Utilising multimodal neuroimaging to investigate reward system deficits and to develop novel therapeutics in individuals with substance use and behavioural addictions

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A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Statement of originality

I, Rayyan Raja Zafar, declare that I have presented original work in my thesis “Utilising multimodal neuroimaging to investigate reward system deficits and to develop novel therapeutics in individuals with substance use and behavioural addictions’ which was completed by myself over the course of my PhD at Imperial College London.

Where I have consulted published work, I have clearly cited the authors and where I have directly quoted from others work the source has always been given. Other than direct quotations, the thesis is entirely my own work.

The projects presented in this thesis were large complex studies where collaboration across a team was required. I have outlined which work was conducted by myself and where work was completed by other members of the team.

Copyright declaration

Publications and Conferences

Directly related to my thesis:

- **Zafar R**, Wall M, Siegel M, Nutt D, Erritzoe D. Psychedelics for addiction: Past present and future. Frontiers in Psychiatry 2023
- **Zafar R**, Wall M, Nutt D, Erritzoe D, 2021. Dopamine D3 Receptor antagonism in alcohol dependence. A functional imaging study. Journal of psychopharmacology (European Journal of Neuropsychopharmacology, Conference paper) **(ECNP poster prize 2021)**
- Nutt, Hayes, **Zafar R,** Fonville, Palmer, Paterson, Lingford-Hughes. Neurotoxicity of alcohol dependence (PET/SPECT) – Elsevier book chapter 'Addiction' 2021
- Wall M, Harding R, **Zafar R**, Rabiner I, Nutt D, Erritzoe D. Psychedelic neuroimaging for drug development. Molecular Psychiatry 2023
- **Zafar R**, Barba T, Lingford-Hughes A, Nutt D Erritzoe D. The Dopamine D2/3 Receptor in psychiatry, a systematic review and meta analaysis (In preparation)
- **Zafar R**, Erritzoe D, Lingford-Hughes A, Nutt D. The Dopamine D2/3 Receptor in psychiatry, a systematic review and meta analaysis (In preparation)
- **Zafar R**, Wall M, Nutt D, Erritzoe D. Validation on novel fMRI paradigms in gambling disorder (In preparation)

Oral Presentations:

- SSA 2020 - Dopamine DRD3 antagonism in Alcohol dependence. A combined PET-fMRI investigations
- ECNP 2021 – Dopamine DRD3 antagonism in Alcohol dependence. A combined PET-fMRI investigations
- FENS 2022 – Overview of Validation of novel fMRI paradigms in addiction
- Oxford Psychedelic symposia 2022 – Harnessing clinical equipoise with scientific discovery in neuropsychopharmacology trials of psychedelics
- Psycence 2022 – The role of psychedelics in the treatment of addiction
- Shrewsbury 2022 – Alcohol, the brain and functional alternatives
- Imperial Lates 2023 - Alcohol and the brain

- L’albatross congress 2023 – Psychedelics for addiction, the past present and future
- BAP 2023 – **Public communication prize (2022) lecture** – Communicating neuropsychopharmacology
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Other publications during my PhD studies not related to my PhD:

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- Schlag A, Hindocha C, **Zafar R**, Nutt D & Curran V. 2020. Cannabis-based medicines and cannabis dependence: a critical review of issues and evidence (Journal of Psychopharmacology)
- Schlag A, Sullivan S, **Zafar, R**, Nutt D. 2021. The current state of medical cannabis: recent developments in human clinical applications and potential therapeutics. (Neuropharmacology)
- Verroust, **Zafar R**, Spriggs, 2021. French Translation of a 1959 case study for psilocybin in the treatment of anorexia nervosa
- **Zafar R**, Schlag A, Phillips L, et al 2021. Medical cannabis for severe treatment resistant epilepsy in children: a case-series of 10 patients (BMJ Paediatrics Open)
- **Zafar R**, Sulak, Brambila-Fernandez, Schlag, Nutt. Raising Awareness, the implementation of cannabis and psychedelics in the treatment of advanced metastatic breast cancer. Drug Science, Policy & Law
- Schlag AK, **Zafar R**, Lynskey MT, Athanasiou-Fragkouli A, Phillips LD, Nutt DJ. 2022. The value of real-world evidence: The case of medical cannabis. Front Psychiatry.

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Abstract

Addiction, a mental health condition, suffers from a significant treatment gap, with less than 10% of affected individuals receiving help. Over the past four decades, research has focused on understanding addiction's neurobiological mechanisms, primarily centering on the role of dopamine as a key player in reward processing. Advanced neuroimaging techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) have unveiled functional and molecular dysfunctions in addiction.

This thesis aimed to utilise and advance neuroimaging methods to understand reward system dysfunction in both substance and behavioral addictions. Additionally, it sought to explore the potential of these methods in translational neuropsychopharmacology research for developing innovative addiction therapeutics.

The three thesis chapters delved into the use of neuroimaging in both basic neuroscience and translational neuropsychopharmacology experiments. The first chapter conducted a systematic review and meta-analysis on dopamine receptor function in addiction, revealing the complexity of this role. The second chapter investigated a novel dopaminergic drug's effects in alcohol addiction, demonstrating its dual impact on the brain through fMRI and PET scans. The final chapter identified gambling disorder-specific brain biomarkers, valuable for future translational research.

These findings suggest that simple molecular biomarkers are insufficient for addiction translational research. However, multimodal neuroimaging investigations, as seen in the second chapter, hold promise for clinical drug development in addiction.

In conclusion, the thesis lays the foundation for future research, highlighting the potential of multimodal translational neuropsychopharmacology studies to yield effective interventions for addiction. These methods could revolutionise not only addiction treatment but also have broader implications in psychiatric research and emerging fields like psychedelic drug development.

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Introduction

Addiction

Addiction, characterised as a persistent and recurrent medical condition, presents a substantial global burden, marked by a scarcity of effective treatment options. Estimates indicate that approximately 164 million individuals worldwide are currently grappling with addiction according to the Global burden of disease study (James et al., 2018). Within the Western hemisphere, a notable 5-6% of individuals contend with substance-related issues, while growing concerns surround the prevalence of behavioural addictions (Bowden-Jones et al., 2022). The global prevalence of all substance use disorders (SUDs) has shown a significant uptick between 1990-2016, with alcohol dependence emerging as the most prevalent (Degenhardt et al., 2018a). Moreover, there has been a consistent rise in drug-related fatalities since the turn of the millennium, with 2017 recording a 35% increase in deaths compared to 2000, particularly concerning trends linked to opiate and synthetic drug overdoses, most prominently observed in the United States, Scotland, and England (Degenhardt et al., 2018a) . This disconcerting trend transcends borders, with the WHO documenting increases in drug-related deaths across all continents (WHO, 2017). Furthermore, addiction exacts a staggering economic toll, exceeding $442 billion annually with far-reaching adverse implications impacting employment, productivity, public health and the legal system (Office of the Surgeon General, 2019).

