useful to assess the effects of a motivation-impairing agent in hedonic and cognitive tasks.

Haloperidol, a dopamine receptor antagonist, was used as a control for the EfR assay in Experiment 13 and induced a clear dose-dependent reduction in motivation for reward. Previous evidence has also shown that haloperidol also impairs reward learning in a probabilistic reward task (Negrelli et al., 2020), and given that the choices in this cognitive task required an equal effort expenditure, reduced preference for the more frequently rewarded choice should not reflect a deficit in motivation but rather learning and memory. This also supports other evidence that reward response bias in the PRT is mediated by dopamine function (Der-Avakian et al., 2013). In addition, evidence of haloperidol's effects on hedonic response alone have not been reported to my knowledge, but Morais et al. (2017) showed that haloperidol reversed a stress-induced reduction in sucrose preference in the SPT, thus having a therapeutic effect on hedonic response. This evidence suggests again that hedonic processing can be dissociated from motivational and cognitive mechanisms, but it cannot be concluded that motivational deficits can be present without cognitive deficits. Thus, further exploration of other pharmacological agents in tasks dissociating these three mechanisms may provide additional insight to whether motivational deficits can be isolated from reward learning deficits.

7.2.2. Evaluating the sensitivity and specificity of behavioural assays

In addition to determining whether these mechanisms can be dissociated, this thesis aimed to evaluate whether the behavioural assays used throughout this thesis are appropriate for this purpose.

Flavour m-ABT

As discussed in Chapter 3, although rats were able to form positive biases in the flavour m-ABT, pro-depressant treatment or genetic variation did not produce reliable or consistent results across replicated studies, unlike findings reported in the digging m-ABT (Hinchcliffe et al., 2017; Stuart et al., 2019; Stuart et al., 2017). Thus, this indicates underlying issues with the flavour m-ABT itself that could impact its ability to dissociate reward learning deficits from other aspects of reward processing.

This may be due to underlying differences in flavour preference conditioning compared with associating other stimuli with reward. It has been shown that the amygdala is required for assigning emotional significance or value to stimuli (Weymar & Schwabe, 2016) and to support this, Stuart et al. (2015) showed the amygdala was required to detect changes in

affective bias in the ABT. This study also showed that the amygdala was not required for measuring changes in reward-induced positive biases in the m-ABT, suggesting the amygdala is involved in learning the initial stimulus-reward association and assigning emotional valence to this, whilst recall of this association is mediated by the mPFC. However, evidence indicates that flavour preference learning is also mediated by the amygdala and mPFC (Bodnar, 2018; Dwyer & Iordanova, 2010; Yamamoto & Ueji, 2011), thus the main neural circuits involved in these different tasks should not differ.

Flavour preference learning can often involve repeated pairings (Ueji & Yamamoto, 2012), whereas conditioned taste aversion is faster/stronger (Yamamoto & Ueji, 2011). Although Experiment 2 evaluated varying numbers of pairing sessions and found no difference between 1 session per day and 2 sessions per day in forming a positive bias in healthy rats, the large variability in preferences seen in results throughout Chapter 3 could be due to insufficient pairing sessions, resulting in weaker associations. Although the number of pairing sessions matched the digging version, it is possible that substrate-reward associations are stronger than flavour-reward, perhaps due to learning when digging being a natural behaviour used for foraging and burying food (Makowska & Weary, 2016). Pacchiarini, Berkeley, Fox, and Honey (2020) showed that texture discrimination in digging tasks relies on the presence of whiskers in rodents, thus implicating the barrel cortex in this associative learning, whereas odour discrimination does not rely on this. Thus, some involvement of the barrel cortex in digging tasks could enable more distinct learning between different stimuli, thus strengthening substrate-reward associations in the digging ABT/m-ABT.

Alternatively, there were several differences in the protocol for the digging and flavour tasks. The latter utilised extinction of the reward association in choice tests, i.e., no sucrose was given with the two previously reward-paired flavours, whereas the digging task uses a random reinforcement schedule in this stage. This positive feedback may have been essential in the accurate recall of stimulus-reward associations; however, it should be noted that on average rats from all experiments did show a preference for the high vs low reward-paired flavour. This preference was also generally at a level higher than seen in the digging task, showing that positive biases could be formed and recalled, but the variation in preference between animals was large and drug-induced changes were not reliably detected. Previous evidence has suggested that some rats show behavioural traits of sensitivity to negative and positive feedback (Noworyta-Sokolowska et al., 2019), thus, perhaps some rats in this task were more sensitive to the lack of positive feedback or presence of negative feedback since a reward was expected but not presented.

The flavour tasks also rely on overall consumption as a measurement of bias, which may cause preferences to be too sensitive to minor changes and variations in the total amount consumed. If one rat consumed 3ml of CS-A^{++} and 1ml of CS-B^{+} this gives a preference of 75%, whilst if another rat consumes 3ml of CS-A^{++} and 2ml of CS-B^{+} this reduces the preference to 60%, giving rise to a greater variability of results with small changes in consumption. The digging task is instead based on a set trial number given to all rats, so the total amount of choices made remains the same for all animals and this would be less sensitive to minor variations. In addition, during pairing sessions and choice tests in the digging version, once a choice was made the other bowl was removed from the arena. This removal of the other stimulus may increase attention during learning the substrate-reward associations, whereas in the flavour version rats were free to try both solutions before making a choice. Thus, variability could be due to differing amount of time trying each solution presented. Though the flavour m-ABT was designed and evaluated to be used without specialist equipment, the apparatus used in Chapter 2 and 3 were connected to lickometers measuring the timing of each lick for each bottle. Thus, although not measured in this thesis, it would be possible in future to determine which bottle was approached first and timings between swapping to try the alternative option to address this issue.

Evaluation of the flavour m-ABT highlights potential avenues for future optimisation of this assay, including assessing the addition of equal value reward in the choice tests or removing bottles after a set amount consumed rather than time limit, although there may be limited logistical ability to do this. However, following the extensive evaluation of this assay in this thesis, a flavour preference-based assay for reward learning may not be the most sensitive option to focus on. Instead, potential avenues for future work could aim to increase reliability and reproducibility of the current digging task by investigating ways to automate the detection of choice-making behaviour and removal of access to the other bowl. Alternatively, a lever-based task could be developed in which the lever is covered with different textures to represent the different ‘substrates’ (conditioned stimuli, CS) following the same principles as the digging m-ABT with one texture paired with one pellet and another texture paired with two pellets. This would address possible limitations in the use of pure sucrose solutions identified in the flavour m-ABT, as well as automating the task and providing textures that could be an important aspect of learning in the digging m-ABT.

