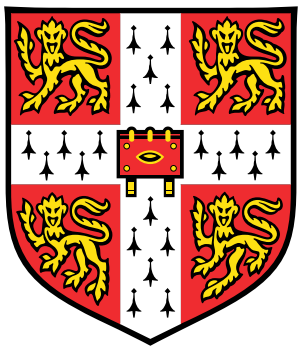
Neurochemical modulation of affective and behavioural control

Models and applications for psychiatry



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To Hisham

Declaration

I hereby declare that this thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the preface and specified in the text, and that it is not substantially the same as any work that has already been submitted before for any degree or other qualification except as declared in the preface and specified in the text. This dissertation does not exceed the prescribed word limit for the Degree Committee for the Faculty of Biology.

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Abstract

Neurochemical modulation of affective and behavioural control: Models and applications for psychiatry

Impairments in emotional reactivity and behavioural flexibility are pervasive across disparate psychiatric conditions as traditionally defined. Here, I provide new evidence on how these processes are altered by neuromodulators in humans, with a primary focus on serotonin (5-HT; 5-hydroxytryptamine). Emotional reactions prepare the body for action. Some emotion is primitive, implicit, and critical for surviving threats, yet can inappropriately persist in times of safety. Other emotions are more complex, self-conscious and important in maintaining harmonious interpersonal relationships. At the same time, learned behaviours that are adaptive in the first instance, may become irrelevant or even disadvantageous as circumstances change. In Chapters 3 through 6, I report on experiments in healthy human volunteers that employed the dietary technique acute tryptophan depletion (ATD). ATD temporarily lowers serotonin synthesis and release by depleting its biosynthetic precursor tryptophan. Chapter 3 is a study of self-reported social emotion. ATD enhanced emotion in response to social injustice non-specifically; however, consideration of personality traits revealed that highly empathic participants reported more guilt under ATD, whereas individuals high in trait psychopathy demonstrated more annoyance. Chapter 4, in contrast, considers evolutionarily ancient automatic emotional reactions to threats. This was assayed instead by an objective measure, the skin conductance response (SCR). Here, ATD conversely attenuated the retention of Pavlovian conditioned emotional memory to threat. Traits again influenced this response: individuals more intolerant of uncertainty displayed the greatest attenuation of emotional reactions. Chapter 5 both extends the studies on emotion and bridges to the remaining empirical work by investigating reversal learning, an index of cognitive flexibility, in two experiments. Individuals again underwent Pavlovian (stimulus-outcome) threat conditioning, whereby one stimulus predicted threat, and another was safe. These contingencies then swapped (reversed). In a separate experiment, participants underwent instrumental (stimulus-response-outcome) conditioning on a deterministic schedule (the correct option was always correct), followed by reversal of the contingencies. ATD impaired both Pavlovian and instrumental reversal learning. Chapters 6 through 8 instead examine instrumental reversal learning that was probabilistic

(the correct option was correct most but not all of the time), rather than deterministic. Chapter 6 expands on previous ATD studies of probabilistic reversal learning (PRL) in the literature, which had not found effects on choice behaviour. Despite nearly tripling the sample size, behaviour here assessed by conventional methods was unaffected, replicating previously published null results. Applying reinforcement learning (RL) models, however, revealed ATD elevated a basic perseverative tendency, referred to as "stimulus stickiness"; behaviour was more stimulus-bound and insensitive to the outcome of actions, consistent with the deterministic instrumental reversal impairment following ATD. Chapters 7 and 8 apply RL models as well, to existing datasets on PRL for comparison. Chapter 7 shows that healthy volunteers under lysergic acid diethylamide (LSD), which acts both at serotonin but also dopamine receptors, showed enhanced learning from positive feedback in particular, which was related to perseveration. Chapter 8 applies computational methods to PRL in clinical populations. RL modelling revealed a computational signature that dissociated PRL in stimulant use disorder (SUD) and obsessive-compulsive disorder (OCD): Individuals with SUD showed heightened stimulus stickiness, as occurred following ATD in healthy volunteers, whereas the OCD group (under serotonergic medication) demonstrated lower stimulus stickiness than healthy controls. Dopaminergic agents remediated a reward learning deficit in SUD, among other measures. The general discussion considers these various findings in terms of theories of central serotonin function, in relation to the animal literature, and its relevance to mental disorder. These results, collectively, advance knowledge of neurochemical and computational mechanisms underlying psychiatric conditions trans-diagnostically, with implications for revised psychiatric classifications in line with the Research Domain Criteria (RDoC).

Jonathan William Kanen

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Nomenclature

Roman Symbols

4-CSRTT Four-choice serial reaction time test (humans)

5,7-DHT 5,7 dihydroxytryptamine

5-CSRTT Five-choice serial reaction time test (non-humans)

5-HIAA 5-hydroxyindoleacetic acid

5-HT serotonin

5-HT1A serotonin type 1A receptor

5-HT2A serotonin type 2A receptor

5-HT2C serotonin type 2C receptor

5-HTP 5-hydroxytryptophan

AAAD aromatic L-amino-acid decarboxylase

ADH aldehyde dehydrogenase

ADHD attention deficit/hyperactivity disorder

AMPA alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AQ autism quotient

ASC altered states of consciousness questionnaire

ASPD anti-social personality disorder

ATD acute tryptophan depletion

AUD alcohol use disorder

BDD body dysmorphic disorder

BDI-II Beck Depression Inventory

BDNF brain-derived neurotrophic factor

BIS Barratt Impulsiveness Scale

CNS central nervous system

CRN caudal raphe nuclei

CS+ conditioned stimulus plus

CS- conditioned stimulus minus

D1 dopamine type 1 receptor

D2 dopamine type 2 receptor

D3 dopamine type 3 receptor

DBS deep brain stimulation

DRN dorsal raphe nucleus

DSM Diagnostic and Statistical Manual of Mental Disorders

fMRI functional magnetic resonance imaging

GABA gamma-aminobutryic acid

HPLC high-performance liquid chromatography

IBS irritable bowel syndrome

IDED intra-dimensional/extra-dimensional set shifting task

IED intermittent explosive disorder

IRI Interpersonal Reactivity Index (empathy scale)

IUS Intolerance of Uncertainty Scale

LNAA large neutral amino acid

LSD lysergic acid diethylamide

LSRP Levenson Self-Report Psychopathy Scale

MAO monoamine oxidase

mCPP meta-chlorophenylpiperazine

MDMA 3,4-methylenedioxy methamphetamine

MINI Mini International Neuropsychiatric Interview

MRN median raphe nucleus

NbN neuroscience-based nomenclature

OCD obsessive-compulsive disorder

OCI-R Obsessive-Compulsive Inventory Revised

OCPD obsessive-compulsive personality disorder

OFC orbitofrontal cortex

PANAS Positive and Negative Affect Schedule

PCA Principal Component Analysis

PET positron emission tomography

PIT Pavlovian-to-instrumental transfer

PRL probabilistic reversal learning

PTSD post-traumatic stress disorder

R receptor

RDoC Research Domain Criteria

RL reinforcement learning

RRN rostral raphe nuclei

SCR skin conductance response

SERT serotonin transporter

SNc substantia nigra pars compacta

SPECT single photon emission computed tomography

SSRI selective serotonin reuptake inhibitor

SSRT stop signal reaction time task

STAI Spielberger Trait Anxiety Inventory

SUD stimulant use disorder

TD learning temporal difference learning

TPH tryptophan hydroxylase

TRP tryptophan

UG ultimatum game

US unconditioned stimulus

VAS visual analogue scale

vmPFC ventromedial prefrontal cortex

VTA ventral tegmental area

Y-BOCS Yale-Brown Obsessive Compulsive Scale

Chapter 1

General Introduction

To survive and thrive in the world an organism must, at a fundamental level, continually maximise rewards and minimise punishments. The brain achieves this through a complex interplay between emotional reactions and behavioural adaptation, psychological processes which depend on signalling by neuromodulators. This thesis centres on neurochemical modulation in relation to two broad themes: emotional reactivity and behavioural flexibility. These processes are principally modelled with fundamental learning paradigms, available across species, that assess Pavlovian (stimulus-outcome) and instrumental (stimulus-response-outcome; operant) learning. The primary emphasis, neurochemically, is on serotonin; however, dopamine is also considered. Most of the empirical evidence contained herein comes from studies of healthy human volunteers that model clinically relevant mechanisms, whereas the final study, described in Chapter 8 (Kanen et al., 2019), is an application to two psychiatric conditions: obsessive-compulsive disorder (OCD; see Box 1) and stimulant use disorder (SUD; see Box 6).

1.1 Dependent measures and their clinical relevance

The dependent psychological variables used in this thesis fall into four categories: subjective self-reported emotion, implicit emotion objectively assessed physiologically, observable behavioural choice, and latent indices of behaviour.

1.1.1 Social emotions

Humans are social animals. Self-conscious, or moral emotions such as guilt and shame help guide or restrain social behaviour so that it accords with societal expectations and is conducive to harmonious interpersonal relationships (Tangney et al., 2007). Excessive guilt, however, is a feature of depression (American Psychiatric Association, 2013) and is classically lacking

in psychopathy (Blair, 2010). Excessive shame, another self-conscious emotion, furthermore, is linked to a wide range of psychopathology (Tangney et al., 2007). Excessive reactions of annoyance or frustration to social conflict, moreover, may contribute to a tendency to behave in an aggressive or anti-social manner. The role of serotonin in social (or moral) emotion, however, has not been studied and is reported on in Chapter 3. Social emotions can be assessed by self-report (Bland et al., 2016; Tangney et al., 2007). I presented individuals with scenarios involving social injustice and probed the feelings elicited when asked to imagine being a particular character involved in the conflict depicted: ratings of guilt, shame, annoyance, and feeling "bad" were collected. To further explore contributors to the experience of social emotions, I also employed validated scales of personality traits, in particular relating to empathy and psychopathy, as proxies for tendencies towards prosocial and antisocial behaviour, respectively (Kanen et al., 2020b).

1.1.2 Threat conditioning processes

Examination of higher-order emotion in Chapter 3 is followed by two experiments (Chapters 4 and 5) on the role of serotonin in primitive, implicit emotion to threat, a survival mechanism that is evolutionarily conserved (LeDoux, 2000). This is modelled across species in an objective manner using Pavlovian (classical) conditioning paradigms, whereby a cue (conditioned stimulus plus; CS+) is paired with an aversive outcome (usually a mild electric shock, as here), and stimulus-outcome contingencies are learned; acting is irrelevant. Subsequent presentations of the cue then automatically evoke a threat response – an emotional reaction that manifests in freezing in rats (LeDoux, 2000) and measurable perspiration in humans referred to as the skin conductance response [SCR] (Hartley et al., 2012; Phelps et al., 2004; Schiller et al., 2008). In human studies, another cue, the CS-, is presented that does not predict shock. Extinction, meanwhile, refers to the repeated presentation of the CS+ without shock, and emotional responses tend to diminish (Bouton 2002). Exposure therapy and exposure and response prevention (ERP) are forms of cognitive behavioural therapy (CBT) that are based on the principle of extinction. Extinction, however, is not unlearning: a safety memory is formed which then competes against the threat memory for expression. The persistence of threat memory despite extinction is a clinically relevant phenomenon and is manifested in laboratory models as a reappearance of conditioned emotion after the passage of time, even in the absence of shock. Indeed, aberrant threat conditioning processes have been reported across numerous psychiatric conditions, particularly those characterised by anxiety (Kim et al., 2011; Marin et al., 2017). Individuals with post-traumatic stress disorder [PTSD] (Milad et al., 2009), OCD (McLaughlin et al., 2015; Milad et al., 2013), and schizophrenia (Holt et al., 2012) have been shown to more strongly retain aversive emotional memories. Pavlovian reversal learning is another

related paradigm whereby following initial conditioning, the contingencies swap such that the CS+ is now safe and the CS- now threatening, requiring flexible updating of emotional reactions. Indeed, this has been tested in individuals with OCD (Apergis-Schoute et al., 2017) and PTSD (Homan et al., 2019) revealing impaired Pavlovian reversal learning. Both the retention and reversal of Pavlovian threat conditioning are also impaired by stress in healthy humans (Raio et al., 2014, 2017). Given exposure therapy is widely used for PSTD (Benedek et al., 2009) and phobias, and ERP is the mainstay of psychological therapy for OCD, elucidating the mechanisms underlying threat conditioning processes is especially important for informing treatment. How serotonin modulates Pavlovian threat memory retention and Pavlovian reversal learning is studied in Chapters 4 and 5, respectively (Kanen et al., 2020a,d).

Obsessive-compulsive disorder (OCD) [Box 1]

Clinical characteristics

OCD is a debilitating psychiatric condition characterised by the presence of obsessions and compulsions (American Psychiatric Association, 2013). Obsessions refer to intrusive and unwanted thoughts, images, or urges that are repetitive and persistent (American Psychiatric Association, 2013) and that are distressing and anxiogenic. Compulsions, also referred to as rituals, are repetitive behaviours that the individual feels compelled to perform, often conforming to rigid rules (American Psychiatric Association, 2013). Most individuals have both obsessions and compulsions, which are excessively time-consuming and cause distress or functional impairment (American Psychiatric Association, 2013). Common themes include fear of contamination or compulsive washing; ordering/symmetry/counting/repeating words; checking behaviour (e.g. of a stove or lock); fear of harm to oneself or others; and taboo thoughts, for instance involving sexual or religious content (American Psychiatric Association, 2013). Hoarding, or accumulating objects, can also occur in relation to obsessions and compulsions in some individuals (American Psychiatric Association, 2013).

Prevalence, development, and course

The 12-month prevalence of OCD internationally is 1.1%-1.8%, and 1.2% in the United States (American Psychiatric Association, 2013). The statistics on OCD cited below are based on the United States population. Note that the lifetime prevalence of schizophrenia is substantially lower, at approximately 0.3%-0.7% (American Psychiatric Association, 2013). Whilst males are more affected in childhood, with nearly 25% of cases of OCD in males emerging before the age of 10, there is a slightly higher prevalence of OCD in females in adulthood (American Psychiatric Association, 2013). One quarter of cases start by 14 years of age, the mean age of onset is 19.5 years old, and it is unusual for onset to occur after age 35 (American Psychiatric Association, 2013). Symptoms typically emerge gradually, and without treatment the course of OCD is typically chronic with symptoms that wax and wane (American Psychiatric Association, 2013). The course of OCD is also shaped by the presence of comorbid psychopathology.

Obsessive-compulsive disorder: comorbidity [Box 2]
Individuals with OCD have high rates of comorbidity, or co-occurring psychiatric disorders. Seventy-six percent of adults with OCD have been diagnosed with an anxiety disorder within their lifetime, and 63% have been diagnosed with a depressive or bipolar disorder (American Psychiatric Association, 2013). Forty-one percent have been diagnosed with major depressive disorder in particular (American Psychiatric Association, 2013). Between 23%-32% of individuals with OCD have an additional diagnosis of obsessive-compulsive personality disorder. Tic disorder is comorbid in up to 30% of individuals with OCD throughout the lifespan. Obsessive-compulsive related disorders such as body dysmorphic disorder, excoriation (skin-picking) disorder, and trichotillomania (hair-pulling disorder) are more common in individuals with OCD than in the general population (American Psychiatric Association, 2013). OCD, at the same time, is markedly more common in individuals with schizophrenia or schizoaffective disorder [12%] (American Psychiatric Association, 2013). The prevalence of OCD amongst individuals with Tourette's disorder, eating disorders, and bipolar disorder is also elevated (American Psychiatric Association, 2013).

1.1.3 Behavioural flexibility

Just as functioning in daily life requires that emotional reactions proportionately reflect threatening or safe circumstances as the environment changes, flexibly adapting learned behaviours to meet new demands is also of critical importance. Behavioural flexibility is widely studied using instrumental reversal learning paradigms (Phillips and Robbins, 2020), which are employed in Chapters 5, 6, 7, and 8. Subjects learn through trial and error the most adaptive action in an acquisition phase (stimulus-response-outcome learning), and the contingencies eventually change such that the previously optimal response becomes disadvantageous, necessitating disengagement with the initially learned behaviour and shifting to a new adaptive response. There are two common varieties of instrumental reversal learning paradigms: deterministic, where the presently correct option is always correct, and probabilistic, where the correct option is correct most but not all of the time. Probabilistic reversal learning (PRL), consequently, requires that one withstand occasional negative feedback in order to obtain the most positive feedback in the long run (Lawrence et al., 1999). Individuals with depression, both medicated and unmedicated, have been shown to succumb to this periodic negative feedback as manifested by switching their behaviour away from the optimal response, referred to as "lose-shift" (rather than "win-stay"), which I will refer to as enhanced sensitivity to negative feedback (SNF) in this thesis. Individuals with OCD have also shown this aberrant behavioural pattern (Ersche et al., 2011; Kanen et al., 2019). Meanwhile, individuals with SUD, have shown a perseverative deficit during PRL (Ersche et al., 2011): upon the reversal of

contingencies, those with SUD maladaptively continued responding to the previously correct stimulus, referred to as perseveration. Whilst win-stay, lose-shift, and perseveration are examples of conventional measures, Chapters 6, 7, and 8 additionally incorporate a different sort of dependent measure, assessing latent (not directly observable) processes underlying choice behaviour, and can be discerned through the application of reinforcement learning models. Measures (parameters) of interest include the rate at which individuals learn from feedback (reinforcement), and the tendency to repeat choices regardless of previously experienced outcomes.

OCD treatment: psychological [Box 3]

The mainstay of psychological treatment of OCD is exposure and response prevention (ERP), the origins of which date back to the 1960s (Fineberg et al., 2020). ERP is based off of principles of exposure therapy, whereby an individual is repeatedly exposed, often in a graded manner, to the feared stimulus (a situation or object, for instance), and must at the same time prevent themselves from performing the compulsion or ritual (Fineberg et al., 2020). The patient should be educated that performing the compulsive rituals strengthens the habit, worsening the condition, and that whilst the initial urge to perform the compulsion will rise initially during ERP, eventually following successful response prevention, the urge will subside and extinguish (Fineberg et al., 2020).

1.2 Neurocognitive underpinnings

The key neuroanatomical systems supporting the psychological processes studied in this thesis can be unified in the following way. The ventromedial prefrontal cortex (vmPFC) has been viewed as an integrative centre involved in the generation of affective meaning (Roy et al., 2012). Its diverse direct projections to subcortical regions including the amygdala, hypothalamus, striatum, periaqueductal grey (PAG), raphe nuclei, among other brainstem structures, as well as to spinal autonomic ganglia, make it unique among cortical regions (Roy et al., 2012). These pathways have been collectively referred to as a visceromotor system involved in coordinating aspects of autonomic nervous system activity (Price and Drevets, 2010; Roy et al., 2012). The vmPFC is additionally monosynaptically connected to the hippocampus, orbitofrontal cortex (OFC), dorsomedial and dorsolateral prefrontal cortices (dmPFC, dlPFC), and the frontopolar cortex (Roy et al., 2012). The vmPFC is therefore anatomically poised to serve as a hub connecting neural systems that support disparate psychological functions, spanning primitive emotional responses, interoception, episodic memory, higher order social cognition, valuation of cues, and goal formation (Roy et al., 2012).

A core feature of mood and anxiety disorders is dysfunctional circuitry involving the

amygdala and orbitofrontal/ventromedial prefrontal cortex [OFC/vmPFC] (Bishop, 2007; Clark et al., 2009; Drevets et al., 2008; Milad and Rauch, 2007; Phillips et al., 2003). Indeed, increased SNF in depression during PRL has been linked to reduced prefrontal control over the amygdala (Taylor Tavares et al., 2008), and neurotoxically disrupting the OFC-amygdala circuit has resulted in impaired PRL in marmoset monkeys (Rygula et al., 2015). Orbitofrontal-striatal function, moreover, has been shown to be altered in OCD during PRL (Remijnse et al., 2006), and abnormalities in OFC metabolism in relation to striatal function have been reported in SUD (Volkow et al., 2001).

Whereas the amygdala is critical for the acquisition and expression of conditioned emotion (LeDoux, 2000), the vmPFC is particularly important in emotion regulation and extinction and its involvement in Pavlovian threat conditioning processes has been studied extensively (Graham and Milad, 2011; Schiller and Delgado, 2010). Indeed, greater conditioned emotional memory expression has been associated with diminished activity of the vmPFC in healthy volunteers (Phelps et al., 2004) and a similar activation pattern has been shown in individuals with PTSD (Graham and Milad, 2011; Milad et al., 2009), OCD (McLaughlin et al., 2015; Milad et al., 2013), and schizophrenia (Holt et al., 2012). The vmPFC has also been linked to successful Pavlovian reversal learning (Schiller et al., 2008), and vmPFC dysfunction has been markedly predictive of impaired Pavlovian reversal learning in OCD (Apergis-Schoute et al., 2017). The OFC and vmPFC have accordingly been implicated in affective processes that guide social conduct (Blair, 2010; Krajbich et al., 2009; Wagner et al., 2011). Whilst the neural substrates of guilt and shame are complex, one study found guilt-related activation which included the lateral OFC as well as dmPFC, a region important for theory of mind, or the ability to take another person's perspective (Wagner et al., 2011). Related to its essentiality in episodic memory, the hippocampus may additionally have a role in moral emotions such as shame (Bastin et al., 2016), as it is thought to be involved in projecting oneself into the past or future (Buckner and Carroll, 2007) and simulating social scenarios (Schafer and Schiller, 2019). Whilst I do not report on brain data in this thesis, the function of these neural substrates depends on the ascending monoamine neurotransmitter systems.

1.3 Serotonin: introduction to its complexities

Serotonin is said to be 'involved in everything but responsible for nothing' (Jacobs and Azmitia, 1992). Indeed, serotonin is involved in mood, emotion, learning, cognition, neural development, emesis, appetite, sex, sleep, pain, migraine, sensation and perception, and gastrointestinal, endocrine, motor, and vascular function (van Donkelaar et al., 2011). Serotonin is thought to have a role in diverse psychiatric conditions including depression, anxiety, panic

(Cools et al., 2008a; Deakin, 2003, 2013; Hood et al., 2005; Ruhé et al., 2007; Young, 2013), obsessive-compulsive disorder (Dersken et al., 2020), and mental phenomena relevant to various disorders such as impulsivity, compulsivity (Phillips and Robbins, 2020), aggression (Bevilacqua et al., 2010; Deakin, 2003; Linnoila et al., 1983), and social cognition (Crockett et al., 2010a, 2008, 2015; Kiser et al., 2012). Many of the most commonly used drugs to treat mental illness act through serotonin (Stahl, 2013). A major clinical paradox is that benzodiazepines alleviate anxiety and reduce serotonin transmission whereas long-term use of selective serotonin reuptake inhibitors (SSRIs) are believed to increase serotonin transmission and can elevate mood, restrain panic, and also ameliorate anxiety (Cools et al., 2008a; Dayan and Huys, 2009; Deakin, 2013). Part of the complexity of understanding serotonin in the central nervous system (CNS) stems from the fact that there are at least 14 different receptor subtypes clustered into seven families (Marin et al., 2020). Additional variation within receptor subtypes comes from the splicing and editing of gene products, the identification of which continues to increase (Vilaro et al., 2020). This is in contrast to five known dopamine receptor subtypes (Sokoloff and Schwartz, 1995). Serotonin receptors can additionally have opposing actions (e.g. 5-HT1 and 5-HT2, which are inhibitory and excitatory, respectively), and can moreover be found as autoreceptors (on the 5-HT-releasing neuron) or heteroreceptors (on the post-synpatic cell). See Figure 1.1 for a cartoon example. Some receptors detect lower concentrations of serotonin, others higher concentrations, conducive to tonic and phasic modes of transmission, respectively (Dayan and Huys, 2009). Serotonin can interact with other neurotransmitters, for instance activating 5-HT1A, 5-HT1B, or 5-HT2A receptors enhances the release of dopamine, whereas 5-HT2C receptor stimulation suppresses dopamine activity (Dayan and Huys, 2009). The current diversity of receptors and their function likely grew out of the fact that serotonin action at its receptors is one of the oldest molecular mechanisms for communicating between cells, dating back to the emergence of simple nervous systems in evolutionary history (Vilaro et al., 2020). CNS serotonergic neurons, furthermore, originate from multiple nuclei (the raphe nuclei) in the brainstem – amounting to serotonin subsystems – and project to virtually all brain regions, depicted in Figure 1.2 (Cools et al., 2008a; Dayan and Huys, 2009). Indeed, there is now clear evidence that no single region of the CNS, including the cerebroventricular cavity, is devoid of serotonergic innervation (Parent and Descarries, 2020). A further complexity is that many serotonin-releasing neurons corelease multiple neurotransmitters and that a minority of neurons in the raphe nuclei do not release serotonin at all (Fischer et al., 2015; Pollak Dorocic et al., 2014). Meanwhile, there are limited means for exploring this intricacy directly in humans.

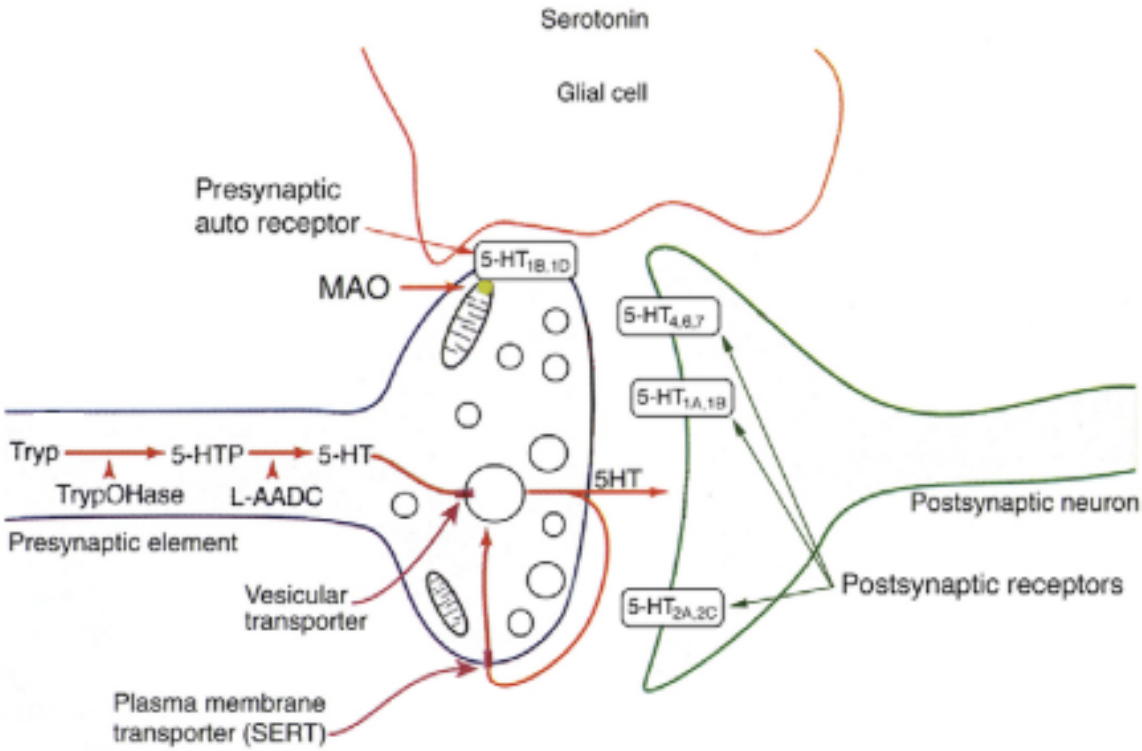

Figure 1.1: Schematic of a serotonin synpase, including a depiction of pre- and post-synaptic receptors. 5-HT1B, serotonin 1B receptor; Tryp, tryptophan; TrpOHase, tryptophan hydroxylase; 5-HTP, 5-hydroxytryptophan; L-AADC, L-amino acid decarboxylase; SERT, serotonin transporter; MAO, monoamine oxidase. Used with permission (Cools et al., 2008a).

1.4 Serotonin: discovery and synthesis

Serotonin was first extracted from the enterochromaffin cells of the rabbit gastrointestinal tract, where it was observed to induce contractions, and consequently named enteramine (Vialli and Erspamer, 1937). It was renamed serotonin after it was found in bovine blood to have vasoconstrictive ('sero-tonin') properties (Rapport et al., 1948). Serotonin is synthesised in two steps from the amino acid tryptophan, which the body cannot produce and therefore must be consumed from the diet. Serotonin synthesis and metabolism will be elaborated upon in Chapter 2. Most serotonin in the body is synthesised in the enterochromaffin cells of the gut, whilst the majority of serotonergic innervation of the brain derives from serotonin synthesising neurons in the raphe ('seam') nuclei which are located near the midline throughout the brainstem and extend rostro-caudally (Hornung, 2003).

1.5 Receptors

All serotonin receptors are G protein-coupled receptors (GPCR) – metabotropic – besides 5-HT3 receptors which are ionotropic (Vilaro et al., 2020). Metabotropic receptors operate through second messengers, which are molecules that send signals inside of cells. Ionotropic receptors, in contrast, operate by allowing certain ions to pass through the cell membrane. The 5-HT1A, 5-HT2A, and 5-HT2C receptors will be introduced first, as they are the most studied in relation to the topics presented in this thesis.

1.5.1 5-HT1A

5-HT1A receptors are located postsynaptically in forebrain regions and presynpatically on the soma and dendrites of rostral and caudal (mesencephalic and medullary) raphe nuclei (Vilaro et al., 2020). The density of 5-HT1A receptors is high in the hippocampus, dorsal and median raphe nuclei (DRN and MRN, respectively, introduced below), cortical areas especially the cingulate and entorhinal cortices, lateral and medial septum, diagonal band of Broca, several amygdala subnuclei, and other brainstem nuclei (Vilaro et al., 2020). Of the 5-HT receptors present in the amygdala, 5-HT1A receptors appear to be the most abundant (Ohuoha et al., 1993). Binding sites of 5-HT1A in the basal ganglia and cerebellum, by contrast, are barely detectable.

1.5.2 5-HT2A

The highest density of 5-HT2A receptors is in the neocortex (Vilaro et al., 2020). Other regions with significantly enriched 5-HT2A receptors include the striatum, substantia nigra, hilus and dentate gyrus of the hippocampus, olfactory tubercle, and some brainstem nuclei especially those of the trigeminal and facial cranial nerves (Vilaro et al., 2020).

1.5.3 5-HT2C

The 5-HT2C receptor is present at very high densities in the choroid plexus (Vilaro et al., 2020). Other regions show comparatively lower densities, and these include the lateral amygdala, striatum, CA1 of the hippocampus, several areas of the cortex, claustrum, central medial thalamic nucleus, anterior olfactory nucleus, olfactory tubercle, and nucleus of the solitary tract (Vilaro et al., 2020).

1.5.4 5-HT1B

Like the 5-H1A receptor, 5-HT1B is found as both a pre- and post-synaptic receptor (Vilaro et al., 2020). There are high densities of 5-HT1B receptors in the ventral pallidum, caudate, putamen, globus pallidus, ventral tegmental area (VTA), substantia nigra, subthalamtic nucleus, entopeduncular nucleus, several cortical areas, superior colliculus, nucleus of the optic tract, and the olivary pretectal nucleus (Vilaro et al., 2020).

1.5.5 5-HT1D, 5-HT1E, and 5-HT1F

Distribution of 5-HT1D receptors in the human brain includes the globus pallidus, substantia nigra, PAG, and spinal cord (Vilaro et al., 2020). High densities of the 5-HT1E receptor, meanwhile, are present in the frontal cortex, amygdala, caudate, putamen, and medial and lateral globus pallidus, with less of a presence in the hippocampus (Vilaro et al., 2020), and evidence for its presence in the hypothalamus and entorhinal cortex as well (Vilaro et al., 2020). 5-HT1E has been identified in humans but does not appear to be present in rats or mice, the most commonly used animal models, thus complicating investigations (Vilaro et al., 2020). The 5-HT1F receptor remains poorly characterised, however closely shares structural and pharmacological properties with the 5-HT1E (Vilaro et al., 2020). High levels of binding have been found in hippocampus and cortex, as well as the caudate and claustrum, and appears to be barely detectable in the substantia nigra (Vilaro et al., 2020).

1.5.6 5-HT3 and 5-HT4

Densities of 5-HT3 are lower in the forebrain and are present in a number of areas of the lower medulla as well all levels of the spinal cord, within the substantia gelatinosa (Vilaro et al., 2020). Regions where 5-HT4 receptors are enriched include the substantia nigra, globus pallidus, nucleus accumbens, caudate, putamen, islands of Calleja, and the olfactory tubercle (Vilaro et al., 2020). 5-HT4 is present at lower densities in cortical areas, hippocampus, amygdala, hypothalamus, thalamus, and a number of structures in the midbrain (Vilaro et al., 2020).

1.5.7 5-HT5 family

Receptors of the 5-HT5 family are less well characterised (Vilaro et al., 2020). There is evidence that 5-HT5A receptors are present in layers II-III and V-VI of the cortex, an area of the ventrolateral amygdala, and parts of the cerebellum and hippocampus (Vilaro et al., 2020). The 5-HT2B receptor, meanwhile, has not been detected in humans but exists in rats and mice (Vilaro et al., 2020).

1.5.8 5-HT6 and 5-HT7

Densitiy of the 5-HT6 receptor is highest in the ventral striatum, caudate, and putamen; and moderate in dorsolateral prefrontal cortex, temporal and visual cortices, hippocampal regions, and parahippocampal gyrus (Vilaro et al., 2020). The 5-HT7 receptor has been found in the DRN, PAG, basolateral amygdala, frontal, entorhinal, and anterior cingulate cortices, hippocampal subregions, several thalamic nuclei, VTA, substantia nigra, hypothalamus, and superior colliculus (Vilaro et al., 2020).

1.6 The raphe nuclei: anatomy

Figure 1.2 includes a simple depiction of raphe nuclei. The raphe nuclei, located along the neuraxis of the brainstem, contain the cell bodies of serotonin-releasing neurons that project throughout the entire CNS (Dayan and Huys, 2009). There are two main groups of raphe nuclei, composed of the caudal raphe nuclei, which contain neurons projecting to the spinal cord, whereas the rostral raphe nuclei have ascending projections (Dayan and Huys, 2009). The rostral group is made up of the DRN and MRN, which differ in a number of properties (e.g. reuptake mechanisms, axon varicosities), especially in their anatomical projections

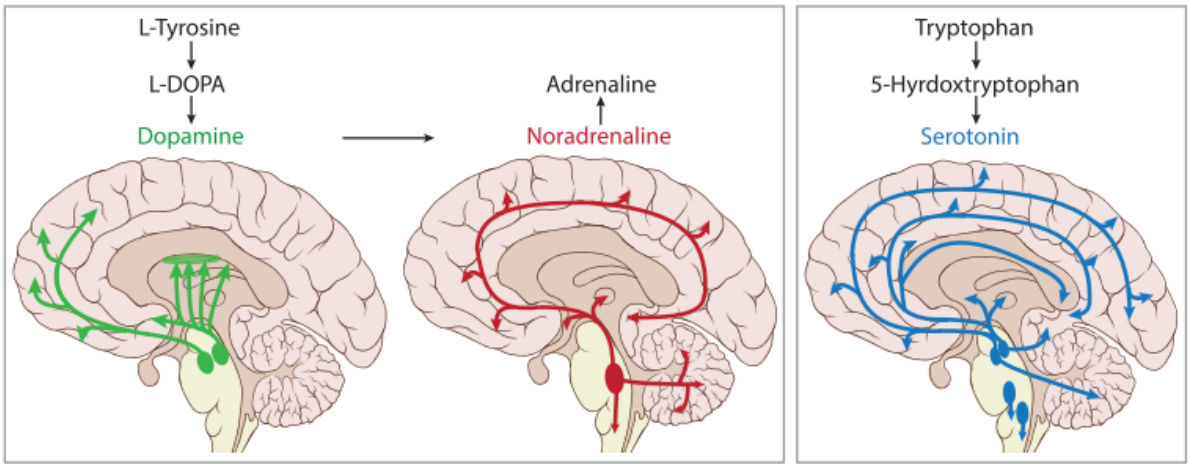

Figure 1.2: Used with permission (Cools, 2019). Synthesis and projection pathways of the three major monoamine neurotransmitters, dopamine, noradrenaline, and serotonin.

(Dayan and Huys, 2009). For unknown reasons (Faulkner and Deakin, 2014), MDMA (3,4-methylenedioxy methamphetamine) has a neurotoxic effect on DRN rather than MRN neurons (Molliver et al., 1990), for instance. Another feature of the rostral raphe nuclei is that there are parallels in innervation patterns between the MRN and noradrenergic projections from the locus coeruleus, and between the DRN and dopaminergic pathways (Deakin, 2013).

The MRN innervates the entire hippocampus, as well as the hypothalamus, the mammillary bodies, several thalamic nuclei, part of the olfactory bulb, the preoptic area, the interpeduncular nucleus (Soiza-Reilly and Gaspar, 2020), and part of the nucleus accumbens shell (Dayan and Huys, 2009), in addition to the amygdala (Deakin, 2003). MRN innervation of the cerebral cortex appears more limited and includes the anterior cingulate cortex and medial PFC (Soiza-Reilly and Gaspar, 2020). The DRN, meanwhile, innervates all major forebrain structures including frontal [especially vmPFC] (Hornung, 2010), insular, entorhinal, motor, and somatosensory cortices (Soiza-Reilly and Gaspar, 2020); the nucleus accumbens core (Dayan and Huys, 2009), caudate, putamen, pallidum; all subdivisions of the amygdala (Parent and Descarries 2020); the extended amygdala (bed nucleus of the stria terminalis); and the dorsal and ventral hippocampus (Soiza-Reilly and Gaspar, 2020). DRN neurons also project to the PAG, habenula, VTA, substantia nigra, locus coeruleus, parts of the thalamus and hypothalamus, superior and inferior colliculi, cerebellum, parabrachial nucleus, and brainstem nuclei including the vagus, trigeminal, cochlear, and facial nerves (Soiza-Reilly and Gaspar, 2020).

1.7 Raphe nuclei: a framework from anatomy to function

1.7.1 Dorsal raphe subsystems

The anatomical organisation of RRN projections lies at the heart of an influential framework that has sought to reconcile the diverse and at times paradoxical effects of serotonin on emotion and behaviour (Deakin, 2013; Deakin and Graeff, 1991). Early work found that antagonising serotonin disinhibited behaviour in pigeons that was otherwise suppressed in the face of foot shock (Graeff and Schoenfeld, 1970), and that raising serotonin promoted behavioural inhibition in rats facing the threat of shock (Wise et al., 1970). It was subsequently proposed that DRN serotonergic neurons were activated by distal cues predictive of aversive outcomes which consequently fostered behavioural inhibition, in contrast to dopaminergic projections that energised behaviour towards incentives (Deakin, 1983).

Electrically stimulating the PAG, meanwhile, elicited escape behaviour [flight] (Kiser et al., 1978), which was exacerbated by (drug-induced) serotonin depletion and alleviated by the serotonin reuptake inhibitor clomipramine, thought to enhance serotonergic neurotransmission (discussed below). It was subsequently shown that targeted microinjections of 5-HT into the PAG restrained escape behaviour in the rat (Schütz et al., 1985). Conversely, reducing 5-HT transmission in humans decreased aversive Pavlovian conditioning (Hensman et al., 1991) and corresponding amygdala activity (Hindi Attar et al., 2012). Serotonin, therefore, appeared to have opposing effects in two different forms of anxiety: to proximal and distal threats (Deakin, 2013). This can be reconciled by viewing serotonergic signalling as involved in anticipatory anxiety: a distal threat would elicit DRN serotonin signalling in the amygdala as well as in the PAG, suppressing reflexive escape behaviour and allowing for the engagement of avoidance strategies, whereas serotonergic restraint over the PAG would be released when a threat becomes imminent or the organism is at risk of death (Deakin, 2013).

Inescapable shocks in rats, meanwhile, have been shown to activate all raphe nuclei (Takase et al., 2004), and lead to characteristically elevated anticipatory anxiety and compromised escape behaviour. This failure to escape, initially studied in dogs, was termed learned helplessness (Seligman and Maier, 1967) and resembled features of depression (Maier and Watkins, 2005). The mPFC, meanwhile, was able to detect when shock was controllable and consequently restrained DRN suppression of escape or avoidance behaviour (Amat et al., 2005).

The accounts put forth by Deakin and Graeff (1991) and Maier and Watkins (2005) posit that overactivity of the DRN could contribute to depressive symptoms through its projections to the amygdala (promoting anxiety), PAG (leading to helplessness), and structures within the striatum also receiving dopaminergic innervation, relating to anhedonia and excessive behavioural inhibition.

1.7.2 Median raphe resilience system

Serotonergic projections from the MRN to 5-HT1A receptors in the hippocampus, moreover, have been posited to confer resilience in the face of repeated aversive experiences, in the event that DRN mechanisms were unable to facilitate avoidance of exposure to such aversion (Deakin, 2013; Deakin and Graeff, 1991). This MRN resilience system would promote disengagement from DRN-signalled anticipatory anxiety, inhibit rumination, and enable normal behaviour to ensue despite the continued presence of chronic adversity (Deakin, 2013). Indeed, classic work has shown that chronic treatment with antidepressants promoted functional enhancement of the MRN-hippocampal pathway (de Montigny and Blier, 1984). The framework of Deakin and Graeff (1991) on DRN and MRN function is discussed in the context of the wider literature below.

1.8 Dopamine: background

1.8.1 Dopamine receptors and anatomy

In contrast to the 14 serotonin receptor subtypes, there are five varieties of dopamine receptors which cluster into two families: D1 and D5, and D2, D3, D4 (Sokoloff and Schwartz, 1995). All are GPCRs. The cell bodies of dopaminergic neurons are primarily located in two regions of the midbrain: the VTA and the substantia nigra pars compacta (SNc), depicted in Figure 1.2. Dopamine neurons are also present in the hypothalamus as well as the olfactory bulb and retina (Collins and Saunders, 2020). There are a four major dopaminergic projection pathways (Collins and Saunders, 2020), three of which are most relevant: the mesolimbic pathway (connecting VTA to ventral striatum, which includes the nucleus accumbens), mesocortical (VTA to PFC), and nigrostriatal (SNc to the dorsal striatum, composed of the caudate and putamen). Dopaminergic neurons in the VTA also project to the amygdala (Collins and Saunders, 2020). The majority of dopaminergic projections from the midbrain synapse in the midbrain (Collins and Saunders, 2020).

1.8.2 Dopamine: from anatomy to function

Dopamine is critically involved in reward learning and behavioural activation, or motivation, and is implicated in a wide range of mental phenomena spanning substance use disorders to Parkinson's disease (Cools et al., 2011; Frank et al., 2004; Hamid et al., 2015; Kish et al., 1988; Schultz et al., 1997; Volkow et al., 1993), anhedonia (Nestler and Carlezon, 2006), mania (Ashok et al., 2017), and psychosis (Kapur, 2003; Murray et al., 2008). One of the most

significant advances in neuroscience has been the observation that the phasic firing of midbrain dopaminergic neurons provides a neuronal substrate of reward prediction errors [RPE] (Schultz et al., 1997). Prediction errors are a mismatch between what was expected and what occurred and represent a critical mechanism by which learning occurs (Schultz et al., 1997); a prediction error can be thought of as a surprise. Rhesus monkeys were presented with a conditioned stimulus (CS) that predicted a rewarding fruit juice. Initially midbrain dopaminergic neurons fired not to the CS, which had not yet acquired meaning, but upon receipt of the fruit juice. Subsequently presentation of the CS elicited phasic dopamine neuron firing, rather than the presentation of the reward itself. When the reward was later omitted, phasic firing dipped, termed a negative (rather than positive) prediction error (NPE). The neurophysiological evidence from rhesus monkeys has been substantiated in rats (Day et al., 2007), and by human neuroimaging studies of the ventral striatum (Knutson and Gibbs, 2007; Pessiglione et al., 2006) and midbrain (D'Ardenne et al., 2008; Murray et al., 2008). Another major discovery from this line of work was that the behaviour of midbrain dopamine neurons conformed to classic algorithms from learning theory, namely the Rescorla-Wagner (Rescorla and Wagner, 1972) and temporal difference [TD] (Sutton, 1988) models. Prediction errors serve as a teaching signal enabling an organism to update prior beliefs about environmental contingencies (associative learning) and are thus seen as an important part of a Bayesian updating processes inherent in the brain (Robbins and Cardinal, 2019). That the neural substrate of reward prediction error could be formalised mathematically has been invaluable for the now burgeoning areas of computational neuroscience and computational psychiatry (Corlett and Fletcher, 2014; Huys et al., 2016; Montague et al., 2012).

These insights have been tied to receptor function and frontostriatal loops in the following way. D1 receptors are excitatory and have been linked with phasic firing, whereas D2 receptors are inhibitory and are associated with tonic dopaminergic activity (Yapo et al., 2017). Moreover, striatal D1 and D2 receptors constitute the direct and indirect cortico-striatal pathways, respectively (Gerfen et al., 1990), and have been suggested to represent go and no/go pathways involved in behavioural activation and inhibition, respectively (Frank et al., 2004). Since positive prediction errors are associated with a transient increase in DA, D1 receptors should be preferentially activated and downstream signalling increased. D2 receptors are consequently thought to respond more to negative prediction errors, where phasic dopamine signalling decreases thus favouring the influence of tonic dopamine and downstream inhibitory signalling should ensue (Yapo et al., 2017).

Evidence in favour of these hypotheses has come from seminal work studying individuals with Parkinson's disease (Frank et al., 2004), where midbrain dopaminergic neurons classically degenerate (Kish et al., 1988). Other evidence comes from healthy humans where it was

shown that D1 and D2 receptors were involved in approach and avoidance, respectively (Cox et al., 2015), and from reversal learning paradigms (most pertinent for this thesis), showing for instance that D2 agonism in rats blocked the effect of negative but not positive feedback (Alsiö et al., 2019). These ideas are discussed further in Chapter 8, in the context of Parkinson's disease and other demonstrations of dopaminergic effects on reversal learning across species.

1.9 Opponency of serotonin and dopamine?

Because serotonin is involved in learning about aversion and is implicated in behavioural inhibition (Deakin, 2013), whilst dopamine is involved in reward learning and behavioural activation, the notion of opponency between serotonin and dopamine has been debated (Cools et al., 2011; Daw et al., 2002). As discussed, phasic mesolimbic dopaminergic signalling has been shown to represent reward prediction errors (Schultz et al., 1997), and it had been hypothesised that serotonin may code for punishment prediction errors (Daw et al., 2002). Whilst the apparent requirement of serotonin signalling in the amygdala for aversive Pavlovian conditioning, shown by Hindi Attar et al. (2012), was suggestive of a neural substrate of punishment prediction error, evidence that followed has pointed to a role of the PAG in aversive prediction errors (Roy et al., 2014). Whilst both findings appear to be compatible with accounts of serotonin and punishment prediction (Deakin and Graeff, 1991), the involvement of serotonin in the purported PAG aversive prediction error signal remains to be tested. Whereas prediction errors implicate phasic firing, tonic dopamine and serotonin have been proposed to represent the slow running average of rewards and punishments, respectively (Cools et al., 2011).

Direct opponency, however, has proven to be an oversimplification, perhaps most obviously because there are now numerous studies supporting the involvement of serotonin in reward processing. Recordings from the DRN (presumably including serotonergic neurons) in monkeys tracked the size of expected and received rewards, whereas dopaminergic neurons in the SNc encoded the discrepancy between received and expected reward, suggesting a role for the DRN in signalling current reward value (Nakamura et al., 2008). Other studies have also linked DRN activity to reward (Bromberg-Martin et al., 2010; Ranade and Mainen, 2009), including Iigaya et al. (2018) who found that optogenetically stimulating the DRN enhanced learning rates for rewards (after a long intertrial interval). A recent study demonstrated that there are subsystems within the dorsal raphe that have distinct and at times opposing functions: both activated to reward but had opposing responses to aversion in mice, underscoring the complexity of serotonin's effects (Ren et al., 2018). Cohen et al. (2015) also found that serotonergic neurons in mice responded to both rewards and punishments, with an additional finding that in contrast to dopaminergic neurons, 5-HT neuron firing patterns differed across

time (Cohen et al., 2015). Matias et al. (2017), in an extension of these results, were able to anchor 5-HT neuron activity in a reinforcement learning framework: DRN 5-HT neuron activation in mice tracked both positive and negative prediction errors during reversal learning. These signals were qualitatively similar to dopaminergic prediction error signalling but differed in their time course: dopaminergic responses to cues were more quickly established and withdrawn. They posited it would follow that as cues result in more positive outcomes, dopaminergic signalling would be favoured temporarily thus invigorating behaviour, and when more negative outcomes emerge (during reversal, for instance) serotonergic signalling would be favoured instead, consequently promoting behavioural inhibition (Matias et al., 2017). This could hypothetically arise from faster signalling of DA neurons to the caudate and slower 5-HT signalling to prefrontal regions, respectively (Chamberlain et al., 2007a; Clarke et al., 2011; Matias et al., 2017; Phillips and Robbins, 2020; Roberts, 2011). Likewise, in marmoset monkeys, there is a body of evidence using instrumental learning paradigms in the appetitive domain showing neurotoxic serotonin depletion resulted in perseveration, impairing the ability to flexibly adapt behaviour to obtain reward from new sources (Clarke et al., 2004, 2007). In humans, a prime example comes from a study by Seymour et al. (2012) who found lowering serotonin, by acutely depleting its precursor tryptophan (ATD, introduced below), impaired the representation of reward both behaviourally and neurally: following ATD, positive reinforcement had less of an impact on discouraging participants to switch their behaviour on a 4-armed bandit task, an effect not present for punished outcomes (electric shocks). Worbe et al. (2015, 2016) likewise showed impairments in the appetitive domain following ATD across two experiments, one of which found opposite effects in the aversive domain in which money could be lost. It should also be noted that Seymour et al. (2012) reported another important finding that was independent of the result on reward: ATD enhanced perseveration on a given bandit (by choosing it) as it yielded less favourable outcomes. These ATD studies will receive additional attention in later sections.

There is also evidence of dopaminergic activity in the aversive domain. Rigoli et al. (2016), for instance, used functional magnetic resonance imaging (fMRI) to show that midbrain dopaminergic areas (VTA/SNc) are involved in avoidance. Avoidance is intuitively tied to the prospect of a negative outcome and would thus be associated with dips in dopamine function below baseline (Schultz et al., 1997). It was proposed, however, that the prospect of safety could be a surrogate for reward (Rigoli et al., 2016).

1.10 Psychopharmacological approaches in humans

I present studies of humans in this thesis and there is a limited array of tools to investigate the function of serotonin, and other monoamines, in humans compared to what can be employed in experimental animals. In humans, drugs are given systemically whereas in non-humans, pharmacological agents can be administered to a specific part of the brain. Likewise, in experimental animals it is possible to dramatically deplete serotonin (or another monoamine) via neurotoxins either globally or in a particular brain region (Clarke et al., 2004; Harrison et al., 1997b). Optogenetic methods in non-humans, furthermore, enable the real-time stimulation of (serotonergic) neural activity in a behaving animal (e.g. Iigaya et al., 2018). Assessing dose-dependent or graded effects strengthen evidence that a correlation is caused, and this is more easily accomplished in experimental animals (e.g. Horst et al., 2019). There are furthermore many pharmacological compounds with specific receptor affinities that are not available for human studies. In any species, a further difficulty of drug administration studies is that (somatodentritic or axon terminal) autoreceptor feedback effects can occur (Horst et al., 2019). It is additionally difficult to discern the specific neural effects of pharmacological manipulations in humans. Pharmaco-fMRI can only assess correlations, and at the same time there is a limited repertoire of ligands for positron emission tomography (PET) to probe various aspects of the serotonin systems (Paterson et al., 2010). Spatial and temporal resolution of these imaging methods represent further limitations.

The two primary methods of studying serotonin in humans are acute tryptophan depletion (ATD) and SSRI administration, which are discussed in the sections that follow. ATD is employed in Chapters 3, 4, 5, and 6. Chapter 7 takes another approach, and tests the effects of the psychedelic compound lysergic acid diethylamide (LSD) on PRL. Perhaps most well-known for acting at 5-HT2A receptors, believed to be the primary mediator of its psychedelic effects, LSD also acts on other receptors including D1 and D2, 5-HT1A/1B/1D, 5-HT2C, 5-HT5A, 5-HT6, 5-HT7, as well as α1- and α2-adrenergic receptors (Nichols, 2004). This thesis, furthermore, examines dopamine pharmacologically in the following ways. Chapter 8 reports on the effects of amisulpride, a D2/3 antagonist, and pramipexole, a D2/3 agonist, on PRL in OCD and SUD. These drugs highlight another important limitation of current human psychopharmacological methods: D2 receptor modulating agents also affect D3 receptors. Clinically, pramipexole is used to treat Parkinson's disease and restless legs syndrome (and fibromyalgia), whereas amisulpride is used to treat schizophrenia and psychosis (Stahl, 2013) and can be an adjunctive medication in OCD (Fineberg et al., 2020), as discussed in Box 4.

1.11 Serotonin reuptake inhibition

SSRIs act to block the reuptake of serotonin released into the synapse back into the presynaptic serotonin-releasing neuron (Figure 1.1). SSRI administration is a core method for studying serotonin in humans (Bauer, 2015; Bui et al., 2013; Chamberlain et al., 2006; Crockett et al., 2010a; Harmer et al., 2004), as well as in experimental animals (Bari et al., 2010; Burghardt et al., 2013, 2004; Lapiz-Bluhm et al., 2009). Drugs of this class are among the most commonly prescribed psychiatric medications (Stahl, 2013). Traditionally referred to as antidepressants, this is a misnomer because SSRIs are used to treat not only depression but also panic disorder, generalised anxiety disorder, PTSD, and OCD (see Box 4), among other conditions (Stahl, 2013). For this reason, there has been an effort to promote neuroscience-based nomenclature (NbN), a framework in which psychiatric drugs are referred to by their mode of action rather than the indication (Zohar et al., 2015). Discrepancies between drug nomenclature and clinical use may instil doubts in patients undergoing treatment, could negatively impact treatment adherence, or promote stigma: "Doctor, why are you giving me an antidepressant for my OCD?" (Zohar et al., 2015).

There are important differences between the acute and chronic effects of SSRIs (Nord et al., 2013). Of foremost clinical relevance is that it generally takes several weeks for any therapeutic benefit to take hold, despite reuptake inhibition, and many patients experience a worsening of anxiety or depression before any improvement occurs (Stahl, 2013). A PET study by Nord et al. (2013) using a novel ligand may help explain this effect: single dose SSRI administration (20mg escitalopram) in healthy humans increased 5-HT1B binding potential in all projection areas studied, which they interpreted as indicative of decreased endogenous serotonin concentrations (i.e. more receptors are available for binding, due to the presence of less serotonin). A parallel result was found in cynomolgus monkeys (Nord et al., 2013). This ostensible paradoxical SSRI effect on serotonin is hypothesised to be attributed to inhibitory 5-HT1A autoreceptors, which may need to be desensitised in order for enhanced serotonin concentration in projection areas to ensue (Gardier et al., 1996; Nord et al., 2013; Owens, 1996).

In the framework of Deakin and Graeff (1991), enhanced serotonin transmission following chronic SSRI administration should restrain the PAG (via DRN) from eliciting panic and the hippocampus from devolving into rumination (via MRN). Indeed, that activation of MRN-hippocampal 5-HT1A receptors, which are more prevalent than 5-HT2 receptors in the hippocampus, contributes to the efficacy of SSRIs is now a widely held view (Carhart-Harris and Nutt, 2017). Whilst it remains to be determined why chronic SSRIs, if they indeed enhance serotonin transmission, would also decrease rather than increase anticipatory anxiety (as can occur early on in treatment), downregulation of 5-HT2C receptors with repeated administra-

tion has been proposed as a possible mechanism (Deakin, 2013). The final common pathway of SSRI treatment may involve glutamatergic neurotransmission (Burghardt et al., 2013; Carhart-Harris and Nutt, 2017). Chronic administration of SSRIs for MDD, furthermore, has been associated with increases in neurogenesis in humans, particularly in the hippocampus (Boldrini et al., 2012, 2009). Remarkably, exercise-induced hippocampal neurogenesis in mice has been shown to require serotonin signalling (Klempin et al., 2013). It should be noted that if SSRIs can treat depression by enhancing serotonin function, this does not automatically imply that decreased serotonin was an etiological factor in that depression (Young, 2013).

OCD treatment: pharmacological [Box 4]

SSRIs are the first line pharmacological treatment for OCD. The American Psychiatric Association guidelines recommend titrating to the maximum approved dose (Koran et al., 2007), which has been shown to be more efficacious (Fineberg et al., 2020). Doses up to three times higher than used in major depressive disorder (MDD) can be necessary to reduce symptoms of OCD, whilst at the same time these high doses in MDD do not improve efficacy and instead increase side effects (Dersken et al., 2020). There is a shortage of head-to-head studies directly comparing the efficacy of different SSRIs for the treatment of OCD (Fineberg et al., 2020). The SSRIs citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline have been consistently found to be efficacious in well-powered, multi-centre, randomised, placebo-controlled trials in adults (Fineberg et al., 2020). Likewise, randomised placebo-controlled trials in children and adolescents with OCD have established efficacy of the SSRIs fluoxetine, fluvoxamine, paroxetine, and sertraline (Fineberg et al., 2020).

Clomipramine was the first effective treatment for OCD, with initial evidence dating back to the 1960s (Fineberg et al., 2020). Whilst clomipramine, like SSRIs, is a potent serotonin reuptake inhibitor, it has other actions including norepinephrine reuptake inhibition, and antagonism of 5-HT2A and 5-HT2C receptors (Stahl, 2013). Clomipramine is of the traditional class of TCAs (tricyclic antidepressants), which have substantially more side effects and greater risk of overdose than SSRIs owing to other pharmacological actions: antihistamine action at H1 histaminic receptors, antimuscarinic action at M1 muscarinic cholinergic receptors, α1 adrenergic receptor antagonism, and weak blockade of voltage-sensitive sodium channels (Stahl, 2013). Whilst SSRIs and clomipramine show equivalent clinical efficacy, SSRIs are better tolerated, are consequently less likely to be discontinued prematurely, and are therefore prioritised (Fineberg et al., 2020).

In cases of SSRI-refractory OCD, adjunctive use of traditional "antipsychotic" drugs have produced the strongest evidence (Fineberg et al., 2020). The term antipsychotic is largely based on knowledge and concepts dating back to the 1960s and is a prime example of the utility of neuroscience-based nomenclature: "Doctor why are you giving me an antipsychotic for my OCD?" (Zohar et al., 2015). First-generation antipsychotics, also referred to as classical, conventional, neuroleptics, or typical antipsychotics, such as haloperidol can be effective however due to the increased risk of side effects, this drug class is reserved as a third-line option. Atypical antipsychotics are used instead. What makes an antipsychotic typical is its blockade of D2 receptors whereas atypical antipsychotics generally have both D2 and 5-HT2A receptor antagonist properties; some are additionally 5-HT2C antagonists (Stahl, 2013). Trials of serotonin-dopamine antagonists including aripiprazole, risperidone, and quetiapine have shown efficacy as adjunctive agents in the treatment of SSRI-refractory OCD (Fineberg et al., 2020). Chapter 8 reports on a behavioural experiment testing the effects of amisulpride, an atypical antipsychotic for which there are no trials on its treatment efficacy for OCD. Amisulpride is distinct from other atypicals in that it has no appreciable affinity for 5-HT2A receptors (Stahl, 2013). Amisulpride is examined in this thesis to elucidate dopaminergic effects on behaviour in individuals with compulsive disorders.

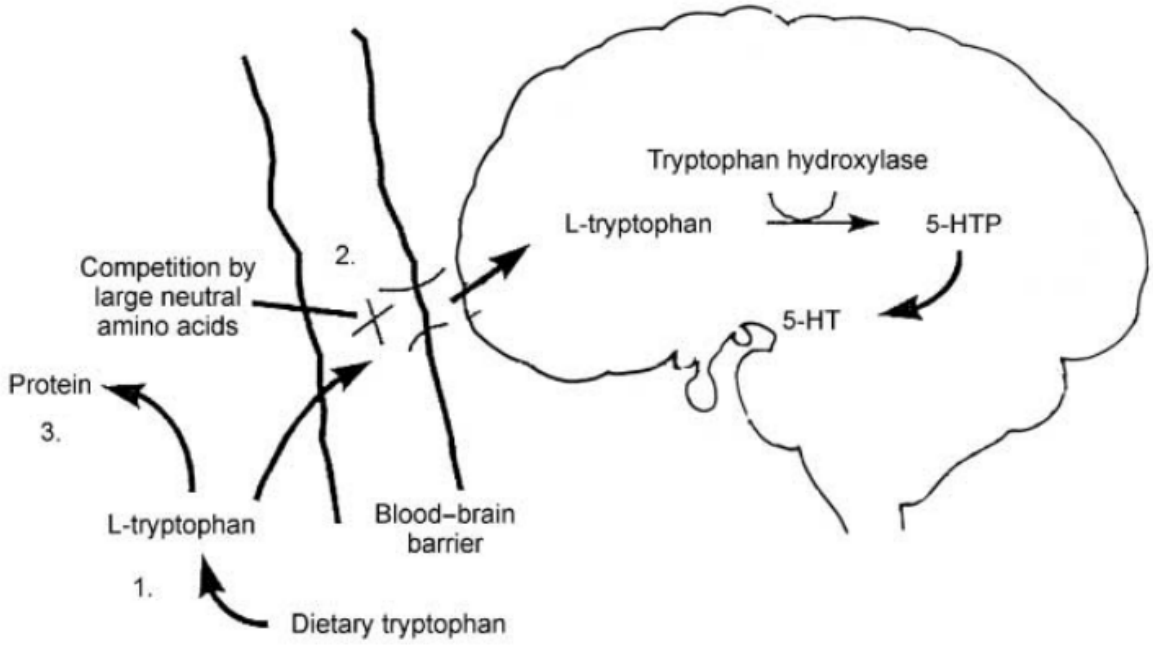

Figure 1.3: The mechanisms of ATD include 1) dietary restriction, 2) increasing competition to cross the blood-brain barrier (BBB) via the large neutral amino acid transporter, and 3) stimulating protein synthesis which consumes tryptophan. 5-HT, serotonin; 5-HTP, 5-hydroxytryptophan. Used with permission (Bell et al., 2001).

1.12 Acute tryptophan depletion

Acute tryptophan depletion (ATD) is the most commonly used technique for studying serotonin in humans (Faulkner and Deakin, 2014) and has been in use for over 30 years (Young, 2013). ATD is the primary neurochemical challenge employed in this thesis. It is widely recognised to lower serotonin synthesis in the CNS (Bell et al., 2005; Crockett et al., 2012b; Faulkner and Deakin, 2014; Hood et al., 2005; van Donkelaar et al., 2011; Young, 2013). ATD ingeniously capitalises on the principles of serotonin synthesis and transport across the blood-brain barrier (BBB). It is non-invasive, non-toxic, reversable, *in vivo* and can be used across species (van Donkelaar et al., 2011). Whilst the methodology of ATD and its specific effects on serotonin will be treated in greater detail in Chapter 2, in this Chapter I will introduce the rationale of ATD and its psychological effects.

1.12.1 Mechanism

There are three core mechanisms by which ATD is believed to reduce serotonin synthesis (Hood et al., 2005) which are represented in Figure 1.3: removing tryptophan from the diet,

inducing protein synthesis in the liver, and increasing competition to cross the BBB. First, tryptophan is an essential amino acid, meaning it must be consumed from the diet as it cannot be synthesised by the body. In the ATD procedure, individuals have fasted for a period of time. They are then asked to ingest a mixture of other amino acids that is devoid of tryptophan. Beyond the omission of tryptophan, loading individuals with a mixture of other amino acids induces protein synthesis in the liver, consequently incorporating extracellular tryptophan already present in the body into proteins in both the liver and other tissues (Harper et al., 1970). Indeed, administration of a protein synthesis inhibitor (in rats) blocked the plasma depletion of tryptophan otherwise induced by ATD (Moja et al., 1991). Standard mixtures include amino acids with "large, neutral" side chains (LNAAs), of which tryptophan is one, which are transported across the BBB via "System L" (Pardridge, 1998). Tryptophan therefore has to compete with the other LNAAs and the availability of tryptophan to the brain depends on the ratio of tryptophan to the sum of the other LNAAs (Hood et al., 2005; Young, 2013). This ratio, consequently, is seen as the best indicator of the degree of tryptophan availability to the brain for the synthesis of serotonin (Hood et al., 2005; Young, 2013).

1.12.2 Mood effects

Delgado et al. (1990) studied depressed individuals on a variety of antidepressants and showed that ATD induced a temporary reappearance of depressive symptoms in most patients – an observation which accelerated the use of ATD as a technique for studying the effects of low serotonin (Young, 2013). Whilst the initial goal of human ATD studies was to see if the relationship between low serotonin and low mood was causal (Young, 2013), the reality is that it depends on the person (Bell et al., 2005; Ruhé et al., 2007). Indeed, there is an interesting gradation of mood effects of ATD, distilled in a meta-analysis by Ruhe et al. (2007): healthy individuals have been consistently unaffected, although those with a family history of major depressive disorder (MDD) have experienced a slight lowering of mood. In drug-free individuals whose MDD was in remission, ATD has induced a moderate lowering of mood (Ruhé et al., 2007). Consistent with previous work (Delgado et al., 1990), the meta-analysis showed that ATD tended to induced relapse in individuals in remission from MDD who used serotonergic medications (Ruhé et al., 2007). The most pronounced effects of ATD on mood in these individuals may occur early in recovery, and the proportion of those affected decreases the longer they have been euthymic (Bell et al., 2005). What differs between individuals who do and do not experience an ATD-induced lowering of mood could possibly be related to regulation of the serotonergic systems, although this remains to be determined (Young, 2013). Because there are known individual differences in the effects of ATD (Bell et al., 2005; Booij et al., 2003; Ruhé et al., 2007), this thesis investigates interactions between baseline person-

ality traits and the serotonin depleted state on emotion in healthy volunteers, in an effort to understand vulnerability factors for psychopathology.