Despite the substantial morbidity associated with SUDs and behavioural addictions, less than 10% of the 22 million Americans identified as needing treatment can access specialized services (Substance Abuse and Mental Health Services Administration, 2016; Blevins, Rawat and Stein, 2018). In the United Kingdom, a mere 9000 individuals sought assistance from specialized gambling treatment clinics in 2019-2020, despite a recent YouGov poll estimating that up to 1.4 million individuals in the UK meet the criteria for problematic gambling (Public Health England, 2023). These statistics lay bare the inadequacies in support provision and treatment accessibility.

The reasons behind this lack of access to appropriate services are multifaceted and extensively detailed in the UK government-commissioned independent drug review by Dame Carol Black (2021). Her report echoes the urgent socio-economic need underscored in NIDA's 2019 medication development priorities paper, emphasizing the imperative for the development of more effective treatments through innovative and mechanistic scientific programs to enhance translation to clinical practice (Rasmussen, White and Acri, 2019).

The prevailing clinical approach to addiction encompasses psychosocial interventions supplemented by pharmacotherapy. However, these existing treatments exhibit limited success, with 20% of individuals experiencing relapse within one month and an additional 40% relapsing within six months (Hayes et al., 2020). Notably, the efficacy of treatments for alcohol addiction ranks among the lowest for all mental health disorders, with only three licensed pharmacotherapies available and only 9% of individuals with this disorder receiving such treatments (Carpenter et al., 2018). The situation is arguably more dire for individuals dealing with other substance or behavioural addictions, as these often lack clinically efficacious medications (Hayes et al., 2020).

Addiction represents a substantial global health challenge characterised by limited effective treatment options. The widespread prevalence, economic burden, and inadequate treatment accessibility underscore the urgency of addressing this issue through innovative approaches. Indeed, a comprehensive response demands interdisciplinary collaboration, encompassing scientific innovation, policy reform, and improved healthcare delivery systems, in order to alleviate the profound suffering associated with addiction on both individual and societal levels. In this PhD I will describe how I aim to contribute to this endeavour, namely through biomedical investigations of the brain and how these can serve to develop novel and effective therapeutics.

History of addiction

Addiction is a biopsychosocial disorder defined behaviourally as an acquired, chronic, relapsing condition that is characterised by powerfully motivating engagement with an activity, despite its adverse psychological and social consequences (American Psychiatric Association and Association, 2013). The word addiction has its roots in ancient Rome, from the Latin word 'Addictus' which means to betray, sell-out or abandon. The word was conceived in a roman mythological story which describes a slave, who, once set free from his master, decided to keep his chains fastened to him as he became overtly familiarised to his pain and suffering.

Since the inception of sentient mammalian civilisation, humans and our close ancestors have used mind-altering substances and had a proximal relationship with psychotropic drugs. Archaeological evidence suggests psilocybin has been used by humans for over 10,000 years, (Pettigrew, 2011) and historical records documents many accounts of negative effects of excessive drug use, for example the Old Testament accounts in 'Genesis' of Noah's drunkenness and the death of Alexander the great in 323 BC which was associated with years of heavy drinking. In more recent centuries and in the roots of the modern medical era, human curiosity to understand the mechanisms surrounding disease have been documented and most explicitly in the 1641, Rembrandt painting 'The anatomy lesson' depicts a Dutch physician attempting to explain the biological loci of loss of control of behaviours, such as excessive alcohol use (Crocq, 2007).

One of the pioneers of developing western medical treatment for addiction was Bill Wilson, the founder of alcoholics anonymous (AA). In 1934, Bill Wilson undertook an experimental treatment, an admixture containing henbane and belladonna; plants that contain tropane alkaloids that have psychedelic-like effects, on his 4th attempt to quit alcohol. During this experience he reported seeing a 'bright white light' and 'feeling great peace' which he interpreted as a self-transcendent and spiritual awakening. After this treatment, he went on to take lysergic acid diethylamide (LSD) with psychotherapy, when it was legally being used and researched for addictions and after this he never drank alcohol again and went on to develop the international mutual aid fellowship Alcoholics Anonymous. On accounting his experience with LSD he said, "If,

therefore, under LSD we can have a temporary reduction, so that we can better see what we are and where we are going — well, that might be of some help. The goal might become clearer. So, I consider LSD to be of some value to some people, and practically no damage to anyone" (Hartigan, 2000). Bill Wilson's creation of the AA set in motion the formalised recognition of addiction as a medical condition which went on to catalyse the development of the first formalised addiction support and treatment groups.

Throughout civilisation, the intimate and primitive relationship humans have had with drugs has been mired in moral debate, stigma, and has been historically politicised which has detracted significantly from our scientific understanding and treatment of the condition (Buchanan and Young, 2000). In more recent times, a more pragmatic medico-scientific approach has emerged which has begun to systematically assess the relative harms of drugs (Nutt et al., 2010) and to investigate some of the science behind the condition in order to develop novel therapies. In very recent developments, novel therapeutics based around psychedelic therapy have shown efficacy in treating addiction and have provided addiction researchers with a novel avenue to explore which will be discussed later in the PhD (Zafar et al., 2023b).

In modern society the term 'addiction' is casually referenced and overused. In the field of western medicine, the current clinical equivalent of 'addiction' according to the diagnostic and statistical manual 5th edition (DSM-5) is the diagnosis of SUD, a term brought into medicine in 2013 by the American Psychiatric Association (APA) (American Psychiatric Association and Association, 2013). This term served to provide a continuum to denote severity of SUD across 11 diagnostic criteria and as mentioned is now annexed with gambling disorder (GD), the only medically recognised behavioural addiction. For the purpose of my PhD, I will be investigating individuals with a clinical diagnosis of addiction as per the DSM-5 or the earlier clinical category of substance dependence as per DSM-IV criteria which are analogous clinical measures.

In the following sections I will introduce a background to our modern understanding of the neurobiological processes related to addiction which centre around reward system dysfunction. In later sections I will outline how an emerging field of experimental

medicine and translational neuropsychopharmacology has developed from insights from basic addiction neuroscience research and how these research techniques can be employed to inform the development of novel treatments. I will finish the introduction by explaining the specific aims of my PhD and how such translational neuropsychopharmacology methods have been applied in my research.

Neurobiology of the reward system

The last sixty years have witnessed the development of a neurobiological understanding of addiction. Through advances in modern biomedical techniques including neuroimaging, DNA and genomic analysis, and preclinical animal models, scientists have been able to develop a better understanding of disease mechanisms underlying addiction. Evidence from early pre-clinical animal models have implicated the central role of the neuromodulator dopamine and its associated circuits in reward, motivation, and addiction. Specifically, one of the first studies found that rats would repeatedly stimulate regions of their brain that were rich in dopaminergic neurons with electrical activity which gave evidence for the reinforcing effects of dopamine and specific neural pathways involved in these effects (Olds and Milner, 1954). Follow-up studies revealed that blocking dopamine receptors with antagonists in the brains of rats and non-human primates arrested the reinforcing effects of psychostimulants (such as cocaine and methamphetamine) that seemed to have an effect on stimulating dopamine activity in this pathway (Wise and Bozarth, 1987). Microdialysis, a way of measuring dopamine concentrations in extracellular brain tissue, later confirmed that a range of drugs, but not all, lead to an increase in dopamine release from the nucleus accumbens (NAc) which is located in the ventral striatum (VS) and is a key brain dopamine locus (Di Chiara and Imperato, 1988). Dopamine was subsequently platformed as the pivotal neuromodulator and neurotransmitter in addiction due to its key roles in reward, motivation and goal directed behaviours (Robinson and Berridge, 1993).