Lick cluster analysis

In Chapter 4, lick cluster analysis (LCA) was used to measure consummatory anhedonia in the chronic CORT and IFN-α models, which had been shown previously to have opposing effects in the sucrose preference test (SPT). As discussed in section 1.4.1., the SPT measures overall consumption preference for a sucrose solution vs plain water, which is

open to influence from other deficits in motivation, motor function, reward sensitivity, and to some extent cognitive flexibility with the use of a choice test, not purely hedonic response (Dwyer, 2012). Experiments 10 and 11 also showed a consistent lack of deficit with chronic IFN-α, supporting previous research with this dose and regimen in the SPT (Stuart et al., 2017).

Chronic CORT in drinking water initially induced a reduction in lick cluster size, indicating a deficit in hedonic response, however this was deemed inconclusive following Bayes analysis. A repetition of this experiment did not show any deficit, but also did not show positive controls for the treatment itself. Thus, a further examination of chronic CORT via subcutaneous injections was undertaken by others in my group, which also showed no deficit but clear positive controls for the treatment. In addition, Clarkson (2019) administered 35µg/mL CORT in drinking water to mice for 28 days and found no deficit in hedonic response to 4% sucrose measured via LCA. Although no evidence was provided for a positive control of body weight change, there was evidence of ruffled and untidy coat state indicating physiological changes. Therefore, these findings together provide a body of evidence that chronic CORT treatment does not impair hedonic response, regardless of administration route, despite consistent evidence in the SPT showing otherwise (Ding et al., 2018; Huang et al., 2011; Lou et al., 2018).

Thus, LCA was potentially advantageous in its specificity to measuring hedonic response as it could be suggested from these findings that chronic CORT must impair other processes that are required for the SPT, such as motivation or general reward sensitivity, rather than hedonic response. However, the initial identification of a deficit in Experiment 8 that was subsequently thought to be a false positive suggests this method may be too sensitive in detecting small changes in licking microstructure that do not accurately represent a hedonic deficit. The variability in lick cluster sizes between animals may also indicate that individual differences between rats can cause variation that presents as a deficit in some cases. To reduce this variability and increase reliability of findings, a within-subjects design could be used to analyse baseline vs post-treatment hedonic response. Clarkson (2019) also demonstrated that consumption and lick clusters size generally increase over time, indicating that learning about the sucrose solution over time may also influence hedonic response, although this also possibly reflects the increase in time spent under food restriction. It therefore may be possible that increasing the time spent in the pretraining stage could help to stabilise consumption and lick cluster sizes to reduce this variation.

Effort-related choice

In Chapter 5, the effort-related choice paradigm (EfR) was used to demonstrate that chronic CORT and IFN-α did not induce any motivational deficits, however there were issues within this experiment that could have reflected upon this. Although it would have been useful to re-run this experiment in a more robust design to understand chronic treatment effects on motivation for reward, this was not possible to do in the timeframe of this PhD. Instead, acute effects of these drugs were analysed. A positive control for the EfR task was found using haloperidol, demonstrating that the lack of deficit seen in CORT and IFN-α models appeared to be a true lack of deficit and not due to a lack of sensitivity or specificity in the way the assay was run.

In addition, this task was able to dissociate an increase in general chow consumption in the acute CORT treated animals from an increase in motivation for reward, since lever presses remained constant. Instead, this identified an increase in general appetite following CORT treatment that could be used to understand the effect of stress on other behaviours related to psychiatric disease. The CACNA1C$^{+/-}$ model showed an unexpected increase in lever presses without chow present, but only in male rats and with no overall interaction or main effects. No differences were seen during the lever press vs chow choice test, which could indicate CACNA1C$^{+/-}$ rats had increased general motivation, or this could have been influenced by other processes such as habit formation, impulse control deficits, or that general acquisition of the lever pressing task is elevated in this model. Other models of schizophrenia have been found to show increased responding in the progressive ratio (PR) task when methamphetamine was used as the reward (Brady, McCallum, Glick, & O'Donnell, 2008), which could indicate schizophrenia models show enhanced reward-seeking behaviours. It is possible that this same increase in lever presses was not seen in the choice test due to animals spending time exploring the new chow bowl in the box and thus attention was not solely focused on the lever-reward.

This task likely relies on some cognitive and hedonic processing such that learning the lever-reward association is required to be able to make a choice between the chow and lever, whilst hedonic response to the chow and pellets is necessary to distinguish between their reward values. Despite this, CORT and IFN-α which do appear to induce affective biases and cognitive biases in processing of rewards do not impair this motivational decision-making, and the CACNA1C$^{+/-}$ model showed deficits in hedonic response and cognitive biases without motivational deficits. Thus, the EfR was successfully able to measure motivation for reward without influence from cognitive or hedonic deficits and may be capable of distinguishing between changes in willingness and motivation to work for a reward from a general change in reward-seeking behaviour.

7.3. Dissociating the underlying neurobiological mechanisms of reward processing

7.3.1. Neurobiological changes following stress, immune response, and genetic risk

Another aim of this thesis was to elucidate the neurobiological changes underpinning reward-related deficits associated with psychiatric disease. Several cohorts of animals were used to assess different biological markers and behaviours, which will be outlined below.

Using exogenous CORT administration, I found that overactivation of the HPA axis appears to increase general appetite but does not influence hedonic response or motivation. This was demonstrated in a cohort of animals administered acute treatment only, and that clearly showed reduced body weight as a positive control. In one cohort of animals who did demonstrate reduced body weight, the flavour m-ABT was able to detect deficits in reward learning following chronic exogenous CORT administration, which supports previous evidence of impairments in cognitive bias and reward learning following dysregulation of the HPA axis in the digging ABT (Hinchcliffe et al., 2020; Hinchcliffe et al., 2017) and the probabilistic reward task (PRT) (Bogdan et al., 2010; Bogdan & Pizzagalli, 2006; Bogdan et al., 2011). However, in a second cohort of animals that did not demonstrate this positive control of body weight, the flavour m-ABT did not replicate this finding. Since a lack of positive control was found here, the alternative evidence can be used to indicate that different biological mechanisms may underpin cognitive, hedonic, and motivational aspects of reward processing based on these combined findings. Chapter 6 also showed no change in neurogenic markers, or glutamatergic receptor subunits in the chronic CORT cohort demonstrating a positive control of reduced body weight, suggesting these factors are not necessary for producing a reward learning deficit. However, the inconsistency in being able to demonstrate a deficit in reward-induced positive bias in this model means this cannot be concluded with certainty.