1.12.3 ATD and panic

Individuals with panic disorder showed enhanced panic responses following ATD when breathing 5% carbon dioxide (CO_2) but diminished ratings of anxiety when anticipating CO_2 administration (Miller et al., 2000). This study was a key affirmation of the DRN-subsystems framework, implicating the PAG and amygdala, respectively. It should be noted that the serotonin-releasing agent fenfluramine had the opposite effect in those with panic disorder: fenfluramine suppressed panic responses to CO_2 inhalation yet enhanced anticipatory anxiety (Mortimore and Anderson, 2000). ATD, however, had minimal effects in healthy volunteers on panic or anticipatory anxiety in relation to the CO_2 procedure. The difference in sensitivity to ATD may relate to the importance of strong serotonergic tone in individuals with panic disorder: their primary fear is having another panic attack, which would in turn enhance serotonin-mediated anticipatory anxiety and PAG-restraint (Faulkner and Deakin, 2014).

1.12.4 ATD and OCD

In a symptom provocation study of individuals with OCD, ATD did not lead to further provocation of symptoms on top of what occurred under placebo; however, ATD did induce an increase in distress from provocation and a lowering of mood (Berney et al., 2006). Other findings on the relationship between serotonin and OCD are presented in Box 5.

1.12.5 Other clinical effects of ATD

ATD has been tested in a number of other clinical conditions, which has provided further evidence of individual differences in its effects (Bell et al., 2005; Booij et al., 2003). ATD reversed the benefit of SSRIs in social anxiety disorder (Argyropoulos et al., 2004). Cox et al. (2011) studied non-dependent cocaine users and found that during cocaine administration, ATD enhanced drug cravings which was accompanied by elevated striatal dopamine responses. Neither of these effects occurred following ATD in the absence of cocaine administration (Cox et al., 2011). There was a small effect of ATD in a study of individuals with bulimia nervosa on mood and the desire to binge, with no effect on food intake, albeit in an experimental setting (Kaye et al., 2000). A study of ATD effects on individuals with schizophrenia showed no effects on mood, symptoms of psychosis, or problems with movement (Golightly et al., 2001)). Across multiple studies of ATD in alcohol use disorder (AUD),

reviewed by Booij et al. (2003), there were no effects on mood, craving, or the amount of alcohol consumed, albeit in an experimental setting. In a small study, ATD increased manic symptoms in individuals in remission and taking lithium, which subsided within five days of the manipulation (Cappiello et al., 1997). Following ATD, drug-free adults with autism spectrum disorder experienced a worsening of emotional symptoms and an increase in repetitive behaviours, or stereotypy (McDougle et al., 1996). ATD has led to impaired cognitive functioning in Alzheimer's disease, suggesting a role for serotonin in combination with the cholinergic deficit in cognitive decline (Porter et al., 2000). ATD additionally induced intermediate levels of symptoms of anxiety and depression in individuals with irritable bowel syndrome [IBS] (Shufflebotham et al., 2006). There is also evidence for increased aggression and impulsiveness following ATD, particularly in individuals with aggressive tendencies (Bell et al., 2005; Booij et al., 2003; Walderhaug et al., 2002; Young and Leyton, 2002).

Serotonin and OCD [Box 5]

Beyond the benefit that drugs with serotonergic modes of action can have in the treatment of OCD, a constellation of findings indicate that there are serotonergic abnormalities in OCD. It should be noted, however, that dopaminergic, glutamatergic, and GABA-ergic dysfunction are also implicated in OCD (Dersken et al., 2020). Some of the evidence from neuroimaging studies using PET and single-photon emission computed tomography (SPECT), and from pharmacological studies is discussed. Chronic administration of SSRIs to individuals with OCD has resulted in increased D2 receptor binding in the ventral and dorsal striatum, as well as decreased striatal dopamine transporter binding, the latter of which was associated with symptom improvement (Kim et al., 2007; Moresco et al., 2007). In contrast to the effects of ATD in OCD (see Section 1.12.4), OCD symptoms have been exacerbated by administering the non-selective serotonin agonist meta-Chlorophenylpiperazine (mCPP) to clinically diagnosed individuals, with some indications of 5-HT3 or 5-HT1D involvement in this effect (Dersken et al., 2020). Indeed, there is some evidence that 5-HT3 antagonism on top of SSRIs may confer clinical benefit (Dersken et al., 2020). Meanwhile, baseline plasma tryptophan levels have been shown not to differ between those with OCD and healthy controls (Dersken et al., 2020). Drug-naïve individuals with OCD have shown lower serotonin transporter (SERT) availability in a large number of brain areas (Derksen et al., 2020). PET has shown decreased SERT availability in the amygdala, anterior cingulate, nucleus accumbens, dorsal parts of the striatum (Hesse et al., 2011), insula (Matsumoto et al., 2010), midbrain, and thalamus (Reimold et al., 2007). SPECT has revealed decreased SERT binding in midbrain-pons (Hasselbalch et al., 2007; Hesse et al., 2005; Stengler-Wenzke et al., 2004), thalamus, and hypothalamus (Hesse et al., 2005; Zitterl et al., 2007). There is additionally some evidence for diminished serotonin transporter availability in the orbitofrontal cortex (Matsumoto et al., 2010). OCD symptom severity, furthermore, has been correlated with lower SERT binding specifically in thalamic and hypothalamic regions, which may relate to therapeutic response to SSRIs (Derksen et al., 2020). The body of evidence on 5-HT receptor binding in individuals with OCD is generally inconclusive, with studies reporting conflicting results on 5-HT2A and 5-HT1B binding (Derksen et al., 2020). Increased serotonin synthesis capacity has been documented in temporal lobe and striatal areas, which has been interpreted as a possible sensitivity to the availability of tryptophan (also see Section 1.12.4 on ATD in OCD) or a compensatory response, for instance to combat decreased serotonergic signalling (Dersken et al., 2020).

1.13 Serotonin and threat conditioning processes

Pavlovian threat ("fear") conditioning paradigms enable the study of anticipatory anxiety, and are sensitive to changes in serotonin (Bauer, 2015), consistent with the Deakin and Graeff (1991) framework. The preponderance of data on the relationship between serotonin and threat conditioning processes has come from rats, with a surprisingly small number of human studies at the intersection of these two otherwise widely studied areas (Bauer, 2015).

Single dose citalopram has potentiated threat conditioning in rats assessed by freezing (Burghardt et al., 2004) and (healthy) humans assessed by the startle response (Grillon et al., 2007), consistent with the common clinical effect of an initial worsening of symptoms with SSRIs before improvement. Chronic administration (22 injections), meanwhile, attenuated conditioning in rats (Burghardt et al., 2004), and two-weeks of citalopram in humans attenuated the startle response when anticipating unpredictable shocks (Grillon et al., 2009). Following threat conditioning off drug, chronic (22 days) but not subchronic (7 days) citalopram impaired extinction learning and downregulated the NR2B subunit of the N-methyl-D-aspartate receptor in the basal and lateral amygdala subnuclei, stimulation of which is critical for plasticity underlying both threat conditioning and its extinction (Burghardt et al., 2013). In healthy humans, meanwhile, administering escitalopram for two weeks had no effect on threat conditioning but facilitated extinction learning (Bui et al., 2013). Fourteen day administration of the serotonin reuptake inhibitor fluoxetine following conditioning diminished the return of threat responses tested after extinction training, presumably reflective of having formed a stronger extinction memory (Karpova et al., 2011); however, this was conducted in mice, whereas rats are the primary animal model used to study Pavlovian threat conditioning processes (Burghardt et al., 2013). Indeed, rats and mice respond differently to another serotonergic drug, MDMA (Battaglia et al., 1988). Consideration of differences in protocol, furthermore, are also likely to be critical in reconciling differences across studies. Hindi Attar et al. (2012) and Hensman et al. (1991), for instance, used SCR in humans to show attenuated threat conditioning following ATD and 5-HT2A/C antagonism, respectively, whereas another study showed startle was unaffected by ATD when anticipating predictable shocks but was potentiated when anticipating unpredictable shocks (Robinson et al., 2012b). Whereas Hindi Attar et al. (2012) were able to show ATD attenuated cue-elicited responses in the amygdala, Robinson et al. (2012b) in the absence of neuroimaging data implicated a different mechanism involving the bed nucleus of the stria terminalis (BNST), referred to as the extended amygdala. Consistent with Hensman et al. (1991) and Hindi Attar et al. (2012), administering meta-Chlorophenylpiperazine (mCPP) intravenously to healthy volunteers elicited bilateral amygdala and caudate activity, which was abolished by pre-treatment with mirtazapine, a 5-HT2C antagonist (McKie et al., 2011).

That 5-HT2A/C antagonism decreased conditioning in humans aligns with the rodent literature: retention of threat conditioning was potentiated by acute citalopram but could be blocked by coadministration of a 5-HT2C antagonist (Burghardt et al., 2007). Moreover, infusing a 5-HT2C agonist into the BLA, or over-expressing 5-HT2C receptors in the BLA have been anxiogenic in rodents (Bauer, 2015), and learned helplessness appears to be mediated by excessive 5-HT2C signalling in the BLA (Christianson et al., 2010). The contribution of 5-HT2C receptors to threat conditioning processes deserves further study, both in humans and experimental animals.

Stimulating 5-HT2A receptors, on the other hand, enhanced threat conditioning when administered in mice immediately after learning, and 5-HT2A agonism given prior to extinction training enhanced extinction learning, as reviewed by Bauer (2015). These results, and those of Hensman et al. (1991), are consistent with the role of 5-HT2A receptors – which are enriched in the amygdala, hippocampus and PFC – in learning and memory across a variety of paradigms, and in facilitating synaptic plasticity via activation of NMDA receptors (Bauer, 2015).

Stimulating 5-HT1A receptors, meanwhile, have been shown to be anxiolytic in both humans and experimental animals (Bauer, 2015) and impair various learning processes (Ögren et al., 2008). For instance, infusing a 5-HT1A agonist into the MRN impairs contextual threat conditioning and its expression (Borelli et al., 2005) – hippocampal lesions have interfered with conditioning to a context and not to a cue, whilst amygdala lesions impair both contextual and cue conditioning (Phillips and LeDoux, 1992). Accordingly, the expression of contextual conditioning was impaired following infusion of a 5-HT1A agonist into the BLA (Li et al., 2006) or BNST (Gomes et al., 2012). Post-synaptic 5-HT1A receptors have been posited to figure more prominently in contextual threat conditioning processes (Bauer, 2015).

The influence of serotonin over Pavlovian threat reversal learning does not appear to have been studied in rats, let alone in humans (Bauer, 2015). This thesis aims to expand the small number of serotonergic manipulations of threat conditioning processes in humans, and focuses in particular on aspects beyond initial learning, namely threat memory retention (Chapter 4) and threat reversal learning (Chapter 5). Given that ATD can have diverse effects across different populations (Booij et al., 2003), and intolerance of uncertainty has been linked to the degree of threat memory retention (Dunsmoor et al., 2015), I additionally examined whether this trait would interact with ATD to influence emotional memory.

1.14 Serotonin and social cognition

There is evidence from non-humans that serotonin is implicated in an array of social phenomena including parental attachment, caregiving, cooperation, social play, mating, and sexual behaviour (Dölen et al., 2013; Ichise et al., 2006; Kiser et al., 2012; Suomi, 2006). Some of this work, however, relates to environmental interactions with polymorphisms in the serotonin transporter gene (5-HTTLPR/SLC64A), a candidate gene approach that is vulnerable to false positives (Border et al., 2019). This limitation therefore underscores the importance of pharmacological manipulations to study serotonin.

In humans, one approach to studying the effects of serotonin on social perceptions (and affective cognition) has been to measure reactions to emotional faces (Elliott et al., 2011). Some studies have found ATD reduced the recognition of fearful faces, whereas acute citalopram has increased fear recognition across multiple studies (Elliott et al., 2011). Seven-day (sub-chronic) treatment with citalopram, moreover, has reduced the identification of facial expression of fear and anger (Harmer et al., 2004). Another study, moreover, found decreased PFC inhibition over the amygdala following ATD when viewing angry faces (Passamonti et al., 2012).

Much of the work on the relationship between serotonin and human social interaction, however, has come from behavioural economic games and decision-making in moral dilemmas. One widely used paradigm is the ultimatum game (UG), in which the participant is required to make a series of decisions to accept or reject fair or unfair proposals from another player to share money (Guth et al., 1982). By rejecting an unfair offer, neither player receives money, which is thus operationalised as retaliatory behaviour. Crockett et al. (2008) found ATD increased retaliatory behaviour on the UG, whereas acute SSRI (citalopram) had the opposite effect, instead decreasing retaliatory behaviour (Crockett et al., 2010a). Acute SSRI also had what was seen as a pro-social effect on a moral judgement task, where individuals were more likely to judge harmful acts as forbidden (Crockett et al., 2010a). Both of these effects were more pronounced in the highly empathic and were interpreted as an effect of serotonin in promoting harm aversion (Crockett et al., 2010a). The norepinephrine reuptake inhibitor atomoxetine, meanwhile, had no effect on these assays (Crockett et al., 2010a). Harm aversion has also been operationalised as reluctance to inflict electric shock on oneself or others. Whilst individuals have tended to be more averse to inflicting shock on others than on themselves, assessed by the amount of money they would require in order to do so, acute SSRI (citalopram) increased aversion for harming oneself and others (Crockett et al., 2015). Administering the dopamine precursor L-DOPA (see Figure 1.2), meanwhile, eliminated the baseline tendency to be more averse to harming another, than oneself (Crockett et al., 2015).

Whilst ATD has reduced cooperative behaviour in the Prisoner's Dilemma (Wood et al.,

2006), the non-selective 5-HT2A agonist lysergic acid diethylamide (LSD) increased pro-social behaviour in a laboratory setting (Dolder et al., 2016). Psilocybin and MDMA are also 5-HT2A agonists, and along with LSD have been reported to enhance empathy (Dolder et al., 2016; Hysek et al., 2014; Pokorny et al., 2017). Indeed, MDMA is referred to as an enactogen or empathogen. Psychedelic drugs, furthermore, have been reported to promote feelings of social connectedness (Forstmann et al., 2020).

Studies that quantify the influence of ATD on social, or moral emotions that may be driving pro- or anti-social behaviour, however, are lacking and is the topic of Chapter 3. That Crockett et al. (2010b) found the highly empathetic were most influenced by acute SSRI served as my motivation to examine whether serotonergic state and baseline traits interacted to differentially affect emotion. The expectation was that empathy in particular may be a marker for susceptibility to the effects of ATD (as with acute SSRI), however other pertinent traits were also tested including psychopathic tendencies.

1.15 Serotonin and behavioural inhibition

Behavioural inhibition, and relatedly impulsivity ("acting without foresight"), are not unitary constructs, and have differing neural and neurochemical substrates (Dalley et al., 2011; Dalley and Robbins, 2017). The importance of fractionating these concepts in order to elucidate the role of serotonin in impulse control has become clear across a vast literature (Dalley and Roiser, 2012; Faulkner and Deakin, 2014).

1.15.1 Serotonin and aggression

Aggression has been traditionally associated with low serotonin, which includes evidence from studies of violent offenders (Brown et al., 1982; Deakin, 2003; Linnoila et al., 1983). Aggression can be either goal-directed (e.g. a premeditated crime), or reactive: an explosive, impulsive response to frustration (Blair, 2010). Whilst many psychiatric conditions increase the risk for reactive aggression, psychopathy, on the other hand, which is classically associated with blunted guilt and empathy, is unique in that there is an increased risk for both reactive and goal-directed aggression (Blair, 2010). Indeed, psychopathic individuals display an analogous pattern of behaviour on the UG (Koenigs et al., 2010) compared with healthy participants under ATD (Crockett et al., 2008). Whilst some, but not all, aggression can be impulsive, aggression and impulsivity are distinct. Indeed, discrete serotonergic circuits modulated aggressive versus impulsive behaviour in mice, both of which implicated the 5-HT1B receptor (da Cunha-Bang et al., 2016).

In a remarkable study, Bevilacqua et al. (2010) examined a founder population in Finland, which facilitates the study of rare alleles (gene variants) because of low genetic variation due to a small number of "founders" and subsequent isolation. A variant in the 5-HT2B receptor gene was highly associated with impulsive unpremeditated violent crimes, in reaction to minor irritations. Whilst most individuals in this sample had diagnoses of anti-social personality disorder (ASPD), borderline personality disorder, and intermittent explosive disorder (IED), alcohol intoxication was a critical driver of their crimes as was male sex (Bevilacqua et al., 2010).

1.15.2 Serotonin and suicidal behaviour

Serotonin has also been heavily implicated in suicidal behaviour, related to its role in impulse control (Åsberg, 1997; Brown et al., 1982). Indeed, Bevilacqua et al. (2010) additionally found that 70% of the males studied who had the rare risk variant of the 5-HT2B receptor gene demonstrated a history of suicidal behaviour. There were no differences in monoamine metabolite levels in the cerebrospinal fluid [CSF] (Bevilacqua et al., 2010). Across other studies, however, there is a consistent association between low CSF levels of the serotonin metabolite 5-hydroxyindolacedic acid (5-HIAA) and suicidal behaviour, which contrasts with the largely inconclusive literature on 5-HIAA and depression (Asperg, 1997; Young, 2013).

1.15.3 Serotonin and action cancellation

The stop-signal reaction time (SSRT) is a classic index of response inhibition, which tests the ability to cease an already initiated behavioural response – "action cancellation" (Dalley and Robbins, 2017; Eagle et al., 2008). Indeed, individuals with OCD and SUD are impaired on the SSRT, as are their first-degree unaffected relatives (Chamberlain et al., 2007c; Ersche et al., 2012). Depleting serotonin in humans (via ATD) and rats (neurotoxically) does not appear to impair motor response inhibition, where there is no prospect of punishment (Clark et al., 2005; Cools et al., 2005; Eagle et al., 2009). These observations have helped refine theories of serotonin function, running contrary to early notions that serotonin may have a role in inhibitory processes that generalise to hedonically neutral circumstances (Deakin, 2013; Faulkner and Deakin, 2014; Soubrié, 1986). Individuals with a family history of alcoholism, however, displayed worsened response inhibition on the SSRT following ATD (Crean et al., 2002). Impaired SSRT in individuals with Parkinson's disease, moreover, where serotonergic dysfunction has been documented (Politis et al., 2010, 2012; Wilson et al., 2019), has been improved by a 30mg dose of the SSRI citalopram (Ye et al., 2014). This improvement under citalopram was furthermore accompanied by increased activity in the right inferior frontal

gyrus (Ye et al., 2014), a region critical for SSRT performance (Aron et al., 2014), and these results parallel those of the selective norepinephrine reuptake inhibitor atomoxetine (Ye et al., 2015), which also improves SSRT in healthy humans (Chamberlain et al., 2006) and the SSRT deficits found in attention deficit/hyperactivity disorder [ADHD] (Chamberlain et al., 2007a).

1.15.4 Serotonin and action restraint

Go/no-go paradigms, meanwhile, partially overlap with the SSRT in that they involve inhibiting a pre-planned motor response, however go/no-go requires behavioural inhibition before the response has been initiated – "action restraint" (Eagle et al., 2008). Critically, in contrast to the SSRT in rats (Eagle et al., 2009) and healthy humans (Clark et al., 2005; Cools et al., 2005), serotonergic manipulations have impaired go/no-go performance in rats (Harrison et al., 1999). There have been a number of null effects of ATD on go/no-go tasks in healthy humans (Evers et al., 2006; LeMarquand et al., 1998; Macoveanu et al., 2013; Rubia et al., 2005). Another study, meanwhile, reported worse performance following ATD in individuals with a strong family history of alcoholism (LeMarquand et al., 1999), suggesting in conjunction with the SSRT results from Crean et al. (2002), that ATD may unearth serotonergic vulnerabilities in certain at-risk populations (Faulkner and Deakin, 2014).

That many previous studies showed ATD did not modulate behavioural inhibition (on the SSRT and go/no-go) in healthy volunteers likely relates to the use of tasks in which behaviour was not reinforced (not rewarded nor punished), which may not sufficiently engage serotonin signalling for ATD to have a detectable impact (Faulkner and Deakin, 2014). Crockett et al. (2009) improved upon previous studies by using a novel go/no-go task that was reinforced, to disentangle the relationship between aversive processing and behavioural inhibition. Indeed, across the animal kingdom aversive stimuli inherently promote behavioural inhibition (Dayan and Huys, 2009). It was found that under conditions of punishment, there was a baseline slowing of behaviour when on placebo, and this punishment-induced inhibitory influence was abolished following ATD (Crockett et al., 2009). Critically, this effect was not present under conditions of reward, advancing the notion that serotonin operates at the intersection of inhibition and aversion (Crockett et al., 2009). Using an altered version of this task, Crockett et al. (2012a) subsequently replicated this behavioural effect and provided evidence that it is mediated by Pavlovian predictions, which are attenuated under ATD. Geurts et al. (2013) provided confirmatory evidence that serotonin has a specific role in linking expectations about aversive events to behavioural inhibition. They used ATD with a Pavlovian-to-instrumental transfer (PIT) paradigm to demonstrate ATD disinhibited maladaptive behaviour in the presence of aversive Pavlovian cues, whereas ATD did not affect the influence of Pavlovian cues on behaviour in the appetitive domain (Geurts et al., 2013). Collectively, these results align with

the observation of attenuated Pavlovian conditioning following ATD (Hindi Attar et al., 2012) and are in keeping with the early work on pigeons (Graeff and Schoenfeld, 1970) and rats (Wise, 1970), indicating a role for serotonin in behavioural inhibition in the face of aversion. Additionally, a theoretical computational framework has been provided for conceptualising serotonin and inhibition in relation to negative mood, which equates thoughts and actions (Dayan and Huys, 2008), and would be remarkably consistent with the notion of a role for serotonin in inhibiting rumination (Deakin, 2013).

1.15.5 Serotonin and impulsive choice

Delay discounting (or temporal discounting/inter-temporal choice) is a form of impulsivity manifested in choosing a smaller reward sooner rather than a larger reward later (delaying gratification). Indeed, individuals with substance use disorders (MacKillop et al., 2011), gambling disorder (Albein-Urios et al., 2014), ADHD (Scheres et al., 2008), borderline personality disorder (Barker et al., 2015), and schizophrenia (Heerey et al., 2007) have been reported to show increased temporal discounting (discounting the value of larger rewards after a delay, in favour of smaller immediate rewards). On the other hand, individuals with anorexia (Steinglass et al., 2012), obsessive-compulsive personality disorder (OCPD), but not OCD (Pinto et al., 2014) have demonstrated lower discounting than healthy controls.

A remarkable series of studies has implicated serotonin in patience. Putative serotonergic neurons in the DRN were recorded when rats waited for delayed rewards, which exibited tonic firing during the wait up until reward delivery, and that firing dipped immediately before instances where rats gave up on waiting (Miyazaki et al., 2011). In a follow-up study, optogenetics was used to selectively stimulate serotonergic neurons in the DRN of mice and this stimulation caused prolonged waiting in anticipation of delayed rewards (Miyazaki et al., 2014). This stimulation, crucially, did not cause a generalised inhibition of behaviour. Several other studies have shown that neurotoxic lesions of the DRN (along with the MRN) in rats yielded results consistent with a smaller sooner over larger later behavioural effect (Al-Ruwaitea et al., 1999; Mobini et al., 2000; Wogar et al., 1993). The implication is that the DRN restrains Pavlovian influences over approaching immediate rewards (Faulkner and Deakin, 2014).

A series of human ATD studies have employed an analogue of the rodent task. One study showed ATD resulted in choosing more smaller sooner rewards and increased temporal discounting (Schweighofer et al., 2007). Another study showed this ATD effect only in relation to the highly neurotic (Demoto et al., 2012), and yet another did not find any differences following ATD (Tanaka et al., 2007); Faulkner and Deakin (2014), however, posit this could relate to these two studies having used fewer trials. More impulsive choice, assessed by delay discounting, has furthermore been correlated with increased retaliatory responses to unfair of-

fers on the UG, which was heightened further by ATD (Crockett et al., 2010b), thus drawing a connection between conventional measures of impulse control and social decision-making. How can this form of behavioural inhibition be unified with instances of punishment-induced behavioural suppression? Computational theories have proposed that serotonin is involved in coding for the opportunity cost of action (not waiting for delayed larger rewards, in this case) (Cools et al., 2011), which could be related to passive avoidance. The cost of acting is higher when it would result in missed opportunity for a larger payoff, and behavioural inhibition would thus be favoured.

1.15.6 Serotonin and waiting impulsivity

The five-choice serial reaction time test (5CSRTT) assesses action restraint when waiting for a reward and has been widely studied in rodents (Dalley and Robbins, 2017). Forebrain serotonin depletion in the rat has increased impulsive responding on the 5CSRTT (Harrison et al., 1997a; Winstanley et al., 2004a), as did 5-HT2C antagonism (Fletcher et al., 2007; Winstanley et al., 2004b), whereas 5-HT2A antagonism decreased impulsive responses (Winstanley et al., 2004b). These effects of 5-HT2C and 5-HT2A were more prominently reproduced by local infusion into the rat nucleus accumbens than into the PFC and are likely related to their differential modulation of dopamine in the nucleus accumbens (Robinson et al., 2008). Lesioning the DRN, moreover, in contrast to MRN lesions, also increased impulsivity on the 5CSRTT in a manner that resembled global forebrain serotonin depletion (Harrison et al., 1997a). The forebrain serotonin depletion effect, moreover, was blocked by D1 antagonism, indicating a role for serotonin in inhibiting dopamine transmission (Harrison et al., 1997a). This paradigm has been translated into humans (4CSRTT). Indeed, ATD has induced waiting impulsivity on the 4CSRTT in healthy individuals (Worbe et al., 2014). These effects on waiting impulsivity, like delay discounting, can be viewed in the framework that a function of serotonin is representing the opportunity cost of acting prematurely (Cools et al., 2011). The clinical relevance is clear: individuals with SUD and AUD also have shown greater waiting impulsivity on the 4CSRTT, as did recreational cannabis users and current cigarette smokers, but not individuals with binge eating disorder (Morris et al., 2016; Voon et al., 2014b). These results collectively implicate a DRN subsystem that modulates dopaminergic function in restraining premature responding to obtain reward (Faulkner and Deakin, 2014).

1.16 From impulsivity to compulsivity

Compulsivity refers to dysfunction of behavioural control characterised by perseverative action that is inappropriate to the situation and persists despite adverse consequences (Phillips and Robbins, 2020). Whilst impulsivity and compulsivity are clinically distinguishable (Hollander and Rosen, 2000), impulsivity is widely seen as a vulnerability factor to the development of compulsive drug-taking, which is supported by abundant cross-species evidence (Dalley and Robbins, 2017).

It has been established that stimulant use disorder, for instance, is characterised by impulsive behaviour, widely demonstrated both in a laboratory setting on the 4CSRTT/5CSRTT, SSRT, in delay discounting, and on self-report scales (Dalley and Robbins, 2017; Ersche et al., 2010; Voon et al., 2014b). Only 16% of those who use stimulants, however, end up meeting the diagnostic criteria for SUD (Dalley and Robbins, 2017). The majority, in other words, are able to quit as negative consequences of drug-taking emerge, and those who lose control over their drug use are in the minority (Pelloux et al., 2012). The remaining 84% generally do not show strong signs of impulsivity, yet on average they tend to be sensation seekers (Dalley and Robbins, 2017). Non-drug using siblings of compulsive users, meanwhile, tend not to be sensation seekers, but are more impulsive than healthy controls without an affected sibling (Dalley and Robbins, 2017; Ersche et al., 2010). Conveniently, about 20% of the normal population of rats display addiction-like behaviour, as defined by the persistence of cocaine seeking behaviour despite the receipt of electric shocks (Belin et al., 2008). Indeed, these rats displayed impulsivity on the 5CSRTT at baseline, in a clear demonstration of the shift from impulsivity to compulsivity in rats (Belin et al., 2008), a behavioural pattern that has been linked to fewer D2/3 receptors in the nucleus accumbens (Dalley et al., 2007). These D2/3 receptor binding results from rats, moreover, resemble findings of decreased D2/3 receptors in individuals with stimulant use disorder (Volkow et al., 2001, 1993).

Stimulant use disorder [Box 6]

Stimulant use disorder (SUD) refers to the use of cocaine, amphetamine-type substances (e.g. amphetamine, dextroamphetamine, and methamphetamine), or other stimulants (e.g. methylphenidate, khat) that leads to distress or impairment that is clinically significant and manifests in at least two of the following ways during a 12-month period: craving or urge to use; desire or unsuccessful efforts to control or cut down use; taking larger amounts of the stimulant or for longer periods than was intended; excessive time spent obtaining, using, or recovering from use; failure to fulfil major life obligations as a result of use; repeated use in hazardous situations; continued use despite continued interpersonal problems related to the effects of the substance; giving up or reducing important life activities because of use; continuation of use despite knowledge of having a psychological or physical problem linked to use; tolerance with continued use as defined by either noticeably decreased effects of the stimulant despite taking the same amount, or a need for noticeably greater amounts to achieve the desired effects; either withdrawal symptoms characteristic of the particular stimulant, or taking the stimulant, or a related substance, in order to mitigate or thwart withdrawal symptoms. The withdrawal symptoms of stimulants include hypersomnia, increased appetite, attention or concentration disturbances, irritability, and symptoms that can resemble a major depressive episode, that typically abate within a week (American Psychiatric Association, 2013). Tolerance or withdrawal occur in most users; however, some individuals who use amphetamine-type stimulants become sensitised resulting in enhanced effects (American Psychiatric Association, 2013). SUD can develop as rapidly as within one week after using amphetamine-type stimulants or cocaine (American Psychiatric Association, 2013). Behaviour can change dramatically and rapidly with use, and when intoxicated individuals typically feel euphoric and confident, and may present with rambling speech among other features (American Psychiatric Association, 2013). With high-dose use, behaviour can become aggressive or violent, intense yet temporary anxiety can occur, and individuals can experience paranoia or psychosis [stimulant-induced psychotic disorder being an extreme] (American Psychiatric Association, 2013). Pavlovian conditioned responses to drug-related cues often develop, which persist after use, are difficult to extinguish, and contribute to relapse (American Psychiatric Association, 2013). SUD is more common in the 12 to 25-year-old age range than in those older than 26 (American Psychiatric Association, 2013). Twelve-month prevalence is greatest in the 18 to 29-year-old age range at 0.4% for amphetamine-type stimulants and 0.6% for cocaine (American Psychiatric Association, 2013). SUD affects all socioeconomic classes (American Psychiatric Association, 2013). Prevalence across the sexes varies based on age, route of administration, and substance, and use across racial/ethnic groups is also variable (American Psychiatric Association, 2013). There are a number of environmental risk factors including early life adversity, which is associated with perturbations of serotonin (Cunningham et al., 2020) and prenatal cocaine exposure (American Psychiatric Association, 2013), as well as temperamental factors such as trait impulsivity (Ersche et al., 2010). There is no consistent evidence for an effective pharmacological agent to treat SUD (Minozzi et al., 2015).

1.17 Serotonin and compulsivity

The aforementioned 20% of rats prone to compulsive drug-taking have furthermore been shown to have diminished forebrain serotonin (assessed via the 5-HT:5-HIAA ratio) in ventral and dorsal PFC, ventral and dorsal striatum, and amygdala (Pelloux et al., 2012). Compulsive cocaine seeking in this subpopulation of rats, remarkably, was remediated by the SSRI citalopram. It should be noted that cocaine, in addition to stimulating dopamine efflux into the synapse and inhibiting its reuptake, is a potent suppressor of spontaneous serotonergic neuron activity in the DRN (Cunningham et al. 2020). Neurotoxic forebrain serotonin depletion in rats with a limited history of cocaine self-administration, furthermore, reproduced the behavioural pattern seen in the high drug-seeking rats, which was remediated by the non-selective 5-HT2A/C agonist mCPP (Pelloux et al., 2012). Drug-seeking behaviour in intact rats with a minimal history of cocaine use, moreover, could be elicited by administration of a selective 5-HT2C antagonist and blocked by selective 5-HT2A anatagonism (Pelloux et al., 2012).

1.17.1 Habitual and goal-directed behaviour

The spiral into addiction in widely viewed as a progression from purposeful actions, to habits, to compulsions, and a corresponding shift from ventral to dorsal frontostriatal loops (Everitt and Robbins, 2005). Two key related tests of the tendency towards habitual responding have been widely studied in humans (Gillan et al., 2015b). Outcome devaluation paradigms involve learning an adaptive response to obtain reward (or avoid punishment), and this goal-directed behaviour (response-outcome) is subsequently devalued: the action is no longer associated with reward, and it is tested whether responding persists even though it has lost its original purpose (stimulus-response). Another paradigm employs a sequential decision-making task that assesses the balance of two computational mechanisms of learning, termed model-based and model-free [MBMF] (Daw et al., 2011). The idea behind model-based learning is that it accounts for a model of the world (or the task), which involves an understanding of the contingencies between actions and outcomes. Model-free learning (free of the task structure) occurs through reinforcing successful responses (Daw et al., 2011). This is a temporal-difference (TD) learning mechanism that occurs through prediction errors. The MBMF task has some similarities to PRL (introduced in Section 1.1.3), a simpler task, in that it demands flexibly adapting responses in a probabilistic environment to maximise reinforcement. In a direct comparison within the appetitive domain, model-based learning was shown to protect against habitual responding on an outcome devaluation task (Gillan et al., 2015b). Indeed, in the appetitive domain ATD has been shown to promote habitual tendencies on both MBMF (Worbe

et al., 2016) and outcome devaluation paradigms (Worbe et al., 2015). This is important clinically because habitual tendencies have been documented in the appetitive domain across OCD, SUD, and binge eating disorder on the MBMF task (Voon et al., 2014a), and on outcome devaluation tests in OCD (Gillan et al., 2011), SUD (Ersche et al., 2016), AUD (Sjoerds et al., 2013), and Tourette syndrome (Delorme et al., 2016). Greater habitual responding during outcome devaluation was also correlated with the severity of nicotine dependence (Luijten et al., 2020), an association observed in AUD as well (Sjoerds et al., 2013).

The MBMF task has also been altered to include a punishment (loss) condition. Behaviour in the aversive domain, remarkably, differed from the appetitive domain in that healthy participants following ATD were instead more model-based (Worbe et al., 2016), as were individuals with OCD (Voon et al., 2015). It should also be noted that the bias toward habitual responding for rewards in OCD assessed with the MBMF task was driven by individuals medicated with chronic SSRIs (Voon et al., 2015) – behaviour in the aversive domain appeared to be unaffected by SSRI treatment – and the study showing habitual responding in OCD upon reward outcome devaluation likewise tested a medicated sample (Gillan et al., 2011). Avoidance habits, tested in a novel outcome devaluation paradigm using shocks, have also been shown to be enhanced in OCD, tested in largely SSRI medicated samples (Gillan et al., 2015a, 2014).

1.17.2 Reversal learning: deterministic reinforcement

Instrumental reversal learning, particularly on a deterministic reinforcement schedule, is a key method used to study compulsivity, as it quantifies rigid, perseverative behaviour (Phillips and Robbins, 2020; Roberts, 2011) – a different form of prepotent responding (introduced in Section 1.1.3). Neurotoxically depleting serotonin with 5,7-dihydroxytryptamine (5,7-DHT) from the marmoset monkey OFC has induced perseveration on deterministic reversal tasks (Clarke et al., 2004), an effect not present after OFC dopamine depletion (Clarke et al., 2007). Indeed, it has been shown that individuals with SUD have decreased serotonin concentration in the OFC assessed post-mortem (Wilson et al., 1996) as well as diminished OFC serotonin transporter (Kish et al., 2009). Conversely, dopamine but not serotonin was required in the caudate nucleus of marmosets for successful deterministic reversal learning (Clarke et al., 2011). Likewise, it has been shown that individuals with SUD have reduced dopamine concentrations post-mortem in the caudate (and nucleus accumbens and putamen), whereas caudate serotonin was not significantly different from controls (Wilson et al., 1996) nor were caudate serotonin transporter levels (Kish et al., 2009).

Serotonin depletion in rats has likewise resulted in deterministic reversal impairments, which were remediated by the SSRI citalopram (Lapiz-Bluhm et al., 2009). Follow-up studies in marmosets demonstrated that the reversal deficits could not be attributed to generalised

disinhibition, as instrumental extinction (Walker et al., 2009) and intra-dimensional/extra-dimensional (IDED) set-shifting was largely left intact under OFC serotonin depletion (Clarke et al., 2005). Depleting OFC serotonin has been proposed to remove descending inhibitory mechanisms that ordinarily bias away from aversive processing, which would also account for the promotion of stimulus-response associations over stimulus-response-reward goal-directed action (Cools et al., 2008b; Roberts, 2011), consistent with the ATD studies of Worbe et al. (2015; 2016) and Seymour et al. (2012). Likewise, reduced reward expectancy following serotonin depletion, may shift action strategies towards exploitation over exploration (Roberts, 2011).

Barlow et al. (2015) examined a subset of rats who were highly perseverative during serial deterministic reversal learning and found they had reduced levels of 5-HT2A receptors and 5-HIAA in the OFC, and decreased expression of monoamine oxidase and tryptophan hydroxylase genes in the DRN. Systemic administration of citalopram, moreover, improved reversal performance (Barlow et al., 2015).

Systemic administration of a 5-HT2C antagonist, meanwhile, has corrected perseverative impairments in reversal learning, with a worsening after 5-HT2A antagonism (Boulougouris et al., 2008). The improvement by 5-HT2C antagonism was furthermore shown to be neuroanatomically specific to the OFC (Alsiö et al., 2015; Boulougouris and Robbins, 2010). Late reversal learning, however, was worsened by 5-HT2C antagonism dosed systemically which has been proposed to relate to increased impulsivity (Alsiö et al., 2015). As discussed, impulsivity following 5-HT2C blockade has been well documented on other tasks [5CSRTT] (Fletcher et al., 2007; Winstanley et al., 2004b). It should be noted that successful reversal learning is thought to involve at least two influences: behavioural inhibition of perseveration and new associative learning, which were found to depend on the rodent lateral OFC and medial PFC, respectively (Chudasama and Robbins, 2003). Systemic drug administration would presumably affect both processes, potentially promoting one and impairing another (Boulougouris and Robbins, 2010). LSD, moreover, has enhanced deterministic reversal learning in rats (King et al., 1974), which aligns with the reversal impairment seen following 5-HT2A antagonism (Boulougouris et al., 2008).

The existing evidence of ATD effects on deterministic reversal learning in healthy humans remains tenuous. There have been some effects on the non-reinforced IDED, which incorporates simple deterministic reversal learning, but its primary use is to test higher order cognitive flexibility (Park et al., 1994; Rogers et al., 1999b). Another lab, however, has tested the same paradigm and showed no effect of ATD (Talbot et al., 2006).

As with literature on other tasks of response inhibition, such as SSRT and go/no-go, the use of non-reinforced (or low salience) reversal learning tasks is likely central in explaining why

ATD has not shown clear effects across studies. This may be a reflection of insufficient motive for updating or restraining action, which consequently does not sufficiently tax serotonergic reserves to result in a detectable change in behaviour (Faulkner and Deakin, 2014). In other words, it is likely that any requirement for serotonin signalling to perform the task at hand was minimal enough to be unaffected even after the (relatively mild) depletion of pools by ATD. Just as Crockett et al. (2009) were able to clarify the role of serotonin in go/no-go by incorporating motivationally salient positive and negative feedback, a deterministic reversal task with highly salient positive and negative feedback is needed. This will be examined in Chapter 5.

1.17.3 Observational reversal learning

Cools et al. (2008b) adapted a deterministic reversal learning paradigm to test whether ATD affected how well participants could predict a rewarded or punished (non-rewarded) outcome. Rather than make a choice and learn from feedback, participants were asked to predict whether a given stimulus would result in reward or punishment. Under placebo, participants were worse at predicting punishment than reward (but during non-reversal blocks only). Following ATD, however, participants were able to predict punished outcomes just as well as rewarded outcomes (Cools et al., 2008b). This result was replicated in a larger (female) sample by Robinson et al. (2012a). The interpretation was that ATD enhanced punishment prediction by removing an inhibitory influence that disengages from aversive stimuli and promotes resilience (Cools et al., 2008b; Robinson et al., 2012a).

How can these findings that ATD enhanced punishment prediction (during observational learning) be reconciled with diminished Pavlovian predictions following ATD (Crockett et al., 2012a), which purportedly underly depletion-induced behavioural disinhibition (Crockett et al., 2009)? It has been hypothesised that phasic serotonin release signals punishment prediction whereas tonic release mediates behavioural inhibition (Cools et al., 2011, 2008b). Removal of tonic 5-HT firing would invigorate behaviour by also disinhibiting dopaminergic signalling in the striatum (Cools et al., 2011). The proposal by Cools et al. (2008b) was that phasic signalling may be less drowned out when tonic firing in the background is reduced by ATD, illustrated in Figure 1.4. In other words, as punishments accrue, (phasic) spikes evolve into enhanced serotonergic tone, and subsequent spikes have less of an impact in light of the elevated tonic firing that gradually developed. This account, however, does not appear to be supported by the fMRI results from Hindi Attar et al. (2012), which indicated reduced phasic aversive predictions under ATD. It is also unclear why tonic serotonergic firing would be more affected by ATD than phasic signalling; however, it is conceivable (Faulkner and Deakin, 2014). Nonetheless, these notions of serotonin conferring resilience are generally compatible

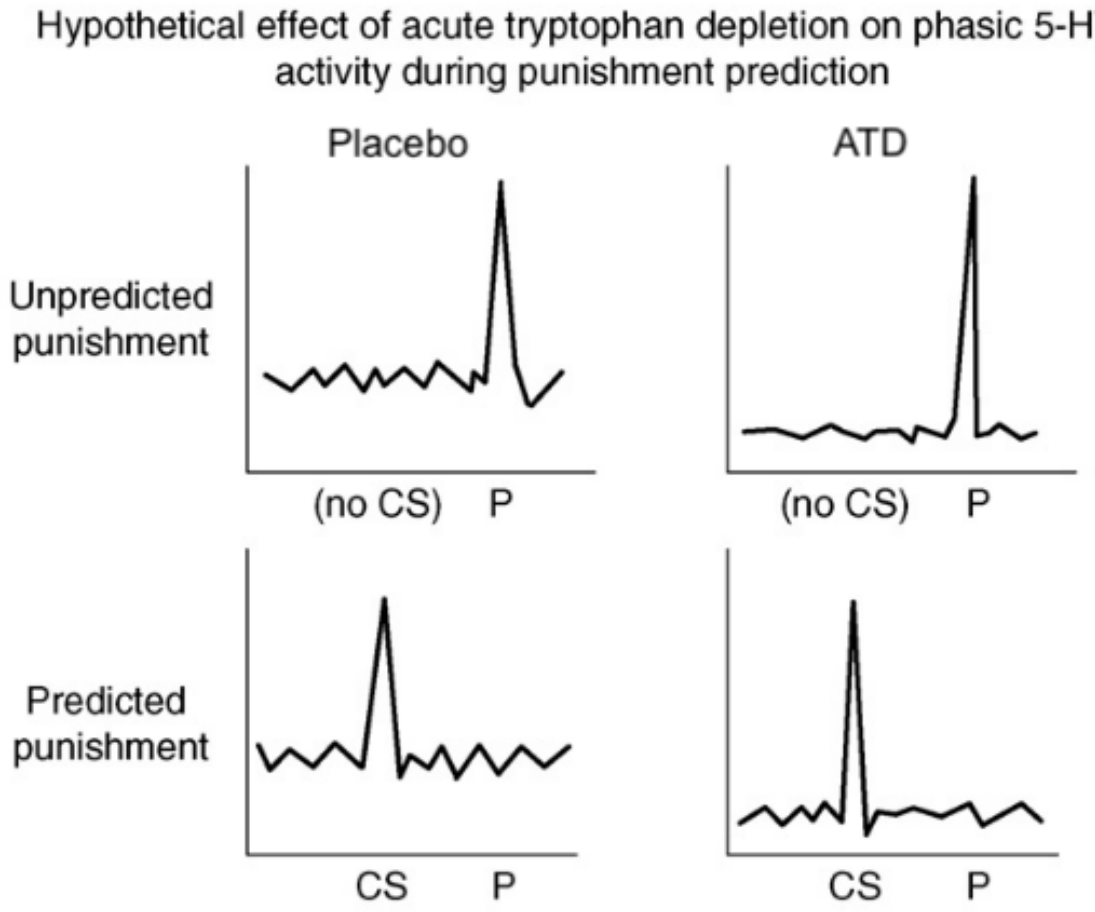

Figure 1.4: CS = conditioned stimulus; P = punishment. Used with permission (Cools et al., 2008a).

with pre-existing thinking, albeit the framework of Deakin and Graeff (1991) implicates the MRN in resilience rather than the DRN, and indeed the MRN-hippocampal system may be more sensitive to ATD (Blier and de Montigny, 1987; Faulkner and Deakin, 2014).

1.17.4 Reversal learning: probabilistic reinforcement

Probabilistic reversal learning allows not only for the assessment of reversal learning but also how organisms respond to potentially misleading feedback. Neurotoxically depleting serotonin from either the marmoset amygdala or OFC, and the rat forebrain has impaired various aspects of PRL (Bari et al., 2010; Rygula et al., 2015). Depletion of the marmoset amygdala (Rygula et al., 2015) and low dose citalopram (Bari et al., 2010) have led to enhanced sensitivity to negative feedback (SNF or lose-shift; see Section 1.1.3). A single high dose of citalopram, meanwhile, reduced SNF perhaps overcoming an autoreceptor effect (Bari et al.,

2010). Repeated SSRI administration in rats, moreover, improved reversal learning and increased win-stay behaviour (Bari et al., 2010).

Single dose administration of SSRIs in healthy humans, meanwhile, has enhanced SNF (Chamberlain et al., 2006; Skandali et al., 2018). Whilst Chamberlain et al. (2006) used 30mg citalopram, Skandali et al. (2018) used a single high dose (20mg escitalopram, equivalent to 40mg of citalopram), in a presumably unsuccessful attempt to overcome an autoreceptor effect and thus diminish SNF on this task. Indeed, the PET study by Nord et al. (2013) showing acute SSRI decreases serotonin signalling in projection areas, used 20mg escitalopram. It is conceivable that the human analogue of the high dose used in rats (Bari et al., 2010) would be above the highest permissible dose for the human studies discussed.

SNF following ATD in healthy humans, however, has been unaffected (Evers et al., 2005; Murphy et al., 2002) and represents a key paradox to be resolved in the human serotonin literature. In light of the PET results from Nord et al. (2013), if ATD decreases serotonin synthesis, why do its effects on SNF not parallel the acute SSRI findings? Whilst human PRL paradigms typically employ non-salient feedback, which could ostensibly be a plausible explanation for null effects following ATD, it is unclear why this condition wouldn't apply to SSRIs as well. Previous ATD studies of PRL have employed small sample sizes and cross-over designs (introducing possible practice effects on a learning task). A larger, between-groups, study may help to work towards a more definitive result. I have therefore conducted such a study and it is presented in Chapter 6.

1.18 Computational psychiatry from rats to monkeys to human health and illness

Computational methods, in addition to conventional measures of PRL, may additionally improve detection of ATD effects, and have yielded new insights in the effects of neurotoxic serotonin depletion during PRL in marmosets (Rygula et al., 2015). Likewise, whilst the study by Seymour et al. (2012) was already highlighted for its novel demonstration that ATD impaired reward-related learning processes, their additional finding on perseveration that was independent of the result on reward is also to be emphasised. Critically, these effects of ATD were brought out by the use of computational modelling: given their 4-armed bandit task characteristically employed probabilistic reinforcement, it suggests that interrogating the influence of ATD on PRL using a modelling approach may bear fruit. This is applied in Chapter 6. As in Seymour et al. (2012), a computational approach enabled Worbe et al. (2016) to discover valence-dependent effects of ATD on the balance between MBMF learning on a probabilistic

task, findings that were discussed previously.

As treated in section 1.17.2, agents acting at 5-HT2A and 5-HT2C receptors have had contrasting effects on indices of compulsivity including deterministic reversal learning in rats (Boulougouris et al., 2008). An area that has yet to be explored, however, is the influence of 5-HT2A receptor agents on PRL in humans, using either conventional or computational measures. Whilst not a selective compound, testing the effects of the 5-HT2A agonist lysergic acid diethylamide (LSD) represents an important comparison to the effects of ATD (Chapter 6) and SSRIs. This is the focus of Chapter 7.

Ultimately the question of paramount importance is: how do these neurochemical studies in healthy humans and experimental animals relate to individuals suffering from mental disorders? Indeed, applying computational modelling to aid in understanding clinical conditions is already underway in other patient populations including AUD (Reiter et al., 2016) and Parkinson's disease (Rutledge et al., 2009), discussed in Sections 8.4.4 and 8.4.5, as well as in schizophrenia (Waltz et al., 2011). Computational approaches have furthermore been applied to behaviour in mood and anxiety disorders on go/no-go and bandit paradigms (Aylward et al., 2019; Mkrtchian et al., 2017), as well as Pavlovian reversal learning in PTSD (Homan et al., 2019). Given my work on reversal learning is most pertinent to disorders of compulsivity, in Chapter 8 I extended investigations into the neurochemical modulation of reversal learning in healthy volunteers to two clinical populations in which serotonergic dysfunction is implicated: OCD (see Box 5) and SUD (see Section 1.16). How D2/3 agents modulated latent mechanisms underlying PRL in these populations was also tested, as dopaminergic dysfunction is implicated in OCD (Perani et al., 2008) and SUD as well (Volkow et al., 2001, 1993), in addition to the fact that dopaminergic drugs can be used in OCD (see Box 4). Critically, head-to-head comparisons of two clinical conditions (use of a positive control group) as reported here are rare and are particularly important for beginning to understand whether certain deficits generalise or are disorder specific. That an assay of fundamental learning processes such as PRL can be tested from rodents to non-human primates to humans, using rigorous analysis techniques, adds further value to mechanistic studies of individuals suffering from mental illness.

1.19 Introduction to computational modelling of reinforcement learning

The application of computational models of reinforcement learning (RL), for instance Q-learning (Watkins and Dayan, 1992), represents another approach to studying learning through

trial and error, as in reversal learning paradigms. These methods have gained remarkable traction, especially following the observations that midbrain dopaminergic neurons code a reward prediction error signal, as introduced in Section 1.8.2 (Corlett and Fletcher, 2014; D'Ardenne et al., 2008; Huys et al., 2016; Montague et al., 2012; Murray et al., 2008; Schultz et al., 1997). Conventional analyses of reversal learning assess sensitivity to immediate reinforcement (Ersche et al., 2011; Murphy et al., 2003) and do not account for the possibility that choice behaviour is influenced by an integration of feedback history from multiple experiences (Rygula et al., 2015). Computational modelling enables the assessment of latent factors underlying task performance, beyond the influence of immediate feedback. Analysing trial-by-trial learning in this manner (as it unfolds) allows for a more detailed and dynamic assessment of learning than was previously possible (Daw, 2011). RL approaches, for instance Q-learning, seek to quantify the expected value of competing choices (on a given trial), incorporating information about choice history (Murray et al., 2019). Quantities such as expectations and their violation would otherwise be subjective but can be estimated (Daw, 2011).

The computational methods employed in this thesis are based on those used by Kanen et al. (2019). Techniques used to analyse behaviour that are rooted in RL describe the behaviour in question – in this thesis, choice – by having a computer simulate putative psychological processes. These include learning from reward or punishment, tending to choose recently chosen stimuli or respond to recently responded-to locations irrespective of outcome, and selecting between alternative actions. These computational processes, for instance a given subject's tendency to learn from reward, or from aversive feedback such as errors in a task, are governed by parameters. In turn, those parameters may be influenced by dynamic pharmacological manipulations, and may vary according to relatively static properties of the subject, such as psychiatric disorders or traits. The most likely values for those parameters are determined by fitting the predictions of a computational RL model (containing parameters) to actual behaviour, in essence testing the goodness of fit of the theory to the raw data (Daw, 2011). The most informative kind of analysis uses a Bayesian hierarchy (Daw, 2011), illustrated in the following example. Subjects are drawn from groups and are influenced by drug manipulations, so the parameters pertaining to a given subject in a given condition (or study session) exist "beneath" the level of groups and drugs; at the lowest level, trial-by-trial data are predicted and compared to behaviour. The best RL model, ultimately, may be selected from a number of competing alternatives according to a formal Bayesian procedure, which penalises models that fit badly or are overly complex, per Occam's razor. Analysing behavioural data using a hierarchical Bayesian RL approach therefore simultaneously allows the best computational model of behaviour to be selected from candidate models – allowing psychological processes to be inferred – and the parameters of that model to be characterised, to uncover the effects of

disorders or pharmacological manipulations, for instance, on those processes. These methods are further introduced in Chapters 6, 7, and 8 where they are employed.

1.20 Objectives of this thesis

The core objectives of this thesis, broadly, are to better understand how emotion and behaviour are modulated neurochemically and to connect these insights with mechanistic abnormalities in psychiatric conditions. Serotonin will be the main focus, neurochemically, however dopamine will also be considered. The literature on ATD, the most common method for studying serotonin in humans, is notably lacking in several areas that will be addressed herein.

Whilst there have been several studies on the influence of ATD on social decision-making, there is a clear need for ATD studies on social or moral emotion. The objectives of Chapter 3 are to investigate whether ATD modulates self-reported social (moral) emotions to scenarios of social injustice, and to determine whether there are individual differences in this response, in particular in relation to trait empathy. Personality traits and temperament shape our view of the world and how we interact with the environment. Consideration of these factors may thus help inform some of the complexities of serotonin function. I predicted that ATD would enhance the magnitude of self-reported moral emotion and that personality traits would impact which distinct emotions were preferentially enhanced.

The next objective is to examine how serotonin influences Pavlovian threat conditioning processes. Conditioning paradigms provide a rigorous way of examining aversive emotional learning and memory across species, with clear clinical relevance. Human studies examining how serotonin modulates threat conditioning processes, however, are surprisingly limited. Chapters 4 and 5 present experiments that advance knowledge on the intersection of serotonin and Pavlovian conditioning. Chapter 4 addresses the following two questions. How does ATD influence the expression of emotional memory for a threatening experience? What is the role of trait intolerance of uncertainty in this effect? I predicted that ATD would modulate the physiological expression of previously acquired aversive conditioning and that this would vary as a function of trait intolerance of uncertainty. Chapter 5 investigates two different aspects of primitive emotion. Does serotonin have different effects on physiological responses when threat is predicted by aggressive facial expressions (social cues) relative to non-social cues? What is the role of serotonin in the ability to shift emotional reactions when a threat moves from one source to another? The latter question is tested using reversal learning, which is the focus of the remaining empirical chapters. I hypothesised that conditioning processes, assessed via SCR, would be impaired by ATD during both the initial acquisition phase as well as following the reversal of contingencies.

Despite widespread demonstrations (mostly in non-humans) that serotonin is critically involved in reversal learning, clear evidence that ATD impairs reversal learning in humans has remained elusive. A key objective of this thesis, therefore, is to study the effects of ATD on reversal learning using new methods (Chapters 5 and 6). In Chapter 5 this is accomplished through two experiments employing salient reinforcement, which should engender increased serotonin signalling more so than in previous human studies. As mentioned above, one of these experiments examined Pavlovian threat reversal learning, which has not been studied under ATD – here, reinforcement was particularly salient (aversive electric shock). Chapter 5 also transitions from the Pavlovian to the instrumental domain: when a learned, previously adaptive, action becomes counterproductive, how does serotonin influence the ability to update behaviour to reflect new circumstances? Chapter 5 addresses this question when the stakes are high yet the best action is clear: an instrumental reversal learning experiment is presented that employed highly salient feedback when performing well or doing poorly, and incorporated a deterministic reinforcement schedule (the correct action was always correct). I hypothesised that deterministic instrumental reversal learning would be impaired when the salience of feedback was highest.

Chapters 6 through 8 examine reversal learning when feedback was instead probabilistic (the correct action was correct most but not all of the time) and with more sensitive measures: computational models of reinforcement learning were applied. The following questions are addressed. To what extent is behaviour during PRL driven simply by repeating the same action as before, regardless of what outcome occurred (stimulus stickiness)? Is behaviour preferentially driven by the receipt of positive or negative feedback? The objectives of Chapters 6 through 8 are to determine how these latent mechanisms are affected by ATD (Chapter 6), LSD (Chapter 7), in OCD and SUD, and by dopaminergic agents (Chapter 8).

I predicted that ATD would not have a detectable effect on PRL when assessed by conventional observable metrics but that the computational parameter stimulus stickiness would be modulated. Another objective of Chapter 6 is to investigate how intolerance of uncertainty and subclinical psychiatric symptoms (of anxiety, depression, and OCD) relate to these computational measures. By examining how reinforcement learning model parameters relate to subclinical symptoms, we can more rigorously discern aspects of psychiatric conditions that may be extremes of normal tendencies. This could furthermore aid in identifying new indicators of vulnerability in healthy individuals.

The human serotonin literature has relied heavily on ATD and SSRIs. To extend the work on ATD (and SSRIs), I acquired a PRL dataset which enables a comparison with the effects of the non-selective 5-HT2A agonist LSD. Indeed, the effect of any psychedelic drug on PRL has not been investigated in humans. Understanding how LSD affects learning could have

implications for appreciating its therapeutic potential. I suspected that LSD would modulate observable measures of either lose-shift behaviour or perseveration. Computationally, I hypothesised that LSD would affect either stimulus stickiness or learning rates.

Ultimately these approaches need to be applied to clinical problems. Therefore, to compare mechanisms of PRL under ATD and LSD to behaviour during PRL in clinical populations, a reanalysis of a large dataset on OCD and SUD was conducted. In this study, reported on in Chapter 8, the effect of modulating D2/3 receptors on PRL was additionally assessed, enabling further comparison to the effects of serotonin on cognitive flexibility. By directly comparing two disorders of compulsivity, I sought to identify a computational profile that could distinguish behaviour in OCD and SUD during PRL. I predicted that learning rates and measures of stickiness may differ between individuals with OCD and SUD and relative to healthy controls. Chapter 9, the general discussion, considers these various findings in terms of the animal literature, psychiatric disorders, and theories of central serotonin function, topics which are also emphasised throughout.

Chapter 2

General Methods

2.1 Serotonin synthesis pathway

The serotonin synthesis and metabolism pathway is depicted in Figure 2.1. Serotonin is synthesised from L-tryptophan, the initial substrate, in two steps. Tryptophan (TRP) is first converted to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase (TPH), in a hydroxylation reaction. This product is then converted into 5-hydroxytryptamine (5-HT; serotonin) by aromatic L-amino-acid decarboxylase (AAAD), in a decarboxylation reaction. 5-HT (serotonin) is eventually broken down into its inactive metabolite 5-hydroxyindolacedic acid (5-HIAA) by monoamine oxidase (MAO) and aldehyde dehydrogenase (ADH). Because it is essential that tryptophan be consumed from the diet, as the body cannot synthesise it, the primary rate-limiting factor in the synthesis of serotonin is the bioavailability of tryptophan (van Donkelaar et al., 2011). As discussed in Chapter 1, the technique acute tryptophan (ATD) takes advantage of the fact that tryptophan is an essential amino acid. I now discuss further considerations for the ATD technique.

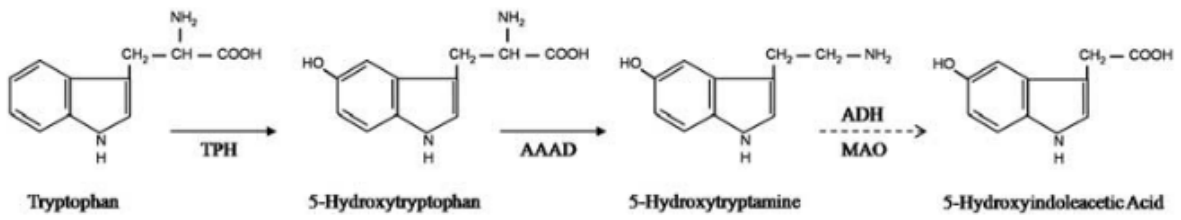

Figure 2.1: Serotonin synthesis and metabolism pathway. From van Donkelaar et al. (2011), with permission.

2.2 ATD: methodological considerations

2.2.1 Measuring the effects of ATD on serotonin

ATD was initially developed in rats (Biggio et al., 1974), and was found to rapidly and substantially lower tryptophan, serotonin, and 5-HIAA in rat brain tissue, as well as plasma tryptophan (Young, 2013). ATD results in a reduction in the ratio of plasma tryptophan to the sum of the other large neutral amino acids (LNAA), confirmed in Chapters 3-6 of this thesis, which is believed to be most reflective of the extent of brain 5-HT depletion (Fernstrom, 1979; Hood et al., 2005; Young, 2013). Whilst ATD has been reported to decrease the serotonin metabolite 5-HIAA in the cerebrospinal fluid (CSF) in humans (Carpenter et al., 1998; Hood et al., 2005), evidence from the rat has indicated that serotonin metabolism is not necessarily correlated with serotonin neuron firing and release (Crespi, 1990). ATD, therefore, has been seen as a more direct way to study the effects of low serotonin than measuring the serotonin metabolite 5-HIAA in CSF (Young, 2013). Indeed, levels of 5-HIAA in the CSF tend to be very similar between depressed and nondepressed individuals (Young, 2013). There are even reports of elevated CSF 5-HIAA in people with depression, and lower levels in the nondepressed (Young, 2013). CSF levels of 5-HIAA have been associated with suicidal behaviour more so than with depression (Åsberg, 1997; Young, 2013).

A number of studies have provided evidence of decreased serotonin release in rats following ATD using in vivo microdialysis (Bel and Artigas, 1996; Fadda et al., 2000; Stancampiano et al., 1997). van Donkelaar et al. (2011) criticised these studies for their use of an SSRI in the dialysate fluid in order to improve sensitivity to detect changes in efflux. Whilst systemically administered SSRIs can decrease serotonin concentration (Nord et al., 2013), presumably through 5-HT1A autoreceptors, Crockett et al. (2012b) noted that the SSRI doses used in the dialysate fluid to increase sensitivity appear to be too low to sufficiently engage this 5-HT1A autoreceptor feedback mechanism. Indeed, adding a 5-HT1A receptor antagonist on top of an SSRI in the dialysate fluid did not increase efflux (Sharp et al., 1996).

Moreover, whilst one study did not use an SSRI in the dialysate fluid and did not find a decrease in serotonin release (van Der Plasse et al., 2007), this was only examined in the rat prefrontal cortex and thus it cannot be concluded that other untested areas were not affected (Crockett et al., 2012b). Indeed, regulation of serotonin release can differ across brain regions (Young, 2013). Rather than assessing basal efflux, measuring serotonin release during a task known to engage serotonin signalling, as confirmed on microdialysis, would be a more appropriate test of how ATD affects serotonin efflux (Crockett et al., 2012b).

Acquiring direct evidence in humans that ATD decreases serotonin release is hampered by inadequate PET ligands to detect such an impairment (Paterson et al., 2010). There is,

however, evidence that ATD reduces the rate of serotonin synthesis in healthy volunteers by more than 85% in all brain regions measured in a PET study by Nishizawa et al. (1997), an effect that was more pronounced in women. Irrespective of ATD, the biochemical and electrophysiological factors influencing serotonin release in general are remarkably complex and many open questions remain (Young, 2013).

2.2.2 Other biochemical considerations

Without direct evidence in humans that ATD impairs serotonin release, the method has been subjected to criticism particularly by van Donkelaar et al. (2011). They have speculated on a number of possible mechanisms that could contribute to the effects of ATD. It should be noted, however, that van Donkelaar et al. (2011) hold the position that ATD does not affect other monoamines (e.g. dopamine or norepinephrine) aside from serotonin. Indeed, it has been shown that ATD in rats did not affect the concentration of dopamine or norepinephrine in any brain region examined, which led the authors to conclude that any cognitive or behavioural changes following ATD are likely due to alterations in the serotonin systems (Ardis et al., 2009).

van Donkelaar et al. (2011) argued that stress caused by implementing the ATD procedure is a confound. Whilst it is true that stress can alter serotonin function (Lapiz-Bluhm et al., 2009), this stress is matched between the placebo and depletion groups. In other words, avoiding any confounding influence of unpleasant aspects of the procedure is precisely why ATD is conducted in a placebo-controlled design. van Donkelaar et al. (2011), moreover, focus their arguments on rodents: whilst the ATD procedure is likely more stressful for a rodent than a human, elements such as taking blood samples, giving injections, and food restriction are used in other pharmacological manipulations in rats besides ATD.

Given serotonin is a potent vasoconstrictor, as mentioned in Chapter 1, and serotonergic neuron fibres have been identified that innervate cerebral arteries, veins, and arterioles, van Donkelaar et al. (2011) suggested cerebrovascular effects may be a confound; however, to support this claim they pointed to a rat study that not only showed decreased blood flow (opposite of what would be expected by removing a vasoconstrictor) but also found no change in brain serotonin, tryptophan, or 5-HIAA (Young, 2013). ATD, moreover, does not lower blood serotonin in humans, most of which is stored in platelets, suggesting the duration of a typical ATD study is not long enough to deplete the pool of platelet serotonin whose function includes vasoconstriction (Young, 2013). Furthermore, if cerebrovascular changes occurred following ATD, then these effects should apply to SSRIs as well.