Subsequent research has found dopamine to be a key neurotransmitter in the brain with a role in modulating executive function in the cortex (Vijayraghavan et al., 2007), motivational salience and reward in the basal ganglia, movement and motor function, and a regulator of prolactin release (Vijayraghavan et al., 2007). Unsurprisingly,

dysregulation of dopamine has been implicated in several neuropsychiatric disorders including schizophrenia (Howes et al., 2012) and Parkinson's disease amongst others (Damier et al., 1999). Neurochemically, dopamine signals act throughout the brain through interaction with its endogenous receptors. Dopamine receptors (DR) are a class of G protein-coupled receptors with the existence of at least 5 subtypes; DRD1, DRD2, DRD3, DRD4 and DRD5. The functionality of the downstream messenger signalling cascades of these receptors split the family into two distinct categories based on their ability to either activate or inhibit the enzyme adenylyl cyclase. The DRD1-like family, including DRD1 and DRD5 are shown to activate adenylyl cyclase whereas the DRD2-like family, consisting of DRD2, DRD3, and DRD4 inhibit the respective enzyme leading to variable levels of cyclic AMP (cAMP) (Cools and Van Rossum, 1976).

The mesocorticolimbic system

With dopamine being the historical candidate and molecule involved in addiction, many neurobiological models for this condition have centred on the role and function of dopamine's key neurocircuitry network which is the mesocorticolimbic system – or the reward pathway of the brain (figure 1).

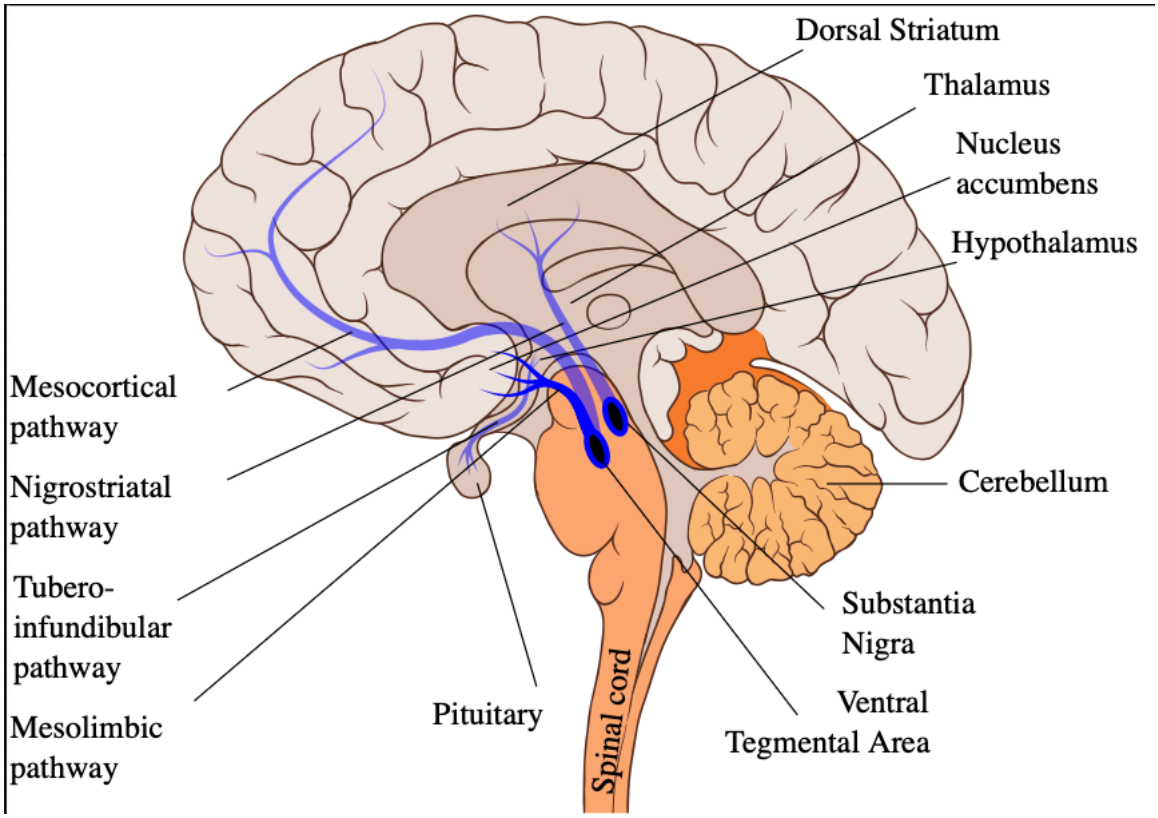


Figure 1: Mesocorticolimbic pathway

Figure 1: Figure demonstrating the neuroanatomical pathways of the mesocorticolimbic system. Figure adapted from (Patrick J. Lynch; CC BY-SA 4.0 https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons)

This pathway is evolutionarily preserved across many species and functions to facilitate survival and reproduction by assessing environmental rewards and punishments (amongst other functions) and driving behaviours to seek these out (Blum et al., 2018). This network harnesses two opposing forces of the human condition: inhibition and consumption. Anatomically, these processes are localised throughout the brain covering the prefrontal cortex, ; the basal ganglia – , and the limbic system

The advent of sophisticated neuroimaging techniques in the 1990's aimed to establish the molecular and functional neuroadaptations occurring in the brains of addicted individuals. Particularly, efforts have been focussed on identifying objective biomarkers of addiction.

In 'healthy' brains, we see a marked increase in firing of dopaminergic cells in the ventral-tegmental area (VTA) in response to naturally rewarding stimuli including food and sex (Kelley and Berridge, 2002). The stimulation of the VTA is mediated by inputs from the body's endogenous pharmacopeia including acetylcholine, glutamate, gamma-aminobutyric acid, noradrenaline, anandamide, serotonin, and dopamine itself (Nestler, 2005). This in turn signals to the Nucleus Accumbens (NAc) located in the Ventral Striatum (VS) to release dopamine. This is one of the neural mechanisms involved in hedonic pleasure (Drevets et al., 2001). Parallel signalling events occur as a result of this process within the amygdala, hippocampus and other cortical networks, where the salience and memory of the stimulus is encoded. The amygdala communicates to the hippocampus to learn and store the reward memory and the stimulus motor experience for the next time (Baler and Volkow, 2006). This is (briefly) how reward learning is integrated into the brain and serves to guide future behaviour.

Individuals addicted to drugs, or addictive behaviours (such as gambling), are hypothesised to lead to develop a chronic overstimulation of this circuit leading to excessive dopamine release in this pathway. In this process the hippocampus begins to learn about non-rewarding cues associated with drug consumption. Early work by Shultz et al characterised how during the development of addiction, dopaminergic neurons began to fire in response to the prediction of, rather than the receipt of a reward (Schultz, 1998). This pre-emptive signalling is what is theorised to drive motivation to engage in reward, colloquially analogous to craving or wanting. This work repurposed the role of dopamine. It no longer represented the often ill-interpreted hedonic neurochemical; it became the mythological chains of Addictus.