IFN-α treatment similarly did not appear to influence hedonic response or motivation but may impair cognitive bias and reward learning, suggesting the stress and immune response influence similar pathways to produce reward-related deficits. However, Chapter 6 showed that chronic IFN-α treatment elevated the expression of neurogenic markers as well as NR2B, which were not changes present in the chronic CORT model. This implies that cognitive biases are not dependent upon changes in neurogenesis and NR2B. Previous literature has shown IFN-α induces deficits in the forced swim test (e.g. Makino, Kitano, Komiyama, & Takasuna, 2000), the SPT - although this evidence has been variable (see 4.1), and also induces a negative bias in the ABT (Stuart et al., 2017). To some extent, these tasks all involve cognitive processing, and given the links between neurogenesis or

glutamatergic receptors with cognition (Costa, Lugert, & Jagasia, 2015; Higgins, Ballard, Enderlin, Haman, & Kemp, 2005) this could imply IFN-α targets the neurobiological pathways underpinning cognitive processes to induce a depression-like state. However, higher doses of IFN-α should still be further evaluated in the EfR task to conclude a lack of motivational deficit.

The CACNA1C$^{+/-}$ model appeared to show a deficit in reward-induced positive bias, though again this was inconclusive given the limitations in the flavour m-ABT, as well as evidence of a hedonic deficit, with no motivational deficit. This implies functional voltage-gated calcium channels are important in the cognitive and hedonic processing of rewards, but not motivation. My findings demonstrating deficits in reward learning in this model supports previous literature implicating the human CACNA1C risk variant in impairments in other cognitive domains such as facial emotion recognition (Nieratschker, Brückmann, & Plewnia, 2015) and structural and functional changes in brain regions related to cognitive processing (Bigos et al., 2010), as well as studies demonstrating deficits in associative learning in the rodent CACNA1C$^{+/-}$ model (Sykes, Clifton, et al., 2018; Sykes, Haddon, et al., 2018). Further evaluation of these three models could therefore examine overlapping changes in the brain that may identify the exact pathways involved in cognitive processing of rewards, where changes only identified in the CACNA1C$^{+/-}$ model could highlight potential pathways involved in hedonic processing.

7.3.2. Limitations and evaluation of preclinical models

The three preclinical models investigated in this thesis were chosen to represent three major risk factors involved in the development of major depressive disorder (MDD) and schizophrenia, stress, immune response, and genetic variation.

The exogenous CORT administration model was chosen to focus on the biological stress pathway, rather than environmental stress which could influence other biological pathways. Chronic CORT was also administered via drinking water to minimise external stress impacting the overactivation of the HPA axis. However, the reliability of this model was questioned Experiment 4 and 9 failed to produce a positive control for treatment. Other studies have demonstrated that the three main administration routes for CORT differ in their ability to change circulating CORT levels, neurogenic marker expression, and immobility in the forced swim test Kott et al. (2016). This indicates that chronic CORT administered in drinking water may not be a reliable model to measure reward-related deficits.

In addition, the IFN-α model was chosen to demonstrate the effects of changes in immune response on reward processing and is translatable to patients given the direct link between exogenous IFN-α administration and depression side effects (Bonaccorso et al., 2002). The

dosage of IFN-α used throughout this thesis (100IU/kg) was chosen to avoid interference of 'sickness behaviours' with the reward-related deficits measured in these assays. However, the recommended dose for treating chronic Hepatitis B patients is $10x10^6$ units three times per week (Group, 1994), which is equivalent to $1.61x10^6$ IU/kg of human IFN-α administered to rats (Nair & Jacob, 2016). Although the efficacy and subsequent equivalent dosage may differ between human and rat recombinant IFN-α, this does suggest the dose of IFN-α administered to human patients that may induce depression side effects is much higher than the dose investigated here. It is therefore possible that other reward-related deficits may be present if a higher dose more comparable to that given in humans was used. Thus, future experiments should explore different doses of this drug to fully understand its influence on reward processing.

The CACNA1C$^{+/-}$ model was more reliable in comparison to these other models as it does not rely on pharmacological efficacy, however, the genetic mutation in this rodent model occurs within exon 6 to produce an early stop codon and entirely prevents transcription of one allele of *CACNA1C*. In patients, the genetic variant associated with psychiatric disease is present in intron 3 and has been suggested to decrease expression of *CACNA1C* but does not completely prevent transcription of one allele, and results showing this have been variable (Sykes, Haddon, et al., 2018). Thus, this model may not accurately represent the human genetic risk, however, it still represents a model of reduced *CACNA1C* expression which could similarly be a result of the human variant and still allows understanding of the role of *CACNA1C* in reward-related deficits.

It also should be noted that across the different cohorts of animals there were variations in the animals' body weights and size that could potentially influence findings using consummatory measures such as the behavioural methods discussed in this thesis.

7.4. Future work

The experiments in this thesis provide some evaluation of three assays used to dissociate cognitive, motivational, and hedonic aspects of reward processing.

Further development of assays for reward-induced biases should be explored and used to confirm the involvement of *CACNA1C* in cognitive processing of rewards. The results in Chapter 3 and 5 also highlight potential sex differences in the CACNA1C$^{+/-}$ model with regards to how they learn about rewards in the environment and their goal-direction actions in response to these. Future exploration of this model could investigate how these sex differences may impact the development of psychiatric disease with the presence of the *CACNA1C* genetic variant, for example by repeating these experiments with greater sample

sizes, more reliable assays of reward-induced biases, and comparing males and females in both behavioural and biological changes.

The LCA technique was also able to show that the chronic CORT model did not display hedonic deficits despite this model previously shown to reduce sucrose preference, thus suggesting LCA can dissociate hedonic deficits from other aspects of reward processing and reduce false interpretation. Thus, this assay could be used in other models of psychiatric disease to elucidate the neurobiology of consummatory anhedonia, and whether this deficit is indeed present in current models of psychiatric disease given the limited literature using these sensitive objective measures of anhedonia. LCA also could be used to examine the ability of antidepressant therapeutic agents to reverse this deficit, rather than relying solely on the SPT.

Furthermore, the EfR test appears to be able to dissociate motivational deficits from other factors that may interfere with simple operant tasks such as stimulus-response learning or habit formation. This task can then be used in tandem with the LCA and assays of reward-induced bias to dissociate the different reward-related deficits in other models of psychiatric disease and again be used to examine therapeutic reversal of each deficit.