Likewise, van Donkelaar et al. (2011) speculated that brain-derived neurotrophic factor (BDNF) might influence the effects of ATD indirectly but acknowledged evidence that there

is no direct relationship (Young 2013). Downstream effects of serotonin signalling obviously involve other molecular mechanisms, and this may include BDNF. The most important issue, however, is whether the primary impact of ATD is on serotonin, and not about downstream effects of serotonin (Young, 2013).

Only a small proportion of tryptophan is converted into serotonin, whereas the major part is metabolised via the kynurenine pathway. Two of the metabolites in this pathway bind to ionotropic glutamate receptors: quinolinic acid agonises NMDA receptors, whereas kynurenic acid antagonises the NMDA, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), and kainite receptors. van Donkelaar et al. (2011) suggested that if ATD were to reduce levels of these metabolites it could lead to non-serotonergic cognitive effects. Young (2013), however, has rebutted this argument for the following reasons: baseline concentrations are low enough for further lowering not to have an appreciable effect; CSF concentrations of quinolinic acid are much higher in inflammatory conditions characterised by neurological deficits, not lower; and plasma kynurenic acid levels in humans are not depleted by ATD. Effects of ATD on human plasma quinolinic acid, meanwhile, have not been reported, and whether ATD modulates human CSF levels of kynurenic acid or quinolinic acid remains to be tested (Young, 2013).

van Donkelaar et al. (2011) additionally called attention to two rat studies that used a gelatinous mixture, different to that typically used, and with a different amino acid composition. Decreased citrulline levels in the tryptophan-free condition were found (van Donkelaar et al. 2011). The enzyme nitric oxide synthase converts the amino acid arginine into citrulline and nitric oxide. van Donkelaar et al. (2011) suggested that nitric oxide may therefore have also been lowered, or that nitric oxide synthase activity might have been decreased. They go on to speculate that diminished nitric oxide synthase function could underly ATD-induced object memory impairments in rats. van Donkelaar et al. (2011) acknowledge, however, that the interaction between nitric oxide and serotonin remains enigmatic. Young (2013), moreover, concluded that given the methodology differed and was tested in rats, the relevance for human studies is unclear. Nonetheless, I will note that this thesis did not employ an object recognition task.

2.2.3 Importance of translation

Convergent behavioural effects of ATD in humans and neurotoxic serotonin depletion in non-humans (Winstanley et al., 2004a; Worbe et al., 2014), moreover, substantiate the interpretation that ATD effects result at least in part from serotonin depletion. In this thesis I present new such parallels, providing further substantiation of ATD as a method for studying serotonin in the central nervous system. In sum, the evidence discussed indicates that the most

parsimonious account is that ATD modulates central serotonin (Crockett et al., 2012b).

2.3 ATD: implementation

Chapters 3, 4, 5, and 6 employ ATD. I now provide details of the methodology implemented.

2.3.1 Plasma amino acid analysis

Whole blood was collected in lithium heparin preservative and centrifuged at room temperature using a standard laboratory centrifugation protocol, in this case 3000 rotations per minute (rpm) was used for 15 minutes. Plasma was then aliquoted, avoiding the buffy coat (leukocytes and platelets), and stored at -80 degrees centigrade. Plasma samples were then analysed using high performance liquid chromatography [HPLC] (Crockett et al., 2013; Passamonti et al., 2012). The ratio of (free) tryptophan to large neutral amino acids (TRP:LNAAs; valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine) was then calculated. The index of depletion used in this thesis was the change in the TRP:LNAA ratio between samples taken at baseline and approximately 4.5 hours following administration of the mixture.

2.3.2 Amino acid quantities

The amino acid quantities employed in Chapter 3, Chapter 4, Chapter 5 (Experiment 1), and Chapter 6 were derived from Worbe et al. (2014) and were as follows. The depletion mixture contained 4.10g L-alanine, 3.70g L-arginine, 8.93g L-aspartic acid, 2.00g L-cystine, 2.40g glycine, 2.40g L-histidine, 6.00g L-isoleucine, 10.10g L-leucine, 6.70g L-lysine, 2.30g L-methionine, 4.30g L-phenylalanine, 9.20g L-proline, 5.20g L-serine, 4.90g L-threonine, 3.00g L-tyrosine, and 6.70g L-valine. The placebo mixture was identical but contained 5.20g of L-tryptophan. The mixtures were manufactured by metaX Institut fur Diatetik GmbH with flavouring included. These drinks were prepared by stirring in 500ml tap water.

The amino acid quantities employed in Experiment 2 of Chapter 5 were derived from Crockett et al. (2009) and were as follows. Tryptophan depletion: 4.10g L-alanine, 3.70g L-arginine, 2.00g L-cystine, 2.40g glycine, 2.40g L-histidine, 6.00g L-isoleucine, 10.10g L-leucine, 6.70g L-lysine, 2.30g L-methionine, 9.20g L-proline, 4.30g L-phenylalanine, 5.20g L-serine, 4.90g L-threonine, 5.20g L-tyrosine, and 6.70g L-valine. The placebo mixture was the same as above, plus 3.00g of L-tryptophan. For females, 20% reductions in the above quantities were used to account for lower body weight. The mixtures were manufactured by SHS International, Liverpool, UK. These drinks were prepared by stirring the mixtures and adding lemon-lime flavouring into 200ml tap water.

2.3.3 Inclusion/exclusion criteria

2.3.3.1 Chapter 3, 4, 5 (experiment 1), 6

Participants were screened to be medically healthy and free from any psychiatric conditions, determined by the Mini International Neuropsychiatric Interview [MINI] (Sheehan et al., 1998). Individuals who reported having a first-degree relative (parent or sibling) with a psychiatric disorder were excluded upon screening as well. Exclusion criteria also encompassed neurological disorders, pregnancy, past use of neurological, psychiatric, or endocrine medication, use of St. John's Wort, or current use of any regular medication besides contraceptive pills. The cut-offs for drug use were smoking more than five cigarettes per day, regular consumption of more than 38 units of alcohol per week, cannabis use more than once per month, and the lifetime use of recreational drugs besides cannabis more than five times. Other medical exclusion criteria: cardiac or circulation issues, respiratory problems including asthma; gastrointestinal, renal, or thyroid conditions; bleeding disorders, diabetes, and head injury.

2.3.3.2 Chapter 5 (experiment 2)

Participants were eligible if they did not have a personal or family history of major depressive disorder, bipolar disorder, or any other psychiatric illness. Other exclusion criteria included medication use, a history of neurological, cardiac, gastrointestinal, hepatic, pulmonary, or renal disorders.

2.3.4 General procedure

Ethical approval was granted by the Cambridge Central Research Ethics Committee, and all participants provided written informed consent and were financially compensated. Research was conducted at the National Institute for Health Research / Wellcome Trust Clinical Research Facility at Addenbrooke's Hospital in Cambridge, England.

2.3.4.1 Chapter 3, 4, 5 (experiment 1), 6

Participants arrived in the morning having fasted for at least 9 hours prior, gave a blood sample, and ingested either the placebo or ATD drink. To assess mood and other feelings including alertness, I used a 16-item visual analogue scale (VAS) at the beginning, middle, and end of the day-long testing session. In the afternoon participants completed computerised neuropsychological tasks. A second blood sample was collected after approximately 4.5 hours after participants consumed the drink, after depletion had occurred but prior to the nadir of plasma tryptophan, per the time course reported by Carpenter et al. (1998).

2.3.4.2 Chapter 5 (experiment 2)

Blood samples were collected at baseline and before the task to verify tryptophan depletion. Participants completed questionnaires including the Positive and Negative Affect Schedule [PANAS] (Watson et al., 1988) assessing self-reported mood state. Data were collected inside of a functional magnetic resonance imaging (fMRI) scanner, but the fMRI data are not reported here. Participants returned for a second session, where they received the other drink condition and also completed different computerised tasks which have been published elsewhere (e.g. Crockett et al., 2013; Passamonti et al., 2012). It is important that participants are naïve to conditioning paradigms, and therefore the data reported here were acquired in the first of two testing sessions spaced at least one week apart.

2.4 Frequentist statistics

Data were analysed using MATLAB (MathWorks) and SPSS (IBM).

2.4.1 Homogeneity of variance

Homogeneity of variance in t-tests was verified with Levene's test, and degrees of freedom were adjusted when this assumption was violated. The Greenhouse-Geisser correction was used where applicable, in designs with within-subjects factors, to correct for violation of the sphericity assumption as determined by Mauchly's test.

2.4.2 Multiple comparisons

Correction for multiple comparisons (controlling for false discovery rate, or false positives), where relevant, was conducted using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995). The critical value for false discovery rate was set a priori (McDonald, 2014) at q = .15 (Genovese et al., 2002; Skandali et al., 2018).

2.5 Bayesian statistics

Models were fitted using Hamiltonian Markov Chain Monte Carlo sampling via Stan 2.17.2 (Carpenter et al., 2017). Convergence was checked with the potential scale reduction factor measure R^ (Brooks and Gelman, 1998; Gelman et al., 2012), which approaches 1 for perfect convergence; values below 1.2 are typically used as a guideline for convergence and a cut-off

of <1.1 is a stringent criterion for convergence (Brooks and Gelman, 1998). The use of multiple simulation runs with measurement of convergence is an important check for simulation reliability (Wilson and Collins, 2019) and is an intrinsic part of Stan. Parameter recovery from simulated data for this modelling approach has been confirmed by Kanen et al. (2019).

Models were compared using a bridge sampling estimate of the marginal likelihood (Gronau et al., 2017a) via the "bridgesampling" R package (Gronau et al., 2017b). This procedure directly estimates the marginal likelihood, and thus the posterior probability of each model, given the data, prior model probabilities, and the assumption that the models represent the entire family of those to be considered. All models were assumed to have equal prior probability.

In addition to the estimated parameters, comparisons of interest (e.g. between pharmacological conditions or clinical groups) were also sampled directly to give a posterior probability distribution for each quantity of interest. Posterior distributions were interpreted using the 95% highest posterior density interval (HDI), the Bayesian "credible interval". Identical priors were used for all groups and conditions.

Chapter 3

Effects of tryptophan depletion on social emotions: role of personality traits

3.1 Introduction

A unified function for serotonin (5-hydroxytryptamine; 5-HT) has, perhaps unsurprisingly, proven to be elusive. It is hypothesised to have a role in many psychiatric disorders (Dayan and Huys, 2009), and is implicated in a wide range of mental functions including aversive processing, impulse control, and social behaviour (Cools et al., 2008a). These domains can be viewed under a unified framework by considering how serotonin impacts Pavlovian (stimulus-outcome, including emotional) and instrumental (stimulus-response-outcome; behavioural) processes that underlie both social and non-social functions (Gesiarz and Crockett, 2015). Much recent work on the relationship between serotonin, aversive processing, and human social behaviour has focused on instrumental action in the context of behavioural economic games and moral dilemmas (Crockett et al., 2010a,b, 2008). Here I examined emotional reactions to social scenarios depicting unjust harm.

Studies of healthy volunteers have primarily employed two techniques to investigate serotonin function: acute tryptophan depletion (ATD), described in Chapters 1 and 2, to lower brain serotonin function (Bel and Artigas, 1996; Biggio et al., 1974; Crockett et al., 2012b; Hood et al., 2005; Nishizawa et al., 1997), and treatment with single doses of selective serotonin reuptake inhibitors (SSRIs). SSRIs are generally assumed to increase extracellular serotonin, however it should be noted that single rather than chronic doses can paradoxically decrease serotonin in projection areas (Cools et al., 2008a; Nord et al., 2013). ATD studies have revealed disinhibition of retaliatory behaviour in the face of perceived injustice (Crockett et al., 2008), modelled using the Ultimatum Game [UG] (Guth et al., 1982), summarised in Figure

3.1, whilst single dose SSRI has had pro-social effects (Crockett et al., 2010a).

Morally relevant social emotions, meanwhile, lie at the interface between moral standards and socially appropriate behaviour (Tangney et al., 2007). Moral standards prohibit behaviours that are likely to have negative consequences for the well-being of others. Emotions are in part Pavlovian in nature, and while ATD has been shown to modulate Pavlovian processes in non-social domains (Crockett et al., 2012a; Hindi Attar et al., 2012), here I tested the influence of ATD on emotion in a social context. I asked whether ATD would enhance morally relevant negative emotions evoked by social scenarios in which a person was unjustly harmed: these served as Pavlovian cues. Using a novel task, I prompted participants to reflect on the situations and specifically assessed emotions involving annoyance, guilt, shame, and feeling "bad". Reporting one's emotional reactions after mentally simulating hypothetical social scenarios implicitly calls on autobiographical memories, which should result in a diversity of subjective experiences tied to personal qualities and expectations about social behaviour. I therefore also tested the influence of three personality traits – empathy, psychopathy, and impulsivity – on how serotonin would affect emotion.

Research on moral emotions has focused mostly on the self-conscious negative emotions guilt and shame (Tangney et al., 2007). Guilt often relates to a negative appraisal of a specific behaviour, whereas shame tends to involve a negative evaluation of the self (Tangney et al. 2007): "If only I hadn't" as opposed to "If only I weren't" (Niedenthal et al., 1994). Whilst guilt is part of the diagnostic criteria for depression (American Psychiatric Association, 2013), proneness to shame most consistently relates to an array of psychiatric conditions, including symptoms of depression (Ashby et al., 2006; Crossley and Rockett, 2005; Stuewig and McCloskey, 2005), anxiety (Crossley and Rockett, 2005), post-traumatic stress disorder (Brewin et al., 2000; Feiring and Taska, 2005; Leskela et al., 2002; Orsillo et al., 1996), eating disorders (Murray et al., 2000; Sanftner et al., 1995), as well as more specific symptoms such as low self-esteem (Feiring et al., 2002), suicidal ideation (Bryan et al., 2013), anger (Harper and Arias, 2004; Tangney et al., 1996), and aggression (Tangney et al., 1996). Importantly, guilt is thought to become maladaptive primarily when it is fused with shame (Tangney et al., 2007).

Evidence from patients with damage to the ventromedial prefrontal cortex (vmPFC) and incarcerated individuals with psychopathy provides a plausible connection between moral emotions and social behaviour, outlined in Figure 3.1. The vmPFC is an area central to emotion regulation (Schiller and Delgado, 2010), with dense serotonergic innervation (Hornung, 2003). Individuals with vmPFC damage also show increased retaliatory behaviour to unfairness on the UG (Koenigs and Tranel, 2007), which mirrors the ATD findings (Crockett et al., 2010b, 2008). This is furthermore analogous to the UG results from psychopathic individuals (Koenigs et al., 2010), where vmPFC dysfunction is a feature (Motzkin et al., 2011), as is

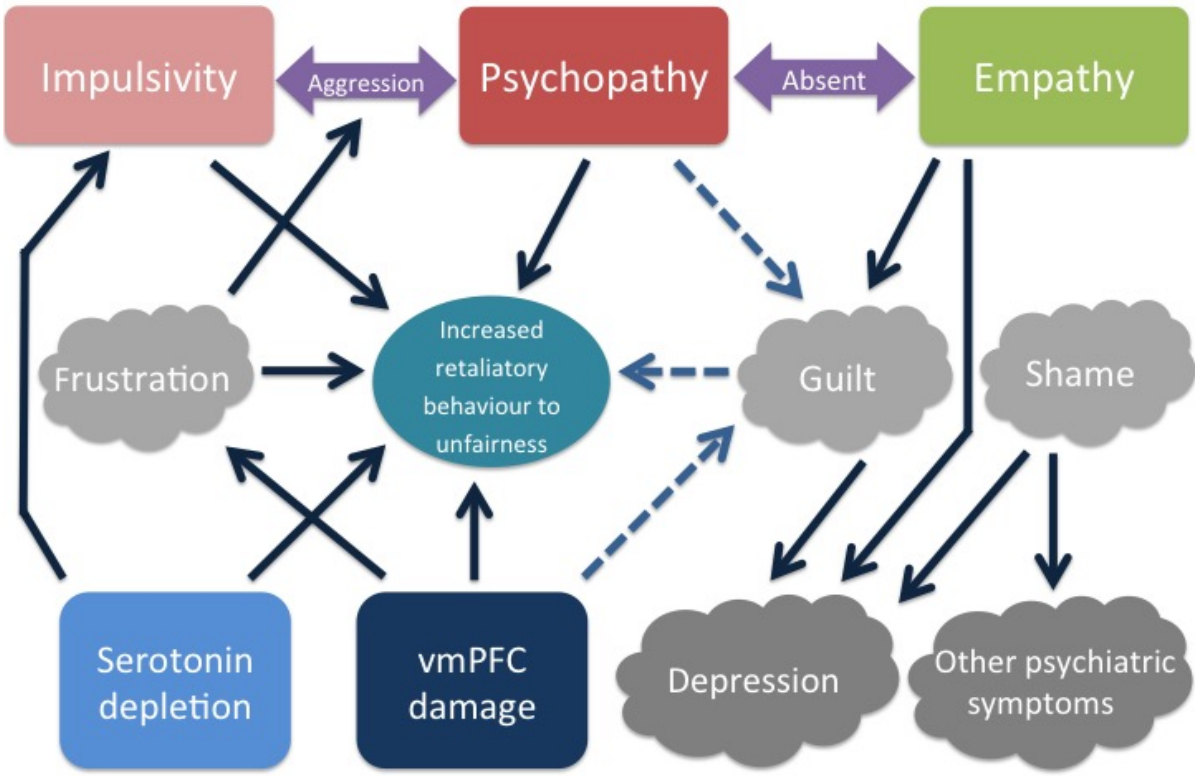

Figure 3.1: Background information about relationships among constructs invoked. Dark blue solid arrows signify a positive relationship, light blue dashed arrows a negative association (see main text). vmPFC = ventromedial prefrontal cortex. Note that guilt and shame are distinct yet guilt can at times be laden with shame. I conceptualise frustration as closest to annoyance, measured by the present task. Frustration here can also be related to anger (Koenigs and Tranel, 2007; Pillutla and Murnighan, 1996). Note the form of aggression depicted is impulsive/reactive, but aggression can also be non-impulsive and goal directed (Blair, 2010). The studies on retaliatory behaviour to unfairness employed the Ultimatum Game as a laboratory model (Crockett et al., 2010a,b, 2008; Koenigs et al., 2010; Koenigs and Tranel, 2007; Krajbich et al., 2009): One player, programmed by the experiment, proposes to split a sum of money with the study participant who can either accept or reject the offer. Both players are paid accordingly if the offer is accepted; however, neither player is paid if the offer is rejected. Participants tend to reject offers that are less than 30% of the total sum even though such retaliatory behaviour to unfairness is costly. Healthy individuals reject more unfair offers under ATD (Crockett et al., 2010b, 2008) and accept more unfair offers on a single dose of an SSRI (Crockett et al., 2010a).

reduced guilt (Blair, 2010). Impaired moral behaviour following damage to the vmPFC has meanwhile been conceptualised as a manifestation of diminished guilt (Krajbich et al., 2009). The implication here is that guilt is related to inhibition of anti-social behaviour, as modelled by restraint in behavioural economic games like the UG. Studying the effects of ATD on guilt could therefore inform how moral emotion and behaviour are integrated.

Proneness to guilt consistently correlates with empathy, which refers to the ability to share the affective experiences of others (Tangney et al., 2007). Empathy, while not a discrete emotion, is a morally relevant emotional capacity (Tangney et al., 2007) and trait (Davis, 1980). Guilt appears to foster reparative action, promote empathy (Tangney et al., 2007), and increase altruistic acts (O'Connor et al., 2007). Elevated empathy, moreover, has been correlated with severity of depression, and proposed as a risk factor for its development (O'Connor et al., 2002). Importantly, empathy is classically absent in psychopathy (Blair, 2010). These relationships from the literature are summarised in Figure 3.1.

I was especially interested in empathy because moral decision-making in individuals high in trait empathy has been shown to be particularly sensitive to manipulations of serotonin (Crockett et al., 2010a). Furthermore, the serotonin 2A (5-HT2A) receptor agonists lysergic acid diethylamide (LSD), psilocybin, and 3,4-methylenedioxy methamphetamine (MDMA), have all been shown to enhance empathy (Dolder et al., 2016; Hysek et al., 2014; Pokorny et al., 2017). To extend these findings I therefore tested the hypothesis that ATD would interact with trait empathy to amplify morally relevant social emotion. Given the consistent correlations between guilt-proneness and empathy (Tangney et al., 2007), I predicted guilt would be the most likely emotion to be affected.

Conversely, psychopathy is characterised by emotional dysfunction reflected in reduced guilt and empathy (Jones et al., 2010), and an increased risk for aggression, outlined in Figure 3.1. Aggression can be either goal-directed (e.g. a premeditated crime), or reactive: an explosive, impulsive response to frustration (Blair, 2010). Many psychiatric conditions increase the risk for reactive aggression, however psychopathy is unique in that there is an increased risk for both reactive and goal-directed aggression (Blair, 2010). Aggression is in turn traditionally associated with low serotonin (Deakin, 2003), including evidence from studies of violent offenders (Linnoila et al., 1983). I explored whether psychopathic traits are likewise related to morally relevant emotions, and whether ATD modulates this relationship. I predicted that psychopathic traits might have the most pronounced effect on feeling annoyed, which invokes the notion of frustration.

While some, but not all, aggression can be impulsive, aggression and impulsivity are distinct. Indeed, discrete serotonergic circuits modulate aggressive versus impulsive behaviour in mice (Nautiyal et al., 2015). ATD can induce "waiting impulsivity" (diminished action

restraint whilst waiting for a reward) and "impulsive choice" (accepting small immediate rewards over larger delayed ones) in healthy individuals (Crockett et al., 2010b; Worbe et al., 2014). More impulsive choice has been correlated with increased aggressive impulses to perceived injustice on the UG, which was heightened further by ATD (Crockett et al., 2010b). I therefore asked whether trait impulsivity on the Barratt Impulsiveness Scale (BIS; Patton et al., 1995) was related to increased annoyance following ATD.

To test my hypotheses I employed ATD in a double-blind, randomised, placebo-controlled, between-groups design, in healthy volunteers. I predicted that ATD would enhance negative emotion overall, and that individual differences in empathy, psychopathy, and impulsivity would influence how ATD modulated the profile of emotion. In line with the traditional disconnection between psychopathy and empathy, I predicted that there would be dissociation between how trait empathy and psychopathy interact with neurochemical status to modulate annoyance, guilt, shame, and feeling bad. Given the established connection between serotonin and impulsivity (Bevilacqua et al., 2010; Dalley and Roiser, 2012; Worbe et al., 2014), and retaliatory behaviour (Crockett et al., 2010b), I also hypothesised that high trait impulsivity would be related to increased feelings of annoyance, which would be further potentiated by ATD in these individuals.

3.2 Methods

3.2.1 Participants

Seventy-three participants completed the study [mean age 24.6]. Thirty-seven underwent tryptophan depletion (20 males), whilst the remaining 36 received placebo (19 males). The ATD procedure is described in Chapter 2. I assessed several personality traits and psychological symptoms in part to ensure the placebo and depletion groups were matched. There were no differences in depressive symptoms ($t_{(71)}$ = -1.258, p = .212) using the Beck Depression Inventory [BDI-II] (Beck et al., 1996); trait anxiety ($t_{(71)}$ = -0.872, p = .386) using the Spielberger Trait Anxiety Inventory [STAI] (Spielberger et al., 1983); psychopathic traits ($t_{(71)}$ = 1.132, p = .261) assessed with the Levenson Self-Report Psychopathy Scale [LSRP] (Levenson et al., 1995); autistic characteristics ($t_{(71)}$ = -0.112 , p = .911) using the Adult Autism Spectrum Quotient [AQ] (Baron-Cohen et al., 2001); empathy ($t_{(63)}$ = 0.442, p = .660; Levene's test $F_{(71)}$ = 4.569, p = .036) using the Interpersonal Reactivity Index [IRI] (Davis, 1980); and impulsivity ($t_{(71)}$ = .444, p = .658) assessed with the Barratt Impulsiveness Scale [BIS] (Patton et al., 1995). Participants in each group, additionally, did not differ in their years of education ($t_{(71)}$ = 0.634, p = .528).

3.2.2 Moral emotions task

I employed a novel task, part of the EMOTICOM neuropsychological testing battery (Bland et al., 2016; Savulich et al., 2018), to measure feelings of guilt, shame, annoyance, and feeling "bad". Using a touchscreen computer, participants were presented with cartoons of social scenarios – Pavlovian cues – in which someone was unjustly harmed, either intentionally or unintentionally. I then interrogated emotional reactions to these scenarios by asking participants to reflect, and report how they would feel if they were the victim or agent of harm. An example of one trial is depicted in Figure 3.2. There were 28 randomised trials, composed of 14 different cartoons, which were each presented twice – once where participants were prompted to identify as the victim, and once where they were asked to identify as the agent. The specific instruction was, "If this was you, please indicate below how you would feel by touching the line." The four different emotions were measured using four unnumbered touchscreen scales, with seven rungs to choose from, where the first rung was labelled "not at all", scored as 1, and the seventh labelled "extremely", scored as 7. Half of the scenarios involved an intentional harm. In the other half, the harm committed was accidental. The task was self-paced.

3.3 Results

3.3.1 Effects of ATD on mood ratings

Mood ratings were unaffected by ATD. I collected rating data from 65 participants (n = 33 depletion) on how happy or sad they were feeling before the task, after depletion had taken effect, and these ratings did not differ from those of participants in the placebo group ($t(63) = -0.727$, $p = .47$). There were also no baseline differences in depressive symptoms ($t(71) = -1.258$, $p = .212$).

3.3.2 How serotonin depletion modulated emotional ratings overall

I tested whether serotonin depletion potentiated emotions overall, irrespective of individual differences. To do this I performed repeated measures analysis of variance (ANOVA) incorporating all four emotions measured. The ANOVA had one between-subjects factor, serotonin status, with two levels (placebo and ATD). There were three within-subjects factors: emotion, with four levels (annoyance, guilt, shame, and feeling bad); intentionality, with two levels (intentional and unintentional); and agency (victim or agent). The model also incorporated the following interaction terms: emotion x agency, emotion x intentionality, agency x intention-

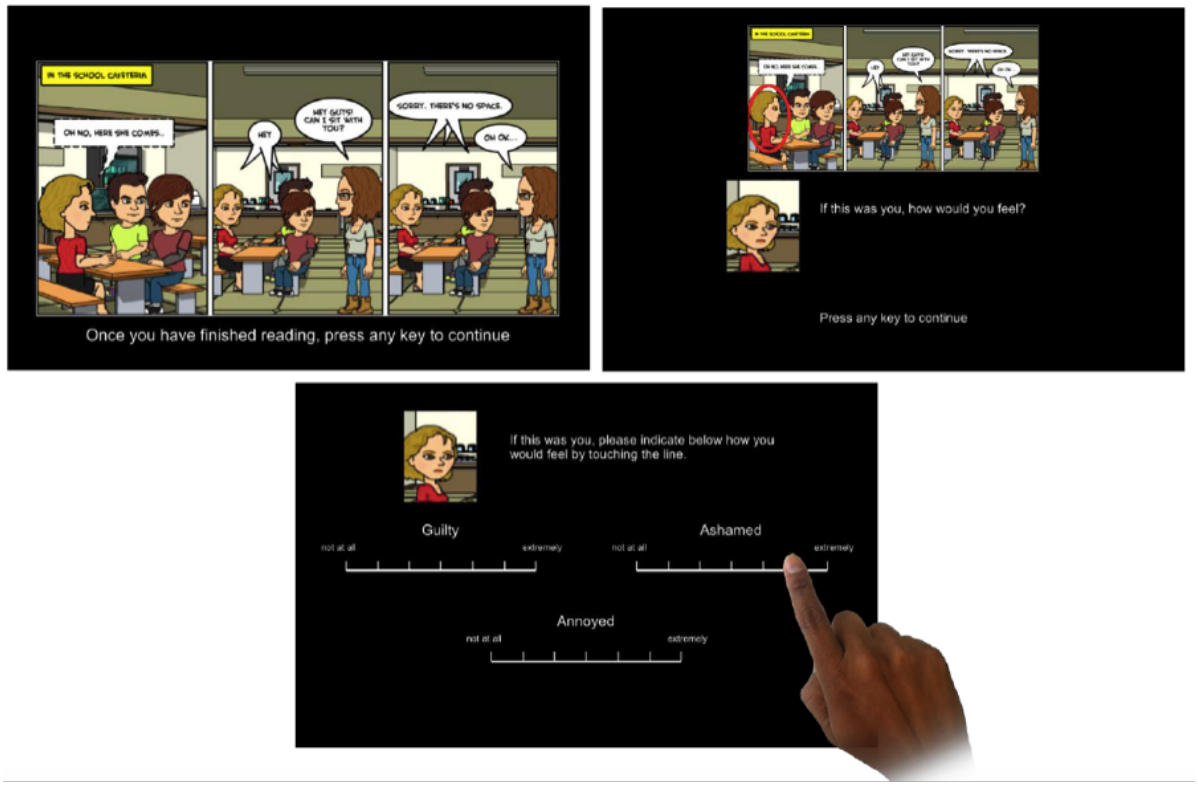

Figure 3.2: Moral emotions task schematic. Three example slides of a trial are shown. Feeling "bad" was assessed with a rating scale on a fourth slide.

ality, and emotion x agency x intentionality. Indeed, serotonin depletion potentiated emotion overall ($F(1,71) = 5.959$, $p = .017$, $\eta_p^2 = .077$), shown in Tables 3.1 and 3.2 and Figure 3.3. There were highly significant main effects for emotion ($F(2,155) = 171.744$, $p = 7.4 \times 10^{-42}$, $\eta_p^2 = .708$), agency ($F(1,71) = 630.212$, $p = 4.9 \times 10^{-37}$, $\eta_p^2 = .899$), and intentionality ($F(1,71) = 11.799$, $p = .001$, $\eta_p^2 = .143$), regardless of serotonergic status, which supports task validity and suggests individuals were attuned to the social context of the scenarios. Emotional ratings were significantly greater when imagining being the agent compared to the victim, and when the harm committed was intentional rather than unintentional. There were no interactions between serotonin status and agency or intentionality (all $p > .05$). Further interactions were present between factors, unrelated to the effects of ATD, as follows. There were significant interactions between emotion and agency ($F(2,148) = 727.007$, $p = 2.090 \times 10^{-78}$, $\eta_p^2 = .911$); emotion and intentionality ($F(3,181) = 37.110$, $p = 7.849 \times 10^{-17}$, $\eta_p^2 = .343$); agency and intentionality ($F(1,71) = 83.911$, $p = 1.188 \times 10^{-13}$, $\eta_p^2 = .542$); and emotion x agency x intentionality ($F(3,178) = 36.860$, $p = 1.978 \times 10^{-16}$, $\eta_p^2 = .342$). I display a breakdown of the means and standard deviations of these factors in Table 3.1. The core results in this study however came from analyses of how trait differences interacted with the serotonin-depleted state to modulate emotion, which now follow.

3.3.3 Correlations between trait measures

First, I ensured that the trait measures of interest were not correlated with one another. Scores on the impulsivity and psychopathy scales were not correlated ($r(73) = -.113$, $p = .342$). Empathy scores were likewise not correlated with impulsivity ($r(73) = .094$, $p = .427$) or psychopathy ($r(73) = .015$, $p = .897$).

3.3.4 How trait empathy modulated emotional effects of serotonin depletion

I was interested in testing whether the relationship between a given personality trait and a given emotional rating was modulated by ATD relative to placebo. To do this, I employed an analysis of covariance (ANCOVA) method, for each emotion separately, in which I defined an interaction term between a categorical variable (placebo versus ATD) and a continuous variable entered as a covariate (personality trait). I additionally controlled for main effects of serotonin status and the personality trait in question, by entering these variables into the model separate from the interaction term.

Given prior evidence that single dose SSRI had a more pronounced effect on social behaviour in highly empathic participants (Crockett et al., 2010a), a central question in my study

Group	Emotion	Circumstances	Mean (SD)
Placebo (n = 36)	Annoyance	total	4.103 (.656)
		intended	4.024 (.686)
		unintended	4.183 (.730)
		agent	2.835 (.997)
		victim	5.371 (.741)
	Guilt	total	3.604 (.396)
		intended	3.615 (.509)
		unintended	3.593 (.351)
		agent	5.756 (.682)
		victim	1.452 (.360)
	Shame	total	3.783 (.650)
		intended	4.095 (.775)
		unintended	3.470 (.636)
		agent	5.323 (.953)
		victim	2.242 (.680)
	Bad	total	2.374 (.431)
		intended	2.256 (.500)
		unintended	2.492 (.484)
		agent	2.214 (.587)
		victim	2.534 (.422)
Depletion (n = 37)	Annoyance	total	4.511 (.729)
		intended	4.444 (.786)
		unintended	4.577 (.760)
		agent	3.367 (1.170)
		victim	5.654 (.604)
	Guilt	total	3.681 (.413)
		intended	3.685 (.441)
		unintended	3.678 (.470)
		agent	5.691 (.633)
		victim	1.672 (.557)
	Shame	total	3.899 (.610)
		intended	4.197 (.662)
		unintended	3.600 (.685)
		agent	5.226 (.871)
		victim	2.571 (.663)
	Bad	total	2.512 (.414)
		intended	2.490 (.526)
		unintended	2.533 (.389)
		agent	2.403 (.494)
		victim	2.620 (.440)

Table 3.1: Summary of results by factor, without consideration of personality traits. SD = standard deviation.

	Annoyance		**Guilt**		**Shame**		**Feeling bad**	
	Placebo	ATD	Placebo	ATD	Placebo	ATD	Placebo	ATD
Impulsivity							**–**	
Psychopathy		↑↑						
Empathy				↑↑	**+**	**+**		

Table 3.2: Summary of results on personality traits. Twelve similar but separate ANCOVAs were conducted to explore the influence of traits on each of the four emotions examined. ↑↑ indicates a significant enhancement of the emotion by ATD at high levels of the personality trait shown; + indicates a significant positive relationship between the personality trait and the emotion that was not significantly modulated by ATD; – likewise indicates a significant negative relationship. Emotions are collapsed across agency and intentionality.

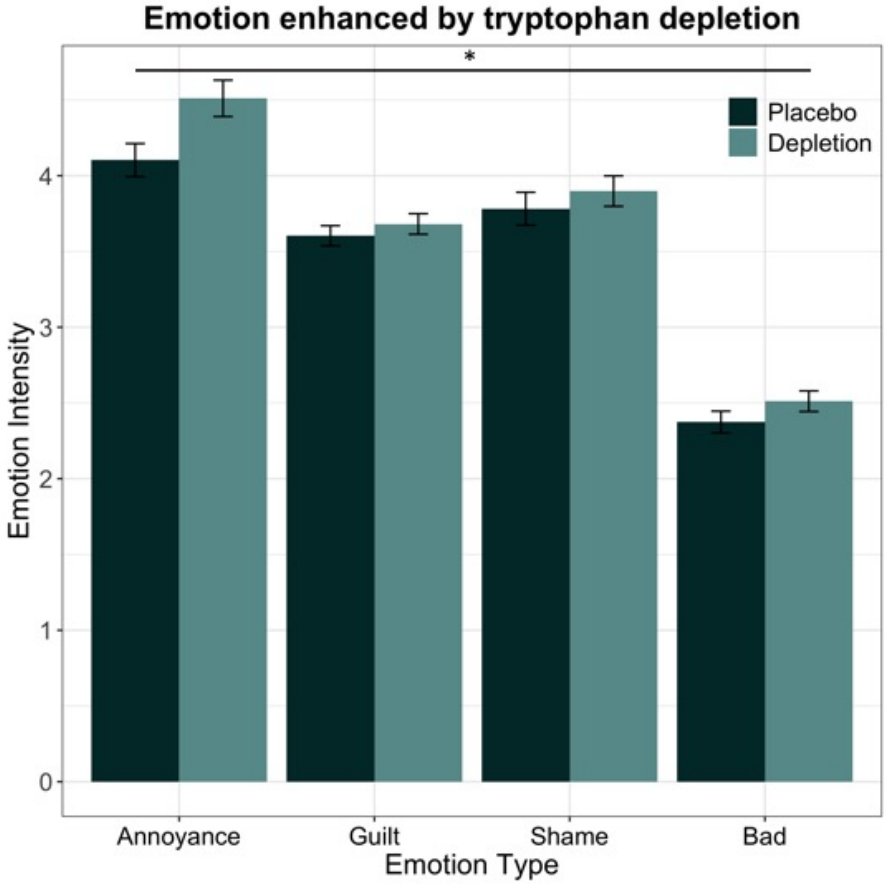

Figure 3.3: Effects of ATD on emotion: serotonin depletion enhanced emotion non-specifically overall (see main text). Each bar represents the average emotion ratings per group, collapsed across agency and intentionality. Error bars indicate 1 standard error. Asterisk indicates main effect, $p < .05$.

was whether the serotonin-depleted state and empathic trait interacted to influence emotion. Indeed, I found that the self-conscious emotion guilt was more sensitive to serotonin depletion in individuals with high trait empathy. I ensured self-reported empathy at baseline was matched between placebo and depletion ($t(63) = 0.442$, $p = .660$; Levene's test $F(71) = 4.569$, $p = .036$). I analysed emotional ratings via ANCOVA, using empathy and serotonin status (ATD versus placebo) as factors in a between-subjects interaction term, controlling for main effects, and agency (agent or victim) and intent (intentional or unintentional) as within-subjects factors. For guilt ratings, this revealed a significant four-way serotonin x empathy x agency x intentionality interaction ($F(1,69) = 5.596$, $p = .021$, $\eta_p^2 = .075$). Guilt ratings were significantly higher in more empathic individuals following serotonin depletion ($r(37) = .385$, $p = .019$), and not under placebo ($r(36) = .265$, $p = .118$), irrespective of agency or intentionality, seen in Figure 3.4a and Table 3.2. Follow-up tests showed that the agency and intentionality effect was driven by a highly significant relationship between empathy and guilt, when imagining inflicting (being the agent of) harm unintentionally ($r(37) = .43$, $p = .008$). There was additionally a three-way interaction between agency, intentionality, and group ($F(1,69) = 4.765$, $p = .032$, $\eta_p^2 = .065$). Critically, however, this three-way interaction disappeared when empathy was not included as a factor in the (otherwise identical) model ($F(1,71) = .608$, $p = .438$, $\eta_p^2 = .008$). I was also able to reproduce this core result on guilt, using a quartiles approach whereby individuals with an empathy score in the top 25% were deemed "high empathy" and the bottom 25% "low empathy". ANCOVA with empathy (high versus low) and serotonin status (ATD versus placebo) as factors in a between-subjects interaction term, controlling for main effects, and agency (agent or victim) and intent (intentional or unintentional) as within-subjects factors, again revealed a significant four-way serotonin x empathy x agency x intentionality interaction effect on guilt ($F(1,36) = 7.028$, $p = .012$, $\eta_p^2 = .163$). Whilst the ANCOVA approach, as above, did not yield a significant interaction of serotonin and empathy on shame ($F < .42$, $p > .05$, $\eta_p^2 < .007$ for all terms involving serotonin status), empathy and overall shame ratings were highly correlated in both the depletion ($r(37) = .437$, $p = .007$) and placebo groups ($r(36) = .425$, $p = .01$). These data for shame are shown in Figure 3.4b and Table 3.2. The ANCOVA on annoyance was not significant ($F < 2$, $p > .05$, $\eta_p^2 < .03$ for all terms involving serotonin status), as was the case for the ANCOVA on feeling "bad" ($F < 1.2$, $p > .05$, $\eta_p^2 = .02$ for all terms involving serotonin status). Guilt, therefore, was uniquely affected by the interaction between trait empathy and the serotonin-depleted state. Serotonin induced a distinct emotional profile in highly empathic individuals.

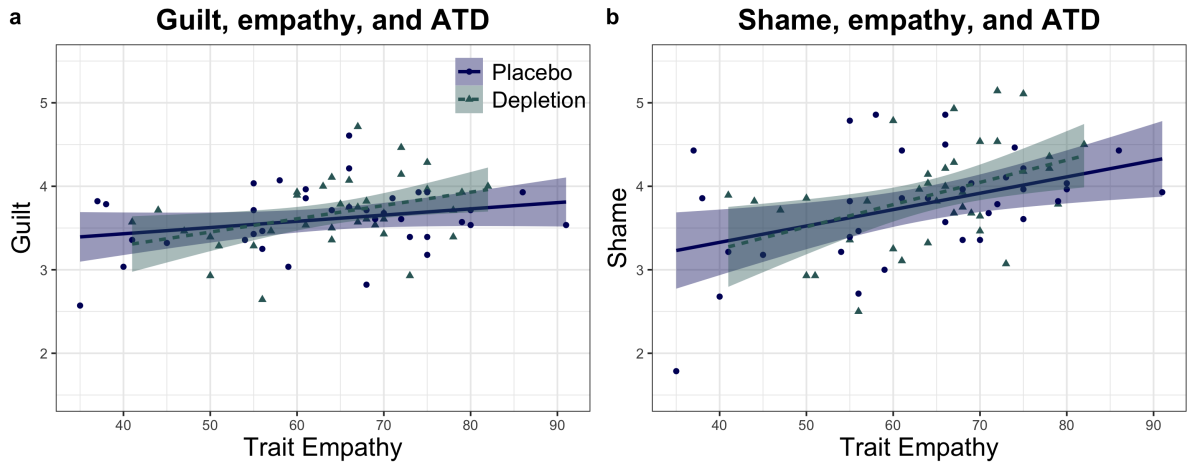

Figure 3.4: Effects of trait empathy on how serotonin depletion influences emotion. Shading indicates 1 standard error. Each point represents the average emotion ratings for each individual, collapsed across agency and intentionality. a) The highly empathic reported more guilt following depletion relative to when on placebo. b) Shame was significantly elevated in individuals high in trait empathy on both placebo and depletion.

3.3.5 How trait psychopathy modulated emotional effects of serotonin depletion

Trait psychopathy also interacted with the serotonin-depleted state to modulate emotion. I ensured psychopathic traits assessed at baseline were matched in the placebo and depletion groups ($t(71) = 1.132$, $p = .261$). ANCOVA with serotonin status (ATD versus placebo) and psychopathy as factors in a between-subjects interaction term, controlling for main effects, and agency and intentionality as within-subjects factors revealed a significant serotonin x psychopathy x intentionality three-way interaction for feelings of annoyance ($F(1,69) = 7.172$, $p = .009$, $\eta_p^2 = .094$). With increasing trait psychopathy, individuals felt even more annoyed following serotonin depletion, seen in Figure 3.5a and Table 3.2. Intentionality significantly interacted with psychopathy to influence annoyance under placebo ($F(1,34) = 5.163$, $p = .03$, $\eta_p^2 = .132$) and not following depletion ($F(1,34) = 2.237$, $p = .144$, $\eta_p^2 = .06$). In other words, the influence of psychopathy on annoyance depended on intentionality when on placebo, but on depletion those high in trait psychopathy were more annoyed regardless of intentionality. There was additionally a significant serotonin x intentionality interaction ($F(1,69) = 7.161$, $p = .009$, $\eta_p^2 = .094$). Critically, however, this two-way interaction disappeared when psychopathy was not included as a factor in the (otherwise identical) model ($F(1,71) = .043$, $p = .836$, $\eta_p^2 = .001$). Next, I assessed guilt using the same ANCOVA approach: there was no serotonin x psychopathy interaction ($F(1,69) < 2.8$, $p > .05$, $\eta_p^2 < .04$ for all terms involving serotonin

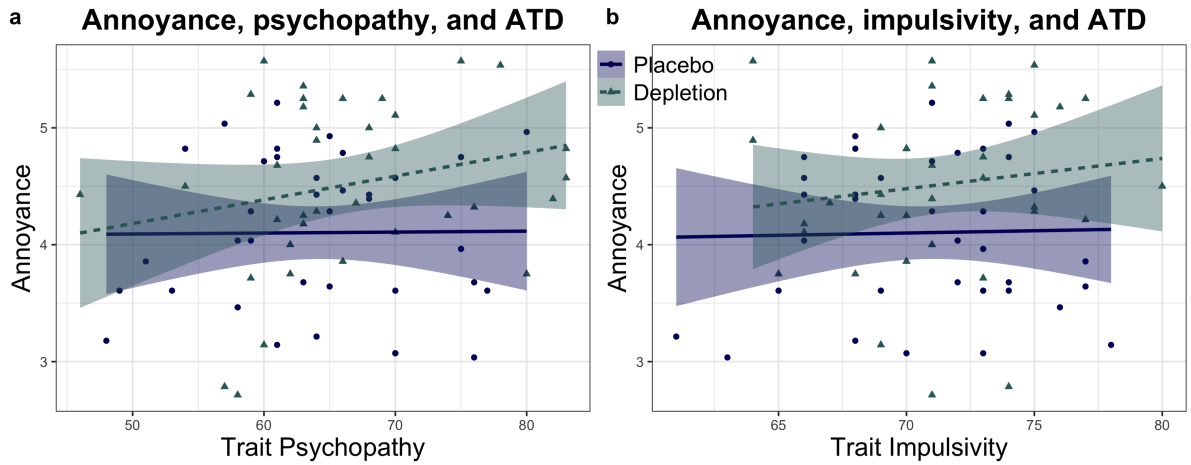

Figure 3.5: Effects of trait psychopathy and impulsivity on emotional effects of serotonin depletion. Shading indicates 1 standard error. Each point represents the average emotion ratings for each individual, collapsed across agency and intentionality. a) Annoyance was potentiated by serotonin depletion in high trait psychopathy. b) Trait impulsivity did not significantly enhance the effects of ATD on annoyance.

status). There was no serotonin x psychopathy interaction for shame ($F(1,69) < 3.8$, $p > .05$, $\eta_p^2 < .055$ for all terms involving serotonin status) nor for feeling bad ($F(1,69) < 2.1$, $p > .05$, $\eta_p^2 < .03$ for all terms involving serotonin status).

3.3.6 Trait impulsivity and emotional effects of serotonin depletion

Trait impulsivity was also matched between groups, and did not interact with the serotonin-depleted state to modulate emotion. Data on impulsivity are summarised in Table 3.2. First, I assessed feelings of annoyance using ANCOVA with serotonin status (ATD versus placebo) and impulsivity as factors in a between-subjects interaction term, controlling for main effects, and agency (agent or victim) and intent (intentional or unintentional) as within-subjects factors: there was no interaction between serotonin and impulsivity ($F(1,69) < 1.7$, $p > .05$, $\eta_p^2 < .025$ for all terms involving serotonin status). These data are shown in Figure 3.5. The same was true for all terms involving serotonin status in the ANCOVAs on guilt ($F(1,69) < .7$, $p > .05$, $\eta_p^2 < .01$), shame ($F(1,69) < .4$, $p > .05$, $\eta_p^2 < .01$) and feeling bad ($F(1,69) < 2.6$, $p > .05$, $\eta_p^2 < .04$).

3.3.7 Principal Component Analysis

I also explored whether there was any structure underlying the task measurements that was not detected by the prior analyses. To do this, I used a principal component analysis (PCA) on the

	Component			
Raw variable	**1**	**2**	**3**	**4**
Annoyed-unintended-victim	.819			
Annoyed-intended-victim	.791	.317		
Bad-unintended-victim	-.736			
Bad-unintended-agent	-.617		-.351	
Bad-intended-victim	-.604			
Guilty-unintended-agent	.587		.403	
Annoyed-unintended-agent		.921		
Annoyed-intended-agent		.897		
Ashamed-intended-agent			.923	
Guilty-intended-agent			.922	
Bad-intended-agent			-.892	
Ashamed-unintended-agent	.396		.455	.417
Ashamed-unintended-victim				.830
Ashamed-intended-victim				.750
Guilty-unintended-victim		.408		.541
Guilty-intended-victim		.504		.521

Table 3.3: Principal components pattern matrix. Rotation method: Oblimin with Kaiser Normalisation. Rotation converged in 19 iterations. Intended = intentional harm; unintended = unintentional harm.

16 outcome variables from the task. The validity of this PCA was confirmed by comparing the number of components, and the variables that clustered together, to a PCA on the same task in the original larger dataset from a non-pharmacological study of 186 healthy participants (Bland et al., 2016). The PCA was performed with oblique rotation (direct oblimin). The Kaiser-Meyer-Olkin (KMO) measure verified the sampling adequacy for the analysis, KMO = .719 which is seen as "good" (Hutcheson and Sofroniou, 1999). Bartlett's test of sphericity indicated correlations between items were sufficiently large for PCA (χ^2 (120) = 700.731, p = 7.9941 x 10^{-83}). I obtained eigenvalues for each component in the data and extracted components with eigenvalues over Kaiser's criterion of one. This resulted in 4 components, which explained 72.354% of the variance. The scree plot showed an inflexion that justified retaining these 4 components, which were then used in the remainder of the analysis. The pattern matrix in Table 3.3 shows the factor loadings after rotation.

I then interpreted how the task measurements from the experiment clustered into the four principal components. Component 1 centred on annoyance with others for having done harm to oneself – in other words, outward frustration. The predominant theme of component 2 was inward frustration, or annoyance with oneself for having harmed another. Components 3 and 4 centred on the self-conscious negative emotions guilt and shame. Component 3 captured these

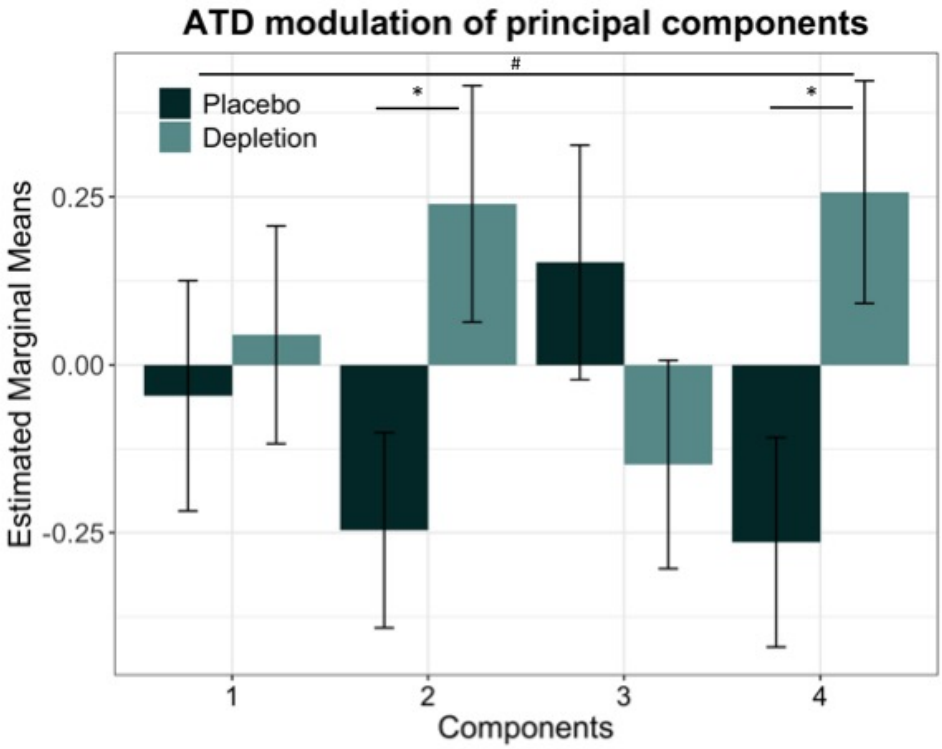

Figure 3.6: ANOVA on four principal components from principal component analysis. Measures contained in components 2 and 4 were significantly increased by ATD (acute tryptophan depletion). Error bars indicate 1 standard error. # indicates interaction at $p < .05$; asterisk indicates significant follow-up t-test at $p < .05$.

emotions when the participant was the agent, component 4 when the participant was the victim of harm. I then used the estimated factor scores for each individual to assess how serotonin depletion modulated the constructs captured by the four components. ANOVA with serotonin status (ATD versus placebo) as a between-subjects factor, and the four components as levels of a within-subjects factor, revealed a significant serotonin-by-component interaction ($F(3,213) = 3.165$, $p = .025$, $\eta_p^2 = .043$). There was no main effect of serotonin depletion ($F(1,71) = 2.187$, $p = .144$, $\eta_p^2 = .030$). Follow up t-tests revealed the values of component 2 ($t(71) = 2.124$, $p = .037$) and component 4 ($t(71) = 2.290$, $p = .025$) were each significantly greater following depletion relative to placebo, as seen in Figure 3.6. Inward frustration, or annoyance, for having harmed another – captured by component 2 – was potentiated by serotonin depletion. When the victim of harm in the task, self-conscious negative emotion was also exacerbated by serotonin depletion (component 4).

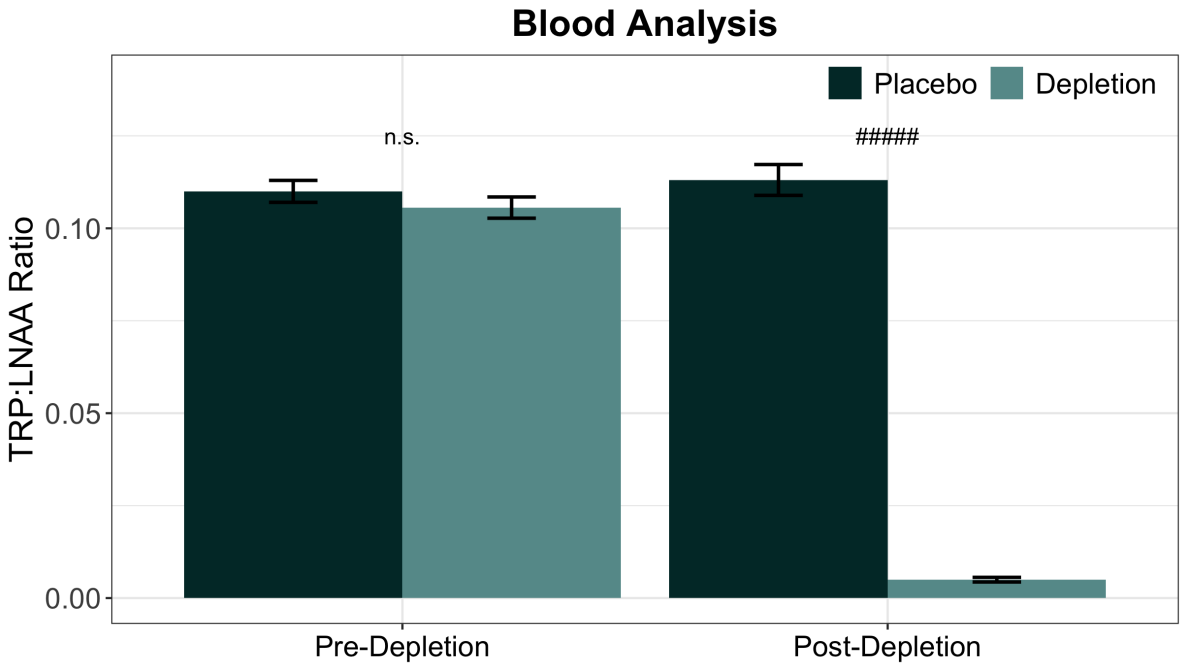

Figure 3.7: Blood analysis results. Error bars represent 1 standard error. Significance at $p < 5 \times 10^{-27}$ is denoted by #####. n.s. denotes not significant.

3.3.8 Blood Analysis

Blood results are shown in Figure 3.7. See Chapter 2 for methods. Plasma levels were unavailable for two participants: one due to a staff processing error, and one due to difficulty with venepuncture. I achieved a robust depletion of tryptophan ($t(60) = -19.163$, $p = 3.01 \times 10^{-27}$).

3.3.9 Correlation analyses

Extent of depletion was not correlated with any particular emotion, reinforcing the importance of accounting for traits to uncover effects of ATD on social emotion. Change in TRP:LNAA ratio was not correlated with annoyance ($r = -.208$, $p = .081$), guilt ($r = -.121$, $p = .314$), shame ($r = -.057$, $p = .636$), or feeling bad ($r = -.088$, $p = .464$). There were correlations between emotion ratings: collapsed across serotonergic status, annoyance, guilt, and shame were all positively correlated with one another ($p < .05$), consistent with the nonspecific enhancement of emotion in the absence of considering traits. Guilt and shame were both negatively correlated with feeling bad ($p < .05$). These correlations are presented in Table 3.4.

	Guilt	Shame	Feeling bad
Annoyance	$r(73) = .481, p = 1.7x10^{-5}$	$r(73) = .403, p = 4.08x10^{-4}$	$r(73) = -.145, p = .220$
Guilt		$r(73) = .779, p = 5.16x10^{-16}$	$r(73) = -.527, p = 2.0x10^{-6}$
Shame			$r(73) = -.451, p = 6.2x10^{-5}$

Table 3.4: Correlations between the factor analysed emotions factors. p-values are uncorrected.

3.3.10 Summary of results

Results are summarised in Table 3.2. Serotonin depletion enhanced emotion overall. Examining trait differences revealed a deeper story. Guilt was significantly enhanced by serotonin depletion in highly empathic participants, which was a distinctive emotional profile. This was driven by guilt when the agent of an unintentional harm. Trait empathy, furthermore, was highly correlated with shame ratings in both the placebo and depletion groups. Greater trait psychopathy following serotonin depletion, meanwhile, was associated with enhancement of annoyance. PCA revealed a component where guilt and shame clustered together when the victim of harm, which was potentiated by ATD.

3.4 Discussion

Using a novel test that cued autobiographical memories, I showed that serotonin depletion heightened emotional reactions when mentally simulating social scenarios involving unjust harm. Whilst emotion was enhanced non-specifically at the group level, harnessing baseline individual differences revealed that personality traits play a critical role in shaping which distinctive types of emotions are affected by serotonin depletion. A key result was that individuals high in trait empathy showed a distinct profile of enhanced guilt following serotonin depletion. This contrasted with how variation in trait psychopathy (classically associated with lack of empathy and guilt) influenced the relationship between serotonin depletion and emotional reactions: only annoyance was potentiated. This dissociation, in other words, mirrored the antithetical nature of empathy and psychopathy. Previous studies have shown that traits can influence the magnitude of effects of serotonin manipulations on social behaviour (Crockett et al., 2010a,b); I now show that traits modulated the quality, as well as the magnitude, of social emotion following serotonin depletion. Traits contribute to an individual's model of the world, and therefore shape prior expectations about social interactions: I propose the influence of traits on how serotonin modulated emotions can be thought of as constituting biological "priors".

Emotions prepare the body for action or inaction (Tooby and Cosmides, 2008). My find-

ings on the serotonergic modulation of social emotion converge with the literature on social decision-making, and I propose that these results represent a Pavlovian influence that can shape social behaviour (Gesiarz and Crockett, 2015). The deployment of empathy has additionally been described as having a Pavlovian character, which can shape behaviour triggered by cues signalling harm (Gesiarz and Crockett, 2015). Whilst I did not measure behaviour, there are some intriguing parallels between my findings on emotional reactions to unjust harm, and studies of retaliatory behaviour to unfairness, as assessed using the Ultimatum Game (Crockett et al., 2010b, 2008) which I highlight below. As was introduced, in the UG individuals must split a sum of money with another, and are given the opportunity to reject unfair offers, in which case neither player gets paid (Crockett et al., 2010b, 2008). Indeed, the UG has been studied under serotonin depletion and in relation to empathy and psychopathy.

Empathy was of central importance to my analysis. This was motivated by the observation that social behaviour in highly empathic participants was especially sensitive to single dose SSRI administration: these individuals showed the greatest reduction in retaliatory behaviour to unfairness (Crockett et al., 2010a). Critically, I found that individuals high in trait empathy had a distinct profile of enhanced emotion: guilt was amplified. Empathy and guilt are consistently correlated (Tangney et al., 2007), and guilt may even promote empathy (Tangney et al., 2007). Feelings of guilt have been associated with real-life altruistic acts (O'Connor et al., 2007). Guilt has been proposed to restrain antisocial behaviour as modelled by laboratory tests: a diminished sense of guilt is thought to contribute to dysfunctional social behaviour following vmPFC damage (Krajbich et al., 2009), and is also a feature of psychopathy (Blair, 2010). The present observation that increased guilt in the highly empathic under ATD was driven by instances of unintentionally inflicting harm in a social setting is entirely consistent with these accounts.

Shame, furthermore, was highly correlated with empathy. This was true in both the placebo and depletion conditions, which could have led to a ceiling effect, leaving less room for further enhancement of shame by ATD in the highly empathic, on top of an already elevated baseline under placebo. Guilt and shame, moreover, clustered together in the PCA when the victim of harm, and this component was enhanced by serotonin depletion regardless of personality traits. Guilt and shame are distinguishable yet can overlap (Tangney et al., 2007), which is also true of their neural correlates (Wagner et al., 2011). Whilst guilt and shame were correlated, the literature indicates that different events can be guilt-inducing or shame-inducing for different people (Tangney et al., 2007). That different types of emotion were enhanced in tandem at the group level, furthermore, does not imply the quality of these emotions cannot be distinguished by the person experiencing them.

Shame-free guilt is seen as possibly adaptive – for instance by promoting reparations –

whilst proneness to shame is seen as a risk for, and is indeed associated with a wide range of psychopathology (Tangney et al., 2007). Importantly, guilt is thought to become maladaptive primarily when it is fused with shame (Tangney et al., 2007). Guilt overlaid with shame is most likely a source of rumination (Tangney et al., 2007). The hippocampus has been reported to be involved in the experience of shame (Bastin et al., 2016), and failure of hippocampal serotonin is suggested to contribute to rumination (Deakin, 2013). SSRIs, meanwhile, improve hippocampal function in depression (Dale et al., 2016). Individuals with hippocampal damage, moreover, appear to show heightened reactive emotionality congruent with their behaviour in moral decision-making tasks, which is antithetical to the pattern seen with vmPFC lesions (McCormick et al., 2016). While the relationship with my results is merely speculative, hippocampal dysfunction is a feature of numerous psychiatric conditions, as is social dysfunction: a recent framework proposes these two well established phenomena can be unified through the purported role of the hippocampus in organising social information (memories), via relational maps that support simulations of social outcomes (Schafer and Schiller, 2019).

Indeed, there are reported links between elevated empathy and depression (O'Connor et al., 2002). Individuals sensitive to distress in others may be more likely to experience personal distress, and this has been highlighted as a vulnerability factor for depression (O'Connor et al., 2007, 2002). At the same time, there is evidence for diminished deployment of theory of mind – non-affective perspective taking – in depression (Wolkenstein et al., 2011), and this combination raises the possibility that sensitivity to distress in oneself and others may become misattributed or inappropriately directed inward.

Whilst mood was unaffected in this study, consistent with the literature on ATD in healthy individuals (Bell et al., 2005; Ruhé et al., 2007), ATD can transiently reinstate low mood in depressed individuals successfully treated with an SSRI (Bell et al., 2005; Ruhé et al., 2007). By using trait measurements and a task that elicited emotions, however, I was able to uncover a pattern reminiscent of depression: more guilt in the highly empathic under serotonin depletion. Indeed, this task has already been used to detect possible latent vulnerabilities in a healthy population with trait paranoia (Savulich et al., 2018). I propose that empathy, which produced a qualitatively unique emotional profile under ATD, may represent an important proxy for sensitivity to changes in serotonin.

Conversely, psychopathic individuals classically have impairments in guilt and empathy, and an increased risk for aggressive behaviour, especially following frustration (Blair, 2010; Jones et al., 2010). This is consonant with my results. I found that the emotional profile following serotonin depletion in healthy individuals high in psychopathic traits dissociated from what I observed in the highly empathic: annoyance was instead amplified following unjust harm. This result is furthermore in line with existing literature on social decision-

making: clinically psychopathic individuals show an analogous pattern of behaviour on the UG (Koenigs et al., 2010) to the disinhibited aggressive impulses seen in ATD studies of healthy volunteers (Crockett et al., 2008), that is also quantitatively similar to how individuals with vmPFC lesions behave on the UG (Koenigs et al., 2010). Critically, vmPFC damage is associated with impaired emotion regulation, and individuals with such lesions tend to exhibit anger and irritability particularly following frustration in their personal lives (Koenigs and Tranel, 2007). Diminished structural and functional connectivity between the vmPFC and amygdala in clinically psychopathic individuals (Motzkin et al., 2011) is indeed thought to be a central mechanism underlying the condition. Interactions between these structures are furthermore sensitive to ATD in healthy individuals viewing facial signs of aggression (Passamonti et al., 2012). That serotonin depletion made participants high in trait psychopathy more annoyed by social injustice may be relevant for understanding how serotonin affects the emotional basis of retaliatory behaviour to unfairness (Crockett et al., 2010b, 2008). This view is supported by work showing that such behavioural reactions are associated with self-reported anger in healthy volunteers (Pillutla and Murnighan, 1996). Trait anger and psychopathy in violent offenders indeed appears to reflect 5-HT1B receptor levels (da Cunha-Bang et al., 2016), which moreover fits with the vast literature implicating serotonergic dysfunction in aggression (Bevilacqua et al., 2010; Deakin, 2003; Frankle et al., 2005).