In unison with the hijacking of the brain's reward system through repetitive exposure to substances of abuse, in addiction there is evidence for drug-induced neurotoxicity represented by brain atrophy in the grey and white matter. Structural imaging studies have consistently found reduced prefrontal cortex (PFC) grey matter density across

addicted populations, particularly in areas such as the dorsolateral prefrontal cortex (DLPFC). Reductions in frontal metabolic activity using positron emission tomography (PET) are also a hallmark neuroimaging finding in addiction (Volkow, 2007). Further, white matter tracts, responsible for connecting and facilitating communication between anatomically divergent regions of the brains have also been found to be impaired. It is thought that these mechanisms lead to a disruption in the reciprocal connectivity and signalling between the frontal executive regions and the deeper limbic regions which are facilitated by the mesocorticolimbic system (Goldstein and Volkow, 2011a).

The result is a dysregulation between regulatory executive 'top-down' control and 'bottom-up' reward and emotional drives. This imbalance, mediated by adaptations to this neural network, gradually leads to a loss of control over impulsive and then compulsive drug seeking behaviours and eventually individuals lose full autonomy over their actions (Droutman et al., 2015a). At this point the person can be said to be addicted - a term that many "addicts" use about themselves.

Models have since developed that have been able to provide overviews of the interaction of these neurocircuits and their role in different parts of the addiction life cycle (Koob and Volkow, 2016). These studies have begun to understand the mechanisms underlying addiction by identifying various neural circuits involved in the transition through the three stages of its progression (Koob and Volkow, 2016). The (VTA and VS) seem to be key mediators of the first stage of the addictive process: the binge/intoxication stage, but they have also been implicated in the decision-making impairments associated with reduced sociality and risk taking (Poisson et al., 2021) observed in the other stages. The extended amygdala, which is involved in the stress systems (corticotropin-releasing factor and norepinephrine) activated by the negative emotional state associated with dependence (Koob, 2021) seems to mediate the second stage of the addictive process: withdrawal/negative affect. The last stage, preoccupation/anticipation, seems to conjure a network involving the orbitofrontal cortex (involved in craving), cingulate gyrus, dorsolateral prefrontal, medial frontal and inferior frontal cortices (involved in disrupted inhibitory control) (see review by (Koob and Volkow, 2016, Volkow and Morales, 2015). The identification of these circuits and their association with the different stages of the addictive process have begun to lead to successful interventions, including the strengthening of executive function via

repeated transcranial magnetic stimulation (rTMS) to the dorsolateral prefrontal cortex in nicotine, alcohol, cocaine and methamphetamine-dependant individuals (Gorelick et al., 2014) as well as identifying novel drug targets that may aim to blunt maladaptive processing. Whilst neuroimaging has been integral to understand the processes and some of the underlying neurobiology of addiction there has been less research understanding how such basic neuroscience can be applied to develop clinically effective interventions for patients.

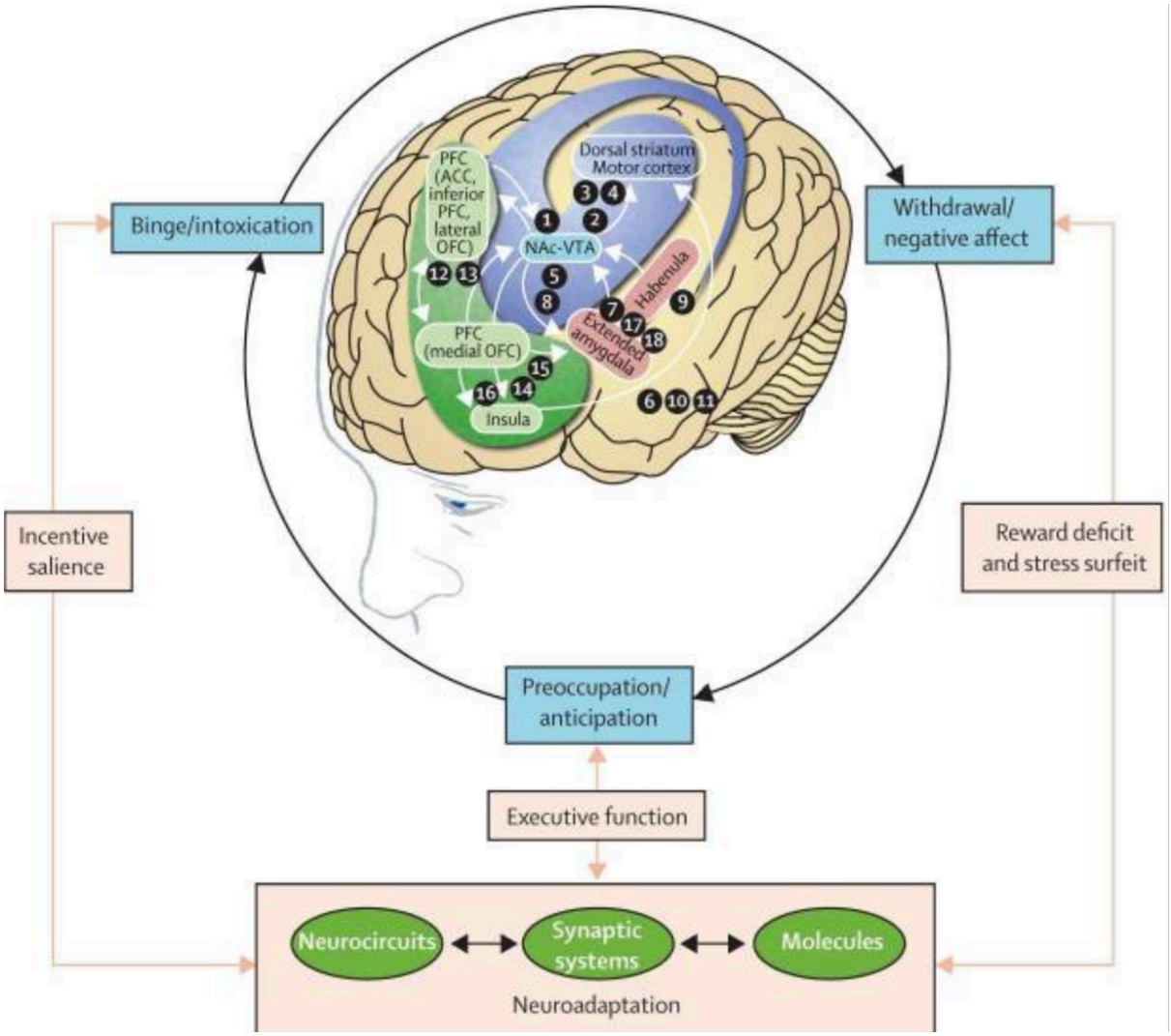


Figure 2: Neurocircuitry model of addiction

Figure 2: adapted from Koob et al 2016: **Model of interacting circuits in which disruptions contribute to compulsive-like behaviours underlying drug addiction. Permission granted from authors.** CC BY-SA 4.0

The overall neurocircuitry domains correspond to three functional domains: binge/intoxication (reward and incentive salience: basal ganglia [blue]), withdrawal/negative affect (negative emotional states and stress: extended amygdala

and habenula (red)), and preoccupation/anticipation (craving, impulsivity, and executive function: PFC, insula, and allocortex (green)). Arrows depict major circuit connections between domains, and numbers refer to neurochemical and neurocircuit-specific pathways known to support brain changes that contribute to the allostatic state of addiction. PFC=prefrontal cortex. ACC=anterior cingulate cortex. OFC=orbitofrontal cortex. NAc-VTA=nucleus accumbens-ventral tegmental area.