In addition, the IFN-α model of depression is not yet fully characterised and the mechanisms by which IFN-α or the immune system induce symptoms of psychiatric disease is not fully understood. This thesis demonstrated elevated neurogenesis following IFN-α treatment, which should be explored further in this model by examining neurogenic changes first without undergoing a behavioural task, then following a learning and memory task to establish whether the effects of IFN-α differ prior to behavioural assays, and the effects of different doses. Aiming to understand how different doses of IFN-α change glutamatergic neurotransmission and neurogenesis, without interference from undergoing behaviour tests, may highlight potential mechanisms by which IFN-α induces deficits in reward learning. Then, investigation of higher doses in the LCA and EfR tasks, whilst monitoring sickness behaviours, could elucidate whether these behaviours are essential for other reward-related deficits to be present in this model.

A major aim of this thesis was to investigate ways to reliably dissociate aspects of reward processing. Although the flavour m-ABT was not deemed as a reliable assay, the LCA and EfR tasks appeared to be capable of dissociating hedonic and motivational deficits. The digging m-ABT can detect deficits but lacks reliability from poor reproducibility of the task. Other assays of reward learning include the PRT, which has shown evidence of reward learning deficits following social defeat and stress-related genetic risk factors (Bogdan et al., 2010; Bogdan et al., 2011; Der-Avakian et al., 2017) but limited evidence in other

psychiatric disease models. In addition, the probabilistic reversal learning (PRL) task similarly investigates reward learning with an additional assessment of cognitive flexibility and has demonstrated impaired reward learning in neurogenesis-deficient rats (Seib, Espinueva, Floresco, & Snyder, 2020), as well as following serotonin antagonist administration (Bari et al., 2010). However, again, investigation of preclinical psychiatric disease models in these tasks are limited. Thus, the PRT or PRL tasks could be used in the future alongside LCA and EfR to potentially dissociate the cognitive aspects of reward processing from hedonic and motivational aspects in preclinical psychiatric disease models.

As discussed in section 1.3, there are several risk factors for psychiatric diseases such as schizophrenia and major depressive disorder (MDD). One major risk factor for both disorders is chronic stress (Yang et al., 2015), and therefore further efforts should be made to understand the contribution of stress to reward-related deficits in psychiatric disease. Chronic CORT administration via subcutaneous injection could be used to model this risk factor more reliably and investigate its effects in the EfR and PRT, since this model was found to show no hedonic deficit in the LCA with clear positive control for weight gain, whilst other environmental stress models such as maternal separation may more accurately reflect the stress experienced in humans.

In addition, inflammation is still a contributing risk factor for these disorders including the direct link between IFN-α administration and depression side effects in humans (Bonaccorso et al., 2002), however, given the variability of findings in this thesis and lack of firm conclusions, future research should aim to match the equivalent human dosage in preclinical models to increase translatability of findings. Thus, a higher dose of IFN-α could be used in the EfR, PRT, and LCA to dissociate the effects of IFN-α on reward processing. Furthermore, other preclinical inflammation models should be investigated to determine whether IFN-α has a specific mechanism of action or if immune activation generally impacts reward-related deficits. For example, maternal immune activation exposes pregnant dams to a pathogen to activate their immune system, which will in turn expose the offspring to a heightened immune response (Brown & Meyer, 2018). Investigation of these offspring in the EfR, PRT, and LCA together could dissociate the reward-related deficits resulting from immune-related developmental changes and elucidate the potential implications of this risk factor in human development.

Finally, although the chronic CORT model did not appear to show any neurobiological changes compared to controls in Chapter 6, the chronic IFN-α model had elevated NR2B expression. As discussed in section 6.5.2, some evidence has related NR2B overexpression with depression-like symptoms, whereas antagonism of NR2B reduces these symptoms. This evidence could implicate a mechanism of IFN-α-induced depression

via NR2B overexpression and thus could be explored further. Future experiments could dissociate the reward-related deficits caused by IFN-α treatment at higher doses using the three assays discussed above, then administer an NR2B antagonist to determine which, if any, of these deficits are reversed in the IFN-α model. This would help to elucidate whether NR2B changes following IFN-α treatment are associated with a particular reward-related deficit and could then be investigated further in other models of psychiatric disease that also induce that reward-related deficit.

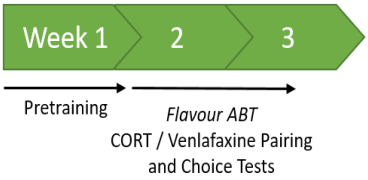
7.5. Concluding remarks

Psychiatric diseases such as MDD and schizophrenia are often characterised by the presence of one or more reward-related deficits, but the number of potential symptoms experienced can differ significantly between individual patients. Currently, pharmacological treatments are prescribed in a ‘one size fits all’ approach, but these individual differences should be considered, and therefore understanding isolated symptoms is imperative to be able to develop personalised treatments. This thesis provides evaluations of novel and recognised behavioural assays to improve preclinical assessments of reward-related deficits in psychiatric disease models, whilst demonstrating limitations of current methods and offering novel insights to the pathophysiology of reward-related deficits.

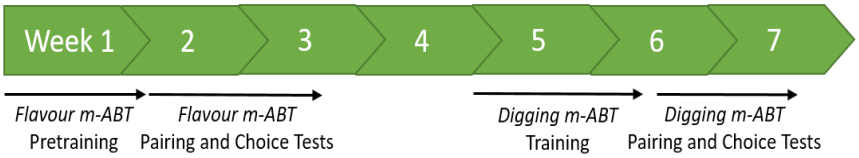
Appendices

Appendix A – Experimental trajectories

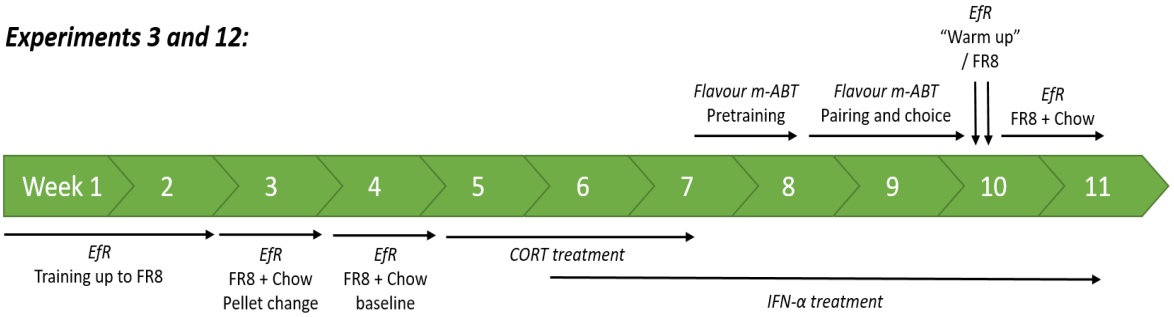
Experiment 1:



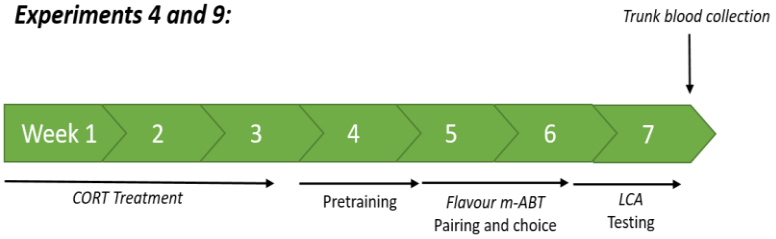
Experiment 2:



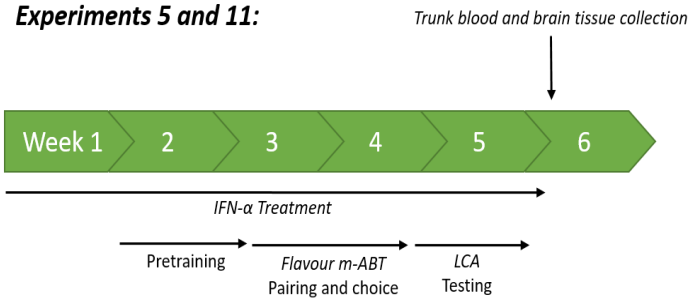
Experiments 3 and 12:



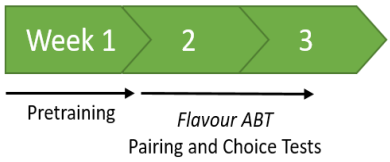
Experiments 4 and 9:



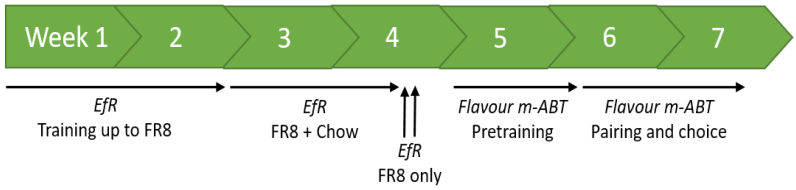
Experiments 5 and 11:



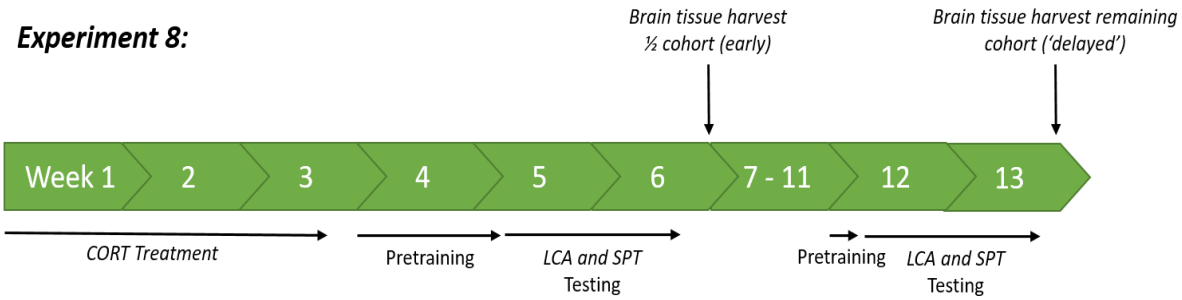
Experiment 6:



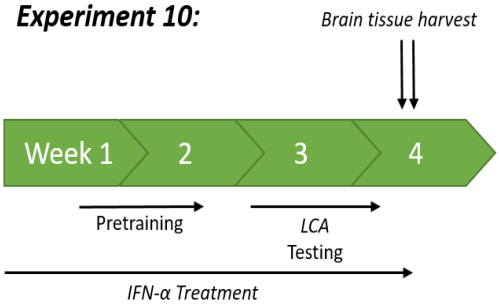
Experiments 7 and 14:



Experiment 8:



Experiment 10:



Experiment 13:

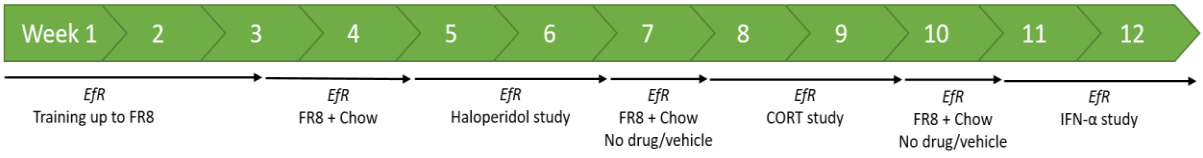


Figure S1. Summaries of each experimental timeline. *"Flavour m-ABT"* = Flavour modified affective bias test discussed in Chapters 2 and 3. *"LCA"* = Lick cluster analysis discussed in Chapter 4. *"EfR"* = Effort-related choice test discussed in Chapter 5.

Appendix B – Drug solutions

Table S1. List of drug solutions and preparation protocols.

Drug	**Preparation**
0.9% Saline w/v	9mg Sodium Chloride (Analytical Grade, Sigma) dissolved in 1000ml distilled water.
50µg/ml Corticosterone (CORT) in 1% ethanol	75mg CORT (C2505, Sigma) fully dissolved in 15ml 100% ethanol, filled to 1500ml.
10mg/kg CORT	80mg CORT (C2505, Sigma) fully dissolved in 0.8ml DMSO (D2650, Sigma). Add 1.6ml Cremaphor (C5135, Sigma) and mix completely before slowly adding 5.6ml 0.9% saline. Inject subcutaneous at 1ml/kg.
Interferon-α (100U/kg)	Dissolve vial containing $1x10^5$ Units IFNα (I8657, Sigma) in 1ml distilled water to give $1x10^5$ Units/ml. Add 9ml saline to this and mix, then aliquot into 0.15ml stock solutions containing $1x10^4$ Units/ml to store at -80˚C. When needed, defrost one stock and re-suspend in 14.85ml saline, giving 100Units/ml. Inject i.p. at 1ml/kg.
Venlafaxine (3mg/kg)	21mg Venlafaxine (2917, Tocris Bioscience UK) dissolved in 7ml saline. Inject i.p. at 1ml/kg.

Appendix C – Immunohistochemistry

Antibody Optimisation

Prior to conducting the full immunohistochemical investigations of Ki67 and DCX expression, optimal primary and secondary antibody dilutions, and DAB development times were investigated using spare brain tissue prepared in the same manner as described in section 6.2.1. The dilutions tested are specified in Table S2.

Table S2. Antibody dilutions and DAB development times investigated for optimisation.