The individual differences I observed in response to a challenge of brain serotonin are likely in part related to the relative contribution of the multiple serotonin subsystems in the brain. Importantly, preferential dysfunction in the median or dorsal raphe nuclei, which innervate, among other regions, the hippocampus and prefrontal cortex, respectively, has been putatively related to phenotypes as divergent as depression and antisocial personality disorder, respectively; both nuclei project to the amygdala (Deakin, 2003). Recent data underscore the complexity of serotonin subsystems, revealing that even within the dorsal raphe there are subsystems that have distinct and at times opposing functions: both activate to reward but have opposing responses to aversion (Ren et al., 2018).

My data from a novel test, that required drawing on autobiographical memories to mentally simulate cued social scenarios, demonstrate that there are important individual differences in the way serotonin influences how we react emotionally to social injustice. This should not come as a surprise given the intricacy of the serotonin systems and the complexities of human emotion and behaviour. Whilst serotonin depletion potentiated the magnitude of emotion non-specifically at the group level, personality traits played a critical role in shaping which distinctive types of emotions were affected. There was a qualitative dissociation in the way trait empathy, relative to psychopathy, amplified social emotion following serotonin depletion. Previous ATD studies on social cognition, by contrast, examined behaviour rather than emotion

and found changes in the magnitude but not the quality of effects (Crockett et al., 2010a,b). I propose that traits in conjunction with the memories the present task evoked represent biological priors, which prime individuals to have different emotional reactions in the social world. These data indicate serotonin would affect the gain. Given emotions are a prescription for action (Tooby and Cosmides, 2008), it follows that these results could represent how serotonin impacts social behaviour via underlying emotional responses, positioned at the nexus of a social Pavlovian influence over action (Pavlovian action selection). When considering apparent paradoxes in the serotonin literature (Cools et al., 2008a; Deakin, 2003) and designing future studies, it is critical to note that the quality and magnitude of effects of a single serotonin manipulation can depend on personality. These data additionally inform the neurochemical basis of psychopathology associated with excessive emotions such as guilt and shame. My findings on the interaction between the serotonin depleted state and personal attributes could help inform which individuals are particularly vulnerable to pathological emotional reactions, and who may be more amenable to serotonin-modulating treatments, with implications for psychiatric classification in frameworks such as the Research Domain Criteria [RDoC] (Cuthbert and Insel, 2013).

Chapter 4

Pavlovian memory expression following tryptophan depletion

4.1 Introduction

The ability to respond emotionally to threats in the environment is critical for an organism to optimise behavior and navigate the world. Once a threat is no longer present, it is crucial to adapt emotional responses flexibly to reflect the safe environment, for normal functioning in daily life to continue. Dysfunction of threat and safety learning lies at the core of post-traumatic stress disorder (PTSD; Milad et al., 2009) and other anxiety disorders (Kim et al., 2011; Marin et al., 2017), and is also a feature of obsessive-compulsive disorder (OCD; Apergis-Schoute et al., 2017; McLaughlin et al., 2015; Milad et al., 2013) and schizophrenia (Holt et al., 2012). PSTD is unique amongst these in that exposure to a traumatic event is a defining feature, and it is characterised by subsequent pathological physiological reactions to cues reminiscent of the event (American Psychiatric Association, 2013). Elucidating the factors that contribute to the persistence of such emotional reactions is essential in order to develop new treatments. Here I tested the influence of the neuromodulator serotonin (5-hydroxytryptamine; 5-HT) on the retention of emotional memory, with a widely used laboratory model of PTSD and related disorders (Graham and Milad, 2011).

Pavlovian threat conditioning paradigms, more commonly known as fear conditioning (LeDoux, 2012; LeDoux and Pine, 2016), involve pairing a previously neutral stimulus with an aversive outcome, such as a mild electric shock. Individuals automatically learn that the cue signals threat, and an anticipatory sympathetic nervous system arousal response occurs. This manifests as measurable perspiration and is known as the skin conductance response (SCR). After an individual has learned that a cue signals threat, the stimulus can later be repeatedly

presented without the aversive consequence (extinction learning) – a model of exposure therapy in the clinic by which a new memory of safety should be formed. These two memories – of threat and safety – then compete for expression upon re-encountering a conditioned stimulus. Threat memories are well known to persist regardless of extinction training, and the re-emergence of the emotional memory after the passage of time is known as spontaneous recovery (Bouton, 2002). Emotional memories for threats can also resurface following re-exposure to an adverse event, known as reinstatement (Bouton, 2002). Understanding what contributes to spontaneous recovery and reinstatement is of great clinical interest, because of its implications for conditions such as PSTD (Graham and Milad, 2011; Milad and Quirk, 2012). One factor that has recently come to light is self-reported intolerance of uncertainty (IUS; Carleton et al., 2007): individuals highly intolerant of uncertainty demonstrated greater spontaneous recovery (Dunsmoor et al., 2015).

Serotonin, meanwhile, is widely implicated in aversive learning (Cools et al., 2008a; Deakin, 2013), and several studies have begun to explore the role of serotonin in threat and safety learning, and aversive memory (Bauer, 2015). Most experiments, however, have been carried out in rodents (Bauer, 2015). The dearth of human studies at the nexus of threat memory and serotonin function is particularly surprising, given that first-line pharmacological treatments of disorders in which threat conditioning processes are impaired – e.g. PSTD, OCD, and other anxiety disorders – modulate serotonin (Stahl, 2013). No one, to my knowledge, has manipulated serotonin experimentally to examine its influence on spontaneous recovery in humans.

Acute tryptophan depletion (ATD) is a commonly used method for studying serotonin function in which tryptophan, the amino acid biosynthetic precursor to serotonin, is temporarily removed from the diet in the presence of other amino acids. This results in decreased serotonin function (Bel and Artigas, 1996; Biggio et al., 1974; Crockett et al., 2012; Nishizawa et al., 1997; Young, 2013). ATD can modulate threat conditioning in humans: Hindi Attar et al. (2012) showed that ATD attenuated aversive conditioning, whilst Hensman et al. (1991) demonstrated that the 5-HT2A and -2C antagonist ritanserin also abolished conditioned threat responses, both assessed by SCR.

Serotonin can also have influences beyond initial learning. Two weeks' administration of the serotonin reuptake inhibitor (SRI) escitalopram in humans did not impact the acquisition of threat memory but facilitated extinction (Bui et al., 2012). Karpova et al. (2011) demonstrated in mice that chronic administration of the SRI fluoxetine, when paired with extinction training, diminished spontaneous recovery and reinstatement. Hartley et al. (2012) used a behavioural genetics approach in humans and found a relationship between spontaneous recovery and normal variation in the serotonin transporter polyadenylation polymorphism (STPP) –

whereas variation in the more widely studied 5-HTTLPR (5-HT-transporter-linked polymorphic region) had no effect. Additionally, neither polymorphism impacted the acquisition or extinction of threat memory. It should be noted, however, that studies of single nucleotide polymorphisms are vulnerable to false positives (Border et al., 2019), which in turn reinforces the importance of pharmacological approaches. The present study of healthy human volunteers investigated two key questions: how does pharmacologically lowering serotonin affect the return of conditioned threat memory? Does self-reported intolerance of uncertainty influence emotion? I employed ATD to investigate this clinically relevant question and predicted that lowering serotonin would modulate the expression of a previously formed threat memory.

4.2 Methods

4.2.1 General procedure

Day 1 comprised a short afternoon session with no pharmacological manipulation. Participants completed the first part of the Pavlovian task (acquisition and extinction phases; see Section 4.2.3) and baseline questionnaires. Conditioning data from Day 1 were evaluated to determine whether participants qualified to continue with Day 2 of the conditioning experiment. Participants were said to have conditioned if they showed greater SCR to both CS+s than to the CS-, averaged across either the entire acquisition phase, the first half of acquisition or the latter half of acquisition. On Day 2 participants received either placebo or ATD and the remainder of the Pavlovian task (spontaneous recovery, reinstatement, reacquisition phases; see Section 4.2.3) was conducted. Forty-seven participants (mean age 25; 29 males) met criterion for Pavlovian conditioning and were included in this analysis.

4.2.2 Skin conductance

Electrodes were attached to the distal phalanges of the index and middle finger on the other arm to the shock electrode, which was counterbalanced. Base to peak increase in SCR to the CSs was valid if an increase began within a window of .5 to 4.5 seconds after stimulus onset. SCR to the CSs and US were low-pass filtered, smoothed, and square root transformed to normalize the distribution. SCR to CSs across all phases were divided by the average SCR to the US on Day 1 to enable between-subjects comparisons (e.g. Hartley et al., 2012).

4.2.3 Pavlovian conditioning task

The task design was adapted from Hartley et al. (2012) and Milad et al. (2009) and is depicted in Figure 4.1. The key dependent measure was SCR, and participants were subjected to the threat of mild transcutaneous electrical stimulation (shock). On Day 1, participants underwent a calibration procedure to determine a shock level that was "uncomfortable, but not painful" to them. They then underwent a Pavlovian threat conditioning procedure: two coloured square images (2 conditioned stimuli [CSs], denoted CS+E and CS+N) were presented for 4 seconds, on 16 trials each, and paired with receipt of shock (unconditioned stimulus; US) on 37.5% (6 of 16) of the presentations, while another coloured square image (CS-) was presented for 10 trials and was never paired with the US. For the CS+ stimuli, the US occurred 3800 milliseconds after stimulus onset, and lasted 200 milliseconds, co-terminating with the image. There was an inter-trial interval that averaged 10 seconds. After a few trials of this procedure it is normal for participants to show an anticipatory arousal response (reflected by mild perspiration of the fingers and measured by SCR) upon viewing the CS+s, relative to the CS-. SCR measurement allowed verification that Pavlovian threat learning had occurred.

Participants then underwent standard extinction training, in which they were repeatedly presented with the CS+E (E for extinguished) and the CS-, both without the US. The other CS+ (the CS+N, N for not extinguished) was not presented during the extinction session.

Participants then returned one day later to receive ATD or placebo and were subsequently tested for the return of conditioned threat memory, using SCR across three phases. In the first phase, participants were first re-exposed to all three CSs (CS+E, CS+N, CS-), again without the US, to assess spontaneous recovery to the CS+E (the return of threat memory after the passage of time; Bouton, 2002). At this stage, the CS+N is a comparator against which spontaneous recovery of the CS+E can be measured. If ATD modulates expression of the original threat memory, it would be expected to alter responses to the CS+N. If it specifically affects the expression of the extinction memory (spontaneous recovery), it would be expected to alter responses to the CS+E but not the CS+N. In phase two, four USs were administered that were not associated with any of the images. Participants were subsequently re-exposed to all three images (reinstatement). The last phase of the experiment was a reacquisition procedure, where the CS+E and CS+N were once again paired with the US on 37.5% of trials (the CS- was also presented, without shock, as before). Greater reacquisition can be reflective of a stronger threat memory (Bouton, 2002). Importantly, the context remained the same across both days.

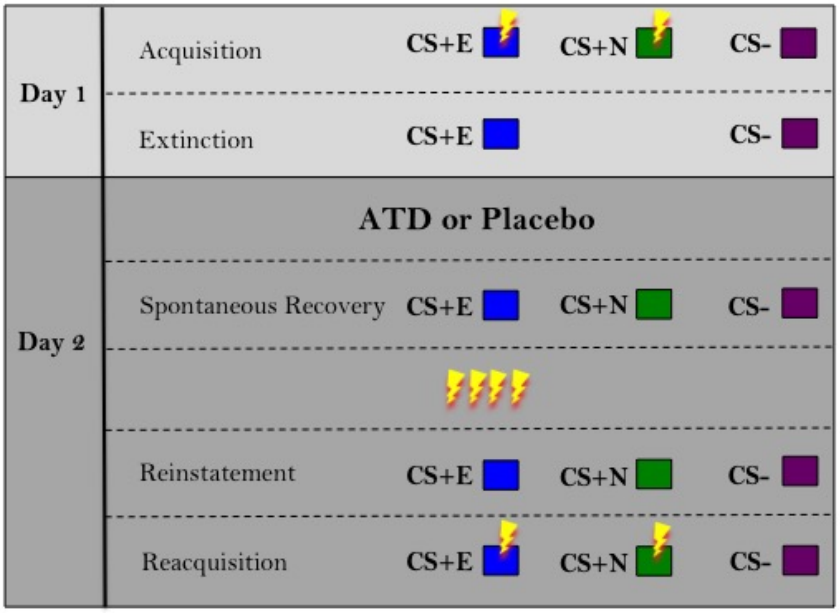

Figure 4.1: Task schematic. Each row represents a different phase of the experiment. Lightning bolts represent shock. CS+E is the CS+ that was presented during the extinction phase. CS+N is the CS+ that was not presented during the extinction phase. The CS- was never paired with shock. ATD = acute tryptophan depletion.

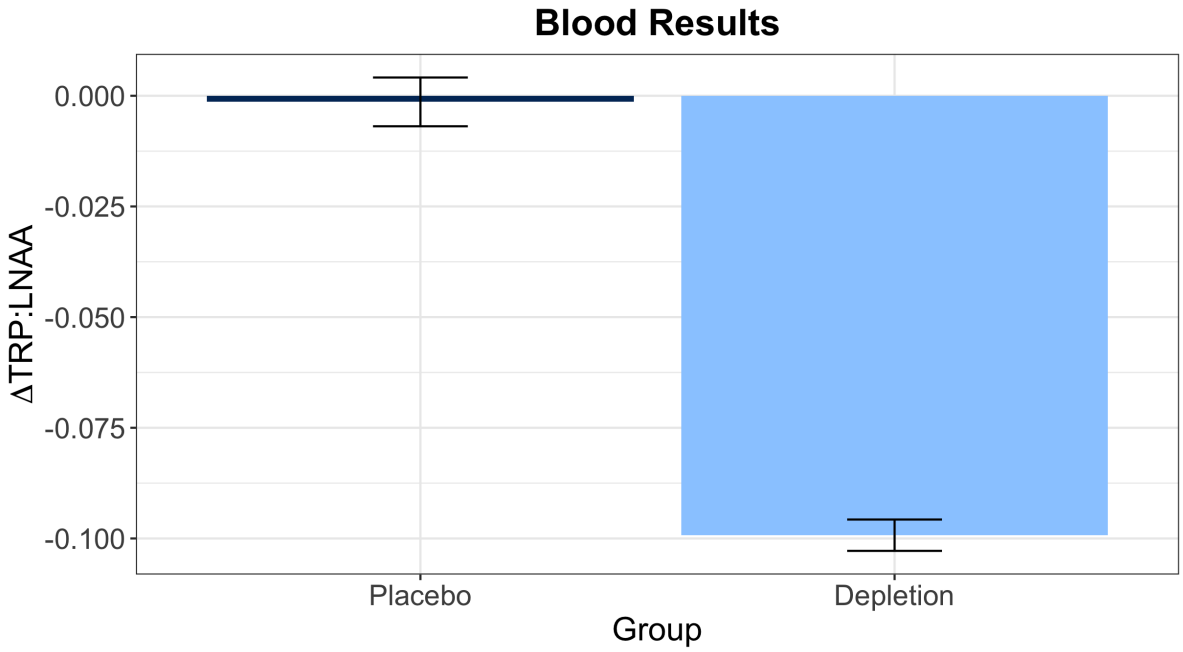

Figure 4.2: Robust tryptophan depletion was achieved, verified via plasma samples. The y-axis represents the change in the ratio between tryptophan and all large neutral amino acids (TRP:LNAA) before and after depletion. More negative bar indicates greater depletion of tryptophan. Error bars indicate 1 standard error.

4.3 Results

4.3.1 Analysis of plasma tryptophan

Twenty-five participants underwent tryptophan depletion, whilst the remaining 22 received placebo. See Chapter 2 for methods. Plasma levels were unavailable for two participants: one due to a staff processing error, and one due to unsuccessful venepuncture. A robust depletion of tryptophan was achieved ($t(43) = -15.317$, $p = 5.05 \times 10^{-19}$), shown in Figure 4.2.

4.3.2 Self-report measures

Rating data was collected from 40 participants (n = 21 depletion) on how happy or sad they were feeling prior to the task, after depletion had taken effect, and these mood ratings did not differ from those participants who received placebo ($t(38) = -1.227$, $p = .228$). At baseline, participants completed a number of other questionnaires: Groups were not different on self-report scales assessing depressive symptoms, anxiety, intolerance of uncertainty, or in other measures reported in Table 4.1.

Group	Placebo	Depletion		
	Mean (SD)	Mean (SD)	t (df)	p
Age	24.23 (5.88)	25.80 (6.24)	0.886 (45)	.380
Edu	17.18 (2.38)	17.28 (2.42)	0.14 (45)	.890
BDI-II	5.23 (4.38)	4.04 (3.92)	-0.981 (45)	.332
STAI	37.36 (7.27)	35.52 (5.96)	-0.955 (45)	.345
IUS	55.73 (13.69)	49.80 (13.44)	-1.495 (45)	.142

Table 4.1: Questionnaires and demographics. NART = National Adult Reading Test (Blair and Spreen, 1989), a proxy for intelligence; BDI-II = Beck Depression Inventory (Beck et al., 1996); STAI = Spielberger Trait Anxiety Inventory (Spielberger et al., 1983); IUS = Intolerance of Uncertainty Scale (Carleton et al., 2007); Edu = years of education; SD = standard deviation; df = degrees of freedom.

4.3.3 Acquisition of threat conditioning before tryptophan depletion

Pavlovian threat conditioning, which was conducted without any serotonergic manipulation, was successfully achieved on Day 1. Importantly there was no difference in conditioning on Day 1 between those who later (on Day 2) received placebo versus depletion, shown in Figure 4.3a. A repeated measures ANOVA with group assignment (future placebo versus future ATD) and stimulus (CS+E, CS+N, CS-; all trials) as factors yielded a main effect of stimulus ($F(1, 61) = 22.031$, $p = 2x10^{-6}$, $\eta_p^2 = .383$), no main effect of group assignment ($F(1,45) = 0.378$, $p = .542$, $\eta_p^2 = .008$) and no group assignment-by-stimulus interaction ($F(1, 61) = 0.658$, $p = .466$, $\eta_p^2 = .014$). Follow up paired t-tests confirmed mean SCR values to the CS+E ($t(46) = -5.315$, $p = 3x10^{-6}$) and CS+N ($t(46) = -4.632$, $p = 3x10^{-5}$) were each significantly greater than the SCR to the CS-.

4.3.4 Extinction before tryptophan depletion

Extinction, which was also conducted before any serotonergic manipulation, was assessed by comparing the mean of the first two and mean of the last two trials, for each of the two stimuli presented during extinction. As expected, there was no difference during extinction on Day 1 between those who later (on Day 2) received placebo versus depletion ($F(1,45) = 0.932$, $p = .340$, $\eta_p^2 = .020$), nor was there an interaction between group and phase (early vs. late trials; $F(1,45) = 0.364$, $p = .549$, $\eta_p^2 = .008$). It is to be expected that by the end of extinction, CS+E responses would no longer be significantly different from CS- responses, however full extinction was not achieved: there was no phase (early vs. late) by stimulus (CS+E vs. CS-) interaction ($F(1,45) = 0.187$, $p = .668$, $\eta_p^2 = .004$) and the main effect of stimulus persisted ($F(1,45) = 11.217$, $p = .002$, $\eta_p^2 = .2$). There was, however, a marginally significant effect

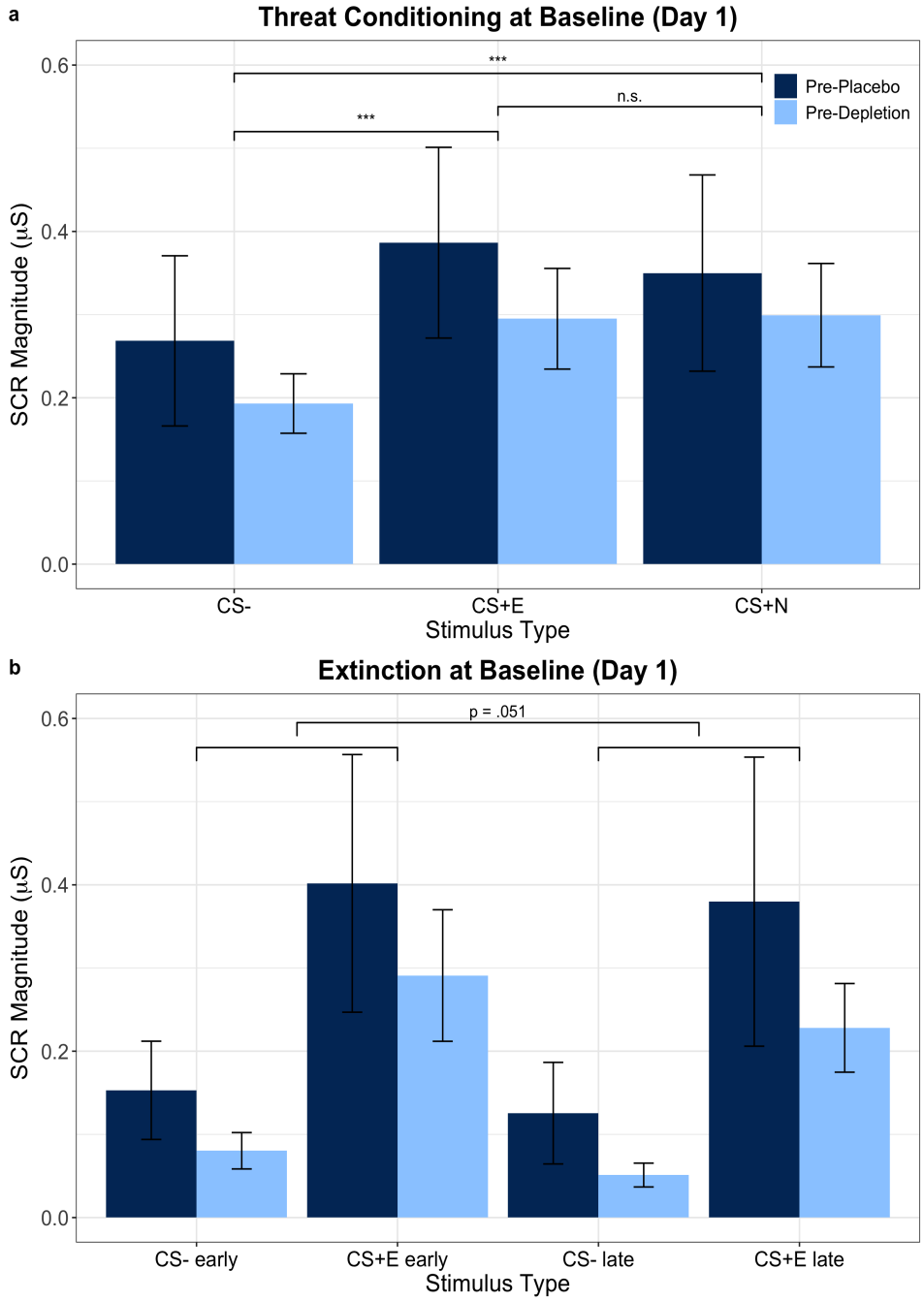

Figure 4.3: Skin conductance responses (SCR) in the **(a)** initial threat conditioning phase (acquisition) on Day 1, conducted before serotonergic challenge. There were no differences between the future placebo and future ATD groups and both groups showed significant threat conditioning to both CS+s compared to the CS–, as predicted. This equivalent baseline conditioning on Day 1 enabled testing the effects of ATD on its retention on day 2. Brackets denote follow-up t-tests contrasting stimuli within group, after observing a main effect of stimulus; *** indicates significance at $p < .001$; n.s. = not significant; error bars indicate 1 standard error. **(b)** SCR in the extinction phase on Day 1. Smaller brackets refer to the beginning and end of extinction, and the larger bracket denotes a marginally significant reduction in SCR in late compared to early extinction; error bars indicate 1 standard error. µS = microsiemens.

of phase (F(1,45) = 4.004, p = .051, η_p^2 = .082), showing lower responding, irrespective of stimulus, in the late trials. This is shown in Figure 4.3b.

4.3.5 Spontaneous recovery following tryptophan depletion

Spontaneous recovery was the critical test in this experiment to address the main hypothesis that serotonin modulates the expression of previously formed Pavlovian threat memories. Indeed, ATD modulated cue-evoked SCRs during the spontaneous recovery phase (Figure 4.4a). To assess whether ATD affected the expression of the conditioned memories from Day 1, SCR during the first half of the spontaneous recovery phase on Day 2 was examined. Repeated measures ANCOVA was conducted with serotonin status (placebo versus ATD) and stimulus (CS+E, CS+N, CS-) as factors, controlling for the strength of initial conditioning, and intolerance of uncertainty. SCR during acquisition was used as a covariate because I was interested in assessing the influence of the pharmacological manipulation on memory expression irrespective of how the strength of the initial memory affected expression a day later. Scores from the intolerance of uncertainty scale (IUS) were used as an additional covariate because this trait can affect threat memory expression (Dunsmoor et al., 2015). The repeated measures ANCOVA yielded a significant main effect of serotonin status (F(1,43) = 8.818, p = .005, η_p^2 = .170), showing that cue-evoked SCRs were significantly attenuated under ATD. There was also a main effect of stimulus (F(2,73) = 3.594, p = .040, η_p^2 = .077). Follow up paired t-tests revealed SCRs to the CS+E and CS+N collapsed across serotonergic status were each significantly greater than responses to the CS- (t(46) = -4.549, p = 3.9×10^{-5} and t(46) = -5.089, p = 7×10^{-6}, respectively), which demonstrated return of threat memory expression occurred irrespective of the serotonergic manipulation. Responses to the CS+E and CS+N did not differ from one another (t(46) = -0.312, p = .756) which is likely because there was not complete extinction of the CS+E on Day 1. There was no serotonin x stimulus interaction (F(1,72) = 1.795, p = .179, η_p^2 = .040), indicating the effect of ATD was not specific to any of the three stimuli. Conditioning to both CS+s from Day 1 was retained on Day 2 in both the placebo and ATD groups; however, overall cue-evoked SCRs were diminished by ATD, irrespective of stimulus. The expression of threat conditioning, as measured by SCR in the spontaneous recovery phase, was not abolished by ATD but was attenuated.

4.3.6 Role of intolerance of uncertainty in the effects of ATD on spontaneous recovery

Next I examined how trait intolerance of uncertainty contributed to the results in the spontaneous recovery phase. Correlation analyses were performed between IUS and SCR to each of

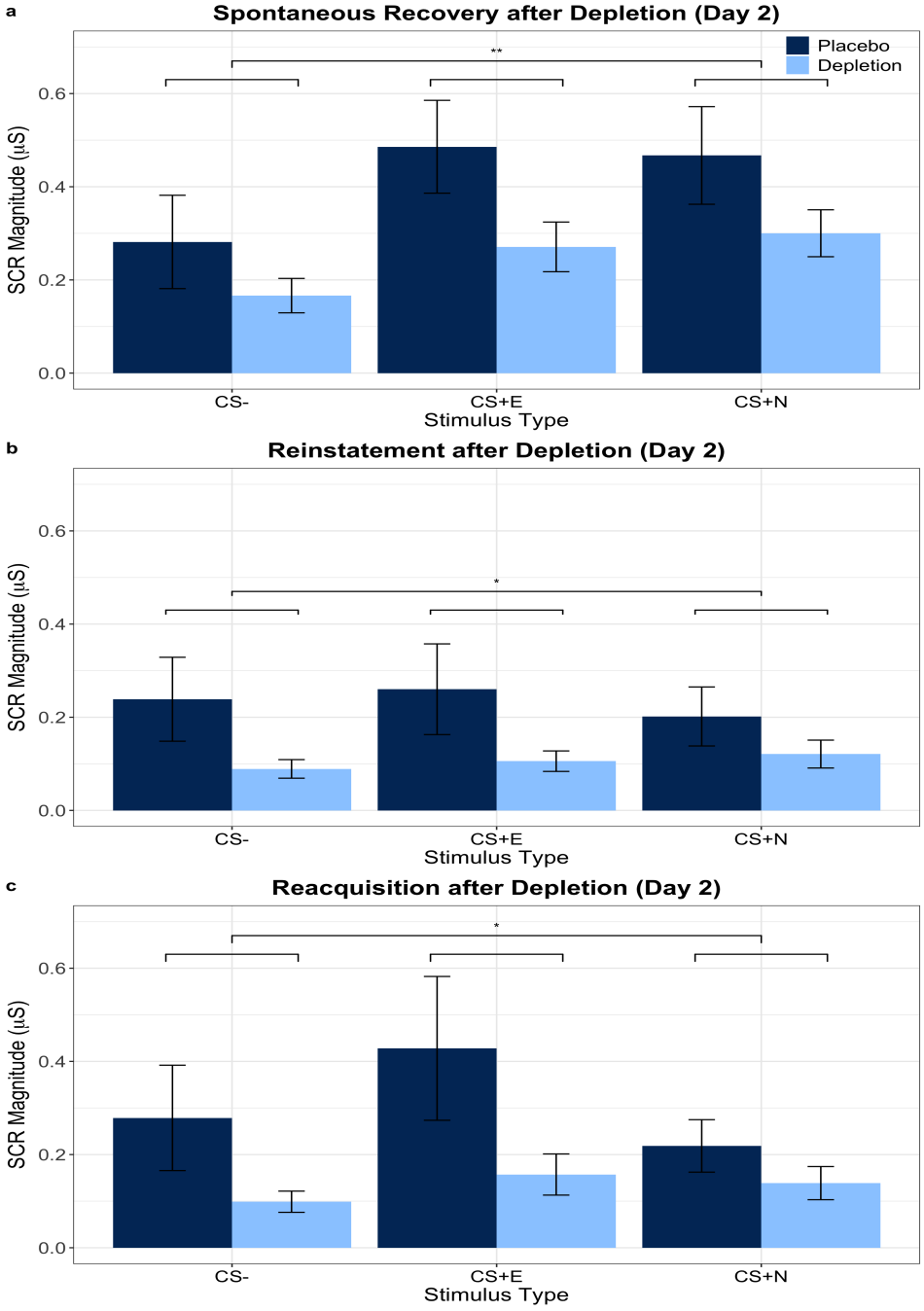

Figure 4.4: Tryptophan depletion reduced expression of conditioning. Skin conductance responses (SCR) are displayed from the **(a)** spontaneous recovery, **(b)** reinstatement, and **(c)** reacquisition phases. Large brackets denote a main effect of stimulus; ** indicates significance at $p < .01$; *indicates a significance at $p < .05$. Error bars indicate 1 standard error. Raw data are displayed, not adjusted values after controlling for intolerance of uncertainty or strength of initial conditioning on Day 1. µS = microsiemens.

the stimuli, controlling for strength of Day 1 conditioning. These analyses revealed significant correlations in the depletion group only: when under depletion, individuals who were more intolerant of uncertainty showed significantly diminished SCRs to the CS+E ($r(25) = -.554$, $p = .004$) and CS+N ($r(25) = -.453$, $p = .023$), as well as the CS- ($r(25) = -.418$, $p = .038$). Under placebo this relationship with IUS was not present for any of the three stimuli: CS+E ($r(25) = -.135$, $p = .549$), CS+N ($r(25) = -.249$, $p = .264$), and CS- ($r(25) = -.109$, $p = .629$). Critically, these results remained significant after being subjected to the Benjamini-Hochberg procedure for six comparisons (see Chapter 2). Next, an interaction term between serotonin and IUS was incorporated into the general linear model used in the initial analysis of spontaneous recovery, in order to examine whether trait intolerance of uncertainty and serotonin status interacted to modulate SCR to specific stimuli. ANCOVA with serotonin and IUS as a between-subjects interaction term, controlling for main effects and strength of initial conditioning, and stimulus (CS+E, CS+N, CS-) as within-subjects factors did not show a two-way interaction between serotonin and IUS ($F(1,42) = .056$, $p = .815$, $\eta_p^2 = .001$) or a three-way interaction between serotonin, IUS, and stimulus ($F(2,69) = 1.379$, $p = .257$, $\eta_p^2 = .032$). Whilst there was no interaction effect between ATD and IUS, the correlation results suggest that ATD modulated the relationship between IUS and SCR to the conditioned stimuli.

4.3.7 Relationship between spontaneous recovery and extent of depletion

The extent of tryptophan depletion significantly correlated with the attenuation of conditioned threat SCRs, but not SCRs for safety memory cues during the spontaneous recovery phase, which is displayed in Figure 4.5. Critically, this substantiated the relationship between depletion and conditioned threat memory expression during spontaneous recovery. Using a partial correlation to control for strength of acquisition and intolerance of uncertainty, there was a significant relationship between degree of tryptophan depletion and the extent to which SCRs to threat memory cues returned. Extent of depletion correlated with SCR to the CS+E and CS+N, and not the CS-, indicating the effect of tryptophan depletion did not generalise to SCRs for safety memory cues: SCRs to threat memory cues were more attenuated, the greater the depletion (CS+E, $r(41) = .409$, $p = .006$; CS+N, $r(41) = .390$, $p = .01$; CS-, $r(41) = .107$, $p = .495$), These results additionally survived the Benjamini-Hochberg procedure for three comparisons (see Chapter 2). There was no interaction between stimulus (CS+E, CS+N, CS-) and plasma results on SCR, however, as assessed by repeated measures ANCOVA with plasma values, intolerance of uncertainty, and strength of initial conditioning as predictors ($F(2,66) = .890$, $p = .397$, $\eta_p^2 = .022$).

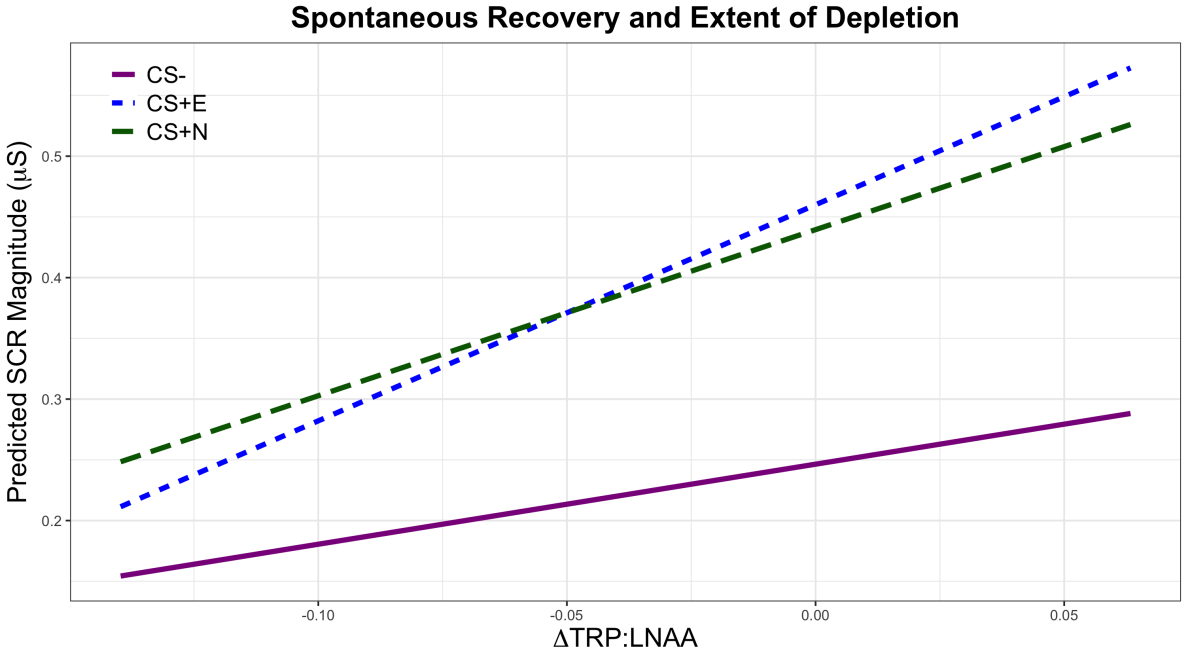

Figure 4.5: Predicted values for spontaneous recovery plotted against the extent of depletion assessed via plasma samples. Lower x-axis values indicate greater depletion. Predicted values were generated based on a univariate ANCOVA where the dependent variable was SCR to each stimulus during the spontaneous recovery phase, and the predictors were intolerance of uncertainty, strength of conditioning on Day 1, and the change (Δ) in the ratio of tryptophan (TRP) to large neutral amino acids (LNAA). μS = microsiemens.

4.3.8 Effects of tryptophan depletion on unconditioned responses

Responses to the unconditioned stimulus were not modulated by ATD. The unconditioned response (UR) to the shock (US) was analysed to evaluate whether ATD reduced skin conductance responsiveness in general, or whether the attenuation was specific to cue-evoked SCR. To do this, SCR to the four US presentations during the reinstatement procedure (where shocks were presented as discrete events, unpaired to any CS) were isolated and averaged. Two participants were not included in this analysis and those that follow: one due to an equipment failure, one due to experimenter error. Responses to the reinstatement USs were not affected by ATD ($t(43) = -0.897$, $p = .375$), which was also the case when controlling for the magnitude of URs on Day 1 (at baseline) and intolerance of uncertainty ($F(1,41) = 1.240$, $p = .272$, $\eta_p^2 = .029$). URs on Day 2, moreover, were not correlated with the extent of depletion, controlling for magnitude of URs on Day 1 and intolerance of uncertainty ($r(39) = .268$, $p = .091$).

4.3.9 Effects of tryptophan depletion on reinstatement

Tryptophan depletion modulated cue-evoked SCRs in reinstatement (Figure 4.4b). The reinstatement phase was analysed in the same way as the spontaneous recovery phase, examining the first half of trials, and controlling for strength of acquisition and intolerance of uncertainty. Repeated measures ANCOVA revealed a significant effect of serotonin status ($F(1,41) = 5.578$, $p = .023$, $\eta_p^2 = .120$), again with lower cue-evoked SCRs under ATD. By this stage in the experiment, however, there was no longer a main effect of stimulus ($F(1, 51) = 0.068$, $p = .849$, $\eta_p^2 = .002$); there was no differential response to the CS+s relative to the CS-, and thus no SCR evidence of reinstatement of the threat memory. Effects in these paradigms are often short-lived (Gershman and Hartley, 2015). There was also no serotonin x stimulus interaction ($F(1, 51) = 1.390$, $p = .251$, $\eta_p^2 = .033$).

4.3.10 Relationship between reinstatement and extent of tryptophan depletion

To further explore the relationship between ATD and SCRs in reinstatement, a partial correlation analysis was conducted. Controlling for strength of acquisition and intolerance of uncertainty, there was a significant correlation between extent of depletion and CS+E and CS- responses, but not CS+N responses (CS+E, $r(39) = .399$, $p = .01$; CS+N, $r(39) = .232$, $p = .144$; CS-, $r(39) = .452$, $p = .003$). This is likely reflective of the fact that at this stage in the experiment there was no longer differential conditioning to the CS+s versus CS-.

4.3.11 Effects of tryptophan depletion on reacquisition

For the final phase of the experiment, the same analysis used in the spontaneous recovery and reinstatement phases was repeated, which examined the first half of trials. Repeated measures ANCOVA showed a non-significant effect of serotonin status ($F(1,41) = 3.442$, $p = .071$, $\eta_p^2 = .077$). There was no main effect of stimulus ($F(1, 60) = 1.915$, $p = .166$, $\eta_p^2 = .045$) nor a serotonin x stimulus interaction ($F(1, 60) = 0.114$, $p = .832$, $\eta_p^2 = .003$). Therefore, there was no evidence of re-conditioning in either the placebo or depletion groups. In both groups, however, the CS+E was numerically greater than the CS-, while the CS+N and CS- were numerically similar. Because effects in these paradigms are often short-lived (Gershman and Hartley, 2015), and participants' SCR tended to habituate later in the experiment, the same analysis was repeated on the first two trials of the re-acquisition phase which then showed a main effect of serotonin status: responses to the CSs were attenuated overall by ATD ($F(1,41) = 6.946$, $p = .012$, $\eta_p^2 = .145$). There was no main effect of stimulus ($F(2,82) = 1.610$, $p = .206$, $\eta_p^2 = .038$), nor a serotonin x stimulus interaction ($F(2,82) = 2.421$, $p = .095$, $\eta_p^2 = .056$), again providing no evidence of re-conditioning in either group. In both groups, however, the CS+E was again numerically greater than the CS-, while the CS+N and CS- were numerically similar. Results are shown in Figure 4.4c.

4.3.12 Relationship between reacquisition and extent of depletion

A partial correlation analysis was performed, as in the prior phases. Focusing on the first half of trials in the reacquisition phase, there were significant correlations between the extent of depletion and responses to each of the three stimuli (CS+E, $r(39) = .338$, $p = .031$; CS+N, $r(39) = .352$, $p = .024$; CS-, $r(39) = .336$, $p = .032$). This partial correlation analysis was repeated, isolating the first two trials, as before. There was a significant correlation between depletion and the SCR response to the CS+E and CS-, but not the CS+N (CS+E, $r(39) = .472$, $p = .002$; CS+N, $r(39) = .227$, $p = .154$; CS-, $r(39) = .367$, $p = .018$). This is again likely reflective of the fact that at this stage in the experiment there was no longer differential conditioning to the CS+s versus CS-.

4.3.13 Relationship with trait anxiety

Intolerance of uncertainty was highly correlated with trait anxiety ($r(47) = .529$, $p = 1.32 \times 10^{-4}$). An additional ANCOVA was therefore conducted, with serotonin status (placebo, depletion) as between-subjects factors, stimulus (CS+E, CS+N, CS-) as within-subjects factors, with trait anxiety, intolerance of uncertainty, and strength of conditioning on Day 1 as co-

variates. Whilst including trait anxiety as an additional covariate in an ANCOVA assessing spontaneous recovery (the primary phase of interest in this study) reproduced the key main effect of serotonin status ($F(1,42) = 8.473$, $p = .006$, $\eta_p^2 = .168$), trait anxiety was not a significant predictor over and above intolerance of uncertainty ($F(1,42) = .742$, $p = .394$, $\eta_p^2 = .017$). Intolerance of uncertainty, on the other hand, was a significant predictor over and above trait anxiety ($F(1,42) = 4.423$, $p = .041$, $\eta_p^2 = .095$). When incorporating STAI into the model, moreover, there was no longer a simple main effect of stimulus ($F(2,71) = 2.634$, $p = .088$, $\eta_p^2 = .059$).

4.3.14 Additional rating data

I collected rating data from 44 participants (n = 25 on depletion) on how much they enjoyed the task each day. Using a repeated measures ANOVA with serotonin status (placebo, ATD) and Day (Day 1, Day 2) as factors, there was no main effect of ATD ($F(1,42) = 1.339$, $p = .254$) nor was there a serotonin-by-day interaction ($F(1,42) = 0.492$, $p = .487$). I also collected rating data from 37 participants (n = 20 on depletion) asking how uncomfortable they found the shocks on each day. Repeated measures ANOVA with group assignment (placebo versus ATD) and Day (Day 1, Day 2) as factors showed there was no main effect of ATD ($F(1,35) = 0.148$, $p = .703$) nor was there a group x Day interaction ($F(1,35) = 0.339$, $p = .564$).

4.3.15 Summary of results

There was successful baseline (Day 1) Pavlovian threat conditioning, which did not differ between groups. There were also no baseline differences in extinction. The key result was that ATD attenuated the expression of previously acquired conditioned SCRs in the spontaneous recovery phase. Accounting for self-reported intolerance of uncertainty (IUS), furthermore, contributed to the prediction of how ATD modulated SCRs on Day 2. Differential conditioning, meanwhile, was not abolished by ATD. Whilst the reduction in SCRs during the spontaneous recovery phase by ATD was not specific to any of the three stimuli at the group level, the greater the extent of depletion, the more the SCRs to the CS+E and CS+N were attenuated, whereas there was no such correlation for SCRs to the CS-. Following tryptophan depletion, individuals more intolerant of uncertainty showed significantly lower cue-evoked SCRs to all three stimuli during the spontaneous recovery phase. Importantly, SCRs to the US were unaffected by ATD. ATD also attenuated SCRs during the reinstatement and reacquisition phases, consistent with the spontaneous recovery phase results, however there was no longer evidence of differential conditioning.

4.4 Discussion

The aim of this study was to advance the understanding of how serotonin influences the retention of conditioned emotional reactions. Here I have provided evidence that pharmacologically modulating serotonin affected the expression of aversive emotional memory in humans. During the key phase of the study – spontaneous recovery – depletion diminished arousal responses to the CS+s and CS- non-specifically and differential conditioning was preserved. Analysis of individual subject plasma samples, however, revealed that a greater degree of depletion was associated with reduced SCRs to the CS+s, with no effect on CS- responses. These plasma level findings suggest that aversive emotional memory was attenuated. Examining self-reported intolerance of uncertainty, a trait measure that has previously been related to spontaneous recovery (Dunsmoor et al., 2015), was critical for uncovering how ATD affected emotion by contributing to the prediction of the general linear model. Individuals who reported being more intolerant of uncertainty at baseline, furthermore, showed even lower responses during spontaneous recovery when depleted. ATD also attenuated cue-evoked SCRs during the reinstatement and reacquisition phases, consistent with the spontaneous recovery results. Importantly, responses to the US were unaffected by ATD, indicating that the effect was specific to learned cues and not a general blunting of arousal encompassing responses to aversion itself. Mood was unaffected by depletion, consistent with previous ATD studies of healthy volunteers (Ruhe et al., 2007). By using a task that elicited physiological reactions, however, it was possible to uncover an effect of serotonin on emotion.

The primary implication of the study is that serotonin transmission is critical for conditioned threat memory expression. This signal may be boosted in individuals highly intolerant of uncertainty, and excessive serotonin signalling may be an important contributor to the persistence of pathological emotional reactions. I have reinforced and extended the observation that intolerance of uncertainty contributes to spontaneous recovery (Dunsmoor et al., 2015): by implicating serotonin signalling in this phenomenon, the present data suggest that trait intolerance of uncertainty may be a latent marker of vulnerability to serotonergic dysregulation.

Surprisingly few studies have investigated the influence of serotonin on threat conditioning processes in humans (Bauer, 2015). The effects of dietary/pharmacological manipulations aimed at lowering serotonin have been studied primarily in relation to the acquisition of threat conditioning in humans (Hensman et al., 1991; Hindi Attar et al., 2012; Robinson et al., 2012b). The current study represents an important extension of this work by addressing a critical clinically relevant question: how does lowering serotonin impact the intensity with which previously formed emotional memories return? Indeed, aberrant spontaneous recovery, assessed using experimental paradigms analogous to the present one, has been demonstrated most notably in PTSD (Milad et al., 2009), as well as in OCD (McLaughlin et al., 2015; Milad

et al., 2013) and schizophrenia (Holt et al., 2012). These disorders are commonly treated with drugs acting on serotonin (Stahl, 2013). This study may therefore inform both pathophysiology and mechanisms underlying treatment.

The directionality of the depletion effects – a reduction rather than enhancement of emotion – may seem counterintuitive. These results, however, are in line with and advance influential theories of serotonin function (Cools et al., 2011; Deakin, 2013; Deakin and Graeff, 1991), and are consistent with an array of experimental data (Bauer, 2015; Bocchio et al., 2016; Deakin, 2013; Hensman et al., 1991; Hindi Attar et al., 2012). Serotonin is thought to be critically involved in predicting punishment, and aversively conditioned cues stimulate serotonin release (Bauer, 2015; Bocchio et al., 2016; Deakin, 2013; Deakin and Graeff, 1991). The present results are most directly comparable to, and therefore substantiated by, two studies on the role of serotonin in healthy volunteers that also employed SCR to measure threat conditioning of neutral cues. Hensman et al. (1991) showed that the 5-HT2C and -2A receptor antagonist ritanserin – which diminishes the effects of serotonin signalling – impaired conditioning. Likewise, Hindi Attar et al. (2012) used ATD to show the same pattern: an attenuation of conditioning. These autonomic responses closely paralleled functional magnetic resonance imaging (fMRI) data indicating that ATD diminished signals in the amygdala and orbitofrontal cortex that were otherwise evoked by cues predictive of aversion (Hindi Attar et al., 2012). The observation of diminished threat responses in the spontaneous recovery phase, in conjunction with the findings from Hensman et al. (1991) and Hindi Attar et al. (2012), therefore strengthens the punishment prediction framework of serotonin function advanced by Deakin and Graeff (1991).

The results of this study appear to agree with what is known about the basic serotonergic innervation of different amygdala subnuclei. The basolateral nucleus of the amygdala (BLA) is critical for storing associations between cues and aversive outcomes (LeDoux, 2000). The central nucleus of the amygdala (CeA), meanwhile, is a major source of output from the amygdala and signals downstream to structures including the hypothalamus and periacqueductal grey that contribute to defensive reactions such as perspiration in humans and freezing in rodents (LeDoux, 2000). Critically, the BLA receives dense serotonergic innervation whilst the CeA receives weak serotonergic input (Bauer, 2015). This is remarkably consistent with the present findings: SCRs to predictive cues (CSs), which should heavily engage serotonin signalling in the BLA, were modulated by ATD, whereas SCR to the aversive outcome itself (shock; US) was unaffected. Indeed, it is the CeA (which receives weak serotonergic input) that responds to aversive outcomes (Michely et al., 2019). That activity associated with aversive expectations occurs in the BLA, but not the CeA, has furthermore been associated with individual differences in trait anxiety in humans (Michely et al., 2019).

One limitation of the study is that complete extinction on Day 1 was not achieved. An experimental design with two CS+s, only one of which was to be extinguished, was used with the goal of comparing retention of conditioning alone (the CS+N; not extinguished) versus the retention of extinction (the CS+E; extinguished), two different processes that ultimately could not be definitively parsed. Whilst the lack of differential response between the CS+E and CS+N on Day 2 is likely due to incomplete extinction on Day 1, it is also possible, based on Bouton (2002), that the inclusion of the CS+N (not extinguished) in all phases of Day 2 cued memory for conditioning on Day 1 – thus enhancing memory expression for CS+E – more so than an extinction memory trace. On Day 2, SCR habituated in the phases after the critical test of spontaneous recovery, which often occurs (Gershman & Hartley 2015), but which made it more difficult to ascertain effects on reinstatement and reacquisition.

The present study contributes to a surprisingly small literature examining how serotonin affects human threat conditioning (Bauer, 2015). I have provided evidence that lowering serotonin attenuates the subsequent return of threat responses that were conditioned prior to depletion. This is a particularly important question from a clinical standpoint, and efforts to determine factors underpinning the retention of pathological emotional memories have been otherwise widespread (Graham and Milad, 2011; Milad and Quirk, 2012). I furthermore demonstrated – extending Dunsmoor et al. (2015) – that individual differences in self-reported intolerance of uncertainty were critical for understanding how serotonin affected spontaneous recovery. Greater intolerance of uncertainty was correlated with even lower spontaneous recovery following depletion. The use of such trait markers may be a valuable tool for refining which individuals may be most affected by serotonergic challenges. Integrating traits and neurochemical state is relevant for understanding vulnerability in healthy individuals, and may inform transdiagnostic mechanisms in clinical populations to refine psychiatric classification (e.g. Research Domain Criteria, RDoC; Cuthbert & Insel, 2013) and help direct pharmacological strategies.

Chapter 5

Pavlovian and instrumental reversal learning under tryptophan depletion

5.1 Introduction

Serotonin is implicated in processing negative events and adapting previously learned responses to reflect new environmental circumstances (Cools et al., 2008a). Behaviour that had been optimal for maximising reward and minimising punishment must at times be adjusted to navigate the world effectively. Likewise, reacting emotionally to threats is critical for survival yet must be modified as danger shifts from one source to another. Behavioural inflexibility is a hallmark of obsessive-compulsive disorder (OCD), for instance, where a learned behaviour persists inappropriately despite adverse consequences (American Psychiatric Association, 2013). Aberrations in threat and safety learning are characteristic of post-traumatic stress disorder (PTSD; Homan et al., 2019; Milad et al., 2009) and other anxiety disorders (Kim et al., 2011; Marin et al., 2017), and are also a feature of OCD (Milad et al., 2013; Apergis-Schoute et al., 2017) and schizophrenia (Holt et al., 2012). Drugs thought to boost serotonin transmission – selective serotonin reuptake inhibitors (SSRIs) – are first line treatments for OCD (Fineberg et al., 2020) and PTSD (Baldwin et al., 2014). Schizophrenia, in which behavioural inflexibility has additionally been documented (Waltz and Gold, 2007), is treated with drugs that modulate serotonin in addition to dopamine, such as risperidone, a non-selective serotonin 2A (5-HT2A) receptor antagonist (Stahl, 2013). Drugs of this class can also be used to augment SSRI therapy in OCD (Fineberg et al., 2020).

Despite broad clinical relevance, the preponderance of evidence on how serotonin impacts behavioural adaptation comes from studies of non-human animals (e.g. Bari et al., 2010; Clarke et al., 2004, 2007; Lapiz-Bluhm et al., 2009), whilst the role of serotonin in human

threat and safety learning has received surprisingly little attention (Bauer, 2015). Here, I studied healthy human volunteers to examine the effects of lowering serotonin on both behavioural and emotional flexibility in two independent experiments employing acute tryptophan depletion (ATD). Depleting tryptophan, serotonin's biosynthetic precursor, decreases serotonin function (Bel & Artigas, 1996; Bell et al., 2005; Biggio et al., 1974; Crockett et al., 2012a; Nishizawa et al., 1997). Reversal learning is a key laboratory assay of cognitive inflexibility that has been translated across species (Cools et al., 2008a). Experiment 1 tested instrumental reversal learning. Individuals acquired an adaptive behaviour through trial and error learning (stimulus-response-outcome), and the correct response subsequently changed multiple times, necessitating cessation of the previous action and performing a new behaviour. Failure to adapt to new contingences is referred to as perseveration. Experiment 2 examined reversal learning in the Pavlovian domain (Apergis-Schoute et al., 2017; Schiller et al., 2008). Participants were presented with two cues (threatening faces, i.e. signs of aggressive conspecifics), one of which was sometimes paired with an electric shock, while the other was not (stimulus-outcome). A reversal phase followed, whereby the originally conditioned face became safe, and the initially safe stimulus was newly paired with shock. Under normal circumstances, anticipatory sympathetic nervous system arousal responses, manifested in perspiration, should track the presently threatening cue (Schiller et al., 2008).

Impairments in human instrumental reversal learning following ATD have been difficult to detect to date (Evers et al., 2005; Finger et al., 2007; Murphy et al., 2002), likely owing in part to its transient and relatively mild depletion in comparison (Worbe et al., 2014) with the profound depletion that is possible in experimental animals (Bari et al., 2010; Clarke et al., 2004; 2007). Given the importance of serotonin in processing both aversive (Crockett et al., 2012a; Deakin, 2013), and rewarding (Cohen et al., 2015; Seymour et al., 2012) outcomes, I used an innovative task (Figure 5.1) incorporating feedback that was markedly more salient than was used in previous reversal tasks (Evers et al., 2005; Finger et al., 2007; Murphy et al., 2002). Prior studies that did not find a perseverative deficit following ATD employed largely probabilistic feedback (Evers et al., 2005; Finger et al., 2007; Murphy et al., 2002) and a single reversal (Finger et al., 2007; Murphy et al., 2002). Meanwhile, evidence of a perseverative deficit following neurotoxic serotonin depletion, in the marmoset monkey orbitofrontal cortex (OFC), comes from a paradigm more similar to that employed in the present study: Clarke et al. (2004) used serial reversals on a deterministic schedule, and found a reversal deficit that emerged only beginning in the second reversal. I was therefore particularly interested in whether focusing on a later reversal phase may be key to uncovering perseveration following ATD in humans.

In previous research on Pavlovian threat conditioning after lowering serotonin signalling

in humans, researchers have focused on initial learning to predict aversion upon pairing previously neutral cues with aversive outcomes [e.g. heat or mild electric shock] (Hensman et al., 1991; Hindi Attar et al., 2012; Robinson et al., 2012b). Here I tested whether instrumental reversal learning impairments additionally extend to the Pavlovian domain in humans following ATD (Figure 5.2). I also expanded upon the few previous Pavlovian studies by examining threat conditioning to facial signs of aggression [innate threat cues] (Öhman, 2009). Whilst similar neural mechanisms are engaged, there are distinctions between circuits that respond to learned threats (e.g. neutral cues), predators (e.g. snakes or spiders), and aggressive conspecifics (Gross and Canteras, 2012). Furthermore, in rodents, serotonin can be engaged differentially by innate versus learned threats (Isosaka et al., 2015) and by the intensity of threat (Seo et al., 2019).

The aim of this study, therefore, was to address the following questions. In Experiment 1: Does ATD induce perseveration in instrumental reversal learning? Do these effects emerge in a later reversal phase, and particularly when feedback is most salient? In Experiment 2: Does ATD impair Pavlovian reversal learning? And does ATD have a different effect on conditioning to threatening cues compared to neutral cues? In the instrumental domain, I hypothesised that ATD would lessen the impact of motivationally salient feedback to guide behaviour, resulting in a perseverative deficit. In the Pavlovian domain, I predicted conditioning processes would be impaired during both the initial acquisition phase as well as following the reversal of contingencies.

5.2 Methods

5.2.1 Participants

Sixty-nine healthy participants (36 males, mean age 24.28) completed the deterministic instrumental reversal learning task and were included in the final analysis. One male participant in the depletion group was excluded because he admitted to responding randomly later in the task. Thirty healthy volunteers (17 females) completed the Pavlovian threat reversal task. Of these, two (1 female) were excluded for an undetectable skin conductance response. Sixteen received depletion. Demographics and questionnaire measures are shown in Table 5.1 for the sample in Experiment 1, and in Table 5.2 for Experiment 2.

5.2.2 Instrumental reversal learning task (Experiment 1)

The task used in Experiment 1 is depicted in Figure 5.1. As an incentive, participants were told that depending on how well they performed the task, they could win a bonus on their

Group	Placebo	Depletion		
	Mean (SD)	Mean (SD)	t (df)	p
Age	24.24 (5.684)	24.31 (4.788)	.063 (67)	.95
Edu	16.97 (2.181)	17.34 (2.376)	.678 (67)	.5
BDI-II	4.76 (4.335)	3.80 (3.701)	-.995 (67)	.323
STAI	37.35 (6.517)	35.71 (5.919)	-1.094 (67)	.278
OCI-R	8.21 (7.543)	7.09 (7.172)	-.632 (67)	.529
BIS	70.41 (4.150)	71.09 (3.673)	.715 (67)	.477

Table 5.1: Demographics and questionnaire measures for Experiment 1. BDI-II = Beck Depression Inventory, version II (Beck et al., 1996); STAI = Spielberger Trait Anxiety Inventory (STAI; Spielberger et al., 1983); OCI-R = Obsessive Compulsive Inventory-Revised (Foa et al., 2002); BIS = Barratt Impulsiveness Scale (Patton et al., 1995); SD = standard deviation; Edu = years of education.

Group	Placebo	Depletion		
	Mean (SD)	Mean (SD)	t (df)	p
Age	23.58 (8.262)	25.21 (2.860)	-.694 (24)	.495
Edu	18.33 (1.923)	18.46 (2.727)	-.135 (23)	.894
BDI-II	3.42 (3.423)	4.06 (4.739)	-.400 (26)	.693
BIS	55.33 (6.827)	59.81 (8.272)	-1.524 (26)	.139

Table 5.2: Demographics and questionnaire measures for Experiment 2. BDI-II = Beck Depression Inventory, version II (Beck et al., 1996); BIS = Barratt Impulsiveness Scale (Patton et al., 1995). SD = standard deviation. Age and years of education (Edu) were unavailable for one participant in the placebo condition. In the depletion condition, age was unavailable for one participant; years of education were unavailable for two participants.

compensation for taking part in the study. In reality, everyone received a small bonus. The instrumental reversal paradigm was designed to increase cognitive load and thus task demands. It had three reversals and a deterministic schedule. Responses were entered via one of two "button boxes" with either the left or right hand (see Figure 5.1). On each trial, the computer screen was framed by a specific colour and displayed five boxes corresponding to five buttons on each button box, one button per finger. The colour indicated the correct hand to respond with, and a black dot inside one of the five boxes on the screen indicated which finger to respond with. Participants were told they needed to learn the colour-hand association by trial and error and that the association would change multiple times within a run. A run consisted of four blocks of 20 trials each: an acquisition block where the initial contingency was established followed by three reversal blocks. The reinforcement schedule was deterministic: the correct option led to positive feedback on 100% of trials, whilst the incorrect response led to negative feedback on 100% of trials. Trial order was randomised. There were four runs in random order, and each contained a unique pair of colours framing the screen which was counterbalanced. All runs contained the same visual feedback cartoon stimuli: a smiling face with "two thumbs up" for correct responses, a face showing disappointment and a "thumbs down" when incorrect, and an analogue alarm clock with a frown if a response was not entered within the allotted time. The salience and valence of feedback across runs was varied using the presence or absence of prominent auditory stimuli. The primary run of interest had the most salient auditory feedback: responding correctly to one colour resulted in reward in the form of a prominent "cha-ching" (slot machine) sound, whilst correct responses to the other colour prevented (avoided) the occurrence of an aversive buzzer noise (reward-punishment run). There was also a reward-neutral run where a correct response to one colour frame resulted in the reward auditory feedback whereas responding correctly or incorrectly to the other colour resulted only in visual (neutral) but no auditory feedback. In the punishment-neutral run incorrect responses to one colour frame were punished with the buzzer noise whereas correct or incorrect responses to the other colour resulted only in visual feedback (neutral). Finally, the task contained a neutral-neutral condition where no auditory feedback was provided and only visual feedback via cartoons was presented.

The experiment began with three training phases, each of which required attaining a programmed criterion to advance to the next stage otherwise the phase would be repeated. The first was self-paced and served to familiarise participants with responding using the button boxes. In the first training phase only, "LEFT" or "RIGHT" was displayed on each trial to instruct the correct hand to use. There was a time limit in the second (short) and third (longer) training phases and participants were told to respond as quickly and accurately as possible. The time window to make responses during the actual experiment was automatically cali-

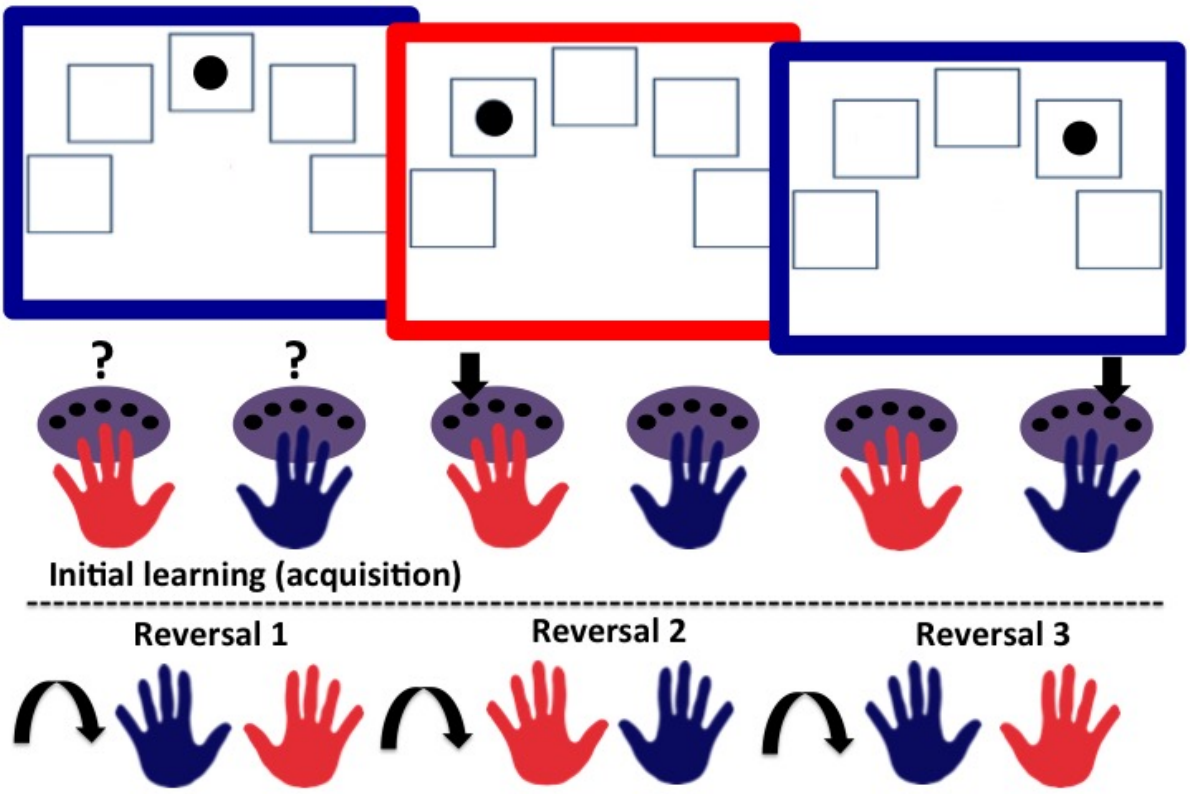

Figure 5.1: Experiment 1 task schematic. TOP: The three rectangles with coloured frames represent three example trials presented in the acquisition phase. Purple ovals symbolise the button boxes. Question marks signify the need to learn the correct hand-colour association by trial and error. Downward pointing arrows indicate the correct hand and button response for that trial. BOTTOM: Curved arrows signify the reversal of colour-hand contingencies, which occurred three times.

brated to each person based on their reaction times during the final practice phase. The task was programmed in E-Prime 2.0 Professional. The primary dependent measure was trials to criterion, which has been used in serotonin depletion studies in marmoset monkeys (Clarke et al., 2004, 2007), which I aimed to translate into humans here. The criterion was defined as making four consecutive correct responses.