Theoretical frameworks of addiction

Based on neuroimaging data as well as preclinical and clinical findings – several theoretical frameworks have been developed that aim to capture how neurobiological deficits fit into models of addiction. In the following sections we will review some of these theories and describe how studies conducted in this PhD have been developed to provide evidence to support these.

Reward deficiency theory

Several theoretical frameworks have been developed to describe neurochemical, molecular and functional deficits that can sustain addiction which centre primarily around the role of the striatum. Patients with addictive disorders have been found to have disrupted striatal processing in response to reward (Gorelick et al., 2014, Volkow and Morales, 2015, Blum et al., 2000a, Bjork et al., 2011). The reward deficiency theory proposed by (Blum et al., 2000a) states that addicted individuals have a hypoactive reward system (Bjork et al., 2011). It is postulated that this is due to affected individuals having an innately lower responsivity to reward due to inherent biologically driven genetic deficits related to their reward system. This in turn may drive an individual to seek out hedonic pleasures which could lead to chronic drug use which in turn could lead to dopaminergic receptor downregulation which further exacerbates the reward deficiency. The overarching theory is that either independently or in synchrony, these processes drive reward- or drug seeking behaviours. Aside from genetic and receptor focussed investigations, a more translational approach of investigating such theories can also be conducted using fMRI tasks such as the monetary incentive delay task (MIDT), which I investigate in chapter 3, or other reward processing tasks such as the novel reward-enriched roulette (RER) which I validate in gambling addiction in chapter 4. This theory can be further investigated using

molecular PET imaging exploring receptor availability which is explored in Chapter 2 and neurotransmission studies which are addressed in chapter 4 briefly.

Incentive salience theory

Conversely, a hyperactive reward system is also described, wherein individuals develop an increased sensitivity to rewards, which in turn drive impulsive behaviours or as a result of the incentive salience from conditioning of drug-associated environmental cues (Robinson and Berridge, 2008). This theory can be addressed using cue-reactivity fMRI which I investigate in chapter 4.

Neuroimaging methods in translational addiction research

Several neuroimaging methods that have been touched upon in the previous sections have been used in translational addiction research, stepping beyond basic neuroscience, with the aim of using neuroimaging to identify novel targets and treatments for addiction. In the following sections I will give an overview of two key neuroimaging modalities namely fMRI and PET that I have used in my PhD

Overview of PET methodology

In-vivo molecular neuroimaging in the living human brain has been made possible by the advent of PET and Single Photon Emission Computed Tomography (SPECT). It is the only direct way to quantify some of the 100 plus different neurotransmitters in the living human brain, and the probe compounds used are known interchangeably as radioligands or radiotracers (Cuevas, 2019). As described, for the last 50 years human neurobiological research in addiction has tried to establish the neurochemical/molecular basis of addiction using neuroimaging tools such as PET. Analytical approaches have included investigation of changes in brain metabolism, neuroreceptor availability and neurotransmitter release capacity (Wiers et al., 2016). .

The radiotracers used in PET contain a short half-life which is dictated by positron emitting radioisotopes. The most commonly used decay with a relatively short half-life (e.g. about 20 min for ^{11}C and 110 min for ^{18}F), allowing administration of doses high enough to provide a strong imaging signal without increasing the health risks of exposure to ionising radiation. PET radioligands have characteristics to enable the quantification of a specific binding signal, such as a suitably high ratio of specific to

non-specific binding and favourable tissue kinetics. These radioligands are very sensitive and are administered in small quantities of less than 5% target occupancy meaning they have no relevant pharmacological activity. Decay of the radionuclide leads to a proton being released which interacts with an electron. This annihilation event leads to the release of two gamma ray photons travelling in opposite directions at 180^{O} to each other. The pair of photons that are emitted travel in opposite directions and are picked up by a ring of detectors on the PET camera. From this, the emission source can be detected and when the scan is complete, the output is a 3D image representing the combination of all these events. To generate the image an attenuation correction is used, this is done using attenuation data from a rotating Germanium-68 source, or a computerised tomography (CT) scan. After reconstructing the data, specific modelling (discussed in the following section) is required to estimate the signal of interest in the brain. PET data is then often co-registered with structural data from CT or MRI to help localise the signal as the spatial resolution of PET, is lower (approximately 3-4 mm) than that achieved by CT or MRI (Pike, 2016)

PET kinetic modelling

Positron Emission Tomography (PET) kinetic modelling is a quantitative method used to analyse dynamic PET imaging data obtained from the administration of radiotracers in living tissues such as the brain. While static PET images provide information about tracer accumulation at specific time points, dynamic PET imaging involves capturing a series of images over time, known as frames. This dynamic data allows researchers to study the kinetics of the radiotracer in various tissues and organs, providing insights into underlying biological processes. PET kinetic modelling involves fitting mathematical models, which are described below, to time-activity curves derived from dynamic PET data. The time-activity curve represents the concentration of the radiotracer in a specific region of interest over time in the brain. These models can then estimate physiological processes, such as cerebral blood flow, metabolism, receptor binding, and transport of proteins.

There are different types of kinetic models, these can be categorised into two main branches including compartmental models and non-compartmental models.

Compartmental models divide the tissue or organ into interconnected compartments, each representing a specific aspect of the kinetics of the tracer. The data collected from the PET scan are then fitted to these compartmental models and the parameters are estimated using various mathematical techniques which will not be discussed in detail here as this goes beyond the aims of this PhD.

The most used compartmental models in PET include the:

1. One-Tissue Compartment Model (1TC): The 1TC model assumes a single compartment representing reversible binding of the radiotracer to a specific target, such as receptors or enzymes. The radiotracer enters the compartment and can bind reversibly to the target or leave it through a process called backflux. The model is characterized by two parameters: the rate constant for the association of the radiotracer with the target (k1) and the rate constant for the dissociation (k2). The ratio of k1/k2 is often referred to as the binding potential (BP), which quantifies the density of the target receptors (Morris et al., 2004)
2. Two-Tissue Compartment Model (2TC): The 2TC model extends the 1TC model by introducing an additional compartment that represents irreversible binding. In this model, the radiotracer can enter the first compartment and bind reversibly to the target (k1, k2) or move into the second compartment and bind irreversibly to the target (k3). The rate constant k3 is associated with the trapping or accumulation of the radiotracer in the target tissue. The 2TC model provides a more accurate estimate of receptor availability or target density (Morris et al., 2004)

Non-compartmental models do not rely on predefined compartments and instead analyse the time-activity data directly. These models often involve data-driven approaches and can be useful when the underlying physiological processes are complex or not well understood.

The Simplified Reference Tissue Model (SRTM) is a popular non-compartmental approach to PET kinetic modelling. It was developed to overcome the limitations of compartmental models that require arterial input function data, which can be

challenging and invasive to obtain in some individuals (Lammertsma and Hume, 1996) such as in individuals with addiction who inject drugs.