Primary Antibody	**Dilutions Tested**	**Final Primary Dilution**	**Secondary Dilutions Tested**	**Final Secondary Dilution**	**Tested DAB Times (s)**	**Final DAB Time (s)**
Ki-67	1:5000, 1:1000	1:1000	1:1000 1:500	1:1000	60, 120	120
DCX	1:5000, 1:1000	1:1000	1:1000 1:500	1:1000	120, 180	180

Solutions

Table S3. List of solutions used for immunohistochemistry and method for preparation. All solutions were mixed thoroughly and *denotes those prepared fresh for each use.

Solution	**Ingredients**
4% Paraformaldehyde (PFA)	1.5L distilled water heated to 45˚C on stirrer. Add 80g Paraformaldehyde (P6148, Sigma) followed by 5ml 2M Sodium Hydroxide (NaOH). Once PFA has dissolved, add 5g Sodium Dihydrogen Orthophosphate $1H_2O$, 28.9g Disodium Hydrogen Orthophosphate $2H_2O$ and 18g Sodium Chloride. Adjust pH to 7.4 with 2M NaOH.
25% Sucrose Solution	25g sucrose added to 1L 0.1M PBS.
0.1M Phosphate Buffered Saline (PBS)	Dissolve 1 Oxoid™ PBS tablet (BR0014G, Thermo Scientific) per 100ml distilled water. Then, pH to 7.4 with 2M NaOH.
Cryoprotectant	300g Sucrose Analytical grade dissolved in 500ml 0.1M PBS. Sprinkle 10g Polyvinyl Pyrrolidone. Once dissolved, add 300ml Ethylene Glycol

0.1M PBS with 0.2% Triton X-100 (PBS-T)	1ml Triton X-100 was thoroughly mixed into 500ml 0.1M PBS.
Quenching Solution*	10ml 30% Hydrogen Peroxidase (H_2O_2) and 10ml Methanol added to 80ml distilled water.
Blocking Solution*	300µl of normal serum (matching the species that the primary antibody was raised in) and 0.2g bovine serum albumin (BSA) dissolved in 10ml PBS-T.
Avidin-Biotin Complex (ABC) Kit*, Vector Labs (PK-6100)	2 drops of A, 2 drops of B (Vector Labs) and 50µl normal serum per 5ml PBS-T.
TRIS Non-Buffered Saline*	6g Trisma base (Sigma-Aldrich) added to 1L distilled water, then pH to 7.4 with 5M Hydrochloric Acid (HCl).
3,3'-Diaminobenzidine (DAB)*, Vector Labs (SK-4100)	2 drops of buffer stock and 4 drops of DAB were added to 5ml distilled water, followed by 2 drops of H_2O_2. Solution was used immediately.

Appendix D – Western blot

Solutions

Table S4. List of solutions used for Western blotting and method for preparation. All solutions were mixed thoroughly and *denotes those prepared fresh for each use.

Solution	Ingredients
10% SDS	10g Sodium dodecyl sulfate (SDS) dissolved in 100ml dH_2O.
SDS/Tris	0.4g SDS dissolved in 100ml 0.5M Tris-HCl with pH 6.8.
3X sample buffer	1.14g Tris base, 6g SDS, 15ml glycerol, 7.5ml β-Mercapoethanol and 1.5ml Bromophenol blue dissolved in 50ml water.
APS	10g ammonium persulfate (APS) dissolved in 100ml dH_2O.
10% Separating gel	3.33ml acrylamide, 1.25ml Tris-HCl, 100µl 10% SDS, 50µl APS and 5µl TEMED were all added to 5.27ml dH_2O and mixed thoroughly on a vortex.
0.1M TBS with 0.2% Triton X-100 (TBS-T)	1ml Triton X-100 was thoroughly mixed into 500ml 0.1M TBS.
5% / 1% milk blotto*	5g / 1g dried skimmed milk powder (Tesco) dissolved in 100ml TBS-T.

Appendix E – Training data

Chapter 2 – Experiment 1

Training for Experiment 1 was split into pairing sessions where CS-A was presented alongside CS-C, or CS-B was presented alongside CS-C, repeated to give 4 total pairing sessions. A summary of the data for mean consumption and percentage preference between the two solutions during pairing sessions is presented in Table S5A and S5B.

A repeated measures ANOVA was conducted on these data with 'flavour' as the within-subjects factor and 'group' as the between-subjects factor. For the CS-A vs CS-C pairing sessions, there was a significant effect of flavour, $F_{1, 46} = 374.32$, $p < 0.001$, with no main effect of group, $F_{1, 46} = 1.12$, $p = 0.29$, and no interaction between group * flavour, $F_{1, 46} = 1.55$, $p = 0.22$.

For the CS-B vs CS-C pairing sessions, there was a significant main effect of flavour, $F_{1, 46} = 355.71$, $p < 0.001$, with no main effect of group, $F_{1, 46} = 0.03$, $p = 0.86$, and no interaction between group * flavour, $F_{1, 46} = 0.001$, $p = 0.97$.

Individual t-tests were conducted on the percentage preferences to compare the two groups. There was no significant difference between the two groups in preference for CS-A vs CS-C, $t(46) = 1.008$, $p = 0.32$, nor in preference for CS-B vs CS-C, $t(46) = 0.19$, $p = 0.85$.

Table S5A.

	CS-A	**CS-C**	**CS-A vs CS-C**
Group	**Mean consumption**	**Mean consumption**	**Preference (%)**
CORT	13.41 ±0.73	0.63 ±0.08	94.9 ±1.2
Venlafaxine	12.08 ±0.86	0.85 ±0.19	90.6 ±4.1

Table S5B.

	CS-B	**CS-C**	**CS-B vs CS-C**
Group	**Mean consumption**	**Mean consumption**	**Preference (%)**
CORT	12.86 ±0.90	0.61 ±0.11	94.2 ±1.5
Venlafaxine	12.99 ±0.85	0.68 ±0.12	93.8 ±1.6

Chapter 3 – Experiment 3

Training for Experiment 3 was split into pairing sessions where CS-A^{++} was presented alongside CS-C, or CS-B^{+} was presented alongside CS-C, repeated to give 4 total pairing sessions. A summary of the data for mean consumption and percentage preference between the two solutions during pairing sessions is presented in Table S6A and S6B.

A repeated measures ANOVA was conducted on these data with 'flavour' as the within-subjects factor and 'group' as the between-subjects factor. For the CS-A^{++} vs CS-C pairing sessions, there was a significant effect of flavour, $F_{1, 45} = 566.25$, $p < 0.001$, with no main effect of group, $F_{2, 45} = 0.88$, $p = 0.42$, and no interaction between group * flavour, $F_{2, 45} = 0.73$, $p = 0.49$.