5.2.3 Pavlovian reversal learning task (Experiment 2)

The Pavlovian reversal task is depicted in Figure 5.2 and had two phases: acquisition and reversal. Two faces (face A and B) were presented in each phase, for four seconds each with an inter-trial interval of 12 seconds (Schiller et al., 2008). The face images were selected from the Ekman series (Ekman and Friesen, 1976). Participants chose a shock level that they felt was uncomfortable but not painful. In the acquisition phase, face A was presented 16 times without a shock (conditioned stimulus plus; CS+) and coterminated with a 200 millisecond shock (unconditioned stimulus; US) on an additional eight trials (CS+US), while face B was presented 16 times and never paired with shock (CS-). In the reversal phase the faces were presented again only the contingencies swapped: face A was presented for 16 trials and was no longer paired with a shock (new CS-), while face B was newly paired with a shock on 8 trials amidst an additional 16 unreinforced trials (new CS+). Trials were pseudorandomized and designation of face A and B was counterbalanced. Reversal was unsignaled and immediately followed acquisition without a break. The dependent measure was the skin conductance response (SCR), which assesses perspiration and is an assay of autonomic nervous system arousal. The primary focus was the SCR to unreinforced trials, to avoid contamination by the shock itself. SCRs were defined as the base-to-peak difference during a seven second interval beginning 0.5 seconds after stimulus onset. SCRs were normalized for each individual participant by dividing values from each trial by their peak amplitude. This task was also programmed in E-Prime. More information about this specific paradigm can be found in Apergis-Schoute et al. (2017).

5.3 Results

5.3.1 Experiment 1

5.3.1.1 Blood Results and Mood

Robust tryptophan depletion was achieved, as verified by plasma samples ($t(64) = -18.725$, $p = 1.161 \times 10^{-27}$). Plasma levels were unavailable for three participants: one due to a staff

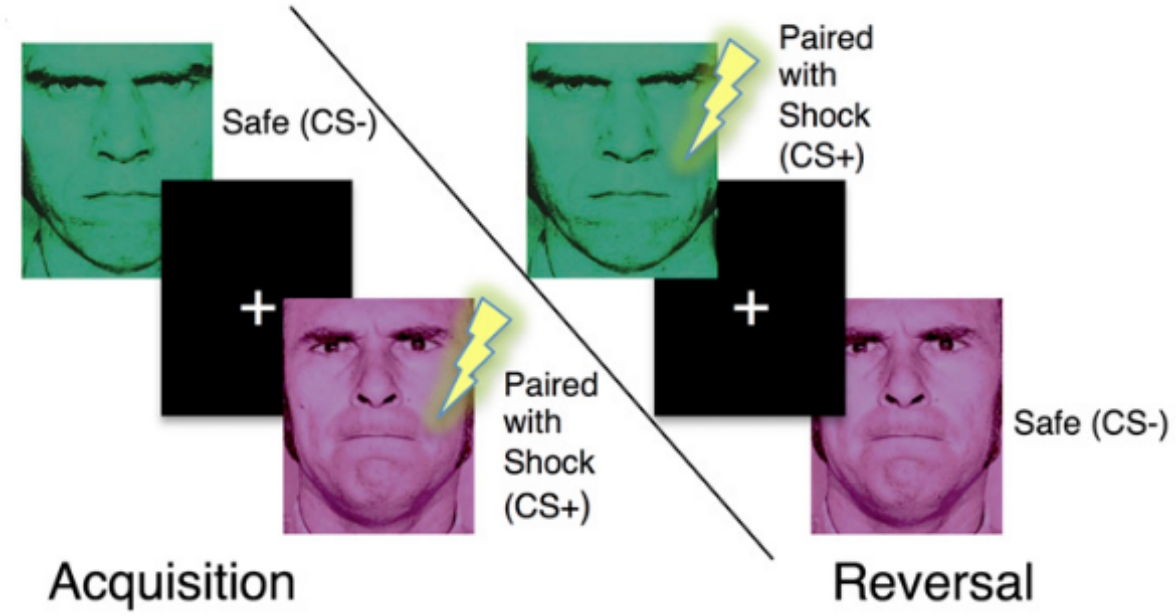

Figure 5.2: Experiment 2 task schematic, from Apergis-Schoute et al. (2017). Face stimuli used with permission from Paul Ekman, PhD/Paul Ekman, LLC.

processing error, and two due to difficulty with venepuncture. Self-reported mood, available for 63 participants, was unaffected by ATD ($t(61) = -.898$, $p = .373$).

5.3.1.2 Group-level instrumental reversal analysis

Instrumental reversal learning was impaired following ATD, and the core deficits are displayed in Figure 5.3. First, omnibus repeated measures analysis of variance (ANOVA) was performed across all valence conditions and blocks. In the most salient condition participants had to make separate responses to obtain reward and avoid punishment (reward-punishment; see Methods). The other conditions incorporated either only neutral feedback (neutral-neutral), or neutral feedback with reward (reward-neutral) or punishment (punishment-neutral). The dependent measure for all analyses was trials to criterion (see Methods). The omnibus ANOVA, with serotonin status (placebo, depletion) as between-subjects factor, valence (reward-punishment, reward-neutral, punishment-neutral, neutral-neutral) and block (acquisition, reversal 1, reversal 2, reversal 3) as within-subjects factors, revealed a significant serotonin-by-valence-by-block interaction ($F(9,603) = 2.024$, $p = .035$, $\eta_p^2 = .029$). There was no main effect of serotonin status ($F(1,67) = 1.869$, $p = .176$, $\eta_p^2 = .027$). Next I verified that this effect was not driven by acquisition learning. Indeed, ATD had no effect on initial discrimination learning in the reward-punishment condition ($t(67) = 1.115$, $p = .269$), reward-neutral ($t(67) = -.325$,

p = .746), punishment-neutral (t(67) = -.688, p = .494) or neutral-neutral conditions (t(64) = .891, p = .376), shown in Figure 5.3. To assess the nature of the reversal learning deficit, the significant three-way interaction was followed up with t-tests in a sequence guided by two key a priori hypotheses. First, serotonin signalling is particularly engaged when responding to motivationally salient feedback (Faulkner and Deakin, 2014), and therefore a reversal learning deficit should be most likely in the highest salience condition (reward-punishment). Second, serotonin depletion in the marmoset monkey OFC has been shown to induce the most pronounced instrumental reversal learning deficit in the second reversal block, without impacting the initial reversal (Clarke et al., 2004). The first follow-up test of reversal learning, therefore, assessed the second reversal of the most salient condition (reward-punishment) and indeed revealed a deficit: participants under ATD required more trials to criterion than on placebo (t(59) = 2.281, p = .026). I then tested whether the effect in the second reversal was present in the other, less salient, conditions. There was a significant deficit under ATD in the reward-neutral condition (t(61) = 2.413, p = .019), and not in the punishment-neutral (t(67) = -.512, p = .61) or neutral-neutral (t(67) = .572, p = .569) conditions. Next I tested whether a deficit was present in the first reversal. Individuals under depletion required more trials to criterion in the reward-neutral condition (t(67) = 2.113, p = .038), but not in the reward-punishment (t(67) = -.528, p = .599), punishment-neutral (t(67) = 1.439, p = .155), or neutral-neutral (t(64) = 1.051, p = .297) conditions. Finally, I assessed whether there was any deficit in the last reversal block. Performance was not impaired in the final reversal phase in the reward-punishment (t(67) = 1.097, p = .277), reward-neutral (t(67) = -.124, p = .902), punishment-neutral (t(67) = 1.348, p = .182), or neutral-neutral (t(67) = -.526, p = .601) conditions. The key deficits, from the reward-punishment and reward-neutral conditions identified in the second reversal block, additionally survived the Benjamini-Hochberg procedure (see Chapter 2), for 12 comparisons (four valence conditions and three reversals), and were therefore the primary drivers of the serotonin-by-valence-by-block interaction.

5.3.1.3 Relationship between instrumental reversal deficits and extent of depletion

More pronounced depletion was significantly correlated with the key reversal deficits, shown in Figure 5.4. To further substantiate the deficits observed upon depletion, correlation analyses between behaviour and individual subject plasma samples were conducted. First, this was performed for behaviour in the second reversal phase during both the reward-punishment and reward-neutral conditions, where significant deficits were found at the group level. Indeed, greater extent of depletion was significantly correlated with the magnitude of these key reversal impairments: more pronounced depletion was related to worse performance in both the reward-punishment condition (r(66) = -.266, p = .031) and the reward-neutral condition (r(66)

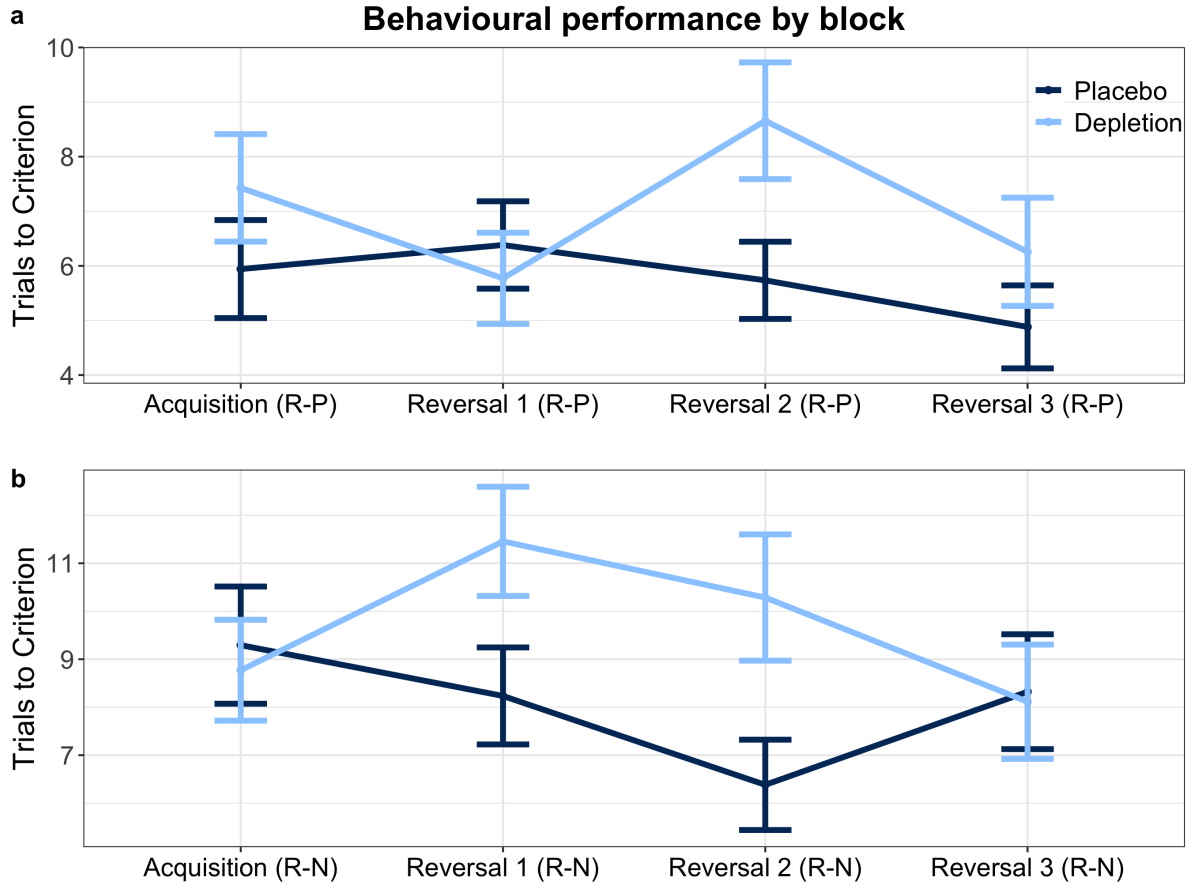

Figure 5.3: Experiment 1. Instrumental reversal learning performance by block. **(a)** R-P = reward-punishment condition. **(b)** R-N = reward neutral condition. Error bars represent 1 standard error of the mean.

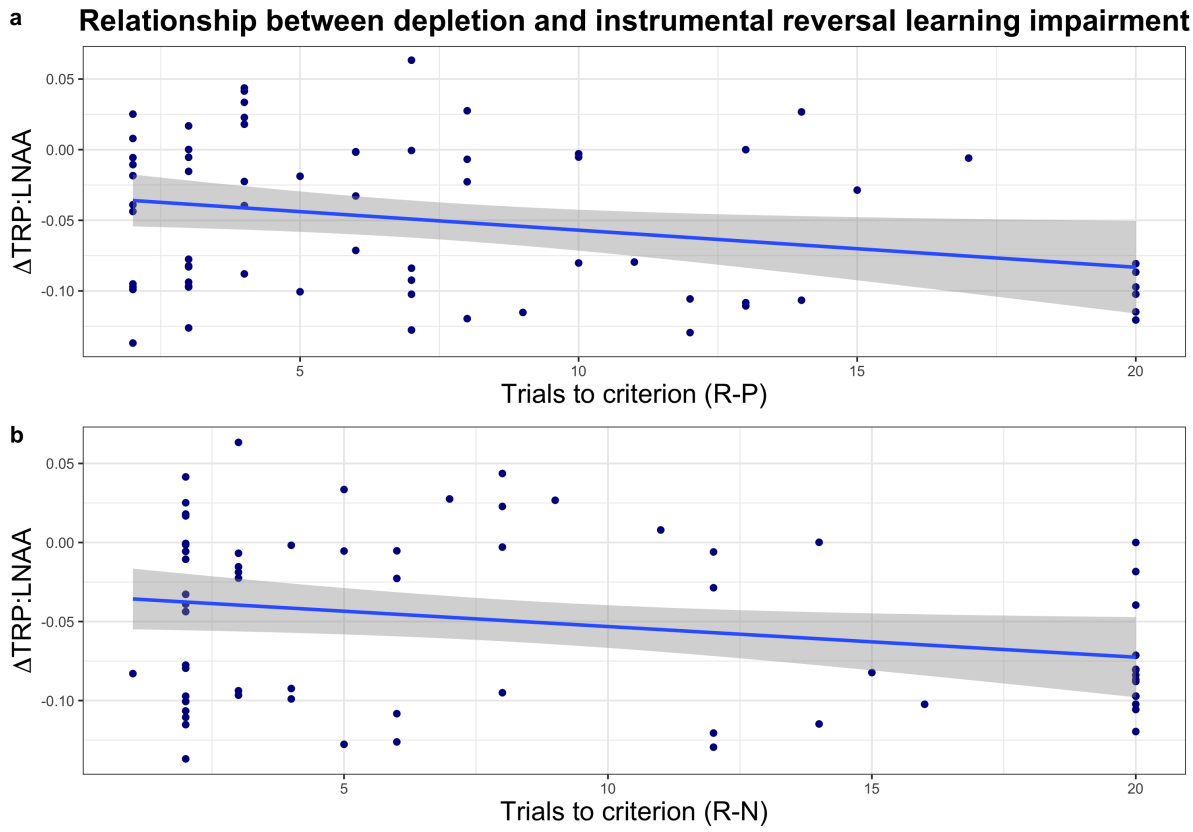

Figure 5.4: Experiment 1. Relationship between extent of depletion and instrumental reversal learning performance. **(a)** Reward-punishment (R-P) condition. **(b)** Reward-neutral (R-N) condition. The y-axis represents the change in the ratio of tryptophan to large neutral amino acids (TRP:LNAA); y = 0 indicates no change. A greater decrease (post-depletion minus pre-depletion blood plasma results) in the TRP:LNAA ratio indicates a more extensive depletion (more negative y-axis values). Reversal learning is indexed here as the number of trials to criterion in the second reversal block. Increasing x-axis values represent more trials to criterion and thus worse reversal performance. Shading indicates 1 standard error (SE).

= -.25, p = .043). These results are displayed in Figure 5.4a and 5.4b, respectively. The other observed behavioural impairment, from the first reversal in the reward-neutral condition, was also significantly correlated with the extent of depletion (r(66) = -.311, p = .011).

5.3.2 Experiment 2

5.3.2.1 Blood analysis and Mood

Robust depletion was also achieved in Experiment 2 (t(17) = -4.907, p = 0.000132). Blood results from one participant were unavailable. Mood, assessed with the Positive and Negative Affect Schedule [PANAS] (Watson et al., 1988) after depletion had taken effect, was

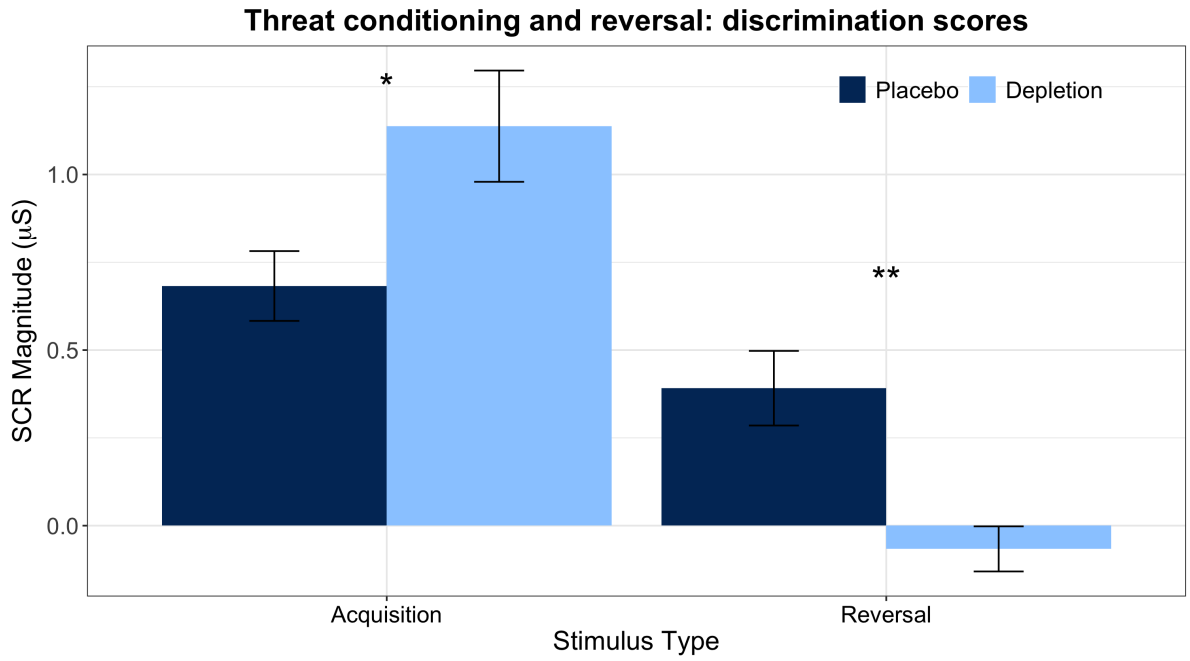

Figure 5.5: Experiment 2. Acquisition and reversal SCR data displaying a difference score of CS+ minus CS-, indicative of the extent of discrimination learning to the two stimuli. Error bars represent 1 standard error (SE). μS = microsiemens.

unaffected: no difference between serotonin status for positive (t(26) = 1.479, p = .151) or negative affect (t(25) = -1.076, p = .292).

5.3.2.2 Acquisition of conditioning

Conditioning data are displayed in Figure 5.5 and 5.6. Differential conditioning (CS+ versus CS-) was attained in both the placebo and ATD groups (paired t-tests: t(11) = 6.866, p = .000027, for placebo; t(15) = 7.181, p = .000003, for depletion). Critically, conditioning was significantly stronger following depletion compared to the placebo group: I calculated a difference score of CS+ minus CS- for each group, and the magnitude of the CS+ relative to the CS- was significantly greater in the ATD group (t(26) = -2.245, p = .034).

5.3.2.3 Reversal of conditioning

The reversal learning results are depicted in Figure 5.5 and 5.6. During the reversal phase, the placebo group successfully conditioned to the new CS+ (t(11) = 3.684, p = .004). The depletion group, however, did not show discrimination between the new CS+ and the new CS- (t(15) = -1.031, p = .319), indicating a reversal learning impairment. Comparing the

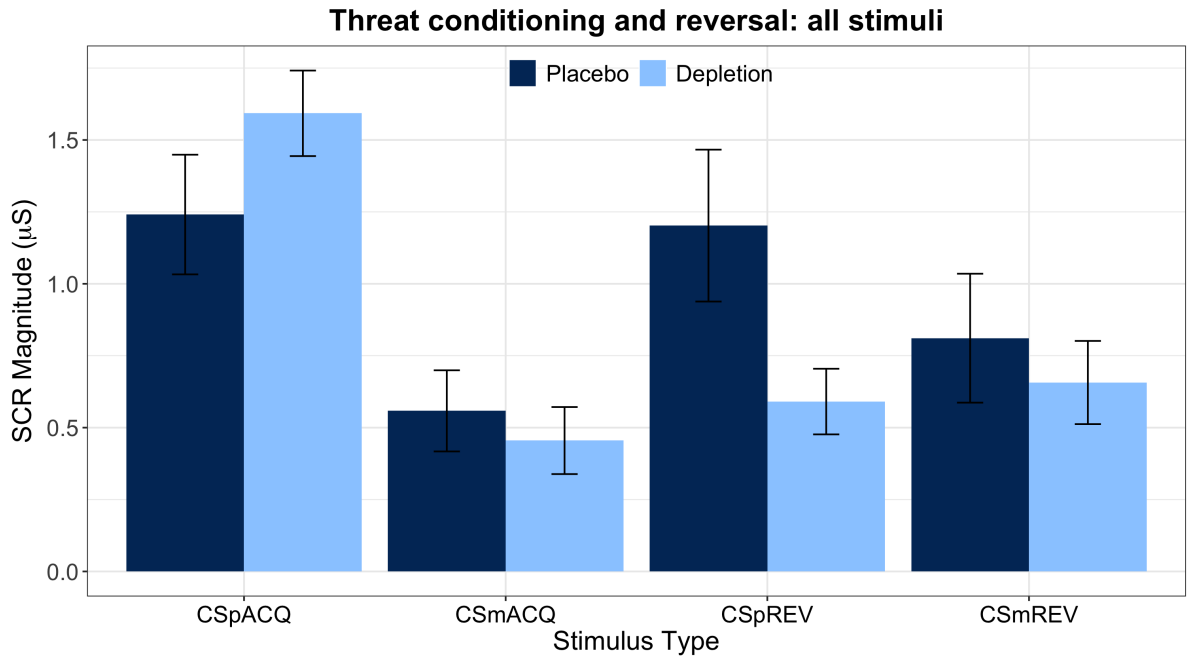

Figure 5.6: Experiment 2. Acquisition and reversal SCR data with all stimuli displayed separately. CSpACQ = (initial) CS+ during acquisition; CSmACQ = (initial) CS- during acquisition; CSpREV = (new) CS+ during reversal; CSmREV = (new) CS- during reversal. Error bars represent 1 standard error (SE). μS = microsiemens.

difference score during the reversal phase (new CS+ minus new CS-) between placebo and ATD also confirmed reversal learning was impaired (t(26) = 3.880, p = .001).

5.3.2.4 Changes in physiological responses to stimuli across phase

I additionally assessed how responses to the stimuli were affected by ATD in each phase, depicted in Figure 5.6. Repeated measures analysis of variance (ANOVA), with serotonin status (placebo, depletion) as a between-subjects factor and phase (acquisition, reversal) and stimulus (CS+, CS-) as within-subjects factors revealed a significant three-way serotonin-by-phase-by-stimulus interaction (F(1,26) = 17.604, p = .00028). Follow-up paired t-tests showed that responding to the initial CS+ extinguished upon reversal both within the placebo group (initial CS+ versus new CS-; t(11) = 2.799, p = .017) and under ATD (t(15) = 6.402, p = .000012). SCR to the initial CS- increased upon reversal in the placebo group (t(11) = -4.172, p = .002) but critically, there was no difference in SCR to the initial CS- in acquisition compared to the new CS+ (old CS-) in reversal (t(15) = -1.370, p = .191). The reversal impairment following ATD was driven by a failure to assign new aversive value, whereas safety learning upon reversal was intact.

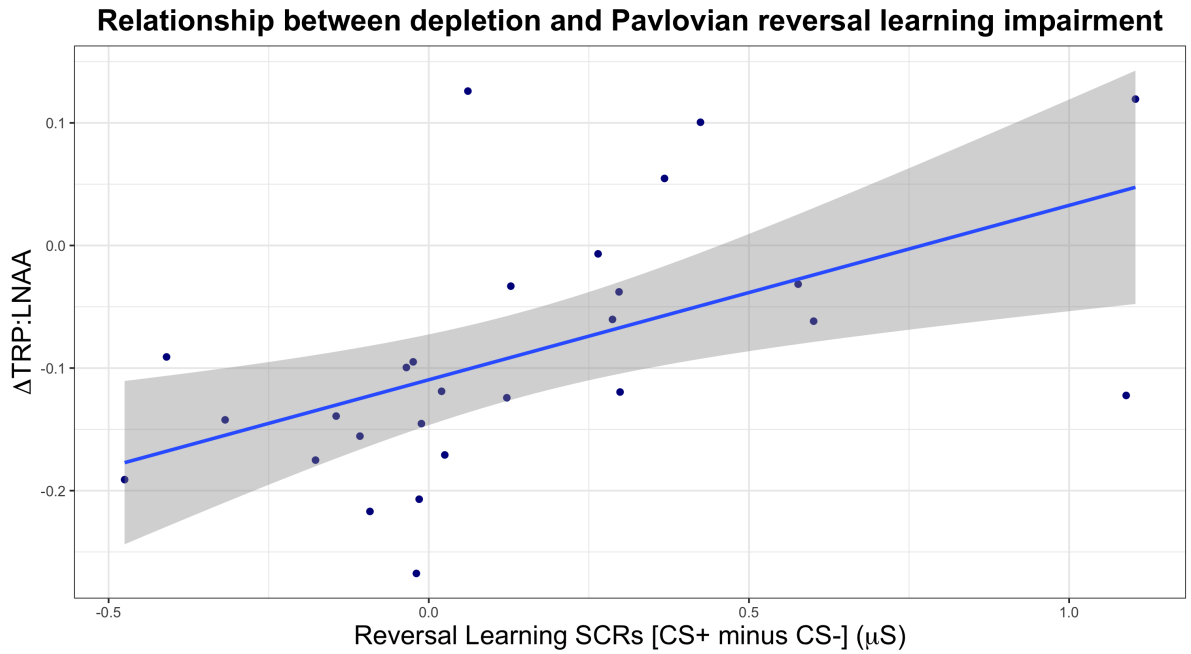

Figure 5.7: **Relationship between extent of depletion and degree of Pavlovian reversal learning impairment** (experiment 2). TRP:LNAA is the ratio of tryptophan to large neutral amino acids; y = 0 indicates no change. A greater change [Δ] (post-depletion blood minus pre-depletion results) in the TRP:LNAA ratio indicates a more extensive depletion (more negative y-axis values). Reversal learning is indexed here as the difference score between CS+ and CS- in the reversal phase. Increasing x-axis values represent better discrimination learning assessed by SCR between the CS+ and CS- in the reversal phase (i.e. better reversal learning). Shading indicates 1 standard error (SE).

5.3.2.5 Correlations between emotional measures and extent of depletion

Next I tested whether the extent of depletion, as assessed via plasma samples, was related to the measures of emotional learning. Extent of depletion was not correlated with the magnitude of the SCR difference score in the acquisition phase ($r(27) = .210$, $p = .294$); however, there was a highly significant correlation between greater depletion and a more pronounced reversal learning deficit ($r(27) = -.536$, $p = .004$), depicted in Figure 5.7.

5.3.2.6 Unconditioned responses

It was then tested whether ATD affected the unconditioned responses (UR), the SCR to the US itself, to demonstrate whether the effect was selective to anticipatory responses to the receipt of shock. ATD had no effect on the URs ($p > .05$).

5.4 Discussion

I have provided convergent evidence from two independent experiments that serotonin depletion effected by acute dietary tryptophan depletion impairs human reversal learning in both the instrumental and Pavlovian domains (Experiments 1 and 2, respectively). The magnitudes of the instrumental and Pavlovian reversal deficits, moreover, were both correlated with the extent of depletion assessed by plasma samples. Both the human instrumental and Pavlovian results are further strengthened by their consistency with studies of experimental animals following neurotoxic serotonin depletion (Bari et al., 2010; Clark et al., 2004, 2007; Lapiz-Bluhm et al., 2009). Remarkably, in rats, marmosets, and humans, the effect of serotonin depletion in the instrumental domain most consistently emerged upon the second reversal of contingencies (Bari et al., 2010; Clarke et al., 2004, 2007). Pavlovian extinction, meanwhile, was intact following serotonin depletion in humans, which is also consistent with data from marmosets following OFC serotonin depletion: (instrumental) extinction was unimpaired (Walker et al., 2009). Initial Pavlovian conditioning here, to innately threatening cues, at the same time, was enhanced under serotonin depletion. This effect has not been seen for conditioning to innately neutral cues (Hensman et al., 1991; Hindi Attar et al., 2012). Mood was unaffected, in line with the ATD literature in healthy humans (Bell et al., 2005).

Perseverative deficits in human instrumental reversal learning following ATD have not been easily captured to date (Evers et al., 2005; Finger et al., 2007; Murphy et al., 2002), owing in part to ATD inducing a transient and relatively mild depletion in comparison (Worbe et al., 2014) with the profound depletion that is possible in experimental animals using 5,7-dihydroxytryptamine (Bari et al., 2010; Clarke et al., 2004, 2007; Walker et al., 2009). These ATD studies employed largely probabilistic feedback (Evers et al., 2005; Finger et al., 2007; Murphy et al., 2002), with a single reversal (Finger et al., 2007; Murphy et al., 2002), and non-salient feedback (Evers et al., 2005; Finger et al., 2007; Murphy et al., 2002). The innovative instrumental task used here was unique in that it incorporated highly salient feedback, multiple reversals on a deterministic schedule, and increased cognitive load. The deterministic schedule with multiple reversals, in particular, aligns with the design of marmoset studies that have provided quintessential evidence that OFC serotonin depletion induces perseveration (Clarke et al., 2004, 2007).

Whilst the instrumental deficits on both the most salient (reward and punishment) and reward-only, but not punishment-only condition, as reported here, may at first seem surprising given the well-established role of serotonin in aversive processing (Cools et al. 2008), this indeed aligns with the literature across species: the key marmoset studies on serotonin depletion and perseveration were conducted in the appetitive domain (Clarke et al., 2004; 2007; Walker et al,. 2009), and Seymour et al. (2012) found human ATD affected the appetitive but

not aversive domain in a 4-choice probabilistic task on which computational modelling also revealed enhanced perseveration.

The Pavlovian reversal findings reported here resemble both the Pavlovian reversal learning impairment reported in OCD (Apergis-Schoute et al., 2017) and what has been demonstrated in healthy volunteers under stress (Raio et al., 2017). The reversal deficit in OCD, indexed by SCR on an identical paradigm, was explained by dysfunctional activity in the ventromedial prefrontal (vmPFC), which receives rich serotonergic innervation (Hornung, 2003). Raio et al. (2017), meanwhile, used SCR and a similar design to that used here (but with neutral cues) and found that upon reversal, stress also attenuated the acquisition of threat responses to the newly threatening (previously safe) stimulus upon reversal, while leaving extinction learning to the previously threatening cue intact. This parallel is striking, and is consonant with data from rats: stress, and separately serotonin depletion, produced comparable deficits in (instrumental) reversal learning (Lapiz-Bluhm et al., 2009). Serotonin release in rats during behavioural testing, moreover, was reduced by stress, and an SSRI given acutely ameliorated the detrimental effect of stress on reversal learning (Lapiz-Bluhm et al., 2009).

The deleterious effects of serotonin depletion and stress on reversal learning can be interpreted as a selective impairment in integrating new information about a change in reinforcement contingencies, needed to update the representation of aversive value appropriately (Raio et al., 2017). These authors invoked the phenomenon of stress-induced dopamine release (Pruessner et al., 2004), which may dampen negative prediction errors evoked by contingency reversal (Cools et al., 2001). The reversal data are consistent with a similar interpretation: ATD may have constrained the negative prediction errors triggered by reversal, which are purported to be associated with serotonin signalling (Cools et al., 2011).

That I found an enhancement of initial Pavlovian conditioning, when employing socially threatening conditioned stimuli (aggressive faces), contrasts with two previous threat conditioning studies that used neutral stimuli: Hensman et al. (1991) showed that the serotonin 2A/2C (5-HT2A, 5-HT2C) receptor antagonist ritanserin attenuated threat conditioning, whilst Hindi Attar et al. (2012) showed the same pattern of results following ATD. The attenuated SCR upon reversal as reported here, however, converges with these previous studies that investigated conditioning alone (Hensman et al., 1991; Hindi Attar et al., 2012). The discrepancy across studies at the initial conditioning phase (Hensman et al., 1991; Hindi Attar et al., 2012) is likely due to the engagement of distinct, yet similar, mechanisms when learning to predict aversion from a neutral cue, compared to responding to innate threats (Gross and Canteras, 2012).

Consideration of rodent studies on the influence of serotonin on specific amygdala subnuclei may inform the human conditioning findings presented here. The central nucleus of

the amygdala (CeA) is the major source of output and its downstream projections ultimately produce defence responses such as perspiration in humans and freezing in rodents (LeDoux, 2000). Critically, cells expressing 5-HT2A receptors in the CeA are differentially engaged by innate versus learned threats (Isosaka et al., 2015). Inhibition of these 5-HT2A-expressing cells upregulates innate threat responses in mice and downregulates learned threat responses (Isosaka et al., 2015). This is remarkably congruent with the current observation that reducing serotonin release potentiates conditioning to innate threats, on the one hand, and findings from previous studies that reduction of serotonin signalling attenuates threat conditioning to learned (neutral) cues. These divergent results may inform therapeutic, and possibly adverse, effects of serotonin modulating drugs. Isosaka et al. (2015) indeed showed that risperidone exacerbated responses to innate threats and alleviated threat responses to previously neutral cues: this latter result is consistent with Hensman et al. (1991). Furthermore, humans with selective damage to the basolateral amygdala (BLA), with the CeA preserved, showed hypervigilant responses to fearful faces (innate threat), which was interpreted as the removal of an inhibitory influence of the BLA over the CeA (Terburg et al., 2012). Indeed, the BLA receives particularly rich serotonergic innervation (Bauer, 2015), which, in conjunction with the role of serotonin in the CeA for innate threats may be important for understanding the present results.

The primary limitation of the present study is the small sample size of Experiment 2, and thus replication will be important, although the effect size was large. It should be noted, however, that one of the small number of studies on Pavlovian conditioning, using pharmacological challenges to lower serotonin, reported data from a sample of just n = 10 per group (Hensman et al., 1991), which the present study has improved upon substantially. Here, fewer participants received placebo than depletion; however the placebo data align with numerous previously published control groups on this paradigm (Apergis-Schoute et al., 2017; Homan et al., 2019; Raio et al., 2017; Schiller et al., 2008).

Whilst some have criticised ATD as a technique for studying serotonin in particular (van Donkelaar et al., 2011), the method has been robustly defended (Crockett et al., 2012a; Young, 2013). Critically, the present findings align with deficits following profound neurotoxic serotonin depletion in both rats (Bari et al., 2010) and marmoset monkeys (Clarke et al., 2004, 2007; Walker et al., 2009). These results build upon other studies, for instance on "waiting impulsivity", that show parallel behavioural effects between neurotoxic depletions in experimental animals (Winstanley et al., 2004a) and ATD in healthy humans (Worbe et al., 2014), thus further bolstering the validity of ATD for studying serotonin. Whilst the neural locus of this impaired neuromodulation is not known, work in the instrumental domain from experimental animals (Clarke et al., 2004, 2007; Walker et al., 2009) and individuals with OCD (Chamberlain et al., 2008; Remijnse et al., 2006) implicate the OFC. The Pavlovian reversal

data from OCD, meanwhile, point to the vmPFC (Apergis-Schoute et al., 2017).

I provided evidence of human reversal learning impairments following serotonin depletion, in both the instrumental and Pavlovian domains, across two independent experiments. Deficits in both domains were underscored by significant correlations showing that a greater extent of depletion, as assessed by plasma samples, was associated with more pronounced reversal impairments. Strikingly, the results align with data from neurotoxic serotonin depletion in experimental animals (Bari et al., 2010; Clarke et al., 2004, 2007; Walker et al., 2009), stress induction in humans (Raio et al., 2017) and rats (Lapiz-Bluhm et al., 2009), and individuals with OCD (Apergis-Schoute et al., 2017). The present results advance knowledge on the neurochemical basis of emotional and behavioural flexibility, which has implications for the understanding and treatment of numerous clinical conditions including OCD.

Chapter 6

Reinforcement learning under tryptophan depletion: role of traits and symptoms

6.1 Introduction

Whereas the preceding chapter examined how acute tryptophan depletion (ATD) modulated instrumental reversal learning on a deterministic schedule, and aversive Pavlovian reversal learning, this chapter introduces a third variant of reversal learning paradigms: probabilistic reversal learning (PRL). Serotonin is critically involved in processing aversive outcomes (Bari et al., 2010; Dayan and Huys, 2009; Deakin, 2013) and adapting behaviour as circumstances change (Lapiz-Bluhm et al., 2009; Rygula et al., 2015). PRL models both: Individuals learn through trial and error the most adaptive action in an acquisition stage, and this rule changes in a reversal phase (Chamberlain et al., 2006; den Ouden et al., 2013; Evers et al., 2005; Murphy et al., 2002; Rygula et al., 2015; Skandali et al., 2018). Severe serotonin depletion via neurotoxins impairs the ability to update actions upon reversal (Bari et al., 2010; Clarke et al., 2004, 2007) and increases sensitivity to negative feedback (SNF) in rats (Bari et al., 2010) and marmoset monkeys (Rygula et al., 2015). SNF is defined here as switching behaviour following spurious negative feedback ("lose-shift"), which should be ignored; increased SNF thus causes subjects to choose the less rewarded stimulus, maladaptively. Indeed, individuals with a diagnosis of depression demonstrate elevated lose-shift/SNF (Murphy et al., 2003; Taylor Tavares et al., 2008). SNF is additionally increased following single dose administration of selective serotonin reuptake inhibitors (SSRIs) in rats (Bari et al., 2010) and healthy humans (Chamberlain et al., 2006; Skandali et al., 2018). These SSRI data are interpreted as paradoxical lowering of serotonin (Bari et al., 2010; Chamberlain et al., 2006; Skandali et al., 2018), presumed to parallel effects following acute tryptophan depletion (ATD), which lowers

serotonin function (Evers et al., 2005; Hood et al., 2005; Murphy et al., 2002). Whilst studies of healthy humans have not found effects of ATD on choice in PRL, their samples have been small and studied only one sex: Evers et al. (2005) included 12 males whereas Murphy et al. (2002) enrolled 12 females. Here I studied a large sample of healthy volunteers, male (n = 33; 17 placebo, 16 ATD) and female (n = 29; 15 placebo, 14 ATD), to better determine whether ATD modulates choice behaviour in PRL (Kanen et al., 2020c). Whereas Evers et al. (2005) and Murphy et al. (2002) used within-subjects designs, here I employed a between-subjects design to avoid practice effects and thus better assess learning.

Previous ATD studies of PRL, furthermore, assessed behaviour using conventional methods and classical statistics (Evers et al., 2005; Finger et al., 2007; Murphy et al., 2002), as they were published before the application of computational modelling of reinforcement learning processes using Bayesian statistics became a more widespread analytical approach (e.g. Huys et al., 2016; Kanen et al., 2019; Rygula et al., 2015). Given previously reported null results (Evers et al., 2005; Finger et al., 2007; Murphy et al., 2002), there is precedent to suspect ATD may not affect the conventional PRL measures of win-stay, lose-shift (SNF), or perseveration. Measures such as win-stay and lose-shift assess sensitivity to immediate reinforcement and do not account for how feedback history is integrated across multiple experiences to influence behaviour (Kanen et al., 2019; Rygula et al., 2015). I was therefore interested in testing whether ATD modulates computations supporting learning beyond the influence of immediate feedback, mechanisms not detectable with conventional measures. In particular, it was assessed whether ATD affects the rate at which individuals learn from reinforcement; whether this differs for positive (rewarding) or negative feedback (nonreward/punishment); and if ATD modulates the extent to which behaviour is driven by the intrinsic tendency to stay or shift, irrespective of the reinforcement environment. The latter tendency is captured by a "stimulus stickiness" parameter, which invokes the notion of whether or not individuals got "stuck" to a stimulus regardless of the result of choosing it. Indeed, this modelling approach uncovered deficits in marmosets during PRL following neurotoxic serotonin depletion, in addition to increased lose-shift/SNF (Rygula et al., 2015). Because serotonin depletion in marmosets led to alterations in stimulus stickiness, and not learning rates among other effects, here I predicted the stimulus stickiness parameter may be affected by ATD in humans.

More recently, an analogous modelling approach revealed learning rate and stimulus stickiness aberrations in obsessive compulsive disorder (OCD) and stimulant use disorder (SUD) (Kanen et al., 2019), beyond what was detectable via conventional means (Ersche et al., 2011). Whilst this is reported on in Chapter 8, Kanen et al. (2019) motivated another set of empirical questions tested here. Individuals with OCD, perhaps counterintuitively, demonstrated diminished perseverative tendencies during PRL (lower stimulus stickiness; Kanen et al., 2019). In

other words, people with OCD showed increased shifting of their choice from what they had done previously, regardless of the outcome of their actions. Is this a manifestation of checking behaviour – i.e. checking the unchosen option? Could intolerance of uncertainty be the driver? Or could this be related to symptoms of anxiety or depression? Here I addressed these questions in healthy volunteers by testing correlations between stimulus stickiness and self-reported obsessive-compulsive symptoms, intolerance of uncertainty, anxiety, and depressive symptoms.

Rygula et al. (2015) additionally showed that impairments in PRL after serotonin depletion in marmosets, assessed by win-stay and lose-shift, were best explained by a reduction in reinforcement sensitivity indicative of a reduced impact of anticipating rewarding and punishing outcomes on behaviour. As this is reminiscent of the overall blunting of affect seen in depression, I also addressed whether elevated symptoms of depression in healthy volunteers was related to diminished reinforcement sensitivity, or whether obsessive-compulsive symptoms, intolerance of uncertainty, and anxiety were instead related.

Kanen et al. (2019) additionally found that individuals with SUD, a characteristically impulsive population (Ersche et al., 2010), showed blunted reward learning (lower learning rates for positive feedback) yet elevated learning rates for negative feedback (nonreward/punishment) during PRL. I therefore tested whether self-reported impulsivity in healthy individuals was likewise correlated with a lower reward learning rate and an elevated punishment learning rate. This chapter addresses the following questions. By using a substantially larger sample size than in previous studies, does ATD enhance SNF or perseveration (conventional measures)? Does ATD modulate latent reinforcement learning mechanisms, in particular stimulus stickiness? Is stimulus stickiness related to intolerance of uncertainty, anxiety, depressive, or obsessive-compulsive symptoms, in a non-clinical sample? How does reinforcement sensitivity relate to these self-report measures? Is trait impulsivity correlated with learning rate parameters? Are any such relationships influenced by ATD?

6.2 Methods

6.2.1 Probabilistic reversal learning task

The task (Chamberlain et al., 2006; Murphy et al., 2002; Skandali et al., 2018) is shown in Figure 6.2 and contained 80 trials: 40 during acquisition and 40 following reversal. For the first 40 trials, one option yielded positive feedback on 80% of trials, the other option on 20% of trials. These contingencies reversed for the latter 40 trials. Eight consecutive correct responses fulfilled the learning criterion. Participants were instructed according to Cools et

al. (2002) and Skandali et al. (2018) as follows: "On each go, the same two patterns will be presented. One of the patterns is correct and the other pattern is wrong and you have to choose the correct pattern on each go. However on some goes, the computer will tell you that you were wrong even if you chose the correct pattern. Your task is to stick to the pattern that is usually correct. So in other words always choose the pattern that is correct more often than the other pattern. At some point during the task, the rule may change so that the other pattern is now usually correct. You then have to follow this new rule and choose the new pattern. It is important that you only start choosing the other pattern when you are sure that the rule has changed." SNF was the primary outcome measure, defined as the observed probability of behaviour switching away from the correct stimulus, following the delivery of spurious negative feedback. I likewise conducted planned comparisons on proportions for win-stay and lose-shift separately for spurious and veracious feedback, and for each phase (Skandali et al., 2018) reported in Figure 6.1. I calculated two measures of perseveration: immediately following reversal (Murphy et al., 2002) and across the reversal phase (den Ouden et al., 2013).

6.2.2 Computational modelling of behaviour

6.2.2.1 Overview

These methods are based on Kanen et al. (2019). Four reinforcement learning (RL) models were fitted to the behavioural data, which incorporated parameters that have been studied previously, using a hierarchical Bayesian method (Kanen et al., 2019). This analysis was conducted in collaboration with Qiang Luo. The priors used for each parameter are shown in Table 6.2.2.1. Trials were sequenced across all 80 trials of the PRL task, and on each trial the computational model was supplied with the participant's identification number and drug condition, whether the trial resulted in positive or negative feedback, and which stimuli were presented. Parameter recovery for this modelling approach has been confirmed previously (Kanen et al., 2019).

6.2.2.2 Models

Model 1 incorporated three parameters and was used to test the hypothesis that ATD would affect how positive versus negative feedback guides behaviour. Separate learning rates for positive feedback (reward) α^{rew} and negative feedback (nonreward/punishment) α^{pun} were implemented. Positive reinforcement led to an increase in the value V_i of the stimulus i that was chosen, at a speed governed by the reward rate α^{rew}, via $V_{i,t+1} \leftarrow V_{i,t} + \alpha^{rew}(R_t - V_{i,t})$. R_t represents the outcome on trial t (defined as 1 on trials where positive feedback occurred), and

Phase	Measure	Feedback	Placebo	Depletion	*p*	Cohen's *d*
Acquisition	Correct responses		38.63 (3.2)	39.27 (1.82)	.34	.25
	Trials to criterion		10.59 (6.76)	9.83 (4.63)	.61	.13
	Win–stay	Veracious	.97 (.07)	.98 (.04)	.326	.18
	Lose–shift	Spurious	.06 (.16)	.03 (.1)	.478	.22
Reversal	Correct responses		33.44 (4.7)	34.13 (4.06)	.536	.16
	Trials to criterion		16.63 (8.57)	15.23 (7.6)	.502	.17
	Win–Stay	Veracious	.93 (.13)	.96 (.1)	.331	.26
	Lose–Shift	Spurious	.11 (.19)	.12 (.17)	.889	.06
	Perseveration, immediate		3.69 (2.1)	3.97 (2.0)	.594	.137
	Perseveration, phase		3.81 (3.68)	3.67 (3.0)	.865	.042
Blood	TRP:LNAA (1)		.11 (.02)	.10 (.02)	.239	.50
	TRP:LNAA (2)		.11 (.03)	.005 (.004)	$\mathbf{4.96 \times 10^{-20}}$	4.9
	Valine (1)		225 (45.10)	230 (46.56)	.662	.11
	Valine (2)		841.30 (238.69)	820.86 (218.96)	.733	.09
	Methionine (1)		29.60 (4.98)	27.62 (6.44)	.191	.34
	Methionine (2)		67.70 (23.97)	63.07 (20.71)	.431	.21
	Isoleucine (1)		64.60 (17.47)	66.14 (17.66)	.738	.09
	Isoleucine (2)		218.13 (92.84)	218.34 (129.83)	.994	.002
	Leucine (1)		128.23 (27.82)	126.76 (30.26)	.846	.05
	Leucine (2)		344.37 (134.99)	342.90 (137.30)	.967	.01
	Tyrosine (1)		67.27 (12.50)	66.45 (18.53)	.843	.05
	Tyrosine (2)		170.40 (41.242)	196.55 (82.14)	.132	.40
	Phenylalanine (1)		59.33 (8.53)	58.93 (9.54)	.865	.04
	Phenylalanine (2)		111.07 (43.94)	120.07 (63.98)	.530	.16
	Tryptophan (1)		61.97 (9.63)	59.21 (9.80)	.280	.28
	Tryptophan (2)		201.53 (77.05)	7.83 (5.33)	$\mathbf{2.68 \times 10^{-14}}$	3.55

Figure 6.1: **Top**: Summary of measures of choice behaviour, planned contrasts assessed by t-test. **Bottom**: Blood results. (1) signifies baseline values; (2) denotes results from sample taken after approximately 4.5 hours. TRP:LNAA is the ratio between tryptophan and all large neutral amino acids, thought to be most reflective of brain serotonin (Hood et al., 2005). Emboldened values indicate statistical significance at $p < .05$. From Kanen et al. (2020c), with permission.

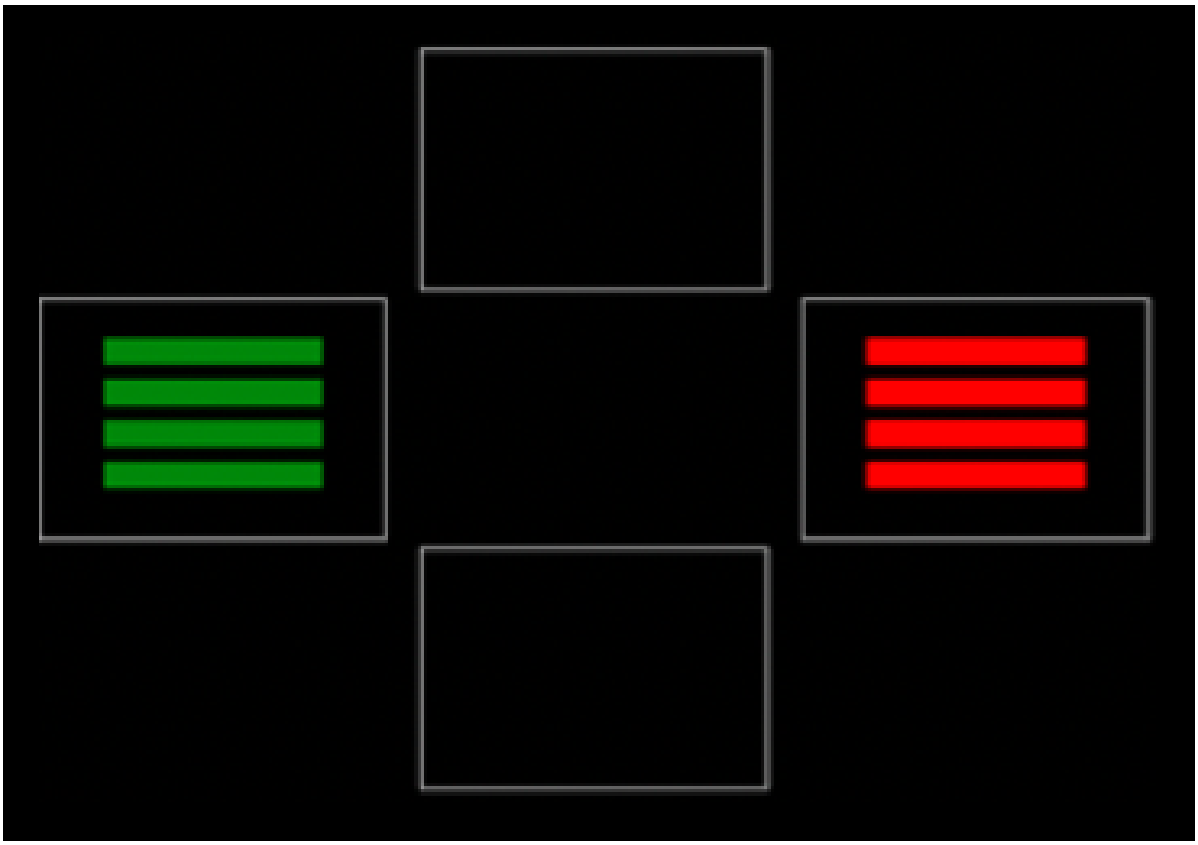

Figure 6.2: Probabilistic reversal learning task schematic. Two stimuli (red and green) appeared in two of four randomised locations on a touchscreen computer. Participants had to learn through trial and error the most adaptive action in the acquisition stage, and this rule changed in the reversal phase.

Model parameter prior distributions			**Reference**
	Models using each parameter	**Prior**	
Model parameters			
reward learning rate, α^{rew}	1, 2	Beta(1.2, 1.2)	den Ouden et al. (2013)
punishment learning rate, α^{pun}	1, 2	Beta(1.2, 1.2)	den Ouden et al. (2013)
combined reward/punishment learning rate, α^{reinf}	3	Beta(1.2, 1.2)	den Ouden et al. (2013)
reinforcement sensitivity, τ^{reinf}	1, 2, 3	Gamma(α=4.82, β=0.88)	Gershman (2016)
stimulus stickness, τ^{stim}	2, 3	Normal(0, 1)	Christakou et al. (2013)
experience decay factor, ρ	4	Beta(1.2, 1.2)	den Ouden et al. (2013)
decay factor for previous payoffs, φ	4	Beta(1.2, 1.2)	den Ouden et al. (2013)
softmax inverse temperature, β	4 [note that β = 1 in all other models]	Gamma(α=4.82, β=0.88)	Gershman (2016)
Intersubject variability in parameters			
Intersubject standard deviations for α^{rew}, α^{pun}, α^{reinf}, τ^{loc}, ρ , φ	As above	Half-normal: Normal(0, 0.05) constrained to $\geq$0	Kanen et al. (2019)
Intersubject standard deviations for τ^{reinf}, β	As above	Half-normal: Normal(0, 1) constrained to $\geq$0	Gershman (2016) but altered from Cauchy to half-normal as per Stan recommendations (Stan Development Team; http://mc-stan.org/)

Table 6.1: *rew* reward, *pun* punishment, *reinf* reinforcement, *loc* location, *stim* stimulus.

$(R_t - V_{i,t})$ the prediction error. On trials where negative feedback occurred $R_t = 0$, which led to a decrease in value of V_i at a speed governed by the punishment rate α^{pun}, according to $V_{i,t+1} \leftarrow V_{i,t} + \alpha^{pun}(R_t - V_{i,t})$. Stimulus value was incorporated into the final quantity controlling choice according to $Q^{reinf}{}_t = \tau^{reinf} V_t$. The additional parameter τ^{reinf}, termed reinforcement sensitivity, governs the degree to which behaviour is driven by reinforcement history. The quantities Q associated with the two available choices, for a given trial, were then input to a standard softmax choice function to compute the probability of each choice:

$$P(action_a) = softmax^a_\beta(Q_1 ... Q_n) = \frac{e^{\beta Q_a}}{\sum_{k=1}^{n} e^{\beta Q_k}}$$

for n = 2 choice options. The probability values for each trial emerging from the softmax function (the probability of choosing stimulus 1) were fitted to the subject's actual choices (did the subject choose stimulus 1?). Softmax inverse temperature was set to $\beta = 1$, and as a result the reinforcement sensitivity parameter (τ^{reinf}) directly represented the weight given to the exponents in the softmax function.

Model 2 was as model 1 but incorporated a "stimulus stickiness" parameter τ^{stim}, which measures the tendency to repeat a response to a specific perceptual stimulus, irrespective of the action's outcome. This four-parameter model served to test whether accounting for stimulus-response learning, in addition to learning about action-outcome associations, would best characterise behaviour under ATD. The stimulus stickiness effect was modelled as $Q^{stim}{}_t = \tau^{stim} s_{t-1}$, where s_{t-1} was 1 for a stimulus that was chosen on the previous trial and was otherwise 0. The final quantity controlling choice incorporated this additional parameter as $Q_t = Q^{reinf}{}_t + Q^{stim}{}_t$. Quantities Q, corresponding to the two choice options on a given trial, were then fed into the softmax function as above.

Model 3 incorporated three parameters and served to test whether a single learning rate α^{reinf}, rather than separate learning rates for positive and negative feedback, optimally characterised behaviour under ATD. Positive reinforcement led to an increase in the value V_i of the stimulus i that was chosen, at a speed controlled by the reinforcement rate α^{reinf}, via $V_{i,t+1} \leftarrow V_{i,t} + \alpha^{reinf}(R_t - V_{i,t})$. R_t represents the outcome on trial t (defined as 1 on trials where positive feedback occurred), and $(R_t - V_{i,t})$ the prediction error. On trials where negative feedback (nonreward/punishment) occurred $R_t = 0$, which led to a decrease in value of V_i. Model 3 also included the stimulus stickiness parameter. The final quantity controlling choice was determined by $Q_t = Q^{reinf}{}_t + Q^{stim}{}_t$.

Model 4 took a different approach, and had three parameters: φ (phi), ρ (rho), and β (beta). Derived from the experienced-weighted attraction model [EWA] (Camerer and Ho, 1999), here it was implemented as in den Ouden et al. (2013) where the EWA model best described behaviour on a nearly identical task. A key difference to the other reinforcement learning

models tested in this study is that here the learning rate can decline over time, governed by a decay factor ρ (rho). The EWA model weighs the value of new information against current expectations or beliefs, accumulated from previous experience.

Learning from reinforcement is modulated by an "experience weight", $n_{c,t}$, which is a measure of how often the subject has chosen a stimulus (i.e. experienced the action), and is updated every time the stimulus is chosen (where c is choice and t is trial) according to the experience decay factor ρ (range $0 < \rho < 1$) and can increase without bounds (den Ouden et al., 2013): $n_{c,t} \leftarrow n_{c,t-1}\ \rho + 1$. The value of a choice is updated according to the outcome, λ, and the decay factor for previous payoffs, φ [range $0 < \varphi < 1$] (den Ouden et al., 2013).

$$v_{c,t} \leftarrow (v_{c,t-1}\ \varphi\ n_{c,t-1} + \lambda_{t-1}) / n_{c,t}$$

The payoff decay factor φ (phi) is related to a Rescorla–Wagner-style (Rescorla and Wagner, 1972) learning rate α (as in Models 1-3), by $\alpha = 1 - \varphi$. A high value of φ means that stimuli keep a high fraction of their previous value and thus learning from reinforcement is slow. When ρ is high, then "well-known" actions (with high n) are updated relatively little by reinforcement, by virtue of the terms involving n, whilst reinforcement has a proportionately larger effect on novel actions (with low n). For comparison to Models 1-3, when $\rho = 0$, the experience weight n, is 1, which reduces to a learning rate α controlling the influence of learning from prediction error. Choice in the EWA model is also governed by a softmax process, only here the softmax inverse temperature β was also a parameter able to vary, in contrast to Models 1-3.

6.3 Results

6.3.1 Participant characteristics and blood results

There were no differences between groups in age, years of education, depressive symptoms, or trait anxiety (all $p > .05$). I achieved a robust depletion of tryptophan ($t(49) = -17.726$, $p = 4.857 \times 10^{-23}$; degrees of freedom (df) were adjusted after Levene's test showed unequal variances; it was not possible to obtain blood samples from 3 participants), without affecting mood ($t(55) = -1.341$, $p = .186$; data unavailable for 5 participants).

6.3.2 Conventional analysis of behaviour

ATD did not affect the core measures of choice behaviour in PRL, summarised in Figure 6.1. After placebo, 31/32 participants attained criterion performance in acquisition; after ATD, 30/30 (Fisher's Exact Test, $p = 1$). On placebo, 29/32 participants reached reversal criterion,

Rank	Name	Parameters	log marginal likelihood	log posterior P(model)
4	Model 1	α^{rew}, α^{pun}, τ^{reinf}	-3438.016	-2181.1517
1	Model 2	α^{rew}, α^{pun}, τ^{reinf}, τ^{stim}	-1257.556	-0.6911
2	Model 3	α^{reinf}, τ^{reinf}, τ^{stim}	-1257.560	-0.6952
3	Model 4	ρ, φ, β	-3437.999	-2181.1341

Table 6.2: Model comparison. *rew* reward, *pun* punishment, *reinf* reinforcement, *stim* stimulus.

28/30 on ATD (p = 1). Analysis of variance (ANOVA) with condition (placebo, ATD) and sex (male, female) as between-subjects factors, and phase (acquisition, reversal) as within-subjects factor revealed no effects of condition or sex on the number of correct responses ($F < 1.60$, $p > .05$, and $\eta_p^2 < .03$ for all terms involving ATD and sex); trials to criterion ($F < 3.10$, $p > .05$, and $\eta_p^2 < .055$); SNF ($F < 3.00$, $p > .05$, and $\eta_p^2 < .050$), shown in Figure 6.3; or win-stay to veracious feedback ($F < 3.10$, $p > .05$, and $\eta_p^2 < .055$). Perseveration was unaffected both immediately following reversal ($t(60) = .536$, $p = .594$, $d = .137$) and across the reversal phase ($t(60) = -.170$, $p = .865$, $d = .042$). Males and females did not differ on either immediate perseveration ($t(60) = 1.535$, $p = .130$, $d = .39$) or phase perseveration ($t(60) = .263$, $p = .793$, $d = .07$). There were no correlations between the extent of depletion and the key measures of interest: SNF, win-stay to veracious feedback, and either measure of perseveration (all $p > .05$). The conventional analyses presented in this chapter have been reported in Kanen et al. (2020c).

6.3.3 Choice of reinforcement learning model

The core modelling results are summarised in Figure 6.4. Computational modelling, unlike the conventional methods applied here thus far, accounts for how behaviour is influenced by an integration of previous choices and feedback history from multiple experiences. Behaviour in the present PRL task was best characterised by a simple reinforcement learning model, as determined by a bridge sampling estimate of the marginal likelihood (Table 6.2). Four reinforcement learning models were fitted and compared. Convergence was nearly perfect with all four models having R^ < 1.001. The winning model included four parameters: 1) positive reinforcement learning rate, the extent to which behaviour is driven by positive feedback learning; 2) nonreward/punishment reinforcement learning rate, the contribution of learning from negative feedback to behaviour; 3) reinforcement sensitivity, which is the degree to which overall outcome of behaviour contributes to choice (how heavily stimulus value learned through reinforcement is weighted); and 4) "stimulus stickiness" which indexes the tendency to get "stuck" to a cue: was the chosen stimulus selected on the previous trial, irrespective of outcome?

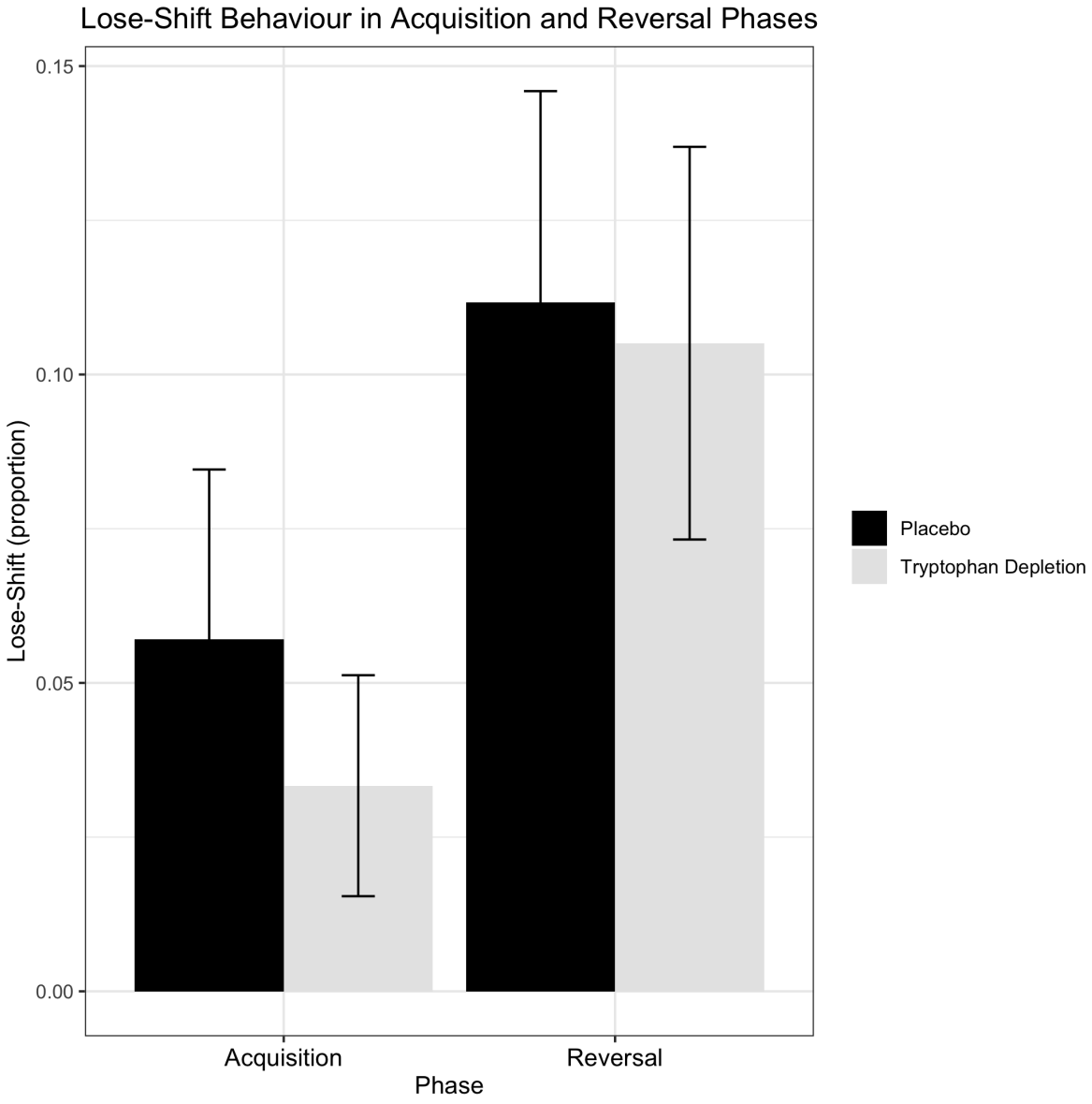

Figure 6.3: Sensitivity to negative feedback. Mean proportion of lose-shift behaviour (probability of shifting) following spurious negative feedback, plotted separately for acquisition and reversal, compared between placebo and ATD groups. Error bars: +/- 1 standard error.