The SRTM is particularly useful when studying reversible binding of radiotracers to receptors in the brain or other tissues. It is widely applied in neuroreceptor imaging to estimate receptor availability, such as the density of specific receptors and some of the data described in this thesis describes data using this analysis method.

The main principle behind the SRTM is to use a reference region in the brain that has negligible specific binding for the radiotracer; this is often the cerebellum. This region serves as a baseline or reference for the unbound radiotracer in the brain. By comparing the time-activity curves of the target region and the reference region, the SRTM can estimate the non-displaceable binding potential (BP_{ND}) or the receptor availability (Lammertsma and Hume, 1996, Innis et al., 2007), without the need for direct arterial input function measurements.

The SRTM has become an invaluable tool in neuroreceptor imaging studies especially in addiction neuroimaging. It is also widely used in both preclinical and clinical research across other neuropsychiatric and neurological conditions, providing critical insights into the molecular mechanisms underlying brain function and dysfunction.

PET kinetic modelling has a wide range of applications in addiction biomedical research though this has not entered clinical practice so far. It aims to better understand biomarkers of addiction, quantify disease mechanisms, monitor treatment responses and evaluate drug interactions. Furthermore, it can play a crucial role in drug development, as it provides valuable information about drug pharmacokinetics and target engagement which will be discussed in later sections and in recent times has been adopted in addiction translational neuropsychopharmacology research.

Overview of fMRI methodology

Advances in fMRI have allowed for the interrogation of different behavioural facets related to addiction such as reward, stress, memory, emotion, and salience processing. Of particular relevance to my PhD, the development of neurocognitive assays measuring reward system reactivity such as cue-reactivity and the monetary-incentive delay task (MIDT) (Knutson et al., 2000) which measure the brains response to salient stimuli and reward processing. Importantly these tools have more recently been used to develop translational theragnostic biomarkers of addiction which may better predict response to treatment, risk of relapse, and uncover the mechanism of action of new treatments. In the following sections I will describe an overview of fMRI methodology and how it has been used in addiction neuroscience research and introduce how I have used fMRI methods in my PhD

fMRI is a non-invasive neuroimaging technique used to measure brain activity by detecting changes in blood flow and oxygenation levels. It allows researchers to observe which areas of the brain are active during specific tasks or under certain conditions. Below is an overview of the fMRI methodology.

fMRI Scanning

fMRI utilises MRI scanners of different strengths denoted by the tesla or (T), which generate high-resolution images of the brain's structure. These images are important for precisely localising the areas of brain activity in subsequent analyses. fMRI relies on the fact that active brain regions require more oxygenated blood to meet their metabolic demands than regions of the brain that are less active. When neurons are active, blood flow to those regions increases to supply the extra oxygen and this increase in blood flow leads to changes in the magnetic properties of blood, which can be detected by MRI scanner. This change is called the Blood Oxygenation Level Dependent (BOLD) signal and is the key measure in fMRI. It reflects the relative amount of oxygenated and deoxygenated blood in the brain, which varies as brain activity changes. The BOLD signal is an indirect measure of neural activity and the relationship between neural activity and the observed BOLD response represents the sum of many neural and molecular signalling events. fMRI cannot directly measure neural activity at the cellular level, which is what PET allows for and therefore When

neurons in the brain become active during a cognitive task or sensory stimulation, they require more oxygen to support their increased metabolic activity. In response to this increased demand, cerebral blood flow to the activated brain regions also increases and it is this rise in blood flow which is known as the hemodynamic response which underlies the BOLD response (Buxton, 2013). By tracking these changes over time, inferences related to brain function can be made based the activation patterns of various brain regions.

Experimental Design

In an fMRI study, tasks or paradigms are designed in order to elicit specific brain activity. Tasks are designed in such a way to ensure that they are reliable in inducing brain activation (Wall, 2019). In this PhD we will explore the use of tasks that probe reward processing in the brain These tasks were designed to elicit changes in brain function through organising the presentation of the stimulus in a specific way that allows the MRI to capture changes in the BOLD signal.

Baseline and Task-Based Contrasts

During an fMRI task, a block design or event-related design is usually employed. In block designs, participants perform specific tasks during designated "on" periods, while in event-related design, individual events are presented randomly. The contrast is then made between the baseline (resting state or neutral condition) and the task or event periods, highlighting the brain regions associated with the given reward related process or stimulation. During task-based fMRI the BOLD signal during the stimulus of interest must be contrasted with the BOLD signal during a neutral stimulus as the BOLD signal is not a quantifiable measure of neural function (Burock et al., 1998). This is due to the complex underlying physiology of the BOLD signal including changes in cerebral blood flow, regional metabolic requirements and oxygenation of haemoglobin all of which may have conflicting effects on the BOLD signal (Buxton, 2013)

BOLD data is analysed using either using a subtraction or activation calculation. This is used to assess relationships between BOLD signal amplitude and experimental conditions. Differences in the BOLD signal amplitude between differing conditions such as the presence vs. absence of a certain stimulus are imputed and. the assessment of these relationships is performed separately at each spatial location or

voxel in the brain, this is referred to as mass univariate analysis and attempts are made post-hoc to aggregate findings across locations.

Preprocessing

To acquire the data to conduct statistical analysis a series of pre-processing steps are required to improve the data quality and remove any non-signal related noise. Pre-processing of fMRI BOLD data usually consist of the following steps which are outlined in (Manjón, 2017). Typically, it involves, filtering for noise, motion correction, spatial smoothing and registration to standard spaces.

Statistical Analysis of fMRI

The General Linear Model (GLM) (Friston et al., 1995) is an analytical method used to identify brain regions showing significant changes in the BOLD signal that is related to the experimental conditions. The GLM assumes that each voxel is independent and its BOLD signal across the volumes of the task can be separated into components representing separate signal sources. Explanatory variables (EVs) are then used as regressors of interest in the analysis and are modelled to reflect the time course of task-related stimuli (based upon the design of the task) and other noise-related variables that may influence the BOLD signal, such as motion (Manjón, 2017). The output from the GLM model in task-based fMRI will typically be a number of single-subject whole brain voxel-wise BOLD contrasts of interest compared between stimuli (e.g., salient cue BOLD > neutral cue BOLD).

Statistical analyses are then carried out using these whole brain BOLD contrasts. In the studies described in this PhD t-tests are conducted either between patients and controls, or correlations with pharmacological, clinical, behavioural, or psychological variables of interest. Covariates can also be added to the model to account for variables such as demographic differences between subjects or groups of subjects . Due to the large numbers of voxels within the GLM and other fMRI statistical analyses, multiple comparison correction is required to reduce the chance of type I errors (false positives).

Neuroimaging in addiction

Dopamine PET receptor density in addiction

In the following sections I will summarise some of the literature that has used PET imaging to understand neuroreceptor availability in addiction. In my thesis, chapter 2 and 3 employ analyses of PET data to determine the relationship between neuroreceptor availability and its subsequent relationship to both addiction and response to novel pharmacological interventions. In Chapter 3, one of my experiments aims to validate a future tool to measure neuroreceptor release capacity combined with fMRI.