For the CS-B^{+} vs CS-C pairing sessions, there was a significant main effect of flavour, $F_{1, 45} = 167.12$, $p < 0.001$, with no main effect of group, $F_{2, 45} = 0.16$, $p = 0.86$, and no interaction between group * flavour, $F_{2, 45} = 0.16$, $p = 0.73$.

A univariate ANOVA was conducted on the percentage preferences to compare the three groups. There was no significant difference between the three groups in preference for CS-A^{++} vs CS-C, $F_{2, 45} = 0.66$, $p = 0.52$, nor in preference for CS-B^{+} vs CS-C, $F_{2, 45} = 1.44$, $p = 0.25$.

Table S6A.

	CS-A^{++}	**CS-C**	**CS-A^{++} vs CS-C**
Group	**Mean consumption**	**Mean consumption**	**Preference (%)**
Control	11.02 ±1.04	0.33 ±0.07	96.2 ±1.4
CORT	12.13 ±0.62	0.34 ±0.06	97.2 ±0.5
IFN-α	10.78 ±0.66	0.25 ±0.06	97.7 ±0.5

Table S6B.

	CS-B^{+}	**CS-C**	**CS-B^{+} vs CS-C**
Group	**Mean consumption**	**Mean consumption**	**Preference (%)**
Control	7.35 ±0.89	0.36 ±0.07	94.4 ±1.2
CORT	6.74 ±0.81	0.39 ±0.05	93 ±1.4
IFN-α	6.58 ±0.85	0.55 ±0.11	90.4 ±2.3

Training for Experiment 4 was split into pairing sessions where CS-A^{++} was presented alongside CS-C, or CS-B^{+} was presented alongside CS-C, repeated to give 4 total pairing sessions. A summary of the data for mean consumption and percentage preference between the two solutions during pairing sessions is presented in Table S7A and S7B.

A repeated measures ANOVA was conducted on these data with 'flavour' as the within-subjects factor and 'group' and 'sex' as the between-subjects factors. Only the main effects and any significant interactions will be fully reported here.

For the CS-A^{++} vs CS-C pairing sessions, there was a significant effect of flavour, $F_{1, 60}$ = 643.13, p <0.001, with no main effect of group, $F_{1, 60}$ = 0.046, p = 0.83, nor of sex, $F_{1, 60}$ = 3.86, p = 0.054. There were no interactions between any of the factors at any level.

For the CS-B^{+} vs CS-C pairing sessions, there was a significant main effect of flavour, $F_{1, 60}$ = 451.24, p <0.001, with no main effect of group, $F_{1, 60}$ = 0.42, p = 0.52, nor of sex, $F_{1, 60}$ = 3.52, p = 0.065. There were no interactions between any of the factors at any level.

A univariate ANOVA was conducted on the percentage preferences to compare the groups and sexes. Preference for CS-A^{++} vs CS-C did not differ between control or CORT groups, $F_{1, 60}$ = 0.73, p = 0.39, nor between males and females, $F_{1, 60}$ = 1.35, p = 0.25, and there was no interaction between group * sex, $F_{1, 60}$ = 0.69, p = 0.41.

Preference for CS-B^{+} vs CS-C did not differ between control or CORT groups, $F_{1, 60}$ = 2.21, p = 0.14, nor between males and females, $F_{1, 60}$ = 0.14, p = 0.72, with no interaction between group * sex, $F_{1, 60}$ = 0.91, p = 0.34.

Table S7A.

		CS-A^{++}	CS-C	CS-A^{++} vs CS-C
Group	**Sex**	**Mean consumption**	**Mean consumption**	**Preference (%)**
Control	Male	11.70 ±1.08	0.65 ±0.28	91.6 ±5.7
CORT	Male	12.33 ±0.85	0.43 ±0.11	96.5 ±0.9
Control	Female	10.83 ±0.64	0.28 ±0.06	97.4 ±0.5
CORT	Female	10.75 ±0.50	0.28 ±0.04	97.4 ±0.4

Table S7B.

		CS-B^{+}	CS-C	CS-B^{+} vs CS-C
Group	**Sex**	**Mean consumption**	**Mean consumption**	**Preference (%)**
Control	Male	9.03 ±0.55	0.67 ±0.21	92.8 ±2.2
CORT	Male	8.94 ±0.82	0.47 ±0.06	94.4 ±0.9
Control	Female	7.22 ±0.81	0.47 ±0.13	88.7 ±5.7
CORT	Female	8.56 ±0.60	0.31 ±0.05	96.3 ±0.6

Chapter 3 – Experiment 5

Training for Experiment 5 was split into pairing sessions where CS-A^{++} was presented alongside CS-C, or CS-B^{+} was presented alongside CS-C, repeated to give 4 total pairing sessions. A summary of the data for mean consumption and percentage preference between the two solutions during pairing sessions is presented in Table S8A and S8B.

A repeated measures ANOVA was conducted on these data with 'flavour' as the within-subjects factor and 'group' and 'sex' as the between-subjects factors. Only the main effects and any significant interactions will be fully reported here.

For the CS-A^{++} vs CS-C pairing sessions, there was a significant effect of flavour, $F_{1, 60}$ = 1620.95, p <0.001, with no main effect of group, $F_{1, 60}$ = 0.075, p = 0.79. There was a significant main effect of sex, $F_{1, 60}$ = 7.4, p = 0.009, with consumption greater in males than females. There was a significant interaction between flavour * sex, $F_{1, 60}$ = 6.23, p = 0.015, where Fisher's LSD post-hoc analysis showed males consumed more flavour A than

females (p = 0.011) but not flavour C (p = 0.16). There were no other interactions between any of the factors at any level.

For the CS-B^+ vs CS-C pairing sessions, there was a significant main effect of flavour, $F_{1, 60}$ = 519.27, p <0.001, with no main effect of group, $F_{1, 60}$ = 1.09, p = 0.30, nor of sex, $F_{1, 60}$ = 1.34, p = 0.25. There were no interactions between any of the factors at any level.

A univariate ANOVA was conducted on the percentage preferences to compare the groups and sexes. There was a significant difference between control and IFN-α rats in preference for CS-A^{++} vs CS-C, $F_{1, 60}$ = 6.39, p = 0.014, where IFN-α rats had lower preference compared to controls. There was no difference between males and females, $F_{1, 60}$ = 0.06, p = 0.81, nor interaction between group * sex, $F_{1, 60}$ = 0.54, p = 0.47.