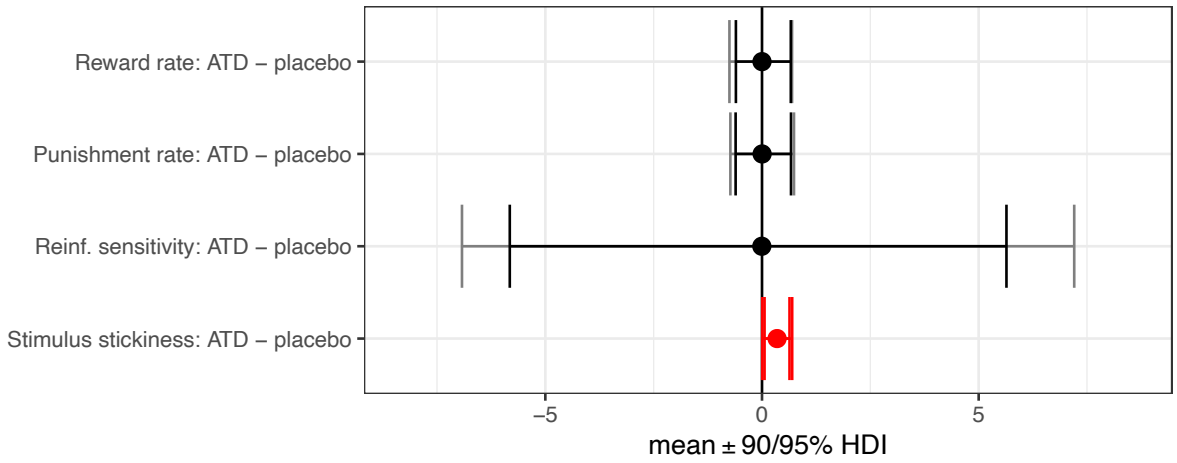

Figure 6.4: Effects of ATD relative to placebo. Enhanced stimulus stickiness under ATD. Red signifies a difference between the parameter per-condition mean according to the Bayesian "credible interval", $0 \notin 95\%$ HDI.

6.3.4 Basic modelling results

Modelling results are summarised in Figure 6.4. The key result was that stimulus stickiness was elevated under ATD compared to the placebo condition (difference in parameter per-condition mean, posterior 95% highest posterior density interval [HDI] excluding zero; $0 \notin 95\%$ HDI). There were no differences between placebo and ATD for the reward rate, punishment/nonreward rate, or reinforcement sensitivity parameters, which all had a 95% HDI containing zero ($0 \in 95\%$ HDI).

6.3.5 Relationship between obsessive-compulsive symptoms and stimulus stickiness

Results are summarised in Figure 6.5. OCD has been associated with diminished stimulus stickiness (Kanen et al., 2019). Here I sought to determine whether reduced stickiness in healthy volunteers is related to elevations in trait anxiety (Spielberger et al., 1983), depressive symptoms (Beck et al., 1996), or intolerance of uncertainty (Carleton et al., 2007), or whether it is instead related to symptoms of OCD in a non-clinical sample [Obsessive-Compulsive Inventory-Revised; OCI-R] (Foa et al., 2002). Stimulus stickiness was not correlated with intolerance of uncertainty ($r(62) = -.032$, $p = .804$), trait anxiety ($r(62) = -.142$, $p = .270$), or depressive symptoms ($r(62) = -.054$, $p = .674$), collapsing across serotonergic status. Stickiness was, however, significantly correlated with obsessive-compulsive (OC) symptoms ($r(62) = -.393$, $p = .002$), such that individuals with more OC symptoms demonstrated lower stimulus

stickiness, consistent with clinical data on OCD (Kanen et al., 2019). Next I asked whether the relationship between traits and stickiness was modulated by serotonergic status. Analysis of covariance (ANCOVA) was performed to test an interaction effect of serotonin and OC symptoms (between-subjects factors) on stimulus stickiness, controlling for main effects. A serotonin × OC interaction was not present, indicating that the relationship between total OCI-R score and stickiness was not modulated by serotonergic status ($F(1,61) = 2.239$, $p = .14$, $\eta_p^2 = .037$). Repeating this ANCOVA approach separately for intolerance of uncertainty, trait anxiety, and depressive symptoms confirmed there were no trait × serotonin interaction effects on stimulus stickiness ($F < 1.4$, $p > .05$, $\eta_p^2 < .03$ for all interaction terms). Because OCD and OC symptoms have numerous manifestations which can be assessed using subscales of tools like the OCI-R, I followed up the significant correlation between stickiness and total OCI-R score by exploring categories of symptoms, collapsed across serotonergic status. Lower stimulus stickiness was significantly correlated with higher scores of checking ($r(62) = -.392$, $p = .002$), ordering ($r(62) = -.261$, $p = .04$), neutralising ($r(62) = -.380$, $p = .002$), washing ($r(62) = -.304$, $p = .016$), and obsessing ($r(62) = -.352$, $p = .005$), but not hoarding ($r(62) = -.129$, $p = .319$). All five significant correlations survived correction for six (subscale) comparisons via the Benjamini-Hochberg method (see Chapter 2). These correlations, furthermore, held up when controlling for BDI, STAI, and IUS (all $p < .05$). Partial correlation analyses, moreover, showed that checking was not the dominant correlation: controlling for washing, for instance, rendered the checking correlation not significant. These relationships were impervious to experimental changes in serotonin, as determined via ANCOVA: interaction effects, between serotonin status and each subscale, on stimulus stickiness were absent for all subscales ($F < 3$, $p > .05$, $\eta_p^2 < .05$ for all interaction terms). This converges with evidence from individuals with OCD, who show aberrantly low stickiness despite serotonergic medication (Kanen et al., 2019).

6.3.6 Relationship between reinforcement sensitivity and self-report

Results are shown in Figure 6.6. Collapsing across serotonergic status, diminished reinforcement sensitivity was correlated with depressive symptoms ($r(62) = -.295$, $p = .02$). The relationship between reinforcement sensitivity and depressive symptoms did not differ between placebo and ATD assessed via ANCOVA ($F = .508$, $p = .479$, $\eta_p^2 = .009$). When collapsing across serotonergic status, reinforcement sensitivity, meanwhile was not correlated with intolerance of uncertainty ($r(62) = -.236$, $p = .064$), trait anxiety ($r(62) = -.201$, $p = .117$), or OC symptoms ($r(62) = -.104$, $p = .42$). The significant correlation between elevated depressive symptoms and diminished reinforcement sensitivity survived the Benjamini-Hochberg procedure for four comparisons. I was then interested in exploring the relationship between

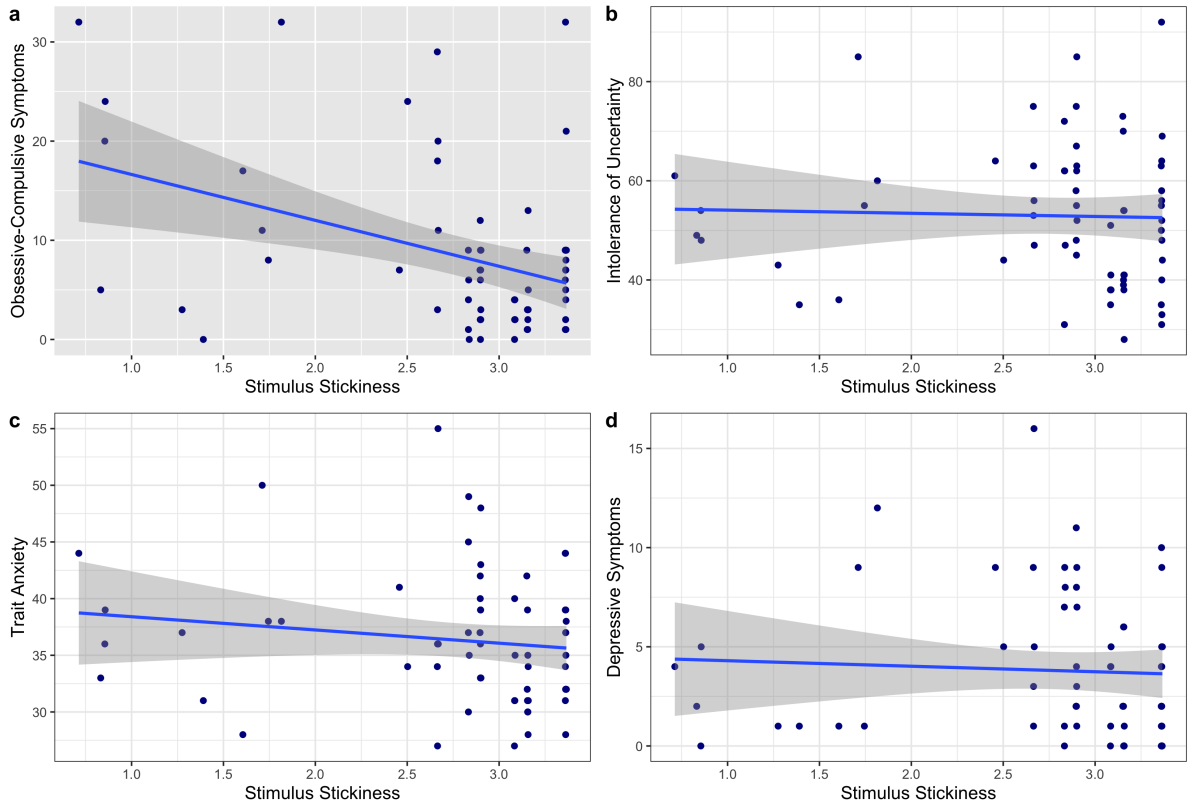

Figure 6.5: **(a)** Elevated obsessive-compulsive symptoms were correlated with lower stimulus stickiness; **(b)** intolerance of uncertainty, **(c)** trait anxiety , and **(d)** depressive symptoms were not correlated with stimulus stickiness. These data are collapsed across both the placebo and depletion groups. Grey background indicates a significant correlation; white background denotes lack of significance. The relationship between obsessive-compulsive symptoms in healthy volunteers and stimulus stickiness is consistent with the diminished stimulus stickiness observed by Kanen et al. (2019) in clinical OCD, which is a focus of Chapter 8.

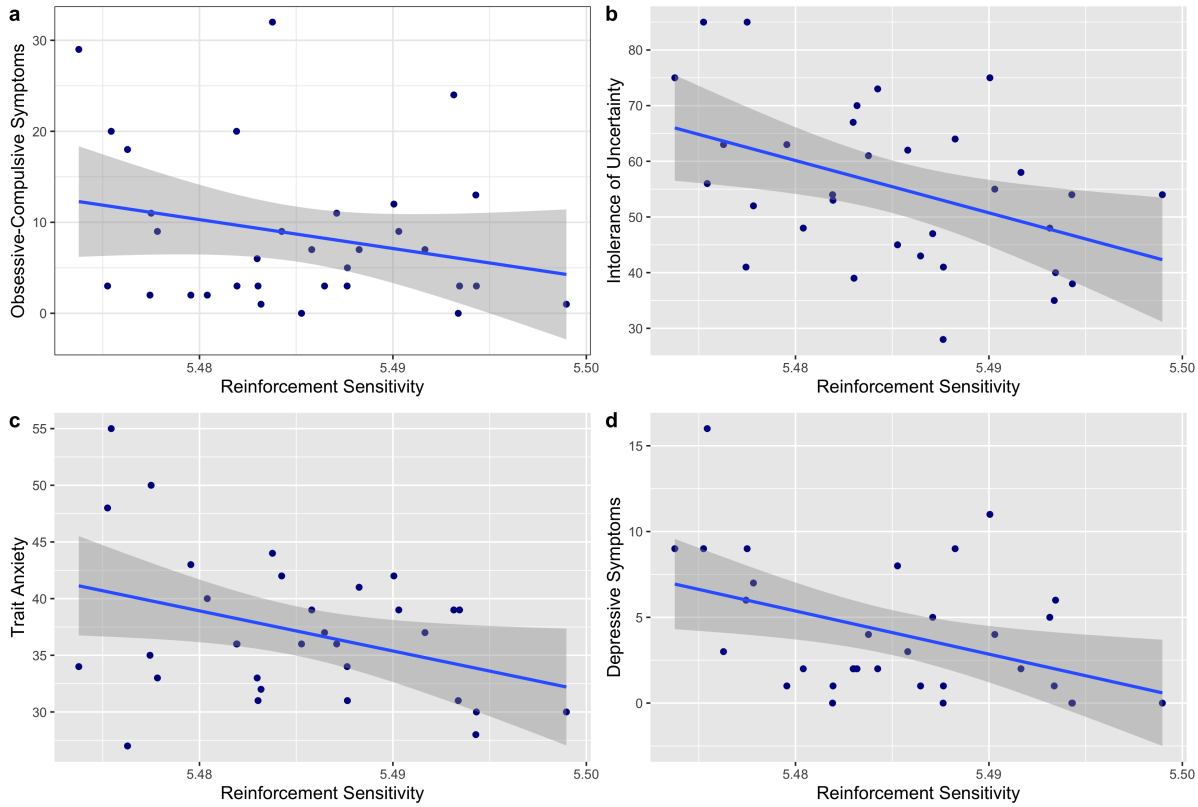

Figure 6.6: Under placebo, diminished reinforcement sensitivity was not related to **(a)** obsessive-compulsive symptoms, but was significantly correlated with **(b)** greater intolerance of uncertainty, **(c)** higher trait anxiety, and **(d)** elevated depressive symptoms.

individual subject parameter estimates and self-report measures in the placebo group only, to characterise these relationships at baseline, when unaffected by ATD, which may have been obscured by including all participants in the same analysis. When isolating the placebo group alone, lower stimulus stickiness remained correlated with elevated OCI-R scores ($r(32) = -.529$, $p = .002$), as was the case for diminished reinforcement sensitivity and elevated depressive symptoms ($r(32) = -.421$, $p = .016$). On placebo, lower reinforcement sensitivity was additionally correlated with greater intolerance of uncertainty ($r(32) = -.433$, $p = .013$) and higher trait anxiety ($r(32) = -.366$, $p = .04$), but not with OCI-R scores ($r(32) = -.246$, $p = .174$). These three significant correlations involving reinforcement sensitivity in the placebo group survived the Benjamini-Hochberg procedure for four comparisons.

6.3.7 Relationship between learning rates and trait impulsivity

Next, I was motivated to test the relationship between learning rates and trait impulsivity, assessed using the Barratt Impulsiveness Scale (BIS; Patton et al., 1995). Results are shown

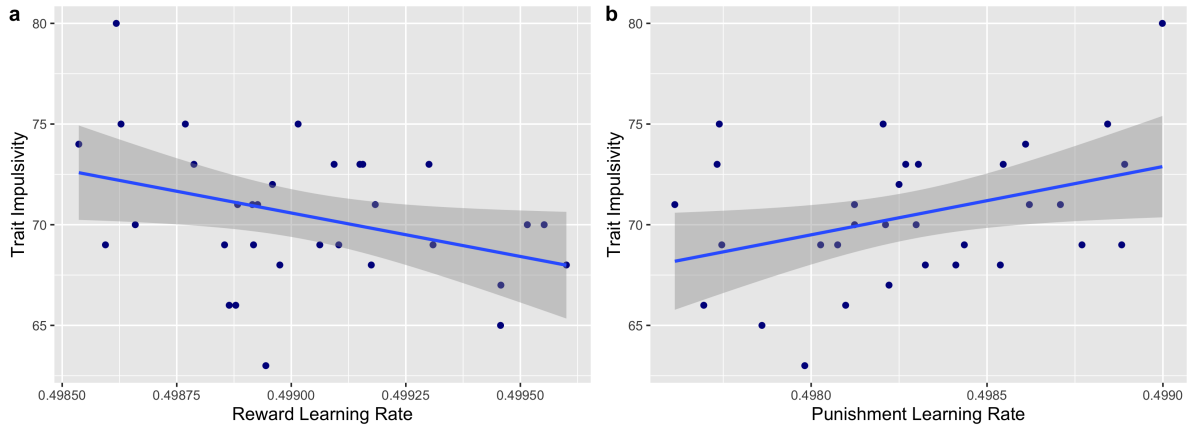

Figure 6.7: Under placebo, higher trait impulsivity was related to **(a)** diminished reward learning rate and **(b)** elevated punishment learning rate. This pattern is consistent with learning rates during PRL in stimulant use disorder (SUD), shown by Kanen et al. (2019), which is a focus of Chapter 8.

in Figure 6.7. This analysis was prompted by Kanen et al. (2019): individuals with stimulant use disorder (SUD), who are characteristically impulsive (Ersche et al., 2010), demonstrated diminished reward and elevated punishment learning rates during PRL. Indeed, analysis of healthy volunteers under placebo revealed that individuals higher in trait impulsivity showed a lower reward learning rate ($r(32) = -.363$, $p = .041$) and a higher punishment learning rate ($r(32) = .375$, $p = .034$). These two significant correlations in the placebo group survived the Benjamini-Hochberg procedure for two comparisons.

6.3.8 Relationship between model parameters and conventional measures

Correlations are summarised in Table 6.3. Correlations were tested between individual subject parameter values from the winning model and the following six conventional measures of behaviour: correct responses in acquisition, correct responses in reversal, proportion of win-stay overall, proportion of lose-shift overall, "immediate perseveration", and "phase perseveration" (see Methods). These tests were performed in the placebo and depletion groups separately. The results were subsequently subjected to the Benjamini-Hochberg procedure. Stimulus stickiness was the only parameter correlated with several conventional measures of behaviour after accounting for 48 comparisons. Significant correlations were followed up by testing whether the relationships were significantly different between the placebo and depletion groups. Stimulus stickiness was highly correlated with correct responses during both the acquisition phase when on placebo ($r(32) = .853$, $p = 5.78 \times 10^{-10}$) and following ATD ($r(30) =$

		Stimulus Stickiness, τ^{stim}
Acquisition	Placebo	↑↑
	ATD	↑
Reversal	Placebo	↑
	ATD	↑
Perseveration	Placebo	↓
	ATD	↓
Lose-Shift	Placebo	↓
	ATD	↓
Win-Stay	Placebo	↑
	ATD	↑↑

Table 6.3: Correlations between conventional behavioural measures and the stimulus stickiness parameter. ↑ denotes a significant correlation. ↑↑ denotes the correlation is significantly stronger compared to the other condition. All correlations displayed survived the Benjamini-Hochberg procedure for multiple comparisons (see section 6.3.8). The conventional measures shown here are correct responses in the acquisition phase, reversal phase; degree of phase (not immediate) perseveration; proportion of lose-shift; proportion of win-stay. *stim* stimulus.

.745, p = 2.0x10^{-6}). The relationship between stimulus stickiness and acquisition performance was significantly stronger under placebo compared to depletion: ANCOVA with serotonin status and stimulus stickiness as a between-subjects interaction term, controlling for main effects, revealed serotonin and stimulus stickiness interacted to modulate acquisition behaviour (F(1,58) = 7.225, p = .009, η_p^2 = .111). Stimulus stickiness was also highly correlated with correct responses in the reversal phase following both placebo (r(32) = .802, p = 3.45x10^{-8}) and ATD (r(30) = .761, p = 1 x 10^{-6}); however, this relationship was not modulated by serotonin status as determined using the same ANCOVA method (F(1,58) = .028, p = .868, η_p^2 = 4.8 x 10^{-4}). Stimulus stickiness was highly correlated with win-stay behaviour both on placebo r(32) = .91, p = 2.44x10^{-18}) and under ATD (r(30) = .946, p = 3.42x10^{-15}), and the correlation was significantly stronger following depletion (F(1,58) = 9.944, p = .003, η_p^2 = .146). Stimulus stickiness was negatively correlated with lose-shift both on placebo (r(32) = -.940, p = 1.50x10^{-15}) and following ATD (r(30) = -.943, p = 6.43x10^{-15}). The relationship between stimulus stickiness and lose-shift was not significantly different between placebo and ATD: ANCOVA showed no stimulus stickiness × serotonin interaction (F(1,58) = .014, p = .905, η_p^2 = 2.5x10^{-4}). Stimulus stickiness was negatively correlated with phase perseveration under both placebo (r(32) = -.554, p = .001) and ATD (r(30) = -.508, p = .004), and these relationships were not significantly different from one another (F(1,58) = .085, p = .771, η_p^2 = .001).

6.3.9 Relationship between model parameters and extent of depletion

Individual subject values of each of the four parameters from the winning model were tested for correlation with the extent of depletion achieved. None of the four parameters were correlated with the change in the TRP:LNAA ratio: reward rate ($r(59) = -.241$, $p = .065$), punishment rate ($r(59) = .151$, $p = .253$), reinforcement sensitivity ($r(59) = -.023$, $p = .861$), and stimulus stickiness ($r(59) = -.057$, $p = .666$).

6.4 Discussion

This study has shown that 1) ATD did not affect the core measures of PRL choice behaviour, as assessed by conventional variables and analyses (Kanen et al., 2020c); 2) computational modelling revealed that ATD increased stimulus stickiness; 3) diminished stimulus stickiness was related to elevated obsessive-compulsive symptoms and not to depressive symptoms, trait anxiety, or intolerance of uncertainty; 4) diminished stimulus stickiness was correlated with all obsessive-compulsive subscales (checking, washing, etc.) except for hoarding; 5) diminished reinforcement sensitivity was related to elevated depressive symptoms, trait anxiety, and intolerance of uncertainty, but not obsessive-compulsive symptoms; and 6) higher trait impulsivity was related to diminished reward and enhanced punishment learning rates.

By nearly tripling the sample size and testing both sexes I considerably extended previous efforts to capture the effects of ATD on PRL and replicated null results on choice using classical statistics and conventional indices of behaviour (Evers et al., 2005; Murphy et al., 2002). I tested additional observable variables using classical statistics, beyond those reported in the previous ATD PRL studies, with no change in conclusions. This contrasts with other serotonergic challenges that have modulated PRL – and SNF in particular – in healthy humans (Chamberlain et al., 2006; Skandali et al., 2018), rats (Bari et al., 2010), and marmoset monkeys (Rygula et al., 2015). The discrepancy is likely due to both differences in the magnitude of change – ATD is mild in comparison to neurotoxic depletion via 5,7-dihydroxytryptamine in rats and marmosets – and the regions preferentially affected. Rygula et al. (2015), for instance, were able to specifically deplete serotonin neurotoxically in the marmoset amygdala or orbitofrontal cortex (OFC), and showed serotonin was required in both structures for normal PRL performance. The implication, in conjunction with previous studies, is that under certain task demands ATD does not necessarily produce effects on conventional measures that parallel acute SSRI or neurotoxic serotonin depletion. It cannot be concluded from the null results that serotonin is uninvolved in processes assayed by conventional means or that ATD did not affect serotonin (Faulkner and Deakin, 2014). It is possible that the feedback employed in this task was not sufficiently salient to elicit behavioural changes following ATD with conventional

measures. Even though the instructions given were based on previous studies (e.g. Skandali et al., 2018 who showed an effect of SSRI), it is possible that the instructions were too explicit (see Methods), making feedback excessively predictable and therefore even less salient. In future, it may be beneficial to simply tell participants "On each go, you must choose one of the two coloured patterns and learn through trial and error which is the best option. The best option may change at some point during the task."

It was reasoned, however, that latent mechanisms underlying learning might be more sensitive to ATD, and computational models of reinforcement learning were therefore applied to the data. The initial motivation to take this approach came from Rygula et al. (2015), who fit analogous models to PRL data in marmosets and found impairments following neurotoxic serotonin depletion. Indeed, computational methods as used in Rygula et al. (2015) revealed, for the first time to my knowledge, an effect of ATD on the latent mechanisms of PRL in healthy humans. The main result on how ATD modulated PRL was that depletion enhanced stimulus stickiness. This is consistent with the findings of Rygula et al. (2015) following neurotoxic serotonin depletion in the marmoset OFC: a variant of the stimulus stickiness parameter was enhanced. That ATD was associated with enhanced stimulus stickiness is also consistent with another study of healthy humans showing, under ATD, elevated choice perseveration on a four-choice task assessed with a different model (Seymour et al., 2012). Rygula et al. (2015) additionally found a decrease in their stimulus stickiness variant, following serotonin depletion in the amygdala, which may be important for understanding the absence of an ATD effect on SNF. Increased SNF in depression, on the one hand, is mediated by amygdala hyperactivity (Taylor Tavares et al., 2008), yet Evers et al. (2005) reported no effect of ATD on the amygdala during PRL. It could be that ATD in health does not sufficiently modulate relevant circuits involving the amygdala to manifest in enhanced SNF, as conventionally assessed in PRL. That Evers et al. (2005) did not report on amygdala is also consistent with the present observation of elevated stimulus stickiness, which may be more related to impaired serotonin function in the OFC (Rygula et al., 2015). That perseverative tendencies were enhanced in the present computational analysis (stimulus stickiness) suggests conventional measures of perseveration may not be sensitive enough to ATD.

Computational modelling not only allowed for a cross-species comparison, but also enabled a comparison with clinical populations reported by Kanen et al. (2019). The elevation of stimulus stickiness in healthy volunteers under ATD is consistent with enhanced stimulus stickiness in individuals with SUD (Kanen et al., 2019). Greater obsessive-compulsive symptoms in healthy volunteers were correlated with lower stimulus stickiness, as in individuals with clinically diagnosed OCD reported by Kanen et al. (2019). That I was able to replicate the OCD result from Kanen et al. (2019) in healthy volunteers with high OC symptoms

further validates the modelling approach undertaken here despite null results on conventional measures. It is worth noting that clinical severity of OCD in Kanen et al. (2019) was not correlated with stimulus stickiness.

Having established the relationship between diminished stimulus stickiness and obsessive-compulsive symptoms in the present healthy sample, I was able to address a series of follow up questions. Kanen et al. (2019) conjectured that the tendency in OCD during PRL to not repeat previous choices could be a manifestation of checking behaviour – checking the previously unchosen option. Here I was able show that it was not only the "checking" subscale of the OCI-R that was correlated with stimulus stickiness, but that all other subscales (neutralising, obsessing, ordering, washing) save for "hoarding" were correlated. Importantly, hoarding disorder is a distinct condition from OCD, albeit in the same DSM5 section: obsessive-compulsive and related disorders (American Psychiatric Association, 2013). That hoarding was not related to diminished stimulus stickiness indicates that this parameter may be an important novel objective measure for distinguishing other related conditions such as body dysmorphic disorder, excoriation (skin-picking) disorder, and trichotillomania [hair-pulling disorder] (American Psychiatric Association, 2013). The significant correlations involving OC symptoms were impervious to changes in serotonin, which is consistent with Kanen et al. (2019): individuals with OCD showed diminished stimulus stickiness despite the fact that most were taking SSRIs. Whilst I also clarified that the stickiness parameter was not related to intolerance of uncertainty, anxiety, or depressive symptoms (regardless of serotonergic status), which I had charted as candidate phenomena underlying this behavioural pattern, the question remains: What does reduced stimulus stickiness mean for understanding OCD?

In subsequent analyses I primarily focused on the placebo group in order to assess baseline relationships between model parameters and self-report measures. There was a striking dissociation in how reinforcement sensitivity as opposed to stimulus stickiness related to self-report measures: Blunted reinforcement sensitivity was related to depressive symptoms, as well as trait anxiety and intolerance of uncertainty, but not to OC symptoms. Remarkably, neurotoxic serotonin depletion of marmosets – in either the amygdala or OFC – resulted in the same pattern: diminished reinforcement sensitivity.

The learning rates of impulsive healthy volunteers, furthermore, aligned with what has been reported in SUD (lower reward and higher punishment learning rates; Kanen et al., 2019), providing additional replications between the non-clinical sample studied here, and clinical populations. It also indicates that alterations in learning rates may precede and potentially predispose to problematic use of stimulants like cocaine and methamphetamine, rather than being a consequence of drug toxicity. That impulsivity is a behavioural endophenotype, or vulnerability trait for stimulant abuse, found also in first-degree unaffected relatives (Ersche

et al., 2010) raises the possibility that learning rates assessed using computational modelling may represent an additional endophenotype warranting further study. It is advantageous that learning rates are objective measures that can be assessed across paradigms, populations, and species.

The present study has, for the first time, identified latent mechanisms supporting behaviour that are modulated by ATD during PRL. That computational methods unearthed effects in the face of null results on conventional measures highlights the sensitivity and promise of applying reinforcement learning models. ATD enhanced a basic perseverative tendency which appears to align with related effects in marmosets following serotonin depletion (Rygula et al., 2015), results from a clinical population (Kanen et al. 2019), and findings from another ATD study in healthy humans (Seymour et al., 2012). Considering a series of self-report measures in a hypothesis-driven approach yielded several important clinically relevant insights. Behavioural patterns in clinical OCD and SUD were replicated using analogous methods in healthy volunteers high in OC symptoms and trait impulsivity, respectively. Applying reinforcement learning models to PRL may hold promise for refining psychiatric classification and identifying vulnerability factors to an array of psychopathologies, including disorders of compulsivity.

Chapter 7

Reinforcement learning under LSD

7.1 Introduction

Research into lysergic acid diethylamide (LSD) as a potential therapeutic agent in psychiatry has been revitalised in recent years (Carhart-Harris and Friston, 2019). Few studies, however, have focused on its behavioural effects in humans. The premise for how psychedelic drugs such as LSD may exert beneficial effects on mental health revolves around learning (Carhart-Harris and Nutt, 2017). LSD acts principally but not exclusively as an agonist at the serotonin 2A (5-HT2A) receptor (Marona-Lewicka and Nichols, 2007; Marona-Lewicka et al., 2005; Nichols, 2016). Indeed, blocking 5-HT2A receptors prevents the psychedelic effects of LSD (Nichols, 2016). The 5-HT2A receptor is involved in plasticity and represents a purported neurobiological mechanism by which the capacity for psychological change in relation to the psychedelic state could occur (Carhart-Harris and Nutt, 2017). Aberrations in 5-HT2A binding have furthermore been reported in numerous psychiatric conditions including depression (Bhagwagar et al., 2006), obsessive-compulsive disorder (OCD; Perani et al., 2008), and schizophrenia (Talvik-Lotfi et al., 2000). LSD, unlike other classic psychedelics such as psilocybin, additionally acts at dopamine type 2 (D2) receptors, among others (Marona-Lewicka and Nichols, 2007; Marona-Lewicka et al., 2005; Nichols, 2004). Indeed, dopamine is well known to play a fundamental role in learning from positive feedback and its omission (Schultz et al., 1997). Psychological change via LSD, salubrious or not, would require learning on some level; however, studies of human learning and cognitive flexibility using objective tests under psychedelic drugs are limited. Here trial and error learning was studied in healthy volunteers on LSD versus placebo and its latent structure was explored by applying reinforcement learning models.

Behaviourally, serotonin is critically involved in adapting behaviour flexibly as environmental circumstances change (Clarke et al., 2004), as well as processing aversive outcomes

(Bari et al., 2010; Chamberlain et al., 2006; Cools et al., 2008a; Dayan and Huys, 2009; Deakin, 2013). Both can be modelled in a laboratory setting using probabilistic reversal learning (PRL) paradigms: Individuals learn through trial and error the most adaptive action in an acquisition stage, and this rule eventually changes in a reversal phase (Lawrence et al., 1999). Profound serotonin depletion via neurotoxins in the marmoset orbitofrontal cortex (OFC) results in perseverative, stimulus-bound behaviour – an impaired ability to update action upon reversal (Clarke et al., 2004; Walker et al., 2009). At the same time, single dose administration of selective serotonin reuptake inhibitors (SSRIs), which can paradoxically lower serotonin concentration (Nord et al., 2013), result in an increased sensitivity to negative feedback in healthy humans (Chamberlain et al., 2006; Skandali et al., 2018) and rats (Bari et al., 2010). Neurotoxic dopamine depletion in the marmoset caudate nucleus, meanwhile, also induced perseveration (Clarke et al., 2011). Additionally, there is a body of evidence across species that D2-modulating agents affect instrumental reversal learning (Boulougouris et al., 2009; Kanen et al., 2019; Lee et al., 2007). LSD and 5-HT2A agonists in experimental animals, meanwhile, have also been shown to modulate associative learning (Harvey, 2003; Harvey et al., 1988; Romano et al., 2010; Schindler et al., 1986).

Indeed, deficits in behavioural flexibility cut across numerous traditional diagnostic categories including schizophrenia (Waltz and Gold, 2007), OCD, and stimulant use disorder (Kanen et al., 2019). Exaggerated negative feedback processing is likewise present across multiple disorders including depression (Murphy et al., 2003; Taylor Tavares et al., 2008) and OCD (Kanen et al. 2019). Here, for the first time, the acute effects of LSD on PRL were tested in a placebo-controlled study of healthy human volunteers. It was predicted that LSD would modulate either sensitivity to negative feedback, or how learning affects subsequent perseverative behaviour (den Ouden et al., 2013).

In addition to conventional analyses, I sought to explore latent mechanisms underlying behaviour by applying computational models of reinforcement learning. Measures such as "staying" or "shifting" after wins or losses assess sensitivity to immediate reinforcement and do not account for how feedback history is integrated across multiple experiences to influence behaviour (Kanen et al., 2019). To do so, the computational methods outlined in Chapter 6 were employed to address the following additional questions. Does LSD alter the rate at which individuals learn from feedback? Is this unique to positive or negative feedback? Does LSD affect the degree to which behaviour is stimulus-driven (stimulus sticky) and independent of an action's outcome? Indeed, serotonergic manipulations have modulated stickiness in marmoset monkeys (Rygula et al., 2015). At the same time, LSD and 5-HT2A agonists have improved associative learning in non-humans (Harvey, 2003; Harvey et al., 1988; Romano et al., 2010; Schindler et al., 1986). Accordingly, I predicted LSD would either modulate

stimulus stickiness or enhance reinforcement learning rates.

7.2 Methods

7.2.1 General procedure

Nineteen healthy volunteers, over the age of 21, attended two sessions at least two weeks apart where they received either intravenous LSD (75μg in 10 mL saline) or placebo (10mL saline), in a single-blind within-subjects balanced-order design. The PRL task was administered approximately five hours post-injection. Participants were blinded to the condition but the experimenters were not. A cannula was inserted and secured in the antecubital fossa and injection was performed over the course of two minutes. Participants reported noticing subjective effects of LSD 5 to 15 minutes after dosing (see Section 7.3.1), and the plateau of effects was generally maintained for approximately four hours. Once the subjective drug effects subsided, a psychiatrist assessed suitability for discharge. All participants provided written informed consent after briefing on the study and screening. Participants had no personal history of diagnosed psychiatric disorder, or immediate family history of a psychotic disorder. Other inclusion criteria were normal electrocardiogram (ECG), routine blood tests, negative urine test for pregnancy and recent drug use, negative breathalyser test for recent alcohol use, alcohol use limited to less than 40 units per week, and absence of a significant medical condition. Participants had previous experience with a classic psychedelic drug (e.g. LSD, mescaline, psilocybin/magic mushrooms, or DMT/ayahuasca) without an adverse reaction, not within six weeks of the study. Screening was conducted at the Imperial College London Clinical Research Facility (ICRF) at the Hammersmith Hospital campus, and the study was carried out at the Cardiff University Brain Research Imaging Centre (CUBRIC). This experiment was part of a larger study, the data from which are published elsewhere (Carhart-Harris et al., 2016). Additional information, including subjective ratings, can be found in Carhart-Harris et al. (2016).

7.2.2 Probabilistic reversal learning task

A schematic of the task is shown in Figure 7.1. Participants could choose from three visual stimuli on 80 trials presented at three of four randomised locations on a computer screen. In the first half of the task, choosing one of the stimuli resulted in positive feedback in the form of a green "smiley" face on 75% of trials. A second stimulus resulted in positive feedback 50% of the time, whilst the third stimulus yielded positive feedback on only 25% of trials. After 40 trials, the most and least optimal stimuli reversed such that the stimulus that initially was

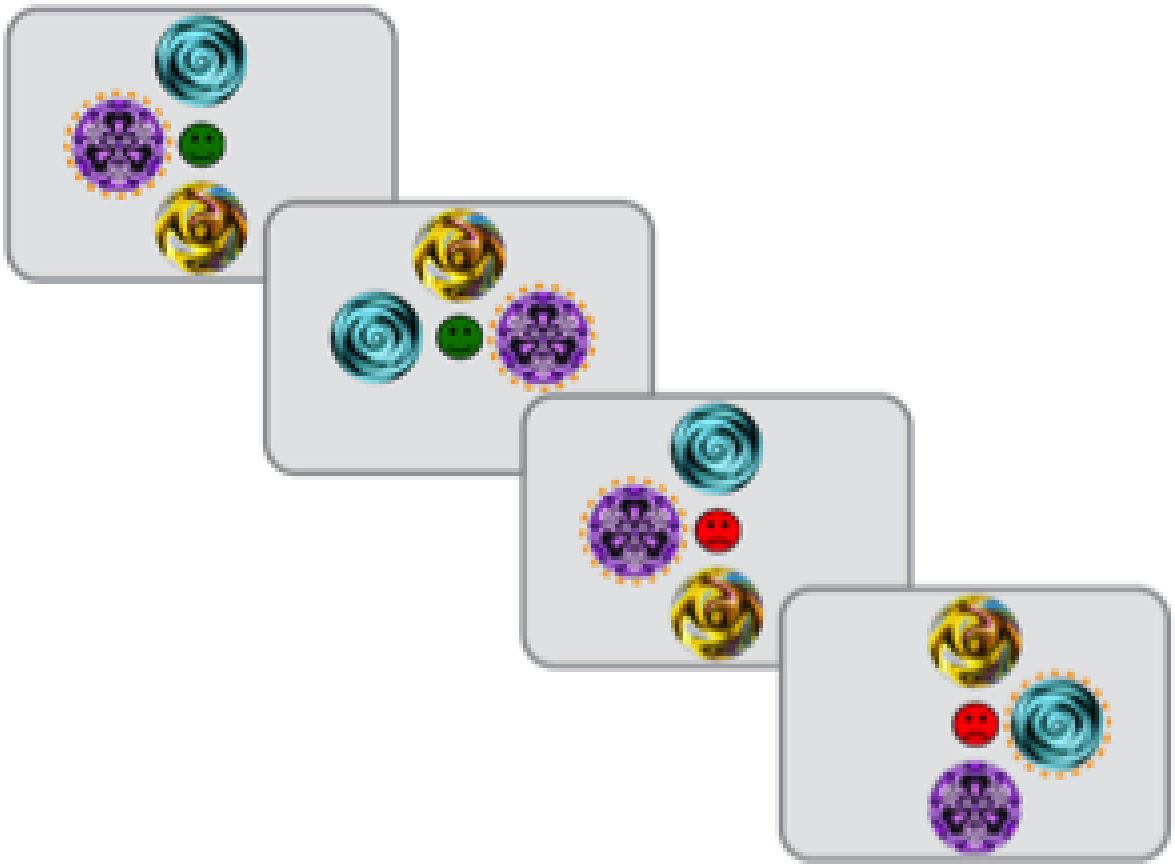

Figure 7.1: Probabilistic reversal learning task schematic. Subjects chose one of three stimuli. One stimulus delivered positive feedback (green smiling face) with a 75% probability, one with 50%, and one with 25%; the probabilistic alternative was negative feedback (red sad face). Midway through the task, the contingencies for the best and worst stimuli swapped.

correct 75% of the time was then only correct 25% of the time, and likewise the 25% correct stimulus then resulted in positive feedback on 75% of trials.

7.2.3 Conventional analysis of behaviour

I measured feedback sensitivity by determining whether participants stayed with the same choice following positive or negative feedback (win-stay or lose-stay). Win-stay was defined as the number of times an individual repeated a choice after a win divided by the number of trials on which positive feedback occurred (opportunities to stay after a win). Lose-stay was calculated in the same manner: number of times a choice was repeated following a loss divided by the total losses experienced. Perseveration was defined according to den Ouden et al. (2013) and was assessed based on responses in the reversal phase. A perseverative error

occurred when two or more (now incorrect) responses were made to the previously correct stimulus, and these errors could occur at any point in the reversal phase. The first trial in the reversal phase (trial 41 of 80), however, was excluded from the perseveration analysis, as at that point behaviour cannot yet be shaped by the new feedback structure.

7.2.4 Computational modelling of behaviour

Models 1, 2, and 3 from Chapter 6 were fitted to the behavioural data. The modelling analysis was conducted in collaboration with Qiang Luo. Trials were sequenced across all 80 trials of the PRL task, and on each trial the computational model was supplied with the participant's identification number and drug condition, whether the trial resulted in positive or negative feedback, and which stimuli were presented.

7.2.4.1 Simulation

Behavioural data from the winning model was simulated to determine how behavioural patterns in the synthetic data compared to the raw data. Simulated data were analysed for win-stay, lose-stay, acquisition performance, and perseveration, as was done for the original raw data analysis. For each condition (placebo and LSD), 100 "virtual subjects" were simulated using the posterior mean parameters from that condition, from the winning model, per Kanen et al. (2019). Each virtual subject performed the PRL task in silico.

7.3 Results

7.3.1 Subjective effects of LSD

The subjective effects of LSD were assessed by visual analogue scale-style (VAS) ratings and the 11-factor altered states of consciousness (ASC) questionnaire (Studerus et al., 2010). VAS items that were rated significantly higher on LSD than on placebo, following Bonferonni correction, included "my imagination was extremely vivid", "the experience had a dream-like quality", "sounds influenced things I saw", "my sense of size and space was distorted", "I felt unusual bodily sensations", "my thoughts wandered freely", "my perception of time was distorted", "I saw geometric patterns", "edges appeared warped", "my thinking was muddled", "I saw movement in things that weren't really moving", "I experienced a sense of merging with my surroundings", "things looked strange", and "I felt like I was floating". Participants rated a control item "I felt entirely normal" higher on placebo than on LSD. All factors on the ASC questionnaire were increased after Bonferonni correction, aside from the anxiety factor,

which included: complex imagery, elementary imagery, audio/visual synaesthesia, meaning, experience of unity, spiritual experience, blissful state, insightfulness, disembodiment, and impaired cognition. Positive mood, furthermore, was significantly increased under LSD as well (also assessed by a VAS). Further details on the subjective effects of LSD in this sample of participants are published in Carhart-Harris et al. (2016).

7.3.2 Learning and perseveration

First, I verified that LSD did not impair participants' ability to perform the task. The number of correct responses did not differ between placebo and LSD via paired-samples t-tests during the acquisition ($t(18) = .842$, $p = .411$, $d = .193$) or reversal phases ($t(18) = .234$, $p = .818$, $d = .054$). I then examined the relationship between learning and perseveration as in den Ouden et al. (2013), shown in Figure 7.2. Simple linear regression showed that when on LSD, relative to placebo, a greater number of correct responses during the acquisition phase (LSD minus placebo) significantly predicted more perseverative errors (LSD minus placebo) in the reversal stage (regression coefficient $\beta = .558$, $p = .002$). Follow up regressions separately for each condition showed that when on LSD, a greater number of correct responses during the acquisition phase significantly predicted more perseverative errors when on LSD ($\beta = .438$, $p = .003$) but not when under placebo ($\beta = .035$, $p = .838$). Drug (placebo versus LSD) and acquisition performance did not, however, interact to modulate perseverative errors, assessed via analysis of covariance ($F(1,18) = 1.487$, $p = .239$, $\eta_p^2 = .012$). Perseverative errors alone did not differ between conditions ($t(18) = .027$, $p = .979$, $d = .007$).

7.3.3 Feedback sensitivity

Feedback sensitivity data are shown in Figure 7.3. I assessed whether LSD influenced how individuals responded immediately to positive and negative feedback – whether participants stayed with the same choice after a win or a loss (win-stay/lose-stay). Paired t-tests showed there were no differences between placebo and LSD for win-stay ($t(18) = .787$, $p = .442$, $d = .179$) and separately lose-stay ($t(18) = -.124$, $p = .903$, $d = .03$).

7.3.4 Choice of reinforcement learning model

The core modelling results are summarised in Figure 7.4. Computational modelling, unlike the conventional methods applied thus far, accounts for how behaviour is influenced by an integration of previous choices and feedback history from multiple experiences. Behaviour in the present PRL task was best characterised by a simple reinforcement learning model, as

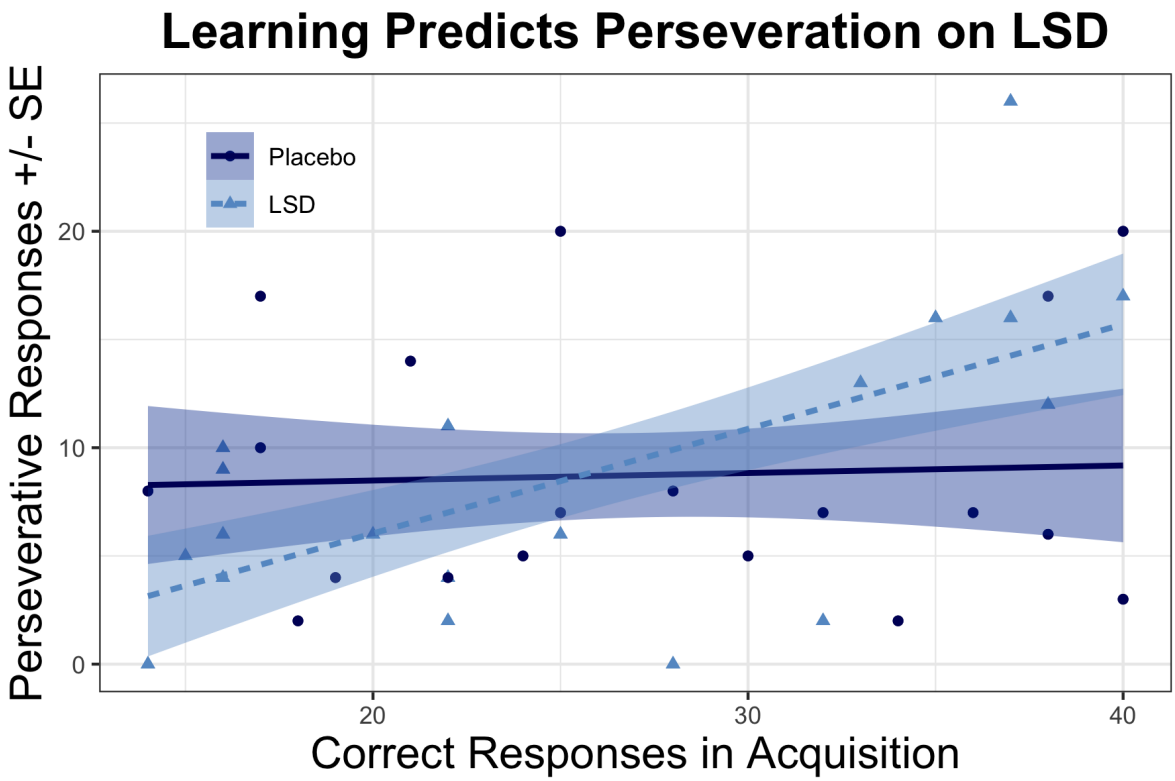

Figure 7.2: Better initial learning was predictive of more perseveration on LSD and not on placebo. Shading signifies 1 standard error (SE).

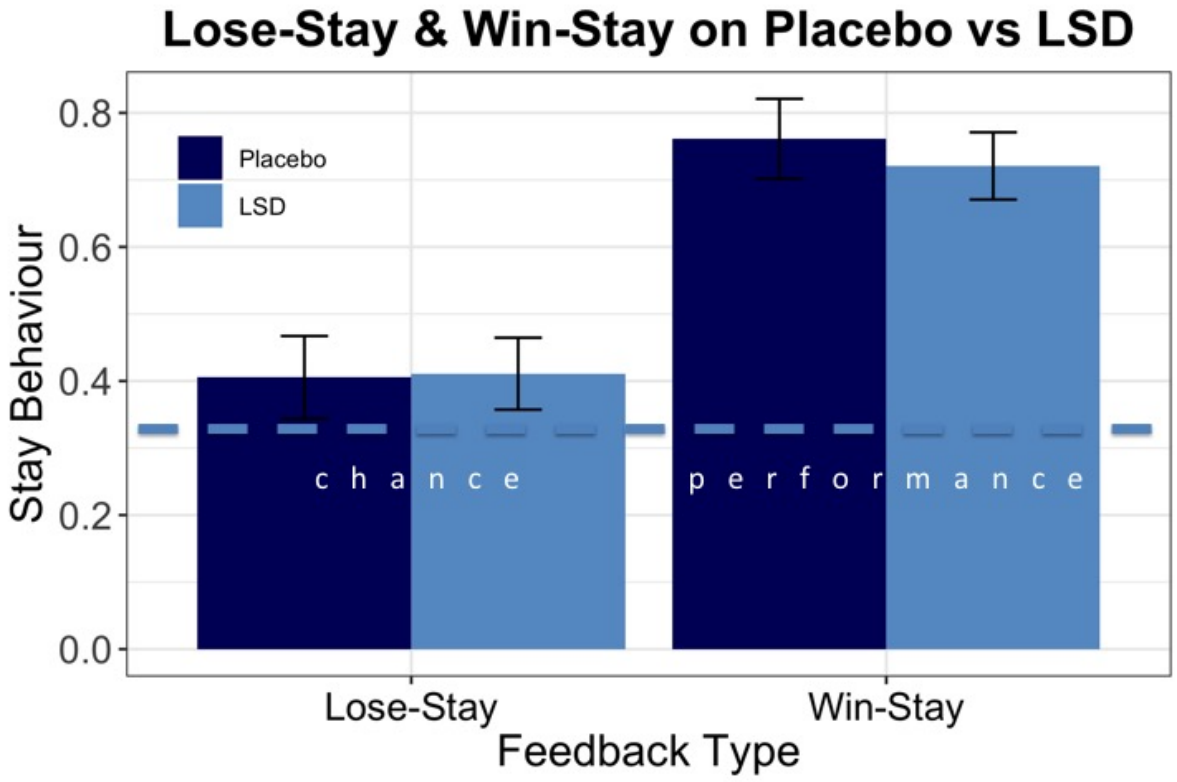

Figure 7.3: Conventional analyses of feedback sensitivity were unaffected by LSD. Dotted line indicates 33% and corresponds to randomly choosing one of the three stimuli. Error bars indicate +/- 1 standard error.

Rank	Name	Parameters	log marginal likelihood	log posterior P(model)
2	Model 1	α^{rew}, α^{pun}, τ^{reinf}	-2401.49	-33.28
1	Model 2	α^{rew}, α^{pun}, τ^{reinf}, τ^{stim}	-2368.21	0
3	Model 3	α^{reinf}, τ^{reinf}, τ^{stim}	-2428.52	-60.32

Table 7.1: Model comparison. *rew* reward, *pun* punishment, *reinf* reinforcement, *stim* stimulus.

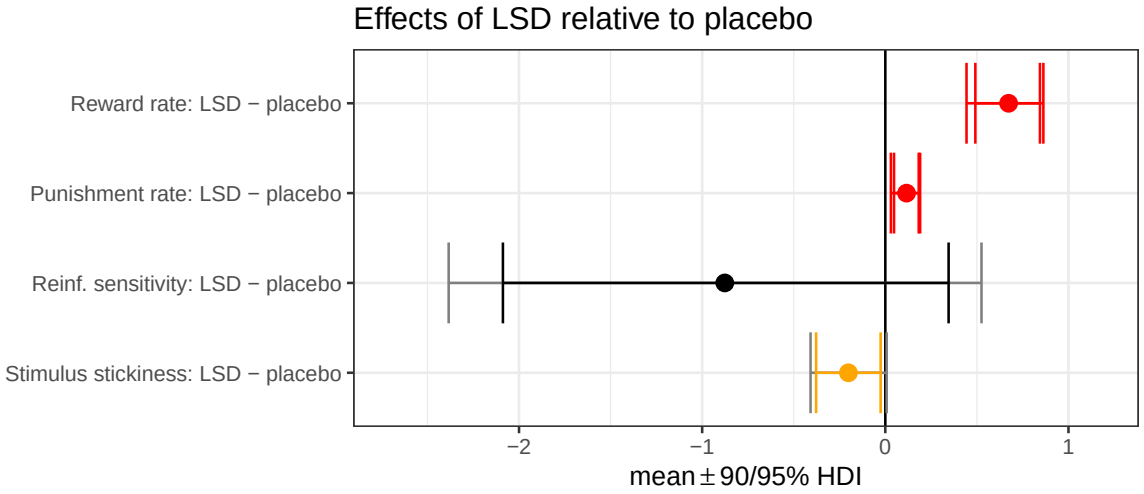

Figure 7.4: Effects of LSD relative to placebo on model parameters. The third row represents a difference of differences scores: ([LSD_Reward - LSD_Punishment] - [Placebo_Reward - Placebo_Punishment]).

determined by a bridge sampling estimate of the marginal likelihood. Three reinforcement learning models were fitted and compared (Table 7.1). Convergence was good with all three models having R^ < 1.2. The winning model included four parameters: 1) reward learning rate, the extent to which behaviour is driven by positive feedback learning; 2) punishment learning rate, the contribution of learning from negative feedback to behaviour; 3) reinforcement sensitivity, which is the degree to which overall outcome of behaviour contributes to choice (how heavily stimulus value learned through reinforcement is weighted); and 4) "stimulus stickiness" which indexes the tendency to get "stuck" to a cue: was the chosen stimulus selected on the previous trial, irrespective of outcome?

7.3.5 Positive and negative feedback-driven learning rates

Results on learning rates are shown in Figure 7.4. The winning model contained separate parameters assessing the rate at which individuals learned from positive feedback (reward) versus negative feedback (nonreward/punishment). Positive feedback-driven learning was el-

evated on LSD compared to placebo (difference in parameter per-drug mean, posterior 99.9% highest posterior density interval [HDI] excluding zero; $0 \notin$ 99.9% HDI). There was also increased negative feedback-driven learning under LSD (drug difference, $0 \notin$ 99% HDI). LSD, furthermore, increased the positive feedback learning rate to a greater extent compared to the negative feedback learning rate ([LSD_Reward - LSD_Punishment] - [Placebo_Reward - Placebo_Punishment]); whilst LSD enhanced both positive and negative feedback learning, the positive learning rate was preferentially increased (drug difference, $0 \notin$ 99% HDI).

7.3.6 Stimulus stickiness and reinforcement sensitivity

The core results on stimulus stickiness and reinforcement sensitivity are depicted in Figure 7.4. The best fitting model also contained a parameter assessing stimulus stickiness, which is the tendency to choose a previously chosen perceptual stimulus again regardless of feedback, and reinforcement sensitivity, which governs the degree to which behaviour is driven by reinforcement history. Neither stimulus stickiness nor reinforcement sensitivity were modulated by LSD at 95% HDI (no drug difference for either parameter, $0 \in$ 95% HDI).

7.3.7 Relationship between model parameters and conventional behavioural measures

Given the initial finding on the relationship between better acquisition learning and perseveration, the primary question here was whether the elevated reward learning rate under LSD was predictive of perseveration. This relationship is depicted in Figure 7.5. Analysis of covariance (ANCOVA) with drug (placebo versus LSD) and reward rate as predictors of perseveration revealed a significant drug × reward rate interaction, controlling for main effects ($F(1,19) = 5.890$, $p = .025$, $\eta_p^2 = .171$). In other words, LSD modulated the relationship between the reward rate and perseveration. Follow-up simple linear regression showed that the interaction was driven by the effects of LSD: under LSD, a higher reward learning rate predicted significantly more perseverative errors ($\beta = 30.218$, $p = .015$), whereas no such relationship was present when the same participants were under placebo ($\beta = -.569$, $p = .948$). Exploratory correlations to understand the relationship between the latent and observable measures are summarised in Table 7.2.

7.3.8 Simulation

Simulation of behavioural data generated using parameter estimates from the winning model were analysed using conventional methods and produced several consistent patterns of be-

	Acquisition		Perseveration		Lose-Stay		Win-Stay	
	Placebo	LSD	Placebo	LSD	Placebo	LSD	Placebo	LSD
Reward Learning Rate, α^{rew}				↑				
Punishment Learning Rate, α^{pun}					↓	↓		
Reinforcement Sensitivity, τ^{reinf}	↑	↑		↑	↑	↑	↑	↑
Stimulus Stickiness, τ^{stim}	↑				↑	↑		↑

Table 7.2: Summary of correlations between conventional behavioural measures and model parameters. Arrows indicate significant correlations tested within each condition, uncorrected. ↑ significant positive correlation; ↓ significant negative correlation. Acquisition refers to correct responses in the acquisition phase. *rew* reward, *pun* punishment, *reinf* reinforcement, *stim* stimulus.

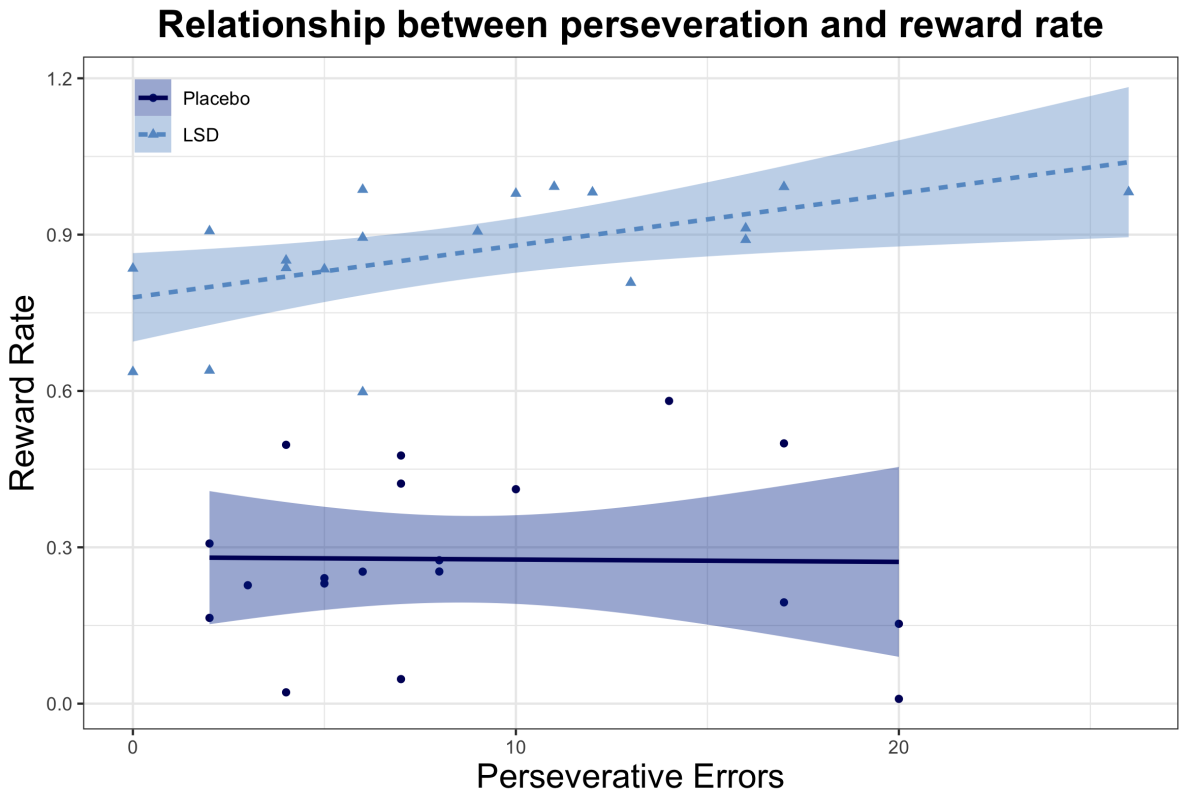

Figure 7.5: Relationship between the reward rate parameter from the computational model and perseveration on placebo and on LSD. Shading signifies 1 standard error (SE).

haviour. This suggests the model captured the essence of the effects of LSD on behaviour. Consistent with the original data, lose-stay was unaffected by LSD in the simulated behaviour ($t(99) = -.374$, $p = .709$, $d = .034$) and acquisition performance was also unaffected ($t(99) = .247$, $p = .805$, $d = .025$). Perseveration was enhanced by LSD in the simulation ($t(99) = -2.235$, $p = .028$, $d = .223$), which differs from yet is in line with the original analyses showing an enhanced relationship between acquisition and perseveration under LSD. Drug condition and acquisition performance did not interact to modulate perseveration in the simulated data ($F(1,115) = 1.602$, $p = .208$, $\eta_p^2 = .012$). Win-stay was diminished under LSD in the simulated data ($t(99) = 11.905$, $p = 8.21 \times 10^{-21}$, $d = 1.193$) whereas it was unaffected by LSD in the raw data analysis.

7.4 Discussion

Here, to my best knowledge, this is the first computational analysis of how a psychedelic drug modulates reinforcement learning in any species. The key result was that LSD magnified the weight of positive feedback learning in guiding the behaviour of healthy human volunteers during PRL. Whilst LSD also enhanced the extent to which negative feedback learning drove behaviour, LSD augmented the positive feedback (reward) learning rate significantly more than the negative feedback (nonreward/punishment) learning rate. Furthermore, the increased positive feedback learning rate under LSD was significantly correlated with perseverative behaviour following the reversal of contingencies. That LSD enhanced learning rates may be particularly important for understanding the mechanisms by which LSD could exert a therapeutic effect in psychiatric conditions.

Investigations of how LSD affects learning, let alone reversal learning, are extremely limited. A recent study of healthy human volunteers showed LSD impaired higher order cognitive flexibility as assessed by the extra-dimensional set-shifting paradigm (IDED), an analogue of the Wisconsin Card Sorting Test (Pokorny et al., 2019). This impairment is in keeping with our result showing that LSD strengthened the association between better learning and more perseveration, a form of cognitive inflexibility, as well as the positive relationship between the reward learning rate and perseveration. The implication of the data reported here is that LSD stamps in initial learning in the acute psychedelic state which may subsequently be harder to update.

The present results on perseveration and the findings of LSD-induced cognitive inflexibility, by Pokorny et al. (2019), ostensibly run counter to the prominent view that psychedelics relax prior beliefs ("priors") and thus facilitate the capacity for change (Carhart-Harris and Friston 2019). The notion of relaxation of priors, meanwhile, is directly compatible with that

of enhanced new learning (reinforcement rates). In these human cognitive flexibility studies all phases of learning took place under the influence of LSD. This is likely to be a critical point, and contrasts with multiple animal studies on the role of the 5-HT2A receptor in reversal learning (Boulougouris et al., 2008; King et al., 1974). King et al. (1974) showed LSD improved (deterministic) reversal learning in rats, although they conducted acquisition on a different day preceding LSD administration, and only reversal took place with LSD onboard. Likewise, Boulougouris et al. (2008) showed 5-HT2A antagonism impaired reversal learning (which was improved by 5-HT2C antagonism), in an experimental design where initial learning once again occurred before drug administration. Collectively, these findings align with the notion that relaxation or overweighting of a prior (belief or expectation) under LSD may depend on whether the prior is formed before or during drug administration. Maladaptive priors, targeted in a therapeutic setting, would have been formed before the psychedelic experience. The prospect of undergoing enhanced new learning from positive feedback under LSD, as documented here, could therefore be conducive to the therapeutic objective.

How might this occur? Whilst it is not possible to discern the specific neurochemical mechanisms underlying the present effects on learning, I will discuss core possibilities, namely involving 5-HT2A and dopamine receptors. The capacity for change purportedly promoted by psychedelics is believed to occur through 5-HT2A-mediated plasticity (Carhart-Harris and Nutt, 2017) via downstream enhancement of NMDA (N-methyl-D-aspartate) receptor transmission (Barre et al., 2016) and BDNF [brain-derived neurotrophic factor] (Vaidya et al., 1997). Co-administration of LSD and a 5-HT2A antagonist such as ketanserin would be one means of testing the specificity of 5-HT2A receptors in the neurocognitive effects of LSD. This was not performed in the present study. Pokorny et al. (2019), however, employed this method and showed that the cognitive inflexibility (and spatial working memory) deficit they reported was rescued by administering ketanserin and LSD in tandem. They were therefore able to conclude that the LSD-induced impairments were mediated by 5-HT2A agonism.

Unlike other classic psychedelics such as psilocybin, LSD acts not only at serotonergic but also at dopaminergic receptors (Nichols, 2004, 2016). LSD preferentially activates D2 over D1 receptors (Nichols, 2004, 2016), its actions at D2 receptors likely being especially pronounced at a later time following drug administration (Marona-Lewicka et al., 2005, 2007). The initial action of LSD at 5-HT2A receptors has been proposed to lead to a sensitisation of dopaminergic systems, which subsequently potentiates the direct dopaminergic effects of LSD (Nichols, 2016). Psychedelics can increase dopaminergic activity in the human caudate and putamen even in the absence of dopamine receptor binding (Vollenweider et al., 1999). Stimulation of 5-HT2A receptors in the prefrontal cortex of the rat, meanwhile, enhances ventral tegmental area (VTA) dopaminergic activity (Bortolozzi et al., 2005). Serotonin-dopamine

interactions therefore represent another mechanism that could underlie the present findings.