Dopamine receptors in addiction

Extensive research has been conducted on the use of dopamine as a biomarker for addiction, particularly concerning dopamine D2/3 receptors ($DRD_{2/3}$) in the striatum, assessed using the [^{11}C] raclopride radiotracer. The results from these studies have yielded mixed findings. For instance, individuals with alcohol and stimulant use disorders have shown reduced availability of $DRD_{2/3}$ receptors compared to control subjects. However, no significant differences between groups were observed in individuals with opiate, cannabis, tobacco, or gambling addictions (Nutt et al., 2015). Nevertheless, in the case of gambling disorder, there was a negative correlation between mood-related impulsivity (referred to as 'Urgency') and [^{11}C]raclopride binding potential (Clark et al., 2012). These studies will be more extensively investigated in chapter two of my thesis.

More novel radiotracers such as [^{11}C]PHNO have been developed to quantify the relative differences in binding of DRD_3 compared with dopamine D2 receptor (DRD_2) in brain regions in order to improve the precision of neuroreceptor identification and gain a deeper understanding of addiction-related biomarkers in alcohol-dependent patients (Erritzoe et al., 2014) and in stimulant use disorder (Ashok et al., 2017). These findings highlight increased hypothalamus and substantia nigra DRD_3 in alcohol dependence and stimulant use disorder respectively and have led to the development of novel interventions such as the DRD_3 antagonist. This specific intervention will be explored in chapter three of my thesis.

PET neurotransmitter release studies in addiction

As referred to previously, addiction has been proposed as a 'reward deficient' state, which can be compensated for with substance use (Blum et al., 2000a) . Radioligands have been developed to assess theories of deficits in neurotransmission and in particular assessment of blunted dopamine, endorphins and serotonin can help to establish the extent of molecular neurotransmitter deficits in addiction to see whether these pathological hallmarks are therapeutically modulated by novel interventions including psychedelics. The methods to assess neurotransmission in the human brain is through conducting neurotransmitter release studies. These rely on the principle that psychostimulants, such as methylphenidate or amphetamines, can reliably increase levels of endogenous dopamine (Volkow et al., 1997), opioids (Colasanti et al., 2012) and more recently serotonin (Erritzoe et al., 2020). Non-pharmacological manipulations to release neurotransmitters include stress, motor tasks, video games, and cue-induced craving. In chapter three we will discuss the development of a specific fMRI behavioural task that aims to measure dopamine neurotransmitter release in future studies (Egerton et al., 2009).

Dopamine release

There is evidence for blunted dopamine release in cocaine, opiate, and alcohol dependence but not in cannabis dependence as assessed with the antagonist radioligand [11C]raclopride (Nutt et al., 2015). A recent review demonstrated that, behaviourally, blunted striatal dopamine transmission could reflect increased impulsivity and altered cost/benefit computations that are associated with addiction. The data suggest that blunted dopamine neurotransmission is a biomarker for increased risk of developing addiction and treatment-resistance, is associated with impulsive behaviour and reduced motivation and is linked to increased drug consumption (Trifilieff et al., 2017). The translational application of such methodologies is to assess whether such observations are sensitive to novel interventions.

fMRI studies in addiction

In the context of addiction, several well-validated fMRI paradigms have been developed that assess the neural responses to reward, punishment, salience attribution, emotions, memory, cognitive, and executive functions and their association with clinical outcomes and relapse vulnerability. In particular, our group have previously successfully developed an fMRI platform that assessed novel candidate drugs on the aforementioned key relapse pathways known to be dysfunctional in individuals with addiction using task-based fMRI (Paterson et al., 2015)

fMRI MIDT in addiction

Reward processing

Substantial evidence points to dysfunction in reward processing in the context of addiction. Research has revealed increased activity in the ventral striatum (VS) in response to observed or actual drug reinforcement, as well as in reaction to monetary rewards, both in individuals with addiction and those without (Sescousse et al., 2013b). This heightened activity is associated with corresponding changes in the activity of more frontal brain regions such as PFC and the OFC, both of which play crucial roles in assessing the balance between rewards and costs, as well as motivation for seeking rewards (Elliott and Deakin, 2005).

To investigate neural responses to monetary reward processing in addiction populations, one of the most used fMRI tasks is the MIDT (Knutson et al., 2000). This task has been instrumental in uncovering pathophysiological aspects of reward processing among individuals with addiction. Meta-analyses of studies employing the MIDT paradigm have consistently revealed reduced activity in the ventral striatum during the anticipation of rewards in individuals with various forms of addiction compared to well-matched healthy controls though in behavioural addictions the responses have been more variable (Luijten et al., 2017).

Furthermore, the activation of the ventral striatum during this task has been employed to evaluate the impact of drugs under development as potential treatments for addiction. This includes substances like DRD3 antagonists (Murphy et al., 2017a), as well as already-approved medications like naltrexone (Nestor et al., 2017) and nalmefene (Quelch et al., 2017). These studies were designed to determine whether

these novel drugs modulate reward processing in individuals with addiction in a manner similar to approved medications, thereby establishing their suitability for clinical development.

Cue-reactivity

fMRI studies have identified disrupted neural responses to video and photo cues in individuals with addiction when compared to well-matched, healthy control subjects. Specifically, these studies have revealed both heightened and diminished activity within the brain's salience, attentional, executive, and memory networks when confronted with addiction-related cues as opposed to naturally rewarding or non-salient visual stimuli (Zilverstand et al., 2018).

Patients with addiction exhibit more robust responses to addiction-related cues and experience increased craving. This phenomenon is indicative of abnormal incentive sensitisation, which is believed to contribute to maladaptive drug-seeking behaviours (Berridge and Robinson, 2016). Moreover, research has also shown atypical fMRI cue-reactivity in individuals with GDs when compared to their matched counterparts. Individuals with GD exhibit heightened reactivity to gambling-related cues in specific brain regions, such as the left insula and the ACC. Furthermore, one study found a positive correlation between the intensity of craving to gamble and the activity in the bilateral insula and ventral striatum in response to gambling cues (Limbrick-Oldfield et al., 2017). As GD is categorized as a behavioural addiction, it offers an opportunity to investigate addictive processes without the potentially complicating factors associated with chronic and excessive drug and alcohol exposure. Consequently, these patterns of dysregulated brain activity may serve as essential and dynamic biomarkers of addiction and could potentially be targeted for the development of innovative interventions.

The use of cue-reactivity fMRI has proven valuable in predicting addiction severity, the risk of relapse, and treatment outcomes (Jasinska et al., 2014). It has also been instrumental in the development of novel therapeutic approaches in the field of addiction (Courtney et al., 2016). Given these findings, cue-reactivity has demonstrated to be a key translational neuroscience tool to utilise in addiction research.