Preference for CS-B^+ vs CS-C did not differ between control or IFN-α groups, $F_{1, 60}$ = 0.29, p = 59, nor between males and females, $F_{1, 60}$ = 2.73, p = 0.10, with no interaction between group * sex, $F_{1, 60}$ = 0.02, p = 0.89.

Table S8A.

		CS-A^{++}	**CS-C**	**CS-A^{++} vs CS-C**
Group	**Sex**	**Mean consumption**	**Mean consumption**	**Preference (%)**
Control	Male	14.3 ±0.73	0.28 ±0.04	98.1 ±0.3
IFN-α	Male	15.0 ±0.63	0.39 ±0.04	97.4 ±0.3
Control	Female	13.2 ±0.52	0.20 ±0.03	98.4 ±0.3
IFN-α	Female	12.6 ±0.76	0.35 ±0.07	97.2 ±0.6

Table S8B.

		CS-B^+	**CS-C**	**CS-B^+ vs CS-C**
Group	**Sex**	**Mean consumption**	**Mean consumption**	**Preference (%)**
Control	Male	9.27 ±0.90	0.47 ±0.14	94.6 ±1.5
IFN-α	Male	10.78 ±0.84	0.69 ±0.19	93.8 ±1.7
Control	Female	9.45 ±0.56	0.33 ±0.04	96.5 ±0.5
IFN-α	Female	9.31 ±0.78	0.35 ±0.05	96.0 ±0.9

Chapter 3 – Experiment 6

Training for Experiment 6 was split into pairing sessions where CS-A^{++} was presented alongside CS-C, or CS-B^{+} was presented alongside CS-C, repeated to give 4 total pairing sessions. A summary of the data for mean consumption and percentage preference between the two solutions during pairing sessions is presented in Table S9A and S9B.

A repeated measures ANOVA was conducted on these data with 'flavour' as the within-subjects factor and 'genotype' as the between-subjects factor.

For the CS-A^{++} vs CS-C pairing sessions, there was a significant effect of flavour, $F_{1, 38}$ = 406.56, p <0.001, with no main effect of genotype, $F_{1, 38}$ = 1.04, p = 0.32, and no interaction between flavour * genotype, $F_{1, 38}$ = 0.88, p = 0.35.

For the CS-B^{+} vs CS-C pairing sessions, there was a significant main effect of flavour, $F_{1, 38}$ = 254.46, p <0.001, with no main effect of genotype, $F_{1, 38}$ = 1.83, p = 0.18, with no interaction between flavour * genotype, $F_{1, 38}$ = 0.83, p = 0.37.

Independent samples t-tests were conducted on the percentage preferences to compare the genotypes. There was no significant difference between genotypes in preference for CS-A^{++} vs CS-C, t(38) = 0.41, p = 0.69, nor in preference for CS-B^{+} vs CS-C, t(38) = -0.63, p = 0.53.

Table S9A.

	CS-A^{++}	**CS-C**	**CS-A^{++} vs CS-C**
Group	**Mean consumption**	**Mean consumption**	**Preference (%)**
Wild Type	12.34 ±0.86	0.21 ±0.06	97.7 ±1.0
CACNA1C$^{+/-}$	11.25 ±0.71	0.29 ±0.07	97.2 ±0.7

Table S9B.

	CS-B^{+}	**CS-C**	**CS-B^{+} vs CS-C**
Group	**Mean consumption**	**Mean consumption**	**Preference (%)**
Wild Type	9.39 ±0.75	0.24 ±0.04	92.1 ±5.8
CACNA1C$^{+/-}$	10.71 ±0.89	0.44 ±0.12	95.4 ±1.2

Training for Experiment 7 was split into pairing sessions where CS-A^{++} was presented alongside CS-C, or CS-B^{+} was presented alongside CS-C, repeated to give 4 total pairing sessions. A summary of the data for mean consumption and percentage preference between the two solutions during pairing sessions is presented in Table S10A and S10B.

A repeated measures ANOVA was conducted on these data with 'flavour' as the within-subjects factor and 'genotype' and 'sex' as the between-subjects factors. Only the main effects and any significant interactions will be fully reported here.

For the CS-A^{++} vs CS-C pairing sessions, there was a significant effect of flavour, $F_{1, 48}$ = 242.12, p <0.001, with no main effect of genotype, $F_{1, 48}$ = 0.16, p = 0.69, nor sex, $F_{1, 48}$ = 3.18, p = 0.08. There were no significant interactions between any of the factors at any level.

For the CS-B^{+} vs CS-C pairing sessions, there was a significant main effect of flavour, $F_{1, 48}$ = 123.99, p <0.001, with no main effect of genotype, $F_{1, 48}$ < 0.001, p = 0.99, nor of sex, $F_{1, 48}$ = 0.44, p = 0.51. There were no interactions between any of the factors at any level.

A univariate ANOVA was conducted on the percentage preferences to compare the genotypes and sexes. Preference for CS-A^{++} vs CS-C did not significantly differ between genotypes, $F_{1, 48}$ = 1.05, p = 0.31, nor between males and females, $F_{1, 48}$ = 3.48, p = 0.068. There was no interaction between sex * genotype, $F_{1, 48}$ = 0.02, p = 0.89.

Preference for CS-B^{+} vs CS-C also did not differ between genotypes, $F_{1, 48}$ = 0.92, p = 0.34, nor between males and females, $F_{1, 48}$ = 0.55, p = 0.46, with no interaction between group * sex, $F_{1, 48}$ = 0.45, p = 0.51.

Table S10A.

		CS-A^{++}	CS-C	CS-A^{++} vs CS-C
Group	**Sex**	**Mean consumption**	**Mean consumption**	**Preference (%)**
Wild Type	Male	8.84 ±0.59	0.24 ±0.04	97.2 ±0.5
CACNA1C$^{+/-}$	Male	8.71 ±1.22	0.36 ±0.08	95.9 ±0.8
Wild Type	Female	6.68 ±0.76	0.29 ±0.06	94.9 ±1.1
CACNA1C$^{+/-}$	Female	7.36 ±1.29	0.38 ±0.04	93.9 ±1.2

Table S10B.

		CS-B^{+}	CS-C	CS-B^{+} vs CS-C
Group	**Sex**	**Mean consumption**	**Mean consumption**	**Preference (%)**
Wild Type	Male	6.13 ±0.71	0.29 ±0.05	92.5 ±2.9
CACNA1C$^{+/-}$	Male	4.51 ±0.71	0.36 ±0.09	91.5 ±2.5
Wild Type	Female	5.15 ±0.71	0.33 ±0.04	92.2 ±1.2
CACNA1C$^{+/-}$	Female	6.55 ±1.29	0.49 ±0.11	86.7 ±6.8

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