Indeed, dopamine is notably involved in the plasticity mediating associative learning (Shen et al., 2008; Yin and Knowlton, 2006). That LSD modulated learning rates may be consistent with a dopaminergic mechanism: using an almost identical model, Kanen et al. (2019) found that D2 agents enhanced both the positive feedback learning rate (in stimulant use disorder) and the negative feedback learning rate (in obsessive-compulsive disorder and healthy volunteers). Several manipulations of serotonin during PRL in marmoset monkeys (Rygula et al., 2015), rats, and healthy humans, meanwhile have shown effects on stimulus stickiness rather than learning rates (Luo et al., personal communication). These studies employed techniques including serotonin depletion via tryptophan depletion or the neurotoxin 5,7-dihydroxytryptamine, and single dose or sub-chronically administered SSRIs (Bari et al., 2010; Luo et al., personal communication; Rygula et al., 2015). Whilst studies of single nucleotide polymorphisms are susceptible to false positives (Border et al., 2019; Bosker et al., 2011; Culverhouse et al., 2018), den Ouden et al. (2013) showed that variation in the dopamine, but not the serotonin transporter, was associated with the same enhanced relationship between acquisition and perseveration as reported here under LSD. Additionally, despite the purported biphasic effects of LSD – initially dominated by action at 5-HT2A receptors and subsequently enhanced activity at D2 receptors (Marona-Lewicka et al., 2005, 2007) – King et al. (1974) showed the effects of LSD on reversal learning were consistent across four different time lags between LSD administration and behavioural testing. It is critical to point out that in addition to binding with high affinity to 5-HT2A, D2, and D1 receptors, LSD acts at numerous other receptors including 5-HT1A/1B/1D, 5-HT2C, 5-HT5A, 5-HT6, and 5-HT7, as well as α1- and α2-adrenergic receptors – in many cases with higher binding affinities than for several of the dopamine receptors (Nichols, 2004). These affinities are summarised in Figure 7.6. Another complexity is that LSD suppresses dorsal raphe serotonin neuron activity (Aghajanian, 1970; Aghajanian and Weiss, 1968; Aghajanian and Vandermaelen, 1982). It also remains to be tested whether these effects of LSD on learning generalise to a psychedelic drug such as psilocybin, which does not act at dopaminergic receptors yet has been shown to increase dopamine release (Vollenweider et al., 1999).

In a future, substantially larger, study it may be fruitful to isolate data from individuals who had a negative LSD experience (a "bad trip") to examine whether the respective balance of learning driven by positive and negative feedback differs, for instance. Accordingly, it would be important to examine whether individual learning rate data during the psychedelic experience relates to any lasting effects. Ideally, the present experiment would serve as a springboard for future investigations, to elucidate how the interplay between psychological and pharmacological processes could be harnessed and refined for therapeutics. The elevated

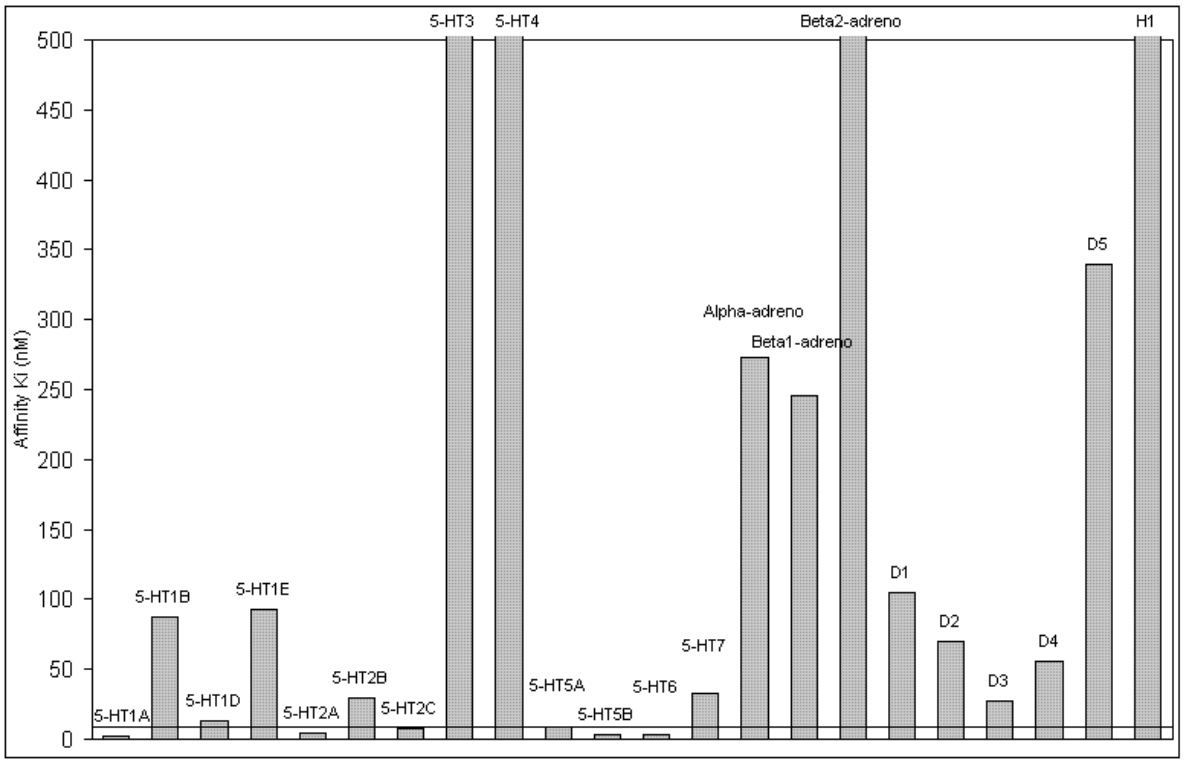

Figure 7.6: LSD receptor affinities. Low black horizontal line represents LSD levels in human plasma during recreational use (Aghajanian and Bing, 1964); lower bars indicate higher affinity. K_i = dissociation constant.

learning rates, in the context of associative learning, for instance, represent a candidate therapeutic mechanism, as the premise for clinical efficacy revolves around the formation of new beneficial associations in the psychedelic state.

In summary, probabilistic reversal learning was examined under LSD in healthy humans, for the first time, and computational models of reinforcement learning were applied. This study additionally represents one of the few applications of objective measures to investigate fundamental cognitive processes in the psychedelic state in humans. The key result was that LSD enhanced the rate at which individuals learned from feedback. Learning rate was most enhanced by LSD when receiving positive feedback, which was furthermore predictive of more perseveration under LSD but not when on placebo. This suggests that LSD may stamp in new learning that occurs in the psychedelic state. The present study builds upon the small number of human and rodent investigations into the influence of psychedelics on learning. These findings on how LSD enhanced learning could have implications for understanding mechanisms by which LSD, and psychedelics in general, may exert a therapeutic effect and thus inform emerging treatment approaches.

Chapter 8

Reinforcement learning in stimulant use disorder and OCD: effects of $D_{2/3}$ agents

8.1 Introduction

Optimal functioning and wellbeing requires flexible adaptation of behaviour to maximise rewards and minimise punishments. Many psychiatric disorders involve aberrant processing of, and responding to, rewarding and aversive experiences. Compulsivity is a hallmark of stimulant use disorder (SUD) and obsessive-compulsive disorder (OCD), where behaviour to obtain reward or avoid punishment, inappropriately persists, resulting in undesirable consequences. In SUD, drug-taking habits prevail despite the risk of family breakup or job loss. Individuals with OCD are unable to desist repetitive behaviours, which can consume large amounts of time and ultimately compromise social or occupational functioning (American Psychiatric Association, 2013).

Deficits in behavioural flexibility can be captured in a laboratory setting using probabilistic reversal learning (PRL) paradigms (Lawrence et al., 1999). Adaptive behaviour involves a trade-off between flexibly updating actions when the environment changes, and ignoring rare events when the environment is stable. PRL models this trade-off. Participants are presented with two choices and learn by trial and error which option is correct most of the time. Ignoring spurious minority feedback leads to more rewards overall, and is thus adaptive. The contingencies are then reversed and participants must update their choices to maximise rewards again. In these experiments, analysed using classical statistics, individuals with SUD show perseverative deficits – impairments in the ability to update behaviour when circumstances change (Ersche et al., 2011, 2008). Whilst patients with OCD also exhibit behavioural inflexibility, the most consistent evidence comes from the extra-dimensional shifting paradigm, which requires

shifting attention from one aspect of a compound stimulus to another, to maximise reinforcement (Chamberlain et al., 2007c). The findings on PRL in OCD, on the other hand, are mixed (Chamberlain et al., 2007b; Ersche et al., 2011; Remijnse et al., 2006). At the same time, individuals with depression (Murphy et al., 2003; Taylor Tavares et al., 2008) instead show hypersensitivity to spurious negative feedback in PRL, manifested by inappropriately changing behaviour following punishment when it is rare. To my knowledge, however, nobody has compared the microstructure of behaviour in PRL between disorders of compulsivity using computational models of reinforcement learning (RL).

Techniques for analysing behaviour that are based on RL describe the behaviour in question – for instance, choice – by having a computer simulate putative psychological processes, such as learning from reward or punishment, tending to choose recently chosen stimuli or respond to recently responded-to locations irrespective of outcome, and selecting between alternative actions. These computational processes are governed by parameters (e.g. a given subject's tendency to learn from reward, or from aversive feedback such as errors). In turn, those parameters may be influenced by dynamic pharmacological manipulations, and may vary according to relatively static properties of the subject, such as psychiatric disorders. The most likely values for those parameters are discovered by fitting the predictions of a computational RL model to actual behaviour. In the most informative kind of analysis (Daw, 2011), a Bayesian hierarchy is used. For example, subjects are drawn from groups, and are influenced by drug manipulations, so the parameters pertaining to a given subject in a given condition (or session) exist "beneath" the level of groups and drugs; at the lowest level, trial-by-trial data are predicted and compared to behaviour. Finally, the best RL model may be selected from a number of competing alternatives according to a formal Bayesian procedure, penalising models that fit badly or that are over-complex (Occam's razor). Analysing behavioural data using a hierarchical Bayesian RL approach therefore simultaneously allows the best computational model of behaviour to be selected from candidate models – allowing psychological processes to be inferred – and the parameters of that model to be characterised, to uncover the effects of disorders or pharmacological manipulations on those processes.

Here I took a transdiagnostic approach to interrogate the computational processes underlying maladaptive behaviour across two disorders of compulsivity: SUD and OCD. RL models were applied in a reanalysis of behavioural data on PRL from Ersche et al. (2011), which enabled a direct comparison of these groups. The original study by Ersche et al. (2011) also investigated the effects of the dopaminergic D2/3 receptor agonist pramipexole, and the D2/3 antagonist amisulpride. Using classical statistics, they showed pramipexole remediated perseverative behaviour in SUD and normalised the corresponding hypoactivity in the head of the caudate; however, their analysis did not detect any further effects of pramipexole or

amisulpride in SUD, OCD, or controls. I additionally sought to deconstruct the influence of dopaminergic agents on computational processes underlying PRL in these groups. Understanding D2/3 receptor involvement in maladaptive behaviour is particularly important given the evidence of reduced striatal D2 receptor availability in cocaine abuse (Volkow et al., 1993), methamphetamine abuse (Volkow et al., 2001), and OCD (Denys et al., 2004; Perani et al., 2008). D2/3 antagonists, additionally, are effective in augmenting first-line selective serotonin reuptake inhibitor (SSRI) therapy in treatment resistant cases of OCD (Fineberg et al., 2020).

The primary aim of the modelling approach was to deepen an understanding of how SUD and OCD differ and overlap, and to do so more robustly and with greater detail than the conventional analyses previously reported. Using data from Ersche et al. (2011), I asked whether behavioural differences could be best accounted for by algorithms describing how rewarding and punishing outcomes drive action, for instance, or if models incorporating additional elements tracking behavioural tendencies independent of action-outcome contingencies – "stickiness" parameters – would yield more optimal characterisations. Experimental data showing abnormalities in processing and flexibility adapting behaviour following rewards and punishments in SUD (e.g. Ersche et al., 2011, 2016) and OCD (e.g. Gillan et al., 2011, 2014) suggest parameters tracking separate reward and punishment learning rates would be of central importance. I predicted separate learning rates, for positive and negative outcomes, would be superior to a single reinforcement rate, and could enable the detection of asymmetries in appetitive and aversive processing – avoiding negative consequences is a key feature of OCD (American Psychiatric Association, 2013), and is not central in SUD, for instance. At the same time, because compulsivity may stem from maladaptive stimulus-response habits, where behaviour persists irrespective of outcome (Everitt and Robbins, 2016; Gillan et al., 2014, 2011), I expected the addition of stickiness parameters would be optimal. Finally, I asked whether these data would instead be better characterised by a different model, used to dissect perseverative behaviour (den Ouden et al., 2013), that tests the balance of how incoming information is valued against current beliefs (based on past experience). I expected that analysing behaviour in this more sophisticated manner would enable me to better differentiate the SUD and OCD groups and characterise their response to dopaminergic agents. The work presented here has appeared in Kanen et al. (2019).

8.2 Methods

8.2.1 Participants

The study included 56 participants, composed of 19 healthy volunteers, 18 patients with SUD, and 19 patients with OCD. Diagnoses of stimulant dependence and OCD were ascertained using the structured clinical interview for the DSM-IV (First et al., 2002). Here I use the term stimulant use disorder (SUD), which is the current nomenclature in the DSM-V (American Psychiatric Association, 2013), rather than stimulant dependence, as used in the DSM-IV-TR (American Psychiatric Association, 2000). Within the SUD group, 10 participants met DSM-IV-TR (American Psychiatric Association, 2000) criteria for cocaine/crack dependence while 8 met criteria for amphetamine dependence. Individuals with SUD had a history of illicit stimulant dependence for a minimum of two years. Participants did not have any other Axis I psychiatric disorder at the time of the study, and were not taking any other medication aside from SSRIs in the OCD group. Both the SUD and OCD groups, however, had elevated depressive symptoms, which is reported in the Results section. Use of illicit drugs, besides in the SUD group, was an exclusion criterion. Participants were assessed for their general health, which included a physical examination and clinical blood tests at baseline, and were excluded if they had a history of any serious medical condition. The study was approved by the Cambridge Research Ethics Committee and all participants provided written informed consent. Further information on the three groups of participants, including their demographics, baseline personality measures, and clinical information are presented in Figure 8.4.

8.2.2 General procedure

Participants attended three sessions, with one week between each session. The task was conducted an hour after a single dose of either placebo, a D2/3 agonist (pramipexole, 0.5mg), or a D2/3 antagonist (amisulpride, 400mg), timed to coincide with peak plasma concentrations. Three individuals with SUD received 1.5mg of pramipexole. All subjects contributed data to the Bayesian analysis. One control participant contributed only placebo data and one participant with OCD contributed only amisulpride and pramipexole data, as they did not complete all three sessions. One subject from the SUD group who contributed data from all three sessions, was excluded from Ersche et al. (2011) due to a behavioural performance cutoff. These three participants were not used for subsequent analyses correlating model parameters with symptoms, and with the key behavioural measures reported in Ersche et al. (2011). The experiment was conducted in an fMRI (functional magnetic resonance imaging) scanner, however the imaging data were not reanalysed here. Further details about the study procedure are

described in Ersche et al. (2011).

8.2.3 Serial probabilistic reversal learning task

Two visual stimuli were presented simultaneously, as shown in Figure 8.1, and participants were prompted to make a choice by pressing one of two buttons. Stimuli were presented for 2000 milliseconds, and if a response was not entered in this period the screen would say "too late". Participants received immediate feedback 500 milliseconds after a response was made, in the form of a green face with a smile or a red face with a frown, and learned by trial and error which stimulus was correct most of the time. A fixation cross appeared between trials for a variable inter-trial interval lasting up to 3000 milliseconds. Participants were told that intermittently they would receive negative feedback even if they made the correct choice, which they should ignore. Ignoring spurious minority feedback leads to more positive feedback overall, and is thus adaptive. They were also informed that the optimal response would reverse several times throughout the task: the initially correct response would lose its value and choosing the other stimulus would then be optimal. There were two runs of 10 sequences, making for 18 response reversals. Participants had to make at least 10 correct responses cumulatively before the contingencies reversed; this criterion varied from 10 to 15 to avoid participants anticipating the occurrence of a reversal. If, however, participants did not reach the required number of correct responses, the task stopped after the 200th trial of that run. Misleading negative feedback to a correct response was provided on about 15% of trials; this varied as a function of when the reversal occurred. Participants completed an initial practice run of 30 trials to familiarise themselves with the task.

Ersche et al. (2011) focused on three main behavioural measures in their conventional analysis: perseverative, probabilistic, and spontaneous errors. A perseverative error occurred when participants made at least one consecutive choice of the previously correct stimulus immediately after the reversal occurred, excluding any error on the first trial of the reversal. They calculated a perseverative error rate by dividing the number of perseverative errors by the number of sequences on which perseverative errors occurred. Probabilistic switches were inappropriate switches from the correct to incorrect stimulus following misleading negative feedback. Spontaneous errors occurred when participants switched from the correct to incorrect stimulus despite receiving veracious positive feedback. More probabilistic switches and spontaneous errors is analogous to more "lose-shift" and less "win-stay" behaviour, respectively – terms used in other studies (e.g. den Ouden et al., 2013; Rygula et al., 2015). Ersche et al. (2011) also reported the average number of trials per sequence.

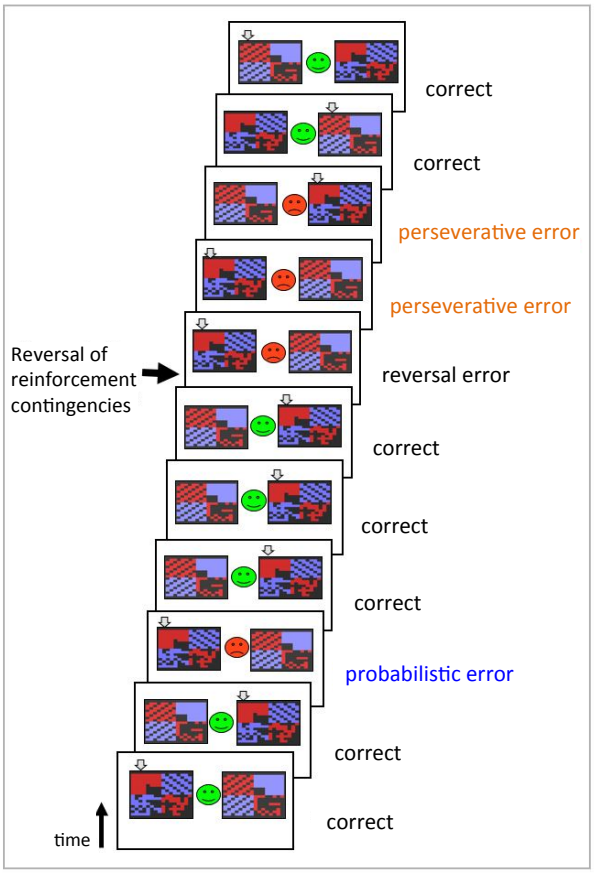

Figure 8.1: Schematic of the serial probabilistic reversal learning task from Ersche et al. (2011) and Kanen et al. (2019), used with permission. Two abstract stimuli were presented on either side of the screen, the participant selected one using a button press, and feedback was immediately given in the centre of the screen in the form of a green smiley face or a red frowning face. A probabilistic error occurred when a participant received spurious negative feedback after making the correct choice, which was rare and should therefore be ignored. A reversal error, on the other hand, was one where feedback was now truly negative, indicating the reversal had occurred, contingencies have thus changed and behaviour should be updated.

8.2.4 Computational modelling of behaviour

8.2.4.1 Overview

Seven RL models were fitted to the behavioural data on PRL from Ersche et al. (2011) using hierarchical Bayesian methods, incorporating parameters that have been studied previously in the RL literature. This work was a collaboration with Rudolf Cardinal (Kanen et al., 2019).

For all models, trials were sequenced across all trials in the PRL task. For each trial, the computational model was informed of the subject's identity, the subject's group and drug condition, which stimuli were presented and where (left or right side of the computer screen), the location (left or right) of the subject's response, and whether the trial was rewarded or unrewarded.

The top level of the Bayesian hierarchy (Figure 8.2) pertained to group and drug: each RL parameter had a group- and drug-condition-specific distribution. The next level involved sessions for individual subjects: RL parameters for each subject in a given (drug) condition were drawn from a normal distribution whose mean was the group/drug mean (from the level above) and whose variance represents inter-subject variability for that parameter (implemented as a subject-specific deviation from the group/drug mean). Through this process, the computer established specific RL parameters for a given set of trials. It then used them to govern an RL model trained by the sequence of stimuli and reinforcement.

Definitions were as follows: t is the trial number, S_t is the stimulus chosen on that trial, L_t is the location chosen on that trial, and R_t is the reinforcement delivered on that trial. Each stimulus was assigned an associated reinforcement-driven value V.

8.2.4.2 Models

Models are listed in Table 8.2.4.2 and Figure 8.3. Figure 8.3 lists the models by order of complexity and nestedness. Priors for the parameters are shown in Table 8.2.4.2.

Model 1 employed two parameters and served to address whether a simple reinforcement learning algorithm was sufficient to best characterise behaviour between groups and under different drug conditions. Reinforcement led to an increase in value V_i of the stimulus i that was chosen, at a speed governed by the reinforcement rate α^{reinf}, according to $V_{i,t+1} \leftarrow V_{i,t} + \alpha^{reinf}(R_t - V_{i,t})$, where R_t represents the reward on trial t (by definition 1 on rewarded trials), and $(R_t - V_{i,t})$ the prediction error. On nonrewarded trials $R_t = 0$, thus leading to a decrease in the value of V_i. Stimulus value contributed to the final quantity controlling choice via ${Q^{reinf}}_t = \tau^{reinf} V_t$. The additional parameter τ^{reinf}, termed reinforcement sensitivity, governs the degree to which a subject is driven by its reinforcement history. The quantities Q associated with the two available choices, for a given trial, were then fed into a standard softmax choice function

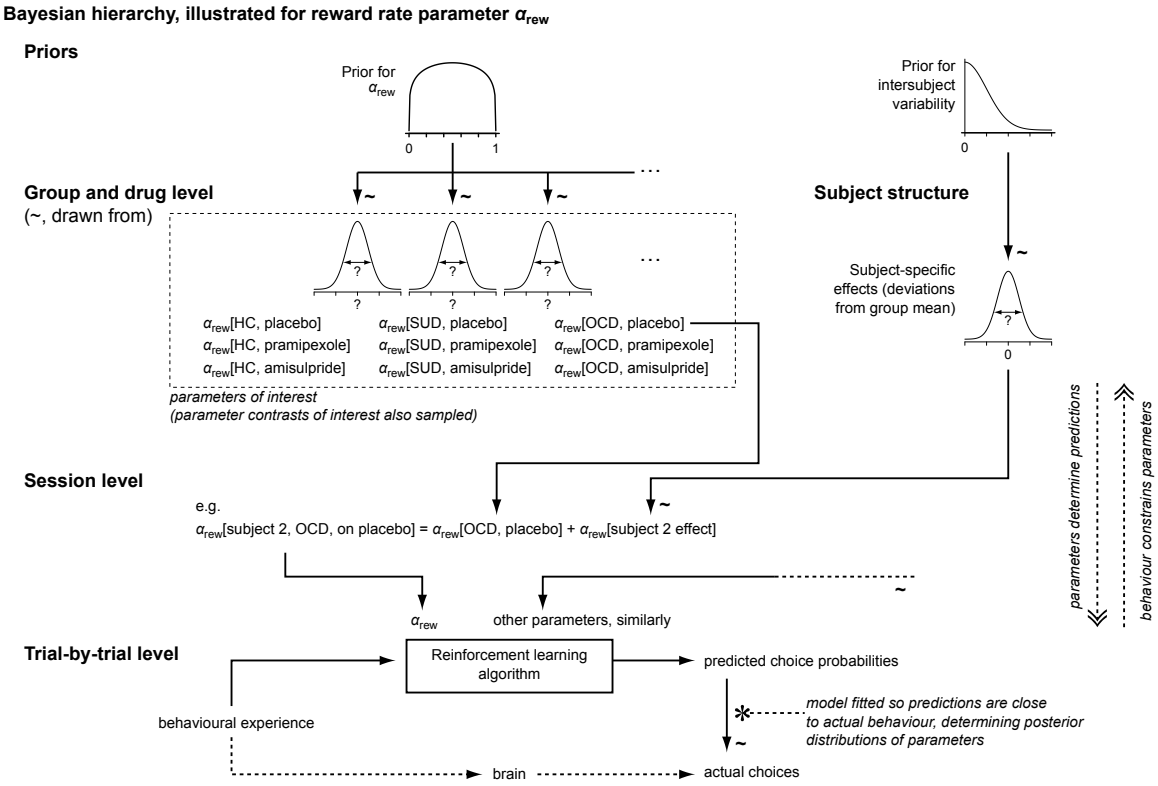

Figure 8.2: Schematic of the Bayesian hierarchy used in this analysis, illustrated here for a single parameter (reward rate). From Kanen et al. (2019).

Rank	Name	Parameters	Log marginal likelihood	Log posterior P (model)
7	Model 1	α^{reinf}, τ^{reinf}	−16984.66	−503.8250
3	Model 2	α^{reinf}, τ^{reinf}, τ^{stim}	−16687.72	−206.8821
6	Model 3	α^{rew}, α^{pun}, τ^{reinf}	−16835.28	−354.4418
4	Model 4a	α^{rew}, α^{pun}, τ^{reinf}, τ^{loc}	−16732.50	−251.6656
2	Model 4b	α^{rew}, α^{pun}, τ^{reinf}, τ^{stim}	−16585.12	−104.2815
1	Model 4c	α^{rew}, α^{pun}, τ^{reinf}, τ^{loc}, τ^{stim}	−16480.83	0.0000
5	Model 5	ρ, φ, β	−16821.35	−340.5171

Figure 8.3: Comparison of model performance. Models are listed in order of increasing complexity and nestedness. Model ranked 1st was the winning model. The log marginal likelihood and log posterior P(model) are comparison metrics used to determine the best model. A numerically larger, i.e. less negative, log marginal likelihood is better. The prior probabilities of all models were equal. Abbreviations: *rew* = reward; *pun* = punishment; *reinf* = reinforcement; *loc* = location; *stim* = stimulus. From Kanen et al. (2019).

to compute the probability of each choice:

$$P(action_a) = softmax^a_\beta(Q_1 ... Q_n) = \frac{e^{\beta Q_a}}{\sum_{k=1}^{n} e^{\beta Q_k}}$$

for n = 2 choices with softmax inverse temperature $\beta = 1$. The probability values for each trial emerging from the softmax function (arbitrarily, the probability of choosing stimulus A) were fitted to the subject's actual choices (did the subject choose stimulus A?). Note that since $\beta = 1$, the τ parameters directly represent weights given to each component in the softmax exponent.

Model 2 was as Model 1 but additionally implementing the concept of "stimulus stickiness", making for three parameters. This describes the tendency of a subject to respond again to a specific perceptual stimulus (regardless of location) that it chose on the previous trial, independent of outcome. A stimulus stickiness parameter, τ^{stim}, was added and this effect was modelled as $Q^{stim}{}_t = \tau^{stim} s_{t-1}$, where s_{t-1} was 1 for a stimulus that was chosen on the previous trial and 0 otherwise. The final quantity governing behaviour now incorporated this new component: $Q_t = Q^{reinf}{}_t + Q^{stim}{}_t$. The quantities Q, associated with the two available choices for a given trial, were likewise fed into a standard softmax function as above.

Model 3 was as Model 1, but instead of using one reinforcement rate α^{reinf}, separate learning rates for rewarded outcomes, α^{rew}, and nonrewarded outcomes, α^{pun} were implemented. These separate parameters enabled a test of the prediction that groups would differ in how positive versus negative feedback guide behaviour, and to assess how dopaminergic agents modulated these processes. Reward led to an increase in value V_i of the stimulus i that was chosen, at a speed governed by the reward rate α^{rew}, according to $V_{i,t+1} \leftarrow V_{i,t} + \alpha^{rew}(R_t - V_{i,t})$, where R_t represents the reward on trial t (by definition 1 on rewarded trials), and $(R_t - V_{i,t})$

the prediction error. Punishment (nonreward) led to a decrease in the value of V_i according to the punishment rate α^{pun}, similarly: $V_{i,t+1} \leftarrow V_{i,t} + \alpha^{pun}(R_t - V_{i,t})$ for $R_t = 0$. Stimulus value contributed to the final quantity controlling choice via ${Q^{reinf}}_t = \tau^{reinf} V_t$. Model 3 therefore had three parameters: α^{rew}, α^{pun}, and τ^{reinf}, as per the "RP" model from den Ouden et al. (2013).

Model 4a was as Model 3, while additionally implementing the concept of "side (location) stickiness", a tendency to repeat responses to the side most recently chosen. This made for four parameters. I asked whether capturing a different perseverative tendency – behaviour bound to a location rather than a specific visual stimulus – in addition to learning from rewarded or unrewarded outcomes would better characterise behaviour. The tendency to choose a location was governed by the location stickiness parameter τ^{loc}, according to ${Q^{loc}}_{l,t} = \tau^{loc} L_{l,t-1}$, where $L_{l,t-1}$ represents the subject's location choice on the previous trial (1 if l was the previously chosen location and 0 otherwise; for the first trial, this was 0 for both sides indicating no "stickiness"). The final tendency to choose a given stimulus at a given location was controlled by the quantity $Q_t = {Q^{reinf}}_t + {Q^{loc}}_t$. This model thus led to quantities Q associated with the two available choices for a given trial.

Model 4b was the same as Model 4a but implemented stimulus stickiness instead of side stickiness, giving four parameters. Given the SUD group from Ersche et al. (2011) perseverated to a particular stimulus, I predicted stimulus stickiness would be more informative than side stickiness.

Model 4c was as Model 3 (α^{rew}, α^{pun}, and τ^{reinf}) with the addition of parameters for both stimulus stickiness and side stickiness, giving five parameters. The final quantity governing behaviour was therefore: $Q_t = {Q^{reinf}}_t + {Q^{loc}}_t + {Q^{stim}}_t$.

Model 5 used a different approach: experience-weighted attraction (EWA; Camerer and Ho, 1999), which was the winning model in den Ouden et al. (2013) who used a single reversal. The EWA model is described in Chapter 6, but in brief, this model balances the value of incoming information against current beliefs (based on past experience). Learning from reinforcement is modulated by an "experience weight" for a stimulus; the experience weight for a stimulus is updated every time it is chosen, and its change over time is governed by a decay factor. In this model, the softmax inverse temperature β was also a parameter able to vary. The learning rate can decline over time in the EWA model. Because the paradigm in the present experiment employed serial reversals, requiring new learning at several points, it is possible the EWA model may be more conducive to PRL with a single reversal.

8.2.4.3 Simulation of behavioural data from winning model

To establish if the winning model was sufficient to reproduce key behavioural phenomena, behavioural data from the winning model was simulated, and analysed per Ersche et al. (2011).

Model parameter prior distributions

	Models using each parameter	Prior	Reference
Model parameters			
reward learning rate, α^{rew}	3, 4a, 4b, 4c	Beta(1.2, 1.2)	(den Ouden et al., 2013)
punishment learning rate, α^{pun}	3, 4a, 4b, 4c	Beta(1.2, 1.2)	(den Ouden et al., 2013)
combined reward/punishment learning rate, α^{reinf}	1, 2	Beta(1.2, 1.2)	(den Ouden et al., 2013)
reinforcement sensitivity, τ^{reinf}	1, 2, 3, 4a, 4b, 4c, 5	Gamma(α=4.82, β=0.88)	(Gershman, 2016)
location (side) stickness, τ^{loc}	4a, 4c	Normal(0, 1)	(Christakou et al., 2013)
stimulus stickness, τ^{stim}	2, 4b, 4c	Normal(0, 1)	(Christakou et al., 2013)
experience decay factor, ρ	5	Beta(1.2, 1.2)	(den Ouden et al., 2013)
decay factor for previous payoffs, φ	5	Beta(1.2, 1.2)	(den Ouden et al., 2013)
softmax inverse temperature, β	5 [note that β = 1 in all other models]	Gamma(α=4.82, β=0.88)	(Gershman, 2016)
Intersubject variability in parameters			
Intersubject standard deviations for α^{rew}, α^{pun}, α^{reinf}, τ^{loc}, ρ , φ	As above	Half-normal: Normal(0, 0.05) constrained to $\geq$0	(Kanen et al., 2019)
Intersubject standard deviations for τ^{reinf}, β	As above	Half-normal: Normal(0, 1) constrained to $\geq$0	(Gershman, 2016) Altered from Cauchy to half-normal as per Stan recommendations (Stan Development Team; http://mc-stan.org/)

Table 8.1: *rew* reward, *pun* punishment, *reinf* reinforcement, *loc* location, *stim* stimulus.

For each group (healthy controls, SUD, OCD) and drug (placebo, amisulpride, pramipexole) combination, 100 identical virtual "subjects" were simulated using the posterior group mean parameters from the winning model. Each "subject" performed the probabilistic reversal learning task in silico. Inter-subject parameter variability (or, therefore, a within-subjects structure) was not simulated, because the purpose of this analysis is to use arbitrarily high power to establish the model's sufficiency to reproduce known behavioural patterns. For a given group/drug combination, variability in the decisions made by each virtual subject is a consequence only of the random process via which choice probabilities are mapped to concrete choices, and the random assignment of stimuli to left/right sides.

To demonstrate the necessity (as well as the sufficiency) of changes in stickiness parameters to explain key behavioural effects, two further simulations were conducted. The first additional simulation fixed the location stickiness parameter, τ^{loc}, so that it did not vary between groups or drugs. The simulation was performed exactly as above except that for all "subjects" in all drug conditions, τ^{loc} was set to its overall posterior mean (taken, for simplicity, as the mean of the 3×3 per-group/per-drug posterior mean parameters). The second additional simulation did the same but fixed τ^{stim} instead, likewise.

8.3 Results

8.3.1 Baseline characteristics

Whilst participants did not have any other Axis I psychiatric diagnosis at the time of the study, the groups differed in their depression scores on the Beck Depression Inventory (BDI-II; Beck et al. 1996; $F(2,50) = 19.782$, $p < .001$). Both the SUD and OCD groups had significantly greater depression scores than controls ($t(18) = -3.759$, $p = .001$ for SUD; $t(18) = -5.960$, $p < .001$ for OCD). Depression scores in the OCD group were also significantly greater than in the SUD group ($t(28) = -3.068$, $p = .005$). Other baseline characteristics on the three groups of participants are presented in Figure 8.4.

8.3.2 Choice of model

Seven reinforcement learning models were fitted and compared (Kanen et al., 2019). Convergence was not perfect, with a maximum R^ = 1.478, but was good, with > 99% of parameters and contrasts having R^ < 1.1. Moreover, all parameters of interest (all group-level mean and distributional parameters and all contrasts), had R^ < 1.121. The winning model, as determined using a bridge sampling estimate of the marginal likelihood (Figure 8.3), included five parameters: 1) positive reinforcement rate, the extent to which behaviour is driven by learning

Group	HC	SUD	OCD	*F*	*df*	*P*
Age (years)	32.7 (± 6.9)	34.3 (± 7.4)	35.4 (± 9.8)	0.49	2.50	0.618
Gender ratio (male/female)	15:3	14:3	7:11			0.318[a]
Ethnic ratio (Caucasian:Afro-Caribbean)	17:1	15:2	18:00			0.308[a]
Verbal intelligence quotient (NART)	108.4 (± 6.0)	108.0 (± 8.3)	107.9 (± 8.8)	0.06	2.50	0.938
Years of education	12.4 (± 1.8)	11.2 (± 1.0)	12.3 (± 2.0)	2.06	2.50	0.082
Dysphoric mood, BDI-II (total score at baseline)	1.1 (± 2.4)	9.8 (± 11.2)	18.5 (± 10.0)	18.07	2.50	< 0.001
Impulsivity, BIS-11 (total score)	62.0 (± 7.2)	81.7 (± 9.7)	66.9 (± 9.7)	22.83	2.49	< 0.001
Compulsivity, Y-BOCS (total score)	0.1 (± 0.5)	–	24.11 (± 13.0)	–	–	–
Compulsivity, OCDUS (total score)	–	26.0 (± 7.8)	–	–	–	–
Age of onset (years) of stimulant abuse or of OCD	–	20.5 (± 5.4)	17.1 (± 11.0)	–	–	–
Duration (years) of stimulant abuse or of OCD	–	11.7 (± 7.4)	18.3 (± 10.6)	–	–	–

Figure 8.4: Demographic, psychological and baseline personality measures for the groups of healthy controls (HC; n=18), individuals with stimulant use disorder (SUD; n=17), and individuals with obsessive-compulsive disorder (OCD; n=18). Reproduced with permission from Ersche et al. (2011) and Kanen et al. (2019). NART = National Adult Reading Test (Blair and Spreen, 1989); BDI-II = Beck Depression Inventory, version II (Beck et al., 1996); BIS-11 = Barratt Impulsiveness Scale, version 11 (Patton et al., 1995); Y-BOCS = Yale-Brown Obsessive-Compulsive Scale (Goodman et al., 1989); OCDUS = Obsessive-Compulsive Drug Use Scale (Franken et al., 2002). [a]Fisher's Exact Test.

from positive feedback; 2) punishment reinforcement rate, or learning from negative feedback; 3) reinforcement sensitivity, which is the overall sensitivity to reinforced stimulus value; 4) "stimulus stickiness", the tendency to repeat choices to recently chosen stimuli, regardless of outcome; and 5) "side (location) stickiness", the degree to which participants responded to, or got "stuck" to, the same side (location) of the computer screen as before, left or right, irrespective of stimulus or outcome. The stickiness parameters, it is worth noting, can be comparable to strategies of exploration versus exploitation (Clarke et al., 2014; Seymour et al., 2012) and may relate to conventional measures of perseveration. Kanen et al. (2019) confirmed the winning model demonstrated parameter recovery from simulated data.

8.3.3 Simulation of behavioural data from winning model

The winning model reproduced key behavioural phenomena found by Ersche et al. (2011). In line with their results, analysis of simulated data revealed a main effect of group for the number of trials per sequence ($F(2,297) = 97.477$, $p = 2.84 \times 10^{-33}$). The SUD group required more trials per sequence to reach criterion compared to both the healthy control group ($t(195) = -8.792$, $p = 7.62 \times 10^{-16}$) and the OCD group ($t(187) = -13.638$, $p = 7.36 \times 10^{-30}$). The groups also differed in the number of spontaneous errors ($F(2,297) = 394.392$, $p = 2.49 \times 10^{-84}$), with the SUD group making more spontaneous errors than both controls ($t(168) = -21.028$, $p = 5.80$

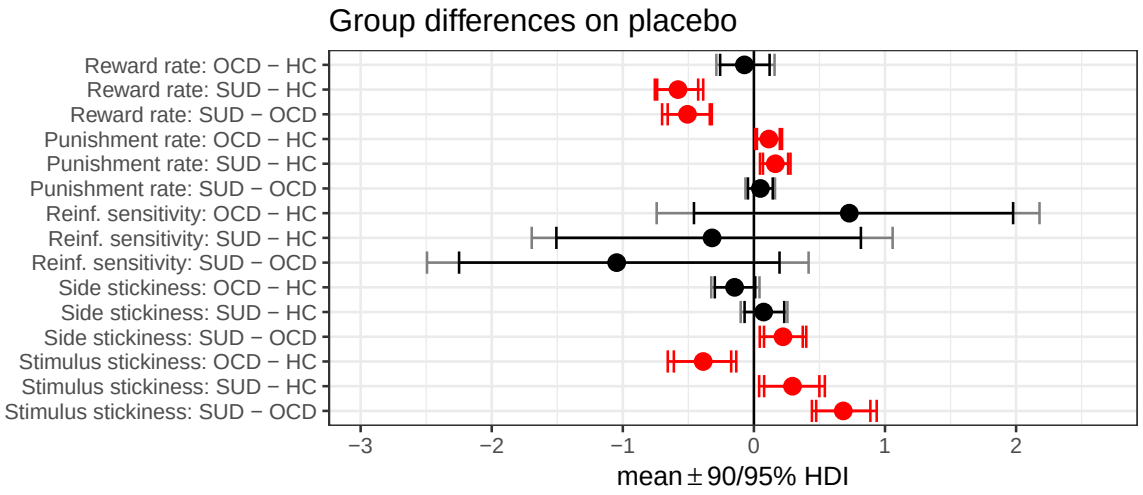

Figure 8.5: Differences between groups on placebo. Abbreviations: Reinf. = reinforcement; HC = healthy controls. The optimal computational model contained parameters measuring (from top to bottom) learning from positive feedback, learning from negative feedback, sensitivity to reinforcement, a tendency to repeat choices on a recently chosen side (side stickiness), and a tendency to repeat choices to a recently chosen stimulus (stimulus stickiness). Differences in parameter per-group means under placebo; posterior $0 \notin 95\%$ HDI signified in red.

x 10^{-49}) and the OCD group (t(148) = -23.446, p = 1.0504 x 10^{-51}).

There was a main effect of group, absent in Ersche et al. (2011), for the number of probabilistic switches (F(2,297) = 26.896, p = 1.84 x 10^{-11}). Both the SUD (t(198) = -4.302, p = 2.7 x 10^{-5}) and OCD groups (t(198) = -7.343, p = 5.31 x 10^{-12}) showed greater probabilistic switching compared to controls, and the OCD group demonstrated more probabilistic switching than the SUD group (t(198) = -3.032, p = .003). I observed a main effect of perseverative error rate (F(2,297) = 36.101, p = 9.24 x 10^{-15}). While this main effect was absent in Ersche et al. (2011), the follow up t-tests are in line with their post-hoc analysis: The perseverative error rate was greater in SUD compared to controls (t(183) = -2.385, p = .018), was greater in SUD compared to OCD (t(173) = 7.967, p = 2.10 x 10^{-13}), and was greater in healthy controls than in OCD (t(198) = 6.611, p = 3.46 x 10^{-10}).

Next, drug effects in the simulated data were assessed, again using the same analyses as reported in Ersche et al. (2011). There was a significant effect of drug on number of trials per sequence (F(2,891) = 6.603, p = .001), and there was also a drug-by-group interaction (F(4,891) = 6.234, p = 6 x 10^{-5}). For spontaneous errors there was also a main effect of drug (F(2,891) = 33.939, p = 6.24 x 10^{-15}) and a drug-by-group interaction (F(4,891) = 22.624, p = 8.09 x 10^{-18}). There was a main effect of drug on probabilistic switches as well (F(2,891) = 10.802, p = 2.3 x 10^{-5}). As in Ersche et al. (2011), there was no drug-by-group interaction

($F(4,891) = 1.630$, $p = .165$) for probabilistic switches. There was a drug-by-group interaction on the perseverative error rate ($F(4,891) = 3.377$, $p = .009$), in line with Ersche et al. (2011). Post hoc analyses on the perseverative error rate revealed a main effect of group for amisulpride ($F(2,297) = 14.939$, $p = 6.58 \times 10^{-7}$) and for pramipexole ($F(2,297) = 20.347$, $p = 5.23 \times 10^{-9}$). In contrast to Ersche et al. (2011), pramipexole was not associated with a change in the perseverative error rate compared to placebo, in SUD ($t(198) = -.071$, $p = .944$). The difference in the perseverative error rate in the SUD group and healthy controls persisted when on pramipexole ($t(178) = -3.933$, $p = 1.20 \times 10^{-4}$). Consistent with Ersche et al. (2011) there was no change in the perseverative error rate on pramipexole relative to placebo in healthy controls ($t(198) = 1.912$, $p = .057$) or in the OCD group ($t(198) = -.980$, $p = .328$). Amisulpride, in line with Ersche et al. (2011), did not significantly alter the perseverative error rate compared to placebo in the healthy control ($t(198) = -1.147$, $p = .253$), SUD ($t(198) = 1.618$, $p = .107$), or OCD ($t(190) = -1.876$, $p = .062$) groups.

8.3.4 Simulation with fixed value for stimulus stickiness

Next, simulated data, generated with a fixed value for the stimulus stickiness parameter, were analysed to determine whether variation in this parameter was necessary to optimally capture the key behavioural phenomena from Ersche et al. (2011). Whilst on placebo, the main effects of group persisted for the number of trials per sequence ($F(2,297) = 88.209$, $p = 8.52 \times 10^{-31}$) and spontaneous errors ($F(2,297) = 911.582$, $p = 1.73 \times 10^{-127}$). The SUD group still required a greater number of trials per sequence than the healthy controls ($t(198) = -10.195$, $p = 6.91 \times 10^{-20}$) and the OCD group ($t(198) = -12.182$, $p = 7.66 \times 10^{-26}$), and this was also true for spontaneous errors: there were more spontaneous errors in SUD compared to controls ($t(178) = -31.738$, $p = 3.09 \times 10^{-75}$) and relative to the OCD group ($t(157) = -36.893$, $p = 2.34 \times 10^{-79}$). There was a main effect of group on probabilistic switches ($F(2,297) = 39.424$, $p = 6.53 \times 10^{-16}$), which was absent in Ersche et al. (2011), but present in the prior simulation analysis which incorporated variation in stimulus stickiness parameter. The pattern of effects, however, changed. In the standard simulation analysis, above, the OCD group showed the most probabilistic switches, followed by the SUD group. When fixing the stimulus stickiness parameter value, however, the SUD group now displayed the most probabilistic switching. There were more probabilistic switches in the SUD group compared to controls ($t(198) = -8.336$, $p = 1.28 \times 10^{-14}$), and in SUD compared to the OCD group ($t(198) = -6.654$, $p = 2.73 \times 10^{-10}$). Probabilistic switches in the OCD group, at the same time, were no longer significantly greater than controls ($t(198) = -1.705$, $p = .09$), which had been the case when incorporating variation in the stimulus stickiness parameter. Critically, the main effect of group on the perseverative error rate, in simulated data generated without variation in the

stimulus stickiness parameter, was completely abolished ($F(2,297) = .546$, $p = .58$). This finding on the perseverative error rate underlines the importance and centrality of the stimulus stickiness parameter in the winning model.

Critically, by fixing the value of the stimulus stickiness parameter, there was no main effect of drug ($F(2,897) = .004$, $p = .996$), nor was there a drug-by-group interaction ($F(4,891) = 2.322$, $p = .055$) on the perseverative error rate. The main findings of Ersche et al. (2011) hinged on presence of this interaction. Variation in the stimulus stickiness parameter in the model, therefore, was necessary to reproduce key patterns of effects on behaviour in relation to drug and disease, seen in the original data.

8.3.5 Simulation with fixed value for side stickiness

I then analysed simulated data generated instead with a fixed value for the side (location) stickiness parameter, with variation in the stimulus stickiness parameter. In contrast to the importance of variation in the stimulus stickiness parameter to capture an effect of group on the perseverative error rate, a fixed value of the side stickiness parameter still produced a highly significant effect of group on the perseverative error rate under placebo ($F(2,297) = 28.519$, $p = 4.68 \times 10^{-12}$). The SUD group had a higher perseverative error rate than both the healthy controls ($t(171) = -4.145$, $p = 5.3 \times 10^{-5}$) and the OCD group ($t(198) = -6.977$, $p = 4.43 \times 10^{-11}$). Controls additionally had a greater perseverative error rate than the OCD group ($t(198) = 3.692$, $p = 2.87 \times 10^{-4}$). The three other key behavioural patterns could also be captured despite a fixed value for the side (location) stickiness parameter: number of trials per sequence ($F(2,297) = 147.069$, $p = 4.06 \times 10^{-45}$) and spontaneous errors ($F(2,297) = 484.526$, $p = 3.09 \times 10^{-94}$) – both consistent with Ersche et al. (2011) – as well as probabilistic switches ($F(2,297) = 27.327$, $p = 1.28 \times 10^{-11}$). In line with Ersche et al. (2011), the SUD group required more trials per sequence than both the healthy controls ($t(191) = -12.046$, $p = 3.19 \times 10^{-25}$), and the OCD group ($t(187) = -15.925$, $p = 1.26 \times 10^{-36}$), and the same pattern was observed for spontaneous errors: the SUD group made more spontaneous errors than the healthy controls ($t(170) = -24.657$, $p = 4.30 \times 10^{-58}$) and the OCD group ($t(169), = -25.448$, $p = 1.02 \times 10^{-59}$). Pairwise comparisons on the number of probabilistic switches produced the same pattern of results as in the first simulation analysis, where neither stimulus nor side stickiness were fixed values: Both the SUD ($t(198) = -4.861$, $p = 2 \times 10^{-6}$) and OCD groups ($t(183) = -7.484$, $p = 2.93 \times 10^{-12}$) showed greater probabilistic switching compared to controls, and the OCD group demonstrated more probabilistic switching than the SUD group ($t(198) = 2.547$, $p = .012$).

Critically, by holding constant the value of the side (location) stickiness parameter, there was no main effect of drug ($F(2,897) = .937$, $p = .392$), nor was there a drug-by-group interaction on the perseverative error rate ($F(4,891) = 2.047$, $p = .086$), demonstrating the importance

Parameter	SUD vs HC			OCD vs HC			SUD vs OCD
	Placebo	Effects of amisulpride	Effects of pramipexole	Placebo	Effects of amisulpride	Effects of pramipexole	Placebo
Reward learning rate, α^{rew}	↓	↑	↑				↓
Punishment learning rate, α^{pun}	↑		↓	↑			
Reinforcement sensitivity, τ^{reinf}		↓	↓				
Location (side) stickness, τ^{loc}		↓	↓				↑
Stimulus stickness, τ^{stim}	↑	↓		↓			↑

Figure 8.6: Summary of between-group effects on parameters from the winning model. Contrasts shown are (left to right) SUD_placebo – HC_placebo; [(SUD_drug – SUD_placebo) – (HC_drug – HC_placebo)] for amisulpride, and separately for pramipexole; OCD_placebo – HC_placebo; [(OCD_drug – OCD_placebo) – (HC_drug – HC_placebo)] for amisulpride, and separately for pramipexole; SUD_placebo – OCD_placebo. Arrows denote an increase or decrease of a parameter in a given contrast. Lack of an arrow indicates no difference. From Kanen et al. (2019).

of variation in this model parameter.

8.3.6 Stimulus stickiness

The first novel result was that the stimulus stickiness parameter differentiated SUD and OCD. Stimulus stickiness assesses stimulus-bound behaviour, a measure of the degree to which choices were driven by the stimulus that was selected in the recent past, irrespective of outcome. Under placebo, individuals with SUD showed significantly increased stimulus stickiness relative to healthy controls (difference in parameter per-group mean, posterior 95% highest posterior density interval (HDI) excluding zero), whereas people with OCD showed decreased stimulus stickiness relative to controls (group difference, $0 \notin$ 95% HDI); see Figure 8.5 and 8.6.

8.3.7 Effects of dopaminergic agents on stimulus stickiness

Amisulpride ameliorated the elevated stimulus stickiness in SUD: there was improvement both compared to their performance on placebo (drug difference, $0 \notin$ 95% HDI; Figure 8.8, 8.7), and relative to the effects of amisulpride on healthy controls (group difference in drug effect, $0 \notin$ 95% HDI; Figure 8.9, 8.6). Stimulus stickiness in OCD, on the other hand, was unaffected: amisulpride did not have a different effect on stimulus stickiness in OCD relative to their performance on placebo (no drug difference, $0 \in$ 95% HDI; Figure 8.10, 8.7) nor

	HC		SUD		OCD	
Parameter	Amisulpride	Pramipexole	Amisulpride	Pramipexole	Amisulpride	Pramipexole
Reward learning rate, α^{rew}			↑	↑		
Punishment learning rate, α^{pun}	↑	↑		↓	↑	↑
Reinforcement sensitivity, τ^{reinf}			↓	↓	↓	
Location (side) stickness, τ^{loc}			↓	↓		
Stimulus stickness, τ^{stim}			↓			

Figure 8.7: Within-group drug effects on parameters from the winning model. All effects are comparisons between drug and placebo within a group. Within group comparisons: HC_amisulpride – HC_ placebo; HC_pramipexole – HC_placebo; likewise for SUD and OCD. Arrows denote an increase or decrease of a parameter in a given contrast. Lack of an arrow indicates no difference. From Kanen et al. (2019).

when compared to the effect of amisulpride on healthy controls (no group difference, $0 \in 95\%$ HDI; Figure 8.12, 8.6). Pramipexole had no effect on stimulus stickiness in SUD or OCD (no differences $0 \in 95\%$ HDI; Figure 8.8, 8.10, 8.11,8.12, 8.6, 8.7). Stimulus stickiness in healthy controls was unaffected by either drug (no drug differences, $0 \in 95\%$ HDI; Figure 8.9, 8.7).

8.3.8 Side (location) stickiness

I also evaluated another type of stickiness: side stickiness, or the tendency to repeat choices to the same side (location) of the computer screen as before, regardless of stimulus type or outcome. Side (location) stickiness was greater in SUD compared to OCD (group difference, $0 \notin 95\%$ HDI; Figure 8.5, 8.6), and dopaminergic agents modulated this parameter in SUD only. Individuals with OCD, on placebo, showed no impairment of side stickiness relative to healthy controls (no group difference, $0 \in 95\%$ HDI; Figure 8.5, 8.6). Both amisulpride and pramipexole reduced side stickiness in individuals with SUD compared to their performance on placebo (drug differences, $0 \notin 95\%$ HDI; Figure 8.8, 8.7). When comparing drug effects in SUD to those in controls, amisulpride reduced side stickiness more in SUD than in controls (group difference, $0 \notin 95\%$ HDI; Figure 8.11, 8.6), but pramipexole did not have a differential effect (no group difference, $0 \in 95\%$ HDI; Figure 8.11, 8.6). In the OCD group, side stickiness was unaffected by either amisulpride or pramipexole compared to placebo (no drug differences, $0 \in 95\%$ HDI; Figure 8.10, 8.7). Amisulpride and pramipexole, additionally, did not differentially affect side stickiness in the OCD group relative to healthy controls (no group differences, $0 \in 95\%$ HDI; Figure 8.12, 8.6).

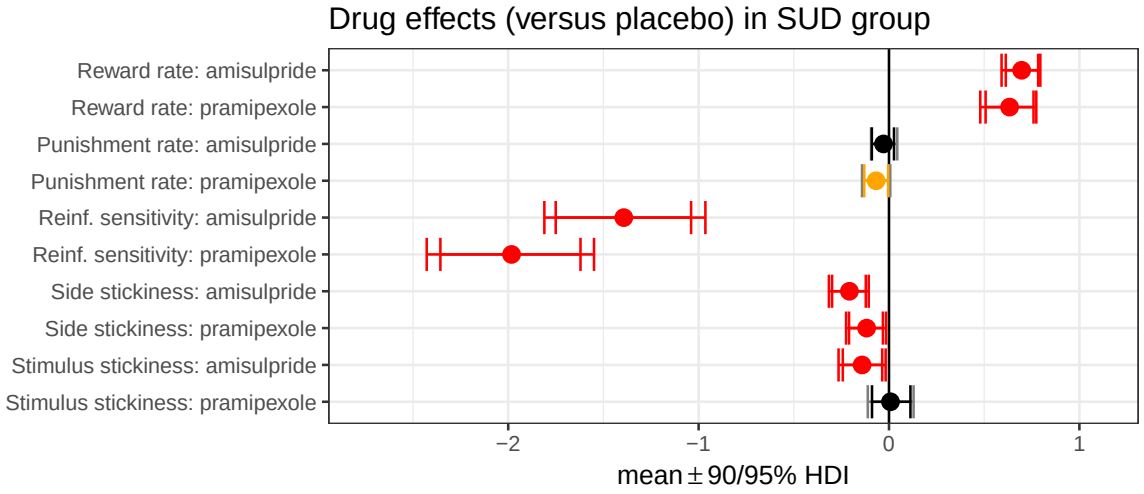

Figure 8.8: Effects of amisulpride and pramipexole (relative to placebo) in stimulant use disorder (SUD). Difference in parameter per-condition means between SUD under amisulpride or pramipexole compared to SUD on placebo, posterior $0 \notin 95\%$ HDI; SUD_drug – SUD_placebo. Reinf. = reinforcement.

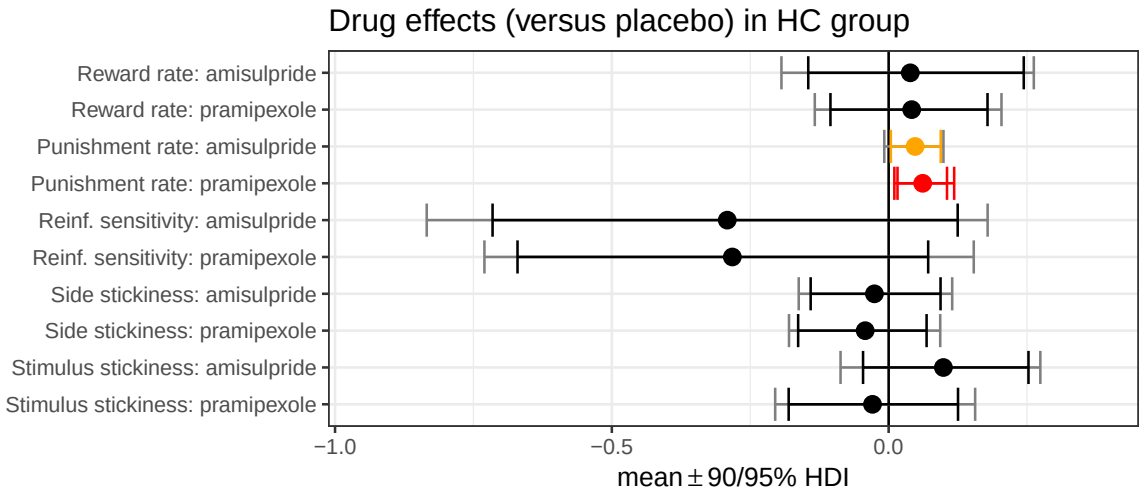

Figure 8.9: Effects of amisulpride and pramipexole (relative to placebo) in healthy controls (HC). Difference in parameter per-condition means between HC on amisulpride or pramipexole compared to HC on placebo; posterior $0 \notin 95\%$ HDI signified in red, $0 \notin 90\%$ HDI in orange; HC_drug – HC_placebo. Reinf. = reinforcement.

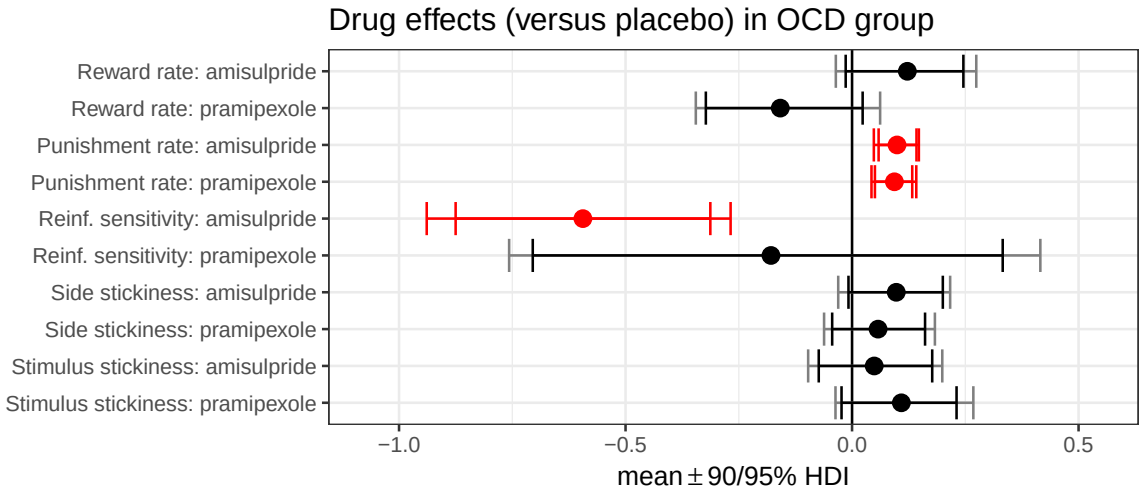

Figure 8.10: Effects of amisulpride and pramipexole (relative to placebo) in OCD. Difference in parameter per-condition means between OCD on amisulpride or pramipexole compared to OCD on placebo, posterior $0 \notin 95\%$ HDI; OCD_drug – OCD_placebo. Reinf. = reinforcement.

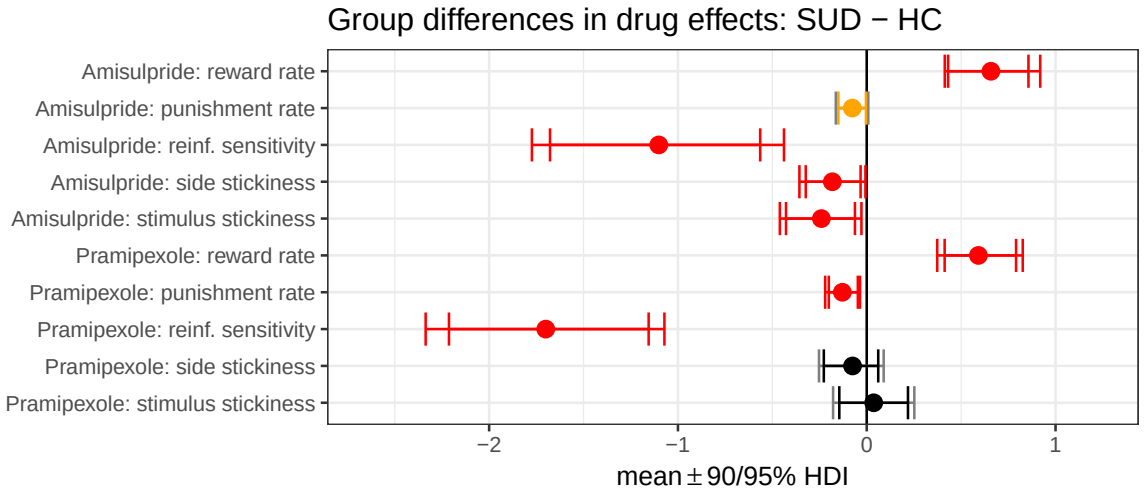

Figure 8.11: Differences in the effects of amisulpride or pramipexole between the SUD group and healthy controls (HC). All contrasts represent the difference between drug X's effect in SUD and its effect in the control group. [(SUD_drug – SUD_placebo) – (HC_drug – HC_placebo)] for amisulpride, and separately for pramipexole; posterior $0 \notin 95\%$ HDI signified in red, $0 \notin 90\%$ HDI in orange. Reinf. = reinforcement.

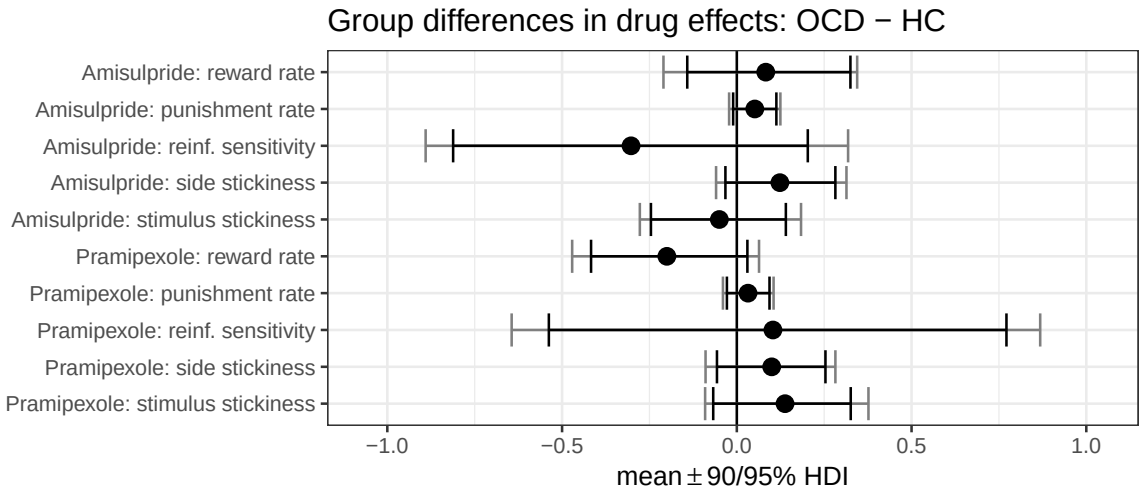

Figure 8.12: Differences in the effects of amisulpride or pramipexole between the OCD group and healthy controls (HC). All contrasts represent the difference between drug X's effect in the OCD group and its effect in the control group. Posterior $0 \in$ 95% HDI denoted in black, indicating no differences. This was a subtraction of [(OCD_drug – OCD_placebo) – (HC_drug – HC_placebo)] for amisulpride, and separately for pramipexole. Reinf. = reinforcement.

8.3.9 Reward-driven learning

I measured the rate at which participants learned from positive feedback in the task. Reward-driven learning was impaired in SUD and not in OCD: On placebo, individuals with SUD showed diminished learning from positive feedback relative to controls (group difference, $0 \notin$ 95% HDI; Figure 8.5, 8.6), while the OCD group was no different from healthy controls in their reward learning (no group difference, $0 \in$ 95% HDI; Figure 8.5, 8.6). Reward learning in SUD was sensitive to dopaminergic agents: Both amisulpride and pramipexole remediated the diminished reward learning seen under placebo, increasing reward-driven learning (drug differences, $0 \notin$ 95% HDI; Figure 8.8, 8.7). This was also the case when comparing the effects of the dopaminergic agents on reward-driven learning in SUD versus healthy controls (group differences, $0 \notin$ 95% HDI; Figure 8.11, 8.6). Neither amisulpride nor pramipexole, meanwhile, altered reward-driven learning in individuals with OCD, both when compared to these drugs' effects in controls (no group differences, $0 \in$ 95% HDI; Figure 8.12, 8.6) and when contrasted with their own performance on placebo (no drug differences, $0 \in$ 95% HDI; Figure 8.10, 8.7). Reward learning in controls was unaffected by amisulpride and pramipexole, compared to placebo (no drug differences, $0 \in$ 95% HDI; Figure 8.9, 8.7).

8.3.10 Punishment-driven learning

Both individuals with SUD and with OCD showed increased learning from negative feedback (punishment in the form of nonreward) on placebo, compared to healthy controls (group differences, $0 \notin 95\%$ HDI; Figure 8.5, 8.6). In individuals with SUD, pramipexole led to a small improvement in their elevated negative feedback learning relative to placebo (drug difference, $0 \notin 90\%$ HDI; Figure 8.8, 8.7). Amisulpride, on the other hand, neither worsened nor corrected the elevated negative feedback-driven learning seen on placebo (no drug difference, $0 \in 95\%$ HDI; Figure 8.8, 8.7). Relative to their baseline performance on placebo, the OCD group showed a further increase in learning from negative feedback on both amisulpride and pramipexole (drug differences, $0 \notin 95\%$ HDI; Figure 8.10, 8.7). The negative feedback learning rate was the only parameter in the model that was modulated in healthy controls by dopaminergic agents. The control group showed an increase in learning from negative feedback on amisulpride (drug difference, $0 \notin 90\%$ HDI; Figure 8.9, 8.7) and more so under pramipexole (drug difference, $0 \notin 95\%$ HDI; Figure 8.9, 8.7). Amisulpride and pramipexole, in fact, increased negative feedback-driven learning in OCD and healthy controls to the same extent (no group differences, $0 \in 95\%$ HDI; Figure 8.12, 8.6). Dopaminergic agents differentially affected negative feedback learning in SUD when contrasted with healthy controls: the SUD group showed a relative decrease in this parameter on amisulpride (group difference, $0 \notin 90\%$ HDI; Figure 8.11, 8.6) and pramipexole (group difference, $0 \notin 95\%$ HDI; Figure 8.11, 8.6), driven in part by the drug-induced elevated negative feedback learning rate in controls.

8.3.11 Reinforcement sensitivity

Reinforcement sensitivity, the overall sensitivity to reinforced stimulus value, was unimpaired in SUD and OCD at baseline but was compromised by dopaminergic agents. Results on this parameter were not different between the OCD, SUD, or healthy control groups under placebo (no group differences, $0 \in 95\%$ HDI; Figure 8.5, 8.6). There were, however, drug-induced effects. Reinforcement sensitivity in individuals with SUD was most impaired by dopaminergic modulation. Both amisulpride and pramipexole reduced reinforcement sensitivity in SUD, relative to placebo (group differences, $0 \notin 95\%$ HDI; Figure 8.8, 8.7). Amisulpride and pramipexole also reduced reinforcement sensitivity in SUD when compared to the drug effects on healthy controls (drug differences, $0 \notin 95\%$ HDI; Figure 8.11, 8.6). In the OCD group, amisulpride induced a deficit compared to placebo (drug difference, $0 \notin 95\%$ HDI; Figure 8.10, 8.7), whereas pramipexole had no effect (no drug difference, $0 \in 95\%$ HDI; Figure 8.10, 8.7). Amisulpride and pramipexole did not differentially affect reinforcement sensitivity in the OCD group compared to controls (no group differences, $0 \in 95\%$ HDI; Figure 8.12, 8.6).

Finally, neither amisulpride nor pramipexole induced an impairment of reinforcement sensitivity in healthy controls compared to placebo (no drug differences, $0 \in 95\%$ HDI; Figure 8.9, 8.7).

8.3.12 Correlations with conventional behavioural measures

I then tested to see how the parameters in the winning model related to the conventional measures of behaviour (see Methods) from Ersche et al. (2011). Because stimulus stickiness is a measure of stimulus-bound perseveration, I asked whether stimulus stickiness was correlated with either conventional measure of perseveration in Ersche et al. (2011), which was their primary focus. I found that in healthy controls on placebo, stimulus stickiness was significantly positively correlated with the number of perseverative errors ($r = .587$, $p = .01$, uncorrected) and the perseverative error rate ($r = .672$, $p = .002$, uncorrected). In SUD and OCD on placebo, however, stimulus stickiness was not correlated with the perseverative error rate or the number of perseverative errors (all $p > .05$). Under amisulpride or pramipexole, moreover, neither the perseverative error rate nor the number of perseverative errors correlated with stimulus stickiness in the healthy control, SUD, or OCD groups (all $p > .05$).

Side stickiness was not correlated with either measure of perseveration under placebo in the healthy control, SUD, or OCD groups (all $p > .05$). There were also no significant correlations between side stickiness and the two measures of perseveration in the SUD or OCD groups on either amisulpride or pramipexole (all $p > .05$). In healthy controls on amisulpride there was a significant correlation between side stickiness and the number of perseverative errors ($r = .773$, $p = 1.69 \times 10^{-4}$, uncorrected), and the perseverative error rate ($r = .473$, $p = .048$, uncorrected). On pramipexole, side stickiness was also correlated in healthy controls with the number of perseverative errors ($r = .499$, $p = .035$, uncorrected).

I also asked whether the diminished reward learning rate I observed in SUD on placebo was related to the increase in spontaneous errors reported in Ersche et al. (2011) – in other words a decrease in staying with the correct choice despite having received a reward (decreased win-stay). Indeed a decreased reward rate was correlated with a greater number of spontaneous errors in SUD on placebo ($r = -.752$, $p = 4.92 \times 10^{-4}$, uncorrected). I then asked whether an increased punishment learning rate was related to increased probabilistic switches in Ersche et al. (2011) – increased switching to the incorrect choice following misleading negative feedback, i.e. lose-shift behaviour. This was indeed the case for all three groups: a greater punishment learning rate was correlated with more probabilistic switches in healthy controls ($r = .748$, $p = 3.58 \times 10^{-4}$, uncorrected), SUD ($r = .815$, $p = 6.7 \times 10^{-5}$, uncorrected), and OCD ($r = .858$, $p = 5 \times 10^{-6}$, uncorrected).

8.3.13 Pairwise tests of probabilistic switches

Because Ersche et al. (2011) did not find a main effect of group on probabilistic switching (lose-shift) behaviour they did not report pairwise comparisons between the groups. I realised conducting these comparisons would be important for multiple reasons: It would enable me to 1) compare the original SUD data from Ersche et al. (2011) to PRL in alcohol use disorder (AUD) from Reiter et al. (2016) who compared only two groups; 2) compare the original SUD data to the results from the simulation analysis; 3) compare the original OCD data to that of Hauser et al. (2017a), who also only compared two groups, and 4) to understand how the original findings in the healthy control, SUD, and OCD groups relate to parameters such as the punishment learning rate. The SUD group showed more probabilistic switching (lose-shift) behaviour compared to healthy controls ($t(33) = -2.119$, $p = .042$). This effect was also marginally significant in the OCD group compared to controls: increased probabilistic switching ($t(23) = -2.054$, $p = .051$). There was no difference between the OCD and SUD groups ($t(27) = -.606$, $p = .549$).

8.3.14 Correlations with questionnaire measures

I tested whether stimulus-bound behaviour – stimulus stickiness – as measured under placebo, was correlated with severity of compulsive symptoms as assessed through questionnaires. Scores on the Obsessive Compulsive Drug Use Scale (OCDUS; Franken et al., 2002) in the SUD group were not correlated with their stimulus stickiness results ($r = -.028$, $p = .914$). There was also no significant correlation between stimulus stickiness in the OCD group and their scores on the Yale-Brown Obsessive Compulsive Scale (Y-BOCS; Goodman et al., 1989; $r = -.395$, $p = .105$). I also tested whether stimulus stickiness in SUD was correlated with years of drug use, which would suggest a drug-induced effect; this correlation, however, was not significant ($r = -.177$, $p = 496$). Because the SUD, OCD, and control groups differed in their depression scores on the BDI-II, I also tested whether this was correlated with stimulus stickiness results on placebo. Depression scores were not correlated with stimulus stickiness in SUD ($r = -.163$, $p = .531$), OCD ($r = .415$, $p = .087$), or healthy controls ($r = -.080$, $p = .754$)

8.4 Discussion

The aim of this study was to uncover the microstructure of behaviour, and its dopaminergic modulation, in SUD and OCD using RL models. It was found that the computational profile of PRL performance differed between SUD, OCD, and healthy controls, and both dopaminergic drugs tested modulated behavioural parameters in all three groups, which considerably

extends the conventional analyses of Ersche et al. (2011). One key result was in regard to stimulus stickiness, which measures a basic perseverative tendency. This one measure differentiated all three groups: individuals with SUD demonstrated increased stimulus stickiness while the OCD group displayed a decrease relative to controls (Figure 8.5). The former result is consistent with Ersche et al. (2011) who showed a perseverative impairment in SUD. The finding of the opposite change in OCD, meanwhile, demonstrates that the stimulus stickiness measure in the model was able to detect subtleties of behaviour across diagnostic categories which were not clearly delineated using conventional methods. Interestingly, stimulus stickiness only correlated with perseveration in the healthy control group, suggesting that this measure is indeed related to perseveration, yet also reinforcing the novelty of stimulus stickiness in assessing these two disorders of compulsivity. This is in consonance with Rygula et al. (2015) who studied PRL in monkeys with different serotonergic lesions: compared to controls they found elevation of stimulus stickiness in one group, reduction in another, and perseveration in neither. A second major set of findings related to basic changes in reward and punishment (nonreward) learning occurring in the SUD and OCD groups, not formalised in the earlier study. The SUD group showed reduced reward learning, whilst both groups demonstrated enhanced learning rates with punishment. These parameters were also sensitive to dopaminergic drug treatments and are discussed in detail below.

8.4.1 Computational modelling procedure

A fully Bayesian process for model comparison and parameter estimation was used. The theoretically optimal method for model comparison is to evaluate the probabilities of each model, given the data; such a process incorporates Occam's razor correctly, penalizing over-complex models (Gronau et al., 2017a; Kruschke, 2011). Bridge sampling (Gronau et al., 2017a,b) allows these probabilities to be estimated directly, in combination with prior probabilities of models; it was assumed that all models were equiprobable a priori. This process eliminated simple reinforcement learning algorithms and selected a model incorporating reinforcement learning with separate rates for reward and punishment (nonreward), in addition to perseverative ("stickiness") behaviour in respect both of the stimulus selected and the response side (location). This model was superior to the EWA model, which incorporates perseverative tendencies in a different way. The model comparison process only selects amongst the models offered for comparison; it is of course inevitable that the true biological processes are more complex than the winning model, and possible that a more complex model that was not considered was better. Nevertheless, simulations demonstrated that the winning model was sufficient (and variation in its stickiness parameters necessary) to capture the key behavioural phenomena found in this dataset by Ersche et al. (2011). Critically, group differences in

perseveration were completely abolished in simulated data generated without variation in the stimulus stickiness parameter. Drug effects on perseveration were additionally absent, which was also true when holding the value of the side stickiness parameter constant. The winning model therefore provides the following interpretation of disease and drug effects in this task.

8.4.2 Dopaminergic modulation of stickiness parameters

I found that amisulpride, but not pramipexole, remediated the elevated stimulus stickiness in SUD, whereas neither drug modulated stimulus stickiness in OCD. This is in contrast to Ersche et al. (2011) who found pramipexole remediated the perseverative deficit in SUD, while their analysis did not detect an effect of amisulpride on behaviour in any group. Both drugs, meanwhile, decreased side (location) stickiness in SUD. These varied pharmacological results are in line with the observation that stimulus stickiness, side (location) stickiness, and perseveration, were distinguishable, yet at times related. Parsing these three conceptually similar measures helps refine the way we study behavioural inflexibility. While the effects of amisulpride and pramipexole on stickiness parameters and perseveration are ostensibly paradoxical, D2/3 agonism and antagonism have each produced deficits in reversal learning – on a deterministic schedule in non-human animals – and the results are multifaceted. D2/3 agonism and antagonism, for instance, affected different aspects of reversal learning (Boulougouris et al., 2008; Lee et al., 2007). D2/3 antagonism, additionally, when co-administered with a D2/3 agonist, protected against the deficit induced by the agonist, and D2 and D3 receptors have been shown to play distinct roles in reversal learning (Boulougouris et al., 2008). Although pramipexole may have higher affinity for the D3 receptor (Camacho-Ochoa et al., 1995), the respective contributions of D2 and D3 receptors cannot be dissected using less selective drugs like amisulpride or pramipexole, as in this study. It is also possible that autoreceptor activity, and the doses used, contributed to the directionality of the effects – notions that apply to all of the present findings on dopaminergic modulation of computational parameters; Horst et al. (2019), for example, have recently shown that the D2/3 agonist quinpirole has triphasic effects on serial deterministic reversal leaning in marmoset monkeys when infused into the caudate nucleus. Performance was impaired at both low and high doses, and improved at intermediate doses; the effects at low doses were likely due to effects of presynaptic autoreceptors. Activation of somato-dendritic autoreceptors versus striatal terminal dopamine autoreceptors, furthermore, may have different effects. Additionally, an important pattern of results raises the notion of baseline-dependency: d-amphetamine induced both response switching and perseveration in the rat (Evenden and Robbins, 1983). Which behavioural pattern was emitted depended not only on dose – perseveration generally occurred at higher doses – and the task structure, but also on baseline behaviour; the effects of d-amphetamine on behaviour were

baseline-dependent. This notion of baseline-dependency likely applies to the present observation of differential effects of dopaminergic modulation in SUD and OCD; the disorders share important baseline features including abnormal OFC functional connectivity, though do not have an identical neural profile (Meunier et al., 2012).

Understanding the nuances of D2/3 receptor involvement in maladaptive behaviour is particularly important given the evidence of reduced striatal D2 receptor availability in cocaine abuse (Volkow et al., 1993), methamphetamine abuse (Volkow et al., 2001), and OCD (Denys et al., 2004; Perani et al., 2008). An analogous pattern has been observed in alcohol (Hietala et al., 1994; Volkow et al., 1996) and opiate dependence (Wang et al., 1997). The studies of cocaine and methamphetamine also tested orbitofrontal cortex (OFC) metabolism, showing an association between lower D2 receptor availability and reduced OFC metabolism (Volkow et al., 2001, 1993). Behaviourally, monkeys with greater D2-type availability indeed exhibited better performance on a (deterministic) reversal learning task (Groman et al., 2011). At the same time, healthy humans carrying the A1 allele of the dopamine D2 receptor Taq1A polymorphism, which is associated with reduced striatal D2 receptor expression, showed deficits in PRL (Jocham et al., 2009).

8.4.3 Perseveration, checking behaviour, and uncertainty

Given SUD and OCD are both disorders of compulsivity, and perseveration can be an indicator of compulsivity, it is logical to hypothesise that people with OCD would also show perseveration in this task. Prior studies of OCD, however, have not found evidence of perseveration during PRL (Ersche et al., 2011; Hauser et al., 2017a; Remijnse et al., 2006). Compulsivity is indeed a complex phenomenon and is difficult to capture in a single measure. To that end, I have presented data that enrich the understanding of the multifaceted nature of the construct. Avoiding negative consequences is a key feature of OCD (American Psychiatric Association, 2013), and is not as central in SUD. This may help explain the divergent task findings in both the conventional perseveration analysis in Ersche et al. (2011) and the computational analyses of stickiness parameters reported here. The probabilistic nature of the task is likely of central importance. The first component of PRL is to learn the optimal behaviour in a stable environment, which requires ignoring rare negative events by not switching choices. Because such probabilistic switching was elevated in the OCD group, I thought it could be related to diminished stimulus stickiness; however, switching was also elevated in SUD. Probabilistic switching (lose-shift), furthermore, was not correlated with stimulus (or side/location) stickiness in SUD or OCD; instead it was correlated with a higher learning rate for negative feedback in healthy controls, SUD, and OCD, supporting the notion of lose-shifting as a behavioural manifestation of hypersensitivity to negative feedback (Murphy et al., 2003; Taylor

Tavares et al., 2008). Moreover, findings from Hauser et al. (2017a), who also studied PRL in OCD, are in agreement with the present diminished stimulus stickiness result: using a different computational model, individuals with OCD showed a decreased likelihood of repeating the same action, regardless of stimulus value. Hauser et al. (2017a), interestingly, did not find elevated probabilistic switching (lose-shift) in their OCD group. The present results in conjunction with Hauser et al. (2017a) strengthen the case that diminished stimulus stickiness provides a novel characterisation of PRL in OCD. The diminished stimulus stickiness seen in OCD may be a manifestation of checking behaviour, a suggestion also made by Hauser et al. (2017a). Checking behaviour, which can be a core symptom in OCD, was indeed modulated by D2/3 agents in rats (Eagle et al., 2014), in a translational laboratory model that has also captured increased checking in OCD (Morein-Zamir et al., 2018). Uncertainty is a feature common to both PRL and the translational model of checking behaviour. When reinforcement is deterministic, as opposed to probabilistic, there is less uncertainty and there is no rare event to ignore. Deterministic reversal learning paradigms may consequently be more sensitive to detect perseveration in OCD. Indeed, while serotonin depletion of the OFC in marmosets induced perseveration on a deterministic reversal learning paradigm (Clarke et al., 2004), which appears to reflect behaviour that has become stimulus-bound (Walker et al., 2009), OFC serotonin depletion also impaired PRL but did not induce conventional perseveration (Rygula et al., 2015).

8.4.4 Reward- and punishment-driven learning

The computational analysis showed diminished learning from positive feedback in SUD, and an increase in learning from negative feedback in both SUD and OCD (Figure 8.5). These results align with the original Ersche et al. (2011) data showing diminished win-stay behaviour (more spontaneous errors) in SUD and increased probabilistic switching (lose-shift) in both SUD and OCD. Ersche et al. (2016), on the other hand, found individuals with SUD were impaired in both reward and avoidance learning, a contrast likely due to several important task differences. Ersche et al. (2016) measured avoidance of electric shock, whereas the present task included negative feedback in the form of a sad red face icon, which is notably less salient and presumably engenders less motivation. The appetitive component of each study, however, was more similar in that the positive feedback was given in the form of points or a happy green face icon. Reinforcement, furthermore, was deterministic in the learning phase of the tasks used in Ersche et al. (2016), which removes an element of uncertainty present in probabilistic paradigms. Ersche et al. (2016) measured reward and punishment learning in two separate tasks without reversals, whereas the results here pertain to learning from positive and negative feedback intertwined in the same task – one affected the other. The salience

of positive and negative feedback in the present task was matched, whereas the salience of the aversive component of Ersche et al. (2016) was greater than the appetitive component. It is possible that the increased learning from negative feedback I observed in SUD was a compensation for the decreased reward learning and increased stimulus stickiness.

How do the present learning rate findings in SUD relate to recent data on PRL in alcohol use disorder (AUD)? Reiter et al. (2016) found increased perseveration (albeit calculated differently) and diminished win-stay behaviour in AUD, both present in SUD as reported by Ersche et al. (2011). Their model, like the winning model here, incorporated separate parameters for reward and punishment (nonreward) rates for the chosen stimulus, but they also included parameters for reward and punishment rates that simultaneously tracked the value of the unchosen stimulus. Of these, the only parameter that differed revealed an AUD group deficit in updating the value of the alternative (rewarded) option, following punishment (nonreward) on the chosen stimulus. Whilst this would be interesting to test, it is difficult to extrapolate to the present data. At the same time, their lack of an effect on learning rates for the chosen option differs from the present findings. I would have expected a diminished reward learning rate in AUD to the chosen option, based on the negative correlation between this parameter and spontaneous errors (increased win-shift) in SUD. Lose-shift behaviour seems to be a key source of divergence in the learning rate results between the two studies: Reiter et al. (2016) did not find a difference in lose-shift between AUD and controls, whereas lose-shift was elevated in the SUD group and correlated positively with the negative feedback learning rate. The lack of lose-shift behaviour in AUD at the outset could be due to task discrepancies – the AUD study used fewer reversals, for instance – or indeed a more general difference in how participants with AUD and SUD learn from negative feedback to guide behaviour in PRL or outside the laboratory.

The increased learning from negative feedback I observed in OCD, meanwhile, is consistent with Gillan et al. (2014) who showed excessive avoidance of electric shock in OCD. Here, it was found that learning from positive feedback was not different between OCD and control participants, in line with Gillan et al. (2011) who also showed no impairment in reward learning, using a similar task to Ersche et al. (2016). Hauser et al. (2017a) reported no difference in learning from reinforcement between healthy controls and OCD on PRL, however their learning rate parameter did not measure positive and negative feedback learning separately, as I report here. Notably, their sample included adolescents with OCD as well as adults, and the neuropsychological profile of OCD in these age groups is not identical (Gottwald et al., 2018). In light of Hauser et al. (2017a), a model with a single learning rate and stimulus stickiness parameter was tested (Table 8.2.4.2), and ranked third best (Figure 8.3), whereas the winning model was not tested in their analysis. The present study additionally used a

bridge sampling estimate of the marginal likelihood to perform model comparison, which has been newly introduced to practical Bayesian inference (Gronau et al., 2017a), and is thought to be an improvement upon the Bayesian Information Criterion (BIC) as used in Hauser et al. (2017a).

8.4.5 Dopamine and reinforcement learning

Dopamine is well known for its central role in learning about rewards. Nonhuman animal studies have shown phasic spiking of dopamine neurons signal positive outcomes that are unexpected or more rewarding than anticipated, known as positive prediction errors, whereas the omission of expected reward – negative prediction error – is associated with a reduction of phasic dopaminergic firing (Schultz et al., 1997). Murray et al. (2019), compared the same set of OCD and control participants as in the present experiment, and found that negative prediction errors were enhanced in OCD and that this was normalised by both amisulpride and pramipexole. Their finding is not only consistent with the role of dopamine in prediction error but also complements the observations that amisulpride and pramipexole produced the same directionality of effects on learning parameters.

In line with the role of dopamine in learning, amisulpride and pramipexole reversed the deficit in learning from positive feedback observed in SUD (Figure 8.8, 8.11, 8.6, 8.7), without affecting this parameter in OCD (Figure 8.10, 8.12, 8.6, 8.7) or healthy controls (Figure 8.9, 8.7). Negative feedback-driven learning was increased in SUD at baseline and was ameliorated by both drugs (Figure 8.8, 8.11, 8.6, 8.7). Both amisulpride and pramipexole increased learning from negative feedback in OCD (Figure 8.10, 8.6) and controls (Figure 8.9, 8.7), and this was the only drug-induced change in healthy individuals detected by the model. This series of results greatly extends the original findings, as Ersche et al. (2011) found no drug effects on spontaneous errors (win-shift) or probabilistic switches (lose-shift). These correlated with the reward and punishment learning rates, respectively, which I show here to be more sensitive to dopaminergic modulation.

The results on learning rates in SUD in particular, and their dopaminergic modulation, align with work on Parkinson's disease. Parkinson's is characterised by dramatic degeneration of dopaminergic neurons in the substantia nigra (Kish et al., 1988), and has therefore been of great import for understanding dopamine function. SUD (cocaine use), at the same time, is associated with lower levels of endogenous striatal dopamine (Martinez et al., 2009). The mainstay of Parkinson's treatment is levodopa (L-DOPA), the biosynthetic precursor to dopamine, and is thought to increase phasic dopamine release (Harden and Grace, 1995; Pothos et al., 1998). Rutledge et al. (2009) studied individuals with Parkinson's on and off of L-DOPA, using a dynamic foraging task with probabilistic and reversal elements. They employed com-

putational modelling and showed L-DOPA increased learning rates to rewards and remediated perseverative deficits. Both of these findings are consistent with the results in SUD on reward learning rates, stimulus stickiness, and their dopaminergic modulation. The perseveration parameter in Rutledge et al. (2009), like the stimulus stickiness measure, was independent of reward history. The results on positive and negative learning rates in SUD are also consistent with conventional analyses by Frank et al. (2004): Individuals with Parkinson's, when off medication, were better at learning from negative feedback, as assessed by probabilistic and deterministic tasks without reversals (Frank et al., 2004). When a reward is omitted, there is ordinarily a dip in dopaminergic firing (Schultz et al., 1997), and in the setting of dopamine depletion in Parkinson's, this mechanism of learning from negative feedback appears to be facilitated, at least when assessed using outcomes of points or money (Frank et al., 2004). On L-DOPA reward learning was enhanced, consistent with Rutledge et al. (2009), and the elevated learning from negative feedback was normalised (Frank et al., 2004).

Another study showed a single 800mg dose of the D2/3 antagonist sulpiride impaired choice performance for probabilistic rewards without affecting responses to punishment in healthy male volunteers – no females were studied (Eisenegger et al., 2014). Superficially, the result from Eisenegger et al. (2014) may seem at odds with the reward and punishment learning findings; however, several key differences between their experiment and the present design appear to account for the discrepancy. While their task was also probabilistic, there were no reversals, and they tested appetitive and aversive learning in separate blocks (gain of money versus nil, or loss of money versus nil). Sulpiride and amisulpride are similar; both are from the benzamine class of atypical antipsychotics. For the treatment of psychosis, a normal dose of amisulpride is 400-800mg per day, whereas the range for sulpiride is 400-2400mg per day (www.medicines.org.uk). Critically, the higher dose used in their study could have led to greater striatal D2 occupancy. Indeed, a single 800mg dose of sulpiride has been reported to occupy ~60% of striatal dopamine D2 receptors (Takano et al., 2006), whereas a single 400mg dose of sulpiride occupies ~30% in healthy volunteers (Mehta et al., 2008). This is especially important because the pharmacological effects in Eisenegger et al. (2014) were driven by participants who achieved higher blood levels of sulpiride, and by individuals with genetic variation associated with diminished D2 receptor expression. Presumably those with lower D2 receptor expression are disproportionately sensitive to D2/3 modulation. In fact, they found no effects on their classical or Bayesian analyses for participants with a low blood sulpiride level, determined by median split. Using classical statistics, Eisenegger et al. (2014) reported a selective impairment on the reward but not punishment component, however this was limited to a late phase after learning had reached asymptote, suggesting behavioural expression rather than learning was disrupted; this complicates comparison with PRL. In line

with this, in a Bayesian analysis, they found sulpiride increased a temperature parameter in the appetitive domain only, which reflects increased choice switching. Eisenegger et al. (2014) also found sulpiride did not affect the learning rate in their appetitive or aversive component, regardless of blood levels or genetics; task design again precludes meaningful comparison. Finally, because their genetic data on the D2 receptor and their effects of a non-selective D2/3 agent aligned, they were able to infer their results were driven by D2 modulation, which was not possible in this study. Differences in the task contingencies and feedback structure between the present task and theirs, the drug dose, and variation of D2 receptor density likely account for the discrepancies between results.

8.4.6 Consideration of medication status

It is important to note that most of those in the OCD group were medicated with SSRIs, which are known to affect dopaminergic function (Pogarell et al., 2005) and also modulate PRL. A single dose of an SSRI, more specifically, has promoted hypersensitivity to negative feedback in rats and healthy humans, and subchronic dosing has improved performance in rats (Bari et al., 2010; Chamberlain et al., 2006; Skandali et al., 2018). The hypersensitivity to negative feedback seen in unmedicated depression (Taylor Tavares et al., 2008) was also present in depressed individuals treated with SSRIs (Murphy et al., 2003), and PRL deficits in OCD persist despite SSRI use as well (Ersche et al., 2011; Hauser et al., 2017a; Remijnse et al., 2006). A possible explanation is that SSRIs do not modulate serotonergic activity in the OFC as readily as in other parts of the frontal cortex (El Mansari et al., 1995), and OFC abnormalities are present in depression (Bremner et al., 2002), as well as in OCD.

8.4.7 Implications for treatment

The present results are important for informing and refining treatment approaches. While there have been no randomised placebo controlled clinical trials of amisulpride for the treatment of OCD, other D2/3 antagonists are often used as an effective augmentation of first-line SSRI therapy in treatment resistant cases of OCD (Fineberg et al., 2020). At the same time, no studies have assessed the clinical efficacy of the D2/3 agonist pramipexole for OCD. There have been numerous studies testing dopaminergic agonists for the treatment of SUD however evidence for their clinical efficacy is currently lacking (Minozzi et al., 2015). To my knowledge there has been no clinical trial of amisulpride for the treatment of SUD. Existing studies testing other D2/3 antagonists have mostly found a lack of clinical benefit for SUD, or even a worsening of symptoms, however multiple studies have found D2/3 antagonists to reduce cravings in those with comorbid psychosis (Zhornitsky et al., 2010).

8.4.8 Conclusion

This study, to my knowledge, is the first comparison of the computational processes underlying two disorders of compulsivity, and their dopaminergic modulation. It was shown that an RL model captured mechanisms that differed between individuals with SUD, OCD, and healthy controls, and also detected changes following dopaminergic drug administration. The parameters in the model revealed subtleties underlying maladaptive behaviour that considerably extend conventional analyses of PRL. One key novel finding was that the stimulus stickiness parameter differentiated all three groups, with opposing effects in SUD and OCD. Behaviour in SUD was driven primarily by a combination of increased stimulus stickiness and an imbalance of learning from positive versus negative feedback – decreased positive and increased negative feedback learning. The altered computations underlying performance in OCD, on the other hand, were a decrease in stimulus stickiness and an increase in learning from negative feedback. D2/3 modulation normalised the stimulus stickiness anomalies in SUD, in particular, and reversed deficits in other parameters as well. The computational analysis allowed for a more nuanced cross-species comparison of the neural basis of PRL, with implications for its neurochemical modulation. The results, taken in the context of the existing literature, highlight the importance of considering how drug dose, receptor subtypes and expression, clinical phenotype, and subtleties of the task environment – the salience of feedback, deterministic or probabilistic reinforcement, single or serial reversals, and the role of uncertainty – may interact to affect behaviour and its underlying computational structure. By using Bayesian hierarchical modelling, we can begin to understand the subtle mechanisms that contribute to maladaptive responses on tests of behavioural flexibility, and their neurochemical basis in health and disease. This may eventually inform susceptibility to illness, diagnosis, and treatment.

Chapter 9

General Discussion

9.1 Summary of results

9.1.1 Serotonin, social emotions, empathy, and psychopathy

The first experiment in this thesis, reported on in Chapter 3, showed ATD enhanced the magnitude of self-reported emotional reactions to social injustice, the quality of which depended on individual trait differences (Kanen et al., 2020b). To date, most studies of serotonin and social phenomena have focused on decision-making. Here, using a novel task (Bland et al., 2015), I have extended the literature by studying the effects of ATD on social emotion. I found that ATD enhanced emotion in reaction to social injustice overall, and the specific emotions enhanced depended on personality traits: the highly empathic felt more guilt following ATD, whilst those high in psychopathic traits reported more annoyance. These ATD results underscore the possibility that certain traits such as empathy may predispose a subset of individuals to pathological emotional reactions such as guilt, which is a diagnostic criterion of depression (American Psychiatric Association, 2013). Likewise, these findings also advance the possibility that empathy is an indicator of sensitivity to serotonergic challenges, which may implicate the MRN-resilience system (Deakin, 2013), in line with indications that MRN-hippocampal interactions (involving 5-HT1A) may be especially sensitive to ATD (Blier and De Montigny, 1987; Faulkner and Deakin, 2014). Additionally, it should be noted that in a recent study, chronic SSRI administration in depressed individuals resulted in diminished empathy for pain in others (Rütgen et al., 2019). That ATD enhanced annoyance, particularly in individuals with psychopathic tendencies, may be relevant for understanding the emotional basis of retaliatory behaviour in the ultimatum game: disinhibition of aggressive impulses has been observed following ATD (Crockett et al., 2008), which resembles the pattern seen in psychopathic individuals (Koenigs et al., 2010) and those with damage to the vmPFC (Koenigs et

al., 2007).

9.1.2 Serotonin, Pavlovian memory expression, and intolerance of uncertainty

The next experiment, reported on in Chapter 4, showed that primitive and implicit, as opposed self-conscious and explicit, emotional reactions to previously learned non-social threats were attenuated by ATD, as indexed by SCR. This was done by testing a Pavlovian threat conditioning paradigm under ATD. To date, there have been surprisingly few studies of how serotonin influences threat conditioning processes in humans (Bauer, 2015), despite the widespread interest in threat conditioning as a translational model (Graham and Milad, 2011) and ubiquitous use of medications with serotonergic effects (Stahl, 2013). Baseline conditioning was equivalent between those destined to receive ATD or placebo. Subsequently, ATD attenuated the expression of emotion that had been conditioned on the previous day. Because self-reported intolerance of uncertainty can modulate the persistence of emotional memory (Dunsmoor et al., 2015), I controlled for this variable, as well as the strength of initial learning, which were indeed critical for isolating the effect of serotonin in particular. ATD, in other words, influenced emotional memory expression even when accounting for other explanatory variables, namely intolerance of uncertainty and strength of initial learning. These results are consistent with, and advance, the prominent view that a major role of serotonin is predicting aversive outcomes and more specifically that DRN signalling is involved in anticipatory anxiety (Deakin, 2013; Hensman et al., 1991; Hindi Attar et al., 2012). My results furthermore correspond with the finding of diminished amygdala activity during aversive conditioning following ATD, as it paralleled a reduction in SCR (Hindi Attar et al., 2012), and the amygdala is a critical structure for emotional memory expression (Phelps et al., 2004); however, this would need to be tested with suitable neuroimaging methods (e.g. pharmaco-fMRI).

9.1.3 Serotonin and Pavlovian reversal learning

ATD impaired another aspect of Pavlovian threat conditioning: reversal learning, which was examined in an experiment presented in Chapter 5. This experiment incorporated two key elements of note: the conditioned stimuli were threatening faces, rather than neutral, coloured squares as in Chapter 4, and after the conditioning phase the contingencies swapped so that the initially threatening cue was now safe and *vice versa*. Both acquisition and reversal phases were conducted during ATD or placebo. The expected response in health and under placebo is for emotional reactions to track the new contingency upon reversal, showing increased SCR to the newly threatening stimulus and extinguishing responses to the previously threatening

(newly safe) stimulus. ATD, however, was associated with impaired acquisition of conditioning to the newly threatening stimulus, yet intact extinction of emotional reactions to the previously threatening stimulus, amounting to impaired reversal learning. Both of these results are consistent with studies from non-humans: compromising OFC serotonin has not impaired instrumental extinction (Walker et al,. 2009) yet impaired instrumental reversal in marmosets (Clarke et al., 2004; 2007) and rats (Lapiz-Bluhm et al., 2009). The extent of the reversal impairment I reported, critically, was correlated with the degree of depletion. Another relevant finding was that individuals showed enhanced initial conditioning to threatening faces under ATD. As this finding contrasts with two previous threat conditioning studies that lowered serotonin, employed non-social cues, and measured SCR (Hensman et al., 1991; Hindi Attar et al., 2012), the present results might indicate a unique role for social versus non-social cues, or innate (here, cues of aggressive conspecifics) versus learned threats (Gross and Canteras, 2012). Accordingly, I propose that one possibility, in the Deakin and Graeff (1991) framework, is that the engagement of or balance between circuits that respond to proximal versus distal threats may differ for innate and learned threats, respectively. Unlike the reversal impairment, however, the observation of enhanced acquisition under ATD was not correlated with the extent of depletion. These findings provide further contributions to the surprisingly limited literature on serotonin and threat conditioning processes in humans.

9.1.4 Serotonin and instrumental deterministic reversal learning

Chapter 5 also reported on an experiment showing that ATD impaired instrumental reversal learning. The extent of the reversal learning impairment, moreover, was correlated with the degree of depletion, in a significant convergence with the Pavlovian reversal impairment. A central focus of the experimental design was on heightening the salience of feedback and testing effects of valence – factors that have frequently been determinants of ATD's effects or lack thereof in previous studies (Faulkner and Deakin, 2014). Indeed, consistent evidence of instrumental reversal learning impairments following ATD have been lacking (Evers et al., 2005; Faulkner and Deakin, 2014; Finger et al., 2007; Murphy et al., 2002; Rogers et al., 1999a,b; Talbot et al., 2006). To this end, I used a novel task that employed salient feedback, a high cognitive load, a deterministic reinforcement schedule, and multiple reversals. As expected, ATD did not modulate instrumental reversal learning when feedback was neutral, whilst there was an ATD-induced impairment when feedback was most salient (needing to simultaneously acquire reward and avoid punishment). Performance on the punishment-only condition, surprisingly, was unaffected whilst at the same time there were impairments in the reward-only phase. This is consistent, however, with the reversal paradigms used in marmosets which are appetitive (e.g. Clarke et al., 2004), as well as with the findings of Worbe et al.

(2016) and Seymour et al. (2012) who found that ATD impaired human choice behaviour in the appetitive domain. Specifically examining blocks beyond the initial reversal proved to be important in identifying effects of ATD, which was motivated by the observation of Clarke et al. (2004) that marmosets with compromised OFC serotonin were not impaired on the initial reversal. These results provide perhaps the strongest evidence to date of an instrumental reversal impairment following ATD and illustrate that key details of the present paradigm, such as salient feedback, should be considered when designing future studies of serotonin function.

9.1.5 Serotonin, instrumental probabilistic reversal learning, and traits

Chapter 6 examined probabilistic instrumental reversal learning (PRL) and showed, for the first time to my knowledge, that ATD enhanced a latent perseverative tendency ('stimulus stickiness') driving behaviour during PRL. The main stimulus stickiness results across experiments presented in this thesis, and from other related studies, are summarised in Table 9.1. Previous literature has indicated that conventional measures of choice behaviour on standard PRL paradigms (Lawrence et al., 1999), with non-salient reinforcement and a single reversal, were unaffected by ATD (Evers et al., 2005; Murphy et al., 2002). Indeed, I confirmed that ATD did not affect the core measures of choice typically tested, namely win-stay, lose-shift, perseveration, and correct responses. Applying a computational model of reinforcement learning, however, revealed that ATD enhanced the tendency to choose the previously chosen stimulus regardless of the outcome of the response (i.e. stimulus stickiness).

A number of other results followed as a result of this modelling approach. Regardless of serotonergic status, more severe, sub-clinical symptoms of OCD were correlated with lower stimulus stickiness. This relationship additionally applied to all the subscales of the OCI-R (checking, washing, etc.) except for hoarding. Stimulus stickiness, at the same time, was not related to self-reported intolerance of uncertainty, trait anxiety, or depressive symptoms. Diminished reinforcement sensitivity was instead correlated with depressive symptoms, self-reported intolerance of uncertainty, and trait anxiety, but not OCD symptoms. Higher trait impulsivity, meanwhile, was correlated with diminished reward learning rates and elevated punishment learning rates. The clinical significance of these correlations will be discussed below, in conjunction with the patient study reported in Chapter 8.

9.1.6 LSD and instrumental probabilistic reversal learning

Chapter 7 reported that the putative serotonergic agonist LSD enhanced learning rates during PRL, in what I believe to be the first application of reinforcement learning modelling to

	Stimulus stickiness
ATD (humans)	↑
SUD	↑
Clinical OCD	↓
Subclinical OCD symptoms	↓
Single dose SSRI (humans)*	↓
Single low dose SSRI (rats)*	↓
Neurotoxic 5-HT depletion: amygdala (marmosets)**	↓
Neurotoxic 5-HT depletion: OFC (marmosets)**	↑

Table 9.1: Summary of stimulus stickiness results. Comparison with acute SSRIs (*Luo et al., personal communication) and neurotoxic 5-HT depletion. **Parameterisation differed slightly in Rygula et al. (2015). ↑ = increased; ↓ = decreased.

behaviour under a psychedelic drug, in any species. Perhaps surprisingly, LSD had a major effect to enhance the weight of positive feedback in guiding behaviour. The negative feedback (punishment/nonreward) learning rate was also elevated under LSD, however enhancement of the positive feedback (reward) learning rate far exceeded the increased punishment learning rate. Conventional analyses using classical statistics releveled that under LSD, better acquisition behaviour was correlated with more perseverative responding across the reversal phase and this appeared to relate to the enhanced reward rate. Whilst the precise neurochemical mechanism underlying this effect remains to be delineated (e.g. 5-HT2A-receptor mediated or otherwise), these results have implications for understanding how or why new learning during the psychedelic state could have lasting beneficial impact therapeutically, and for refining treatment approaches.

9.1.7 PRL in OCD vs. SUD: modulation by dopaminergic agents

Chapter 8 reported on a clinical application of reinforcement learning modelling during PRL which revealed a striking dissociation between two disorders of compulsivity: individuals with OCD showed diminished stimulus stickiness whereas those with stimulant use disorder demonstrated elevated stimulus stickiness (see Table 9.1). In SUD, the reward learning rate was blunted whilst the punishment learning rate was higher in both SUD and OCD. Another finding was that dopaminergic D2/3 agents remediated several reinforcement learning deficits observed under placebo in SUD. Latent mechanisms of behaviour in the OCD and healthy control groups, in contrast, were largely unaffected by dopaminergic modulation. I now discuss the implications of the results presented in this thesis.

9.2 Implications for the role of serotonin in emotion

The present results for emotion are consistent with a role of serotonin in predicting distal threats, as ATD attenuated aversive Pavlovian responses, and also with a contribution of serotonin to inhibiting negative thought processes (Dayan and Huys, 2008), as demonstrated by enhanced self-conscious emotions, including guilt following ATD. Both of these effects, on implicit conditioned and explicit unconditioned emotion, respectively, align with prominent thinking on how serotonin influences different aspects of aversive processing (Deakin, 2013; Deakin and Graeff, 1991).

The social emotions task required participants to mentally simulate a social situation to assess how they would feel if in the same position as an individual depicted in the cartoon. Doing so involves the automatic recall of autobiographical memories. Indeed, there is evidence that ATD induces a negative bias in the delayed recall of emotional material (Elliott et al., 2011; Kilkens et al., 2004; Klaassen et al., 2002; Richell and Anderson, 2001). Individuals with clinical depression also show negative biases in declarative memory, which can manifest as bias towards remembering negative material or away from positive material (Elliott et al., 2011; Roiser et al., 2012). This may be especially important for contextualising the results on guilt and shame: simulations of social scenarios may have incorporated negatively biased memories for past experiences.

The test of Pavlovian conditioned memory expression (Chapter 4) instead showed ATD attenuated amygdala-dependent anticipatory emotion. This contrast is likely an example of an ostensible paradox, common in the serotonin literature, that could hypothetically be reconciled by considering the anatomical organisation of serotonergic neurons (Deakin, 2013). At the most basic level, the fact that the median raphe nucleus heavily innervates the hippocampus and the dorsal raphe has enriched projections to the amygdala is likely important in understanding these two results in concert. Whereas I anticipate the threat conditioning result reflected altered DRN-amygdala signalling, I propose that enhanced guilt and shame following ATD was preferentially associated with modulation of the MRN-hippocampal system. Mentally simulating social scenarios and recalling autobiographical information critically depends on the hippocampus (Schafer and Schiller, 2019). Intact hippocampal serotonin function has been suggested to inhibit rumination and foster resilience to depression (Deakin, 2013). Excessive guilt is part of the diagnostic criteria for depression (American Psychiatric Association, 2013) and is even more closely associated with a range of psychopathologies when laden with shame (Tangney et al., 2007). That ATD enhanced guilt especially in the highly empathic, provides evidence for a role of serotonin function in resilience against disproportionate negative self-conscious emotion and points to a trait that may confer additional vulnerability.

9.3 Contribution of personality traits to serotonin's effects: implications for vulnerability

In this section, I will first recapitulate a key result on ATD in panic disorder, that involved breathing 5% CO_2, which has been used to model panic in a laboratory setting. Following ATD, individuals with panic disorder displayed increased panic yet ratings of anxiety when anticipating CO_2 administration were diminished (Miller et al., 2000). That there were minimal effects on anxiety and panic in healthy individuals under ATD, when inhaling CO_2, was postulated to relate to strong serotonergic tone in individuals with panic disorder due to characteristic fear of panic and thus anticipatory anxiety (Faulkner and Deakin, 2014). This notion of serotonergic tone in certain populations under particular circumstances may critically apply to my findings on personality traits in this thesis. The prospect of harming another person may relate to a baseline increase of serotonergic tone in the highly empathic, that is more easily compromised by ATD. Likewise, it stands to reason that being highly intolerant of uncertainty would result in enhanced serotonergic tone when it is unclear, based on their memory from the day before, whether a shock is to be anticipated. Because intolerance of uncertainty has been reported to be high in individuals with OCD who show checking and repeating behaviours (Tolin et al., 2003), it would be important to discern whether this characteristic was a driver in the Pavlovian threat retention and reversal abnormalities that have been reported in OCD (Apergis-Schoute et al., 2017; McLaughlin et al., 2015; Milad et al., 2013).

Another consideration pertains to the extent to which serotonergic tone is dynamic and over what time course. In panic disorder, for instance, anticipatory anxiety about the prospect of panic would have presumably developed following the experience of a panic attack: serotonergic tone may therefore have changed after, relative to before the onset of panic disorder (aside from baseline vulnerability). Personality traits, by contrast, may instate longstanding prior beliefs about the world and could represent proxies for understanding serotonergic tone over the lifespan in the general population. Furthermore, it could be argued that personality traits dictate how salient a given event is for a given individual, which in turn relates to arousal, motivational drive to approach or avoid, and thus presumably serotonin release, which would also be differentially affected by the available serotonin or tryptophan pools (Young, 2013).

9.4 Implications for the role of serotonin in reversal learning

I have reinforced the importance of serotonin in human instrumental reversal learning. Previous evidence in humans was unclear and the present results therefore represent an important translational step: the deterministic experiment in particular parallels findings from depletion

with 5,7-DHT in marmosets (Clarke et al., 2004; 2007). The data I have reported also demonstrate the importance of serotonin in using motivationally salient feedback to guide behaviour. I have affirmed that, as expected, the salience and valence of reinforcement appear to be key in shaping whether and in what manner ATD modulates conventional measures of behavioural flexibility (Faulkner and Deakin, 2014). Indeed, ATD did not affect conventional measures of choice during PRL (Kanen et al., 2020c), tested using non-salient feedback, which replicated previous null results (Evers et al., 2005; Murphy et al., 2002).

This thesis also demonstrated the utility of methods that assess latent mechanisms of choice to advance the understanding of serotonin function: stimulus stickiness was enhanced during PRL by ATD in the face of null effects on observable indices of choice, contextualised in Table 9.1. Given that this paradigm incorporated non-salient feedback, how can the positive result on stimulus stickiness be reconciled with hypotheses about the salience of reinforcement, serotonin stores and signalling in the context of ATD? I suggest a reason for this difference may lie in the fact that stickiness is by definition unrelated to feedback and is also estimated using an accumulation of choice history trial-by-trial. This experiment also serves as a good example of why it should be emphasised that an absent effect of ATD is not a solid basis on which to reject the involvement of serotonin (Faulkner and Deakin, 2014). The PRL result is strengthened when aligned with Seymour et al. (2012), where computational modelling also revealed increased perseveration following ATD, on a 4-armed bandit task which characteristically incorporates probabilistic feedback. That ATD enhanced a perseverative tendency on PRL accords with data I have presented showing inflexibility following ATD on instrumental deterministic reversal and Pavlovian threat reversal paradigms, and with computational evidence on PRL after 5,7-DHT depletion of the marmoset OFC (Rygula et al., 2015).

It is particularly noteworthy that acute SSRIs have instead been shown to diminish stimulus stickiness in healthy humans, and in rats at a low dose (Luo et al., personal communication). Likewise, ATD had no effect on sensitivity to negative feedback (SNF) yet acute SSRIs, which have been shown to decrease serotonin concentrations in projection areas (Nord et al., 2013), enhanced SNF in healthy humans (Chamberlain et al., 2006; Skandali et al., 2018), as did low dose acute SSRI administration in rats (Bari et al., 2010). If both manipulations lower serotonin, why would there be opposing effects? Whilst neither stickiness effect has been studied with human neuroimaging, I hypothesise that during PRL, ATD had relatively more of an effect on the OFC whereas SSRIs had comparatively more influence over the amygdala. This would accord with the observation that SSRI administration over a longer period of time appears to be needed to influence serotonin in the OFC of guinea pigs: an effect of SSRIs in the OFC was not detected at three weeks but emerged at eight weeks of treatment (El

Mansari et al., 1995). Meanwhile, Evers et al. (2005), who showed no effect of ATD on choice behaviour during PRL in a small sample, employed fMRI and did not report an effect of ATD on the amygdala. Taylor Tavares et al. (2008), moreover, found heightened amygdala and diminished PFC responses in association with elevated SNF in unmedicated depression. Intact serotonin functioning in both the amygdala and OFC, furthermore, is necessary for PRL (Rygula et al., 2015). In sum, the implication is that under the demands of PRL, ATD and acute SSRI differentially affect the circuits recruited.

9.5 Implications for serotonin and reward processing

Whilst the traditional line of thinking about the role of central serotonin revolves around aversive processing, accumulating evidence points to its involvement in reward-related functions as well (Cohen et al., 2015; Matias et al., 2017; Ren et al., 2018; Seymour et al., 2012). I also found new evidence for this. Following ATD, there were deterministic reversal impairments during the conditions involving reward. The neutral condition was unaffected by ATD, as expected. These findings collectively accord with a body of evidence indicating that ATD is preferentially sensitive to motivationally salient feedback (Faulkner and Deakin, 2014). The present effects involving reward additionally align with studies showing deterministic reversal impairment in marmosets, which have used rewarding (milkshake) feedback (Clarke et al., 2004; 2007). Roberts (2011) has suggested serotonin depletion instils a negative bias away from reward contingencies thus promoting stimulus-response associations which in turn manifest as habitual, perseverative, or compulsive behaviour. This interpretation also accords with the finding of enhanced stimulus stickiness following ATD during PRL. The most pronounced effect of LSD on PRL, meanwhile, was a markedly enhanced reward learning rate. Whilst it remains to be determined which receptor(s) contributed to this effect, it has been shown that optogenetic stimulation of DRN 5-HT neurons enhanced reinforcement learning rates in mice for rewards, when presented after a long intertrial interval (Iigaya et al., 2018).

9.6 Implications for disorders of compulsivity

As summarised in Table 9.1, the opposing effects of 5,7-DHT in the marmoset amygdala and OFC on perseverative tendencies (Rygula et al., 2015) nicely align with the demonstration of opposing effects on stimulus stickiness in individuals with OCD and SUD (Kanen et al., 2019). Analogous to what I proposed for ATD and acute SSRI, I posit that the decreased stimulus stickiness seen in the present analysis of clinical OCD, and subclinical OCD symptoms in healthy volunteers, may be related to a serotonergic anomaly in amygdala circuitry that

has a more pronounced effect during PRL. Complementarily, it is possible that the increased stimulus stickiness seen in SUD reflects an abnormality in OFC serotonin that prevails instead in this context. Whilst the neurochemical and neuroanatomical basis of PRL is multifaceted, serotonergic abnormalities in the amygdala and OFC that differ between individuals with SUD and OCD may contribute to the contrasting computational profiles.

It is essential to note that most of those in the OCD group were medicated with SSRIs, which modulate PRL, as discussed, and are also known to affect dopaminergic function (Pogarell et al., 2005). Heightened SNF in unmedicated depression (Taylor Tavares et al., 2008) was also present in depressed individuals treated with SSRIs (Murphy et al., 2003), and PRL deficits in OCD persist despite SSRI use as well (Ersche et al., 2011; Hauser et al., 2017a; Remijnse et al., 2006). Testing whether low stimulus stickiness is absent in depression, despite elevated SNF may inform this discussion. A possible explanation is that SSRIs may not modulate serotonergic activity in the OFC as readily as in other parts of the frontal cortex (El Mansari et al., 1995), as introduced earlier, and OFC abnormalities are present in depression (Bremner et al., 2002), as well as in OCD. This may also relate to why, for unknown reasons, higher doses of SSRIs than prescribed for depression have conferred additional efficacy in OCD (Fineberg et al., 2020). It should be noted that 5-HT2A receptors in the rat OFC have contributed to improved reversal learning following chronic (three weeks) citalopram administration (Furr et al., 2012). How 5-HT2A agonism, for instance with psychedelic compounds such as LSD or psilocybin, affects reversal learning in OCD remains to be tested.

Whereas the stimulus stickiness effect during PRL in OCD appears to be impervious to SSRI treatment, instrumental reversal learning on a deterministic schedule seems to be a different story: using the same exact deterministic reversal learning task as in Chapter 5, Apergis-Schoute et al. (personal communication) showed that unmedicated individuals with OCD were impaired on the punished condition, compared to healthy controls, yet this deficit was not present in the medicated (chronic SSRIs) OCD group. Another complexity to be grappled with is that the negative correlation between subclinical OCD symptoms and stimulus stickiness was unaffected by ATD; however, this is not inconsistent with the OCD stimulus stickiness data in relation to SSRIs. That ATD, as studied by Berney et al. (2006), did not potentiate OCD symptoms upon provocation, and instead lowered mood, may be relevant for reconciling these findings.

With respect to dopaminergic effects on PRL, D2/3 receptor modulation remediated the abnormalities in reward learning rate and stimulus stickiness in SUD. Stickiness in OCD, meanwhile, was insensitive to D2/3 modulation. However, it remains to be determined whether higher and/or chronic dosing would have an influence. Some of the effects observed, for instance, may have related to action at dopaminergic autoreceptors (Horst et al., 2019). It should

also be noted that amisulpride may function as a partial D2 agonist at low doses and as a more conventional D2 antagonist at higher doses (Stahl, 2013).

9.7 Implications for psychiatric classification: fractionating compulsivity

The notion that impulsivity is not a unitary construct has received ample and necessary attention experimentally (Dalley et al., 2011; Dalley and Robbins, 2017). As with impulsivity, there are several well-validated translational laboratory paradigms for studying learning processes important for understanding compulsivity. As introduced in Chapter 1, two widely studied paradigms that assess habitual tendencies versus goal-directed action have captured compulsive tendencies in humans transdiagnostically. Several of these studies have identified strikingly concordant biases towards habitual responding for rewards, between OCD, SUD, and binge-eating disorder in one paradigm (Voon et al., 2014a), and between OCD, SUD, AUD, nicotine dependence, and Tourette syndrome on the other (Delorme et al., 2016; Ersche et al., 2016; Gillan et al., 2011; Luijten et al., 2020; Sjoerds et al., 2013). Whilst identifying objective commonalities across traditional diagnostic boundaries holds enormous potential for psychiatric classification, this thesis (Chapter 8) puts forth a novel transdiagnostic and translational assay for dissociating OCD and SUD from one another, and from healthy controls (Kanen et al., 2019). The stimulus stickiness result on OCD (from serial reversal PRL), furthermore, has been replicated in an independent OCD sample using single reversal PRL (Apergis-Schoute et al., personal communication). Chapter 6, meanwhile, indicates that diminished stimulus stickiness in OCD appears to be an extreme of a normal tendency: healthy individuals with subclinical OCD symptoms also showed the same behavioural pattern (on single reversal PRL), thus providing another replication. Moreover, reduced choice consistency, albeit a different metric to stimulus stickiness, appears to occur in adolescent OCD (Marzuki et al., personal communication). Not only has the stimulus stickiness parameter result been robust across paradigms, its translational value has already been highlighted, spanning from rodents, to non-human primates, to humans in both health and illness.

Whilst enhanced stimulus stickiness in SUD is perhaps a more intuitive result in the context of compulsive disorders (persisting with a previous action irrespective of its outcome, or usefulness), it remains to be determined what low stimulus stickiness means in the context of OCD. I had suspected it might be a reflection of checking behaviour (checking the alternate choice option) or intolerance of uncertainty. Indeed, individuals with OCD who show checking and repeating rituals have been found to be more intolerant of uncertainty than those

with other manifestations of OCD (Tolin et al., 2003). I was able to test these hypotheses in a healthy sample.

I found that diminished stimulus stickiness in a healthy population was not associated with intolerance of uncertainty [using the same scale as Tolin et al. (2003)], or symptoms of anxiety and depression, but instead with OCD symptoms. That result was followed up by showing this stickiness-OCD pattern does not appear to be unique to any specific symptom cluster (checking, washing, etc.). Hoarding tendencies in healthy volunteers, however, were not correlated with stimulus stickiness. Habitual responding in an outcome devaluation paradigm has also been tested in relation to subclinical obsessive-compulsive symptoms in healthy volunteers: Snorrason et al. (2016) used the same self-report scale as reported in Chapter 6 (OCI-R) and found that habitual tendencies were also correlated with all symptom clusters besides hoarding. Importantly, however, when controlling for what they termed negative affect, only the checking subscale remained significantly correlated (Snorrason et al., 2016). This served as motivation to control for symptoms of depression and anxiety in Chapter 6, and the correlations originally reported held up. In other words, whereas the experiment reported in Chapter 6 and Snorrason et al. (2016) aligned in that self-reported hoarding was not correlated with either laboratory measure, stimulus stickiness was not related to checking alone whereas the most definitive result in the outcome devaluation study pertained to checking.

Pathological hoarding is indeed its own disorder, within the obsessive-compulsive and related disorders section of the DSM5 (American Psychiatric Association, 2013), which informs a new hypothesis to test: is stimulus stickiness in individuals with hoarding disorder indeed different to what is seen in clinical OCD? Likewise, the implication of the present results is that assessing stimulus stickiness holds promise for fractionating compulsivity transdiagnostically within the current classification sections of obsessive-compulsive and related disorders and substance use disorders. Body dysmorphic disorder (BDD), for instance, may be interesting to explore. Whilst abnormally low stimulus stickiness is emerging as a signature that may be unique to OCD, more comparator populations are needed. Whilst I have provided evidence that intolerance of uncertainty may not be the driver, individuals with OCD have also demonstrated increased indecisiveness on an objective measure (Hauser et al., 2017b), which may be important to consider in this context.

9.8 Questions from deep brain stimulation

Abnormally low stimulus stickiness in OCD appears to be insensitive to SSRIs and D2/3 modulations at the dosing regimen tested, and is not correlated with symptom severity on the Y-BOCS: what does it mean for the lived experience of individuals suffering from OCD? A

similar question could be asked about the following observation: deep brain stimulation (DBS) of a non-motor component of the subthalamic nucleus remediated higher order cognitive inflexibility on the IDED in OCD (Tyagi et al., 2019), a proposed endophenotype (Robbins et al., 2019); however, symptoms were improved just as much as stimulating the ventral capsule, which in contrast did not correct IDED deficits, yet significantly improved mood (Tyagi et al., 2019). How is remediating IDED in OCD via DBS enhancing daily functioning? Could DBS alleviate the stimulus stickiness deficit? Applied to which circuit? To what real life consequence?

9.9 Implications for acute tryptophan depletion as a method for studying serotonin

The experiments contained within this thesis provide multiple new lines of evidence that substantiate ATD as a method for studying central serotonin. The support for this claim comes from remarkable parallels between the effects of ATD and neurotoxic serotonin depletion, particularly in non-human primates. Because these findings have already been discussed, I provide only a brief summary here. ATD in humans (Chapter 5) and 5,7-DHT depletion of the marmoset OFC (Clarke et al., 2004; 2007) both impaired instrumental deterministic reversal learning. Likewise, neither ATD in humans (Chapter 5) nor 5,7-DHT depletion of the marmoset OFC (Walker et al., 2009) impaired extinction learning, in the Pavlovian and instrumental domains, respectively. Both ATD (Chapter 6) and 5,7-DHT depletion of the marmoset OFC (Rygula et al., 2015) moldulated comparable indices of stimulus stickiness during PRL. Reversal deficits following ATD, moreover, were present across the Pavlovian and instrumental domains, in two independent human samples, both of which correlated with the extent of depletion. Evidence (e.g. from PET) on whether ATD diminishes serotonin release in humans, however, is still needed.

9.10 Future directions

9.10.1 Affective control

The role of traits like empathy and intolerance of uncertainty in vulnerability or treatment response should be considered in future studies of serotonin and emotion, as well as in studies on mood and anxiety disorders. The social emotions task was designed to test negative emotions specifically, and the role of ATD in positive social emotions remains to be tested. Likewise,

how serotonin affects appetitive Pavlovian conditioning, extinction, and retention or reversal remains to be studied and is pertinent to SUD in particular, where conditioned responses to drug cues can precipitate relapse. The effects of ATD on several other aspects of threat conditioning remain to be tested including renewal – the effect of a change in environment on the return of emotional memory (Bouton, 2002) – and threat generalisation (Morey et al., 2015). Future work will be needed to disentangle whether ATD has similar effects on human Pavlovian extinction learning when it is not accompanied by concurrent Pavlovian threat learning, as is the case for understanding the effects of stress on extinction learning (Raio et al., 2017). It also remains to be tested whether the analogous pattern of results between ATD and stress holds true for both innate and learned (as used under stress) threats. It should be noted that the concept of innate threats can furthermore be broken down into predators (e.g. snakes or spiders) or, as here, aggressive conspecifics (Gross and Canteras, 2012). Moreover, because contingency reversal confers uncertainty, accounting for trait intolerance of uncertainty may be important to further elucidate the role of serotonin in Pavlovian reversal learning, a metric not collected in that sample. A computational model characterising Pavlovian threat reversal has gained traction (Homan et al., 2019; Li et al., 2011; Raio et al., 2017), which could be used to assess a related set of latent mechanisms underpinning ATD effects on this paradigm, and likewise in OCD (i.e. Apergis-Schoute et al., 2017).

9.10.2 Reinforcement learning

Generally, it behoves the reinforcement learning community to foster consistency of models tested across studies to facilitate comparisons. Topically, whether reinforcement learning model parameters can be used to forecast the therapeutic effects of LSD, or lack thereof, would likely be of great interest particularly amidst the resurgence of research on psychedelics (Carhart-Harris and Friston, 2019). Likewise, latent mechanisms underlying PRL choice behaviour should be tested on other psychedelic drug manipulations, including psilocybin and microdoses of LSD. Assessing stimulus stickiness across obsessive-compulsive and related disorders, and other substance use disorders could be a valuable contribution to the RDoC framework (Cuthbert and Insel, 2013). Future serotonin studies of instrumental learning should incorporate more salient feedback, specifically shock. Whilst ATD did not potentiate symptom provocation in OCD (Berney et al., 2006), it is unknown how ATD would affect the behaviour of individuals with OCD when assessed via objective laboratory measures.

9.10.3 Pharmacological and imaging approaches

In future, it will be critical to use (or obtain) pharmacological agents with specific 5-HT receptor affinities to study the contribution of the serotonin subsystems emotionally, behaviourally, or neurally. It would be important to determine the influence of 5-HT2C receptor agents, for instance, on laboratory behavioural indices in both healthy humans and individuals with OCD or SUD. As many SSRI studies have employed acute drug administration (Chamberlain et al., 2006; Cools et al., 2008a; Crockett et al., 2010a; Skandali et al., 2018), it would be advantageous to test chronic (high) doses of SSRIs to better reflect regimens used clinically. Neural substrates of the results presented in this thesis were not directly tested and employing multimodal neuroimaging methods will be of great importance, including pharmaco-fMRI and PET. The development of improved PET ligands for imaging the contribution of specific 5-HT receptors will also be essential (Paterson et al., 2010). Directly comparing the effects of ATD and acute (or chronic) SSRI in humans, for instance, on the MRN versus DRN, as well as projection areas would be another crucial aim, to determine their precise effects on regional 5-HT activity.

9.11 Conclusions

The present thesis has covered a wide range of interconnected topics centred on the neurochemical modulation of affective and behavioural control. I have extended the study of serotonergic effects on social behaviour by showing how serotonin can affect socially relevant (moral) emotions, which may be important for understanding the visceral reactions underlying behavioural restraint or aggression in social situations. Across two experiments, I have expanded the surprisingly small existing literature on the influence of serotonin in threat conditioning processes in humans: following depletion, emotion was attenuated when reversing or retaining a learned threat response, advancing the account that serotonin is critically involved in predicting distal threats. I have likewise highlighted personality traits, including empathy, psychopathy, and intolerance of uncertainty, that may be important for understanding vulnerability to pathological emotional reactions. These could furthermore represent proxy measures for who may be most sensitive to serotonergic challenges in different circumstances – either reacting emotionally to an ensuing social conflict or when confronted with a previously threatening experience. I have delineated instances where serotonin depletion induced deficits reminiscent of those seen in patient populations. Showing ATD repeatedly paralleled findings following neurotoxic serotonin depletion, particularly in monkeys, further substantiates ATD as a selective method for studying serotonin. Applying computational modelling enabled a direct and rigorous comparison between the effects of OCD, SUD, LSD, ATD,

and D2/3 modulations on PRL, and additionally allowed for cross-species comparisons. Diminished stimulus stickiness emerged as a core computational profile of clinical OCD and subclinical OCD symptoms. Stickiness dissociated the two disorders of compulsivity tested herein, which was instead increased in SUD, both relative to healthy controls and those with OCD. This contrasts with another previously reported computational approach, which identified common rather than distinct deficits between OCD and SUD (and binge eating disorder; Voon et al., 2014). Whereas D2/3 modulation remediated core latent learning deficits in SUD, individuals with OCD did not show benefit. When the D2/3 results are coupled with a lack of improvement of low stimulus stickiness in OCD by SSRIs, this has implications for understanding the shortcomings of current OCD drug treatments and potential drug targets for SUD. Modelling the effects of LSD on reinforcement learning processes, moreover, uncovered that the weight of learning from feedback was enhanced in the psychedelic state. This too has therapeutic implications, for understanding how plasticity induced by LSD could promote the capacity for change on a psychological level.

That computational modelling can be applied across populations, paradigms, species, and neurochemical manipulations confers potential as a unifying strategy for a psychiatric neuroscience research agenda (Robbins and Cardinal, 2019). In this dissertation, I have demonstrated how neurochemical modulations in humans, and assessment of traits, combined with translational models of affective and behavioural control, can advance the understanding of psychiatric disorder across traditional diagnostic categories, with the goal of informing vulnerability, and refining classification and treatment.

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