Addictions Neuroclinical Assessment (ANA)

In the last decade, conceptual frameworks for understanding psychiatric diseases have slowly moved from a categorical approach towards a more dimensional one. The Research Domain Criteria (RDoC) initiative was proposed as a dimensional alternative to the DSM, moving away from a symptom-based to a more mechanistic-based understanding of psychiatric disease (Insel et al., 2010). In 2016, a similar dimensional framework was proposed for addictive diseases, a neuroscience-based framework called the Addictions Neuroclinical Assessment (ANA), focused on mechanistically relevant domains based on the current neurobiological understanding of addiction. The ANA domains include incentive salience, negative emotionality, the bias for negative affective stimuli and executive function, specifically attention, response inhibition, planning, working memory, behavioural flexibility and valuation of future events (Kwako et al., 2016). This new framework aims to support the discovery and integration of biomarkers into these domains in the hope that it might lead to a more accurate assessment of addictive disorders and might even uncover new biomarkers which pave the way for the development of novel interventions (Kwako et al., 2018). Hence, an objective of this PhD is not only to characterise the addictive state but to explore how neuroimaging can aim to identify novel targets and optimise drug development efforts for addiction medicine.

The following sections briefly review how this approach has been previously implemented in addiction research in the context of drug development and will lay the foundations for the translational neuropsychopharmacology research approach of my PhD.

Neuroimaging in drug development

Translational neuropsychopharmacology harnesses human neuroimaging techniques to advance novel psychopharmacological interventions for psychiatric conditions. Neuroimaging offers unparalleled insights into the pathobiology of disorders such as addiction, enabling the identification of potential functional and molecular biomarkers. These tools also evaluate the impact of innovative interventions, like psychedelics, on addiction biomarkers and their correlation with clinical and behavioural outcomes. Such an approach facilitates a comprehensive exploration of addiction neuropathology and its implications for utilizing psychedelic therapy, encompassing functional, molecular, and structural aspects. Moreover, this approach allows for biomarker-informed prognosis, ultimately paving the way for precision-based patient stratification to tailored treatments, thereby advancing personalised medicine strategies for enhanced patient outcomes.

Drug development

The process of drug development typically commences with the identification of a biological target believed to play a pivotal role in a specific disease. Following this, a comprehensive screening of numerous molecules takes place to assess their chemical properties and their potential to bind with the target in laboratory experiments. From this extensive pool of molecules, a smaller subset undergoes testing in preclinical animal models of the disease. This evaluation includes an assessment of toxicity, pharmacokinetics (PK), pharmacodynamics, bioavailability at the target organ, in vivo target engagement and overall efficacy. This process leads to the selection of lead molecules that will progress to further development stages (Wong et al., 2010).

Subsequently, human clinical trials are conducted. Phase 1 clinical trials encompass a limited number of participants to ensure the drug's safety and tolerability across a range of doses, including those potentially effective in treating the condition. PK and PD responses are closely monitored to inform dose selection for Phase 2 trials. Phase 2 trials involve enrolling anywhere from approximately 50 to several hundred participants, comparing their therapeutic responses with those in placebo or control groups, all while meticulously tracking safety and efficacy. In Phase 3, a larger participant pool, distributed across multiple global sites, is chosen to confirm earlier safety and efficacy findings (Wong et al., 2010).

Throughout this multi-stage process, imaging assays such as fMRI/PET can play a pivotal role in the mechanistic evaluation of drugs and in distinguishing treatment responders from non-responders.

fMRI in translational addiction research

Functional Magnetic Resonance Imaging (fMRI) has been used in numerous studies related to addiction drug development.

Early phase clinical trials

In the context of early-phase clinical investigations, fMRI is commonly employed as a pharmacodynamic or response biomarker. Its purpose lies in the identification of functional central nervous system (CNS) effects resulting from pharmacological interventions, particularly in brain regions pertinent to the compound's mechanism of action and/or the intended target population (Borsook et al., 2013). Although not inherently indicative of direct target engagement, an fMRI signal can indirectly imply such engagement if a biologically plausible correlation can be established between the observed fMRI response and the molecular target being pursued (Nathan et al., 2014). The absence of detectable fMRI signals during early-phase investigations can also contribute to the collective knowledge about the compound, which could inform decisions regarding progression to later-phase studies or the need for a change in research direction.

In a similar vein, the NIMH FAST-FAIL paradigm, originating from neuropsychiatric studies, presents a stringent and transparent strategy. Within this framework, early-phase experimental agents are mandated to induce predetermined biomarker modifications (including fMRI markers), as outlined in preregistered trial protocols. Failure of the compounds to to elicit the specified alterations results in the exclusion of these agents from advancing to subsequent trial phases (Krystal et al., 2019)

This has been used previously in a trial of AUD where positive fMRI results observed during the early phases of one agent (varenicline) influenced the decision to pursue further development, subsequently demonstrating clinical efficacy in mitigating alcohol cravings (Gandhi et al., 2020). Conversely, null early-phase fMRI findings for a different AUD agent (pexacerfont) prompted a redirection of attention toward a

competitor agent (verucerfont (Cannella et al., 2019)). This exemplifies how early-phase fMRI outcomes can significantly shape decisions regarding the progression of agents within therapeutic development pathways.

Later phase

In later phase clinical research, these studies might include fMRI to provide more objective demonstrations of disease modification, thereby increasing the evidence base for a regulatory submission. In 2021, a systematic review (Sadraee et al., 2021) found that that over 109 phase 3 or phase 4 drug interventions with fMRI readouts were registered on ClinicalTrials.gov, or 21.6% of all registered drug interventions with fMRI readouts showing the increasing integration of such tools in drug development. Despite this at present, there is only one such biomarker, under consideration with the European medical agency that utilises fMRI: this a battery of brain MRI measurements that includes two task fMRI paradigms and a resting state acquisition, with a goal of enriching trials in autism spectrum disorder (Loth et al., 2017). Using such an approach in addiction clinical research will enable the design of novel, effective treatments by identifying new brain targets. Precision-medicine algorithms can then be developed to predict individual patient relapse risk and likelihood of treatment response. This translational neuroimaging approach to addiction clinical trials is recommended by a white paper co-authored by over 30 global leading addiction scientists and doctors from the International Society of Addiction Medicine (Verdejo-Garcia et al., 2019).

PET in translational addiction research

PET imaging has played a key role in addiction drug development by providing in-vivo measures of neurobiological dysfunction related to neuroreceptor or neurotransmitter release dysfunction and such insights have helped identify relevant brain targets for drug development.

Monitoring Drug Distribution: PET imaging allows the evaluation of drug distribution in the brain after administration. This information is crucial for understanding how drugs interact with the brain's target areas and for optimising dosing regimens, it is also critical for establishing target engagement by determining the relationship between (functional) target engagement and clinical response in the patient group. Recent research has demonstrated that over 50% of drug programs were not able to conclude if drug mechanism of action had been tested adequately which may be behind many Phase II drug failures (Morgan et al., 2012). Indeed, testing the three pillars of survival has been put forward to ensure (i) proof of distribution; (ii) proof of occupancy, and (iii) proof of function.

Predicting Treatment Outcomes: PET imaging has been explored to develop potential biomarkers of addiction and these findings could be used to predict individual responses to addiction medications. By measuring specific brain biomarkers before treatment, researchers can identify individuals more likely to benefit from a particular drug and personalize treatment approaches. Some studies have been able to demonstrate the validity of this approach with one in cocaine addicted individuals finding those with higher DRD2/3 and higher dopamine release responding to treatment whilst those with lower levels did not (Martinez et al., 2011).

Assessing treatment Efficacy: Clinical assessment scales commonly used in addiction typically require clinicians to assign a patient's condition a score based on an ordinal scale featuring various severity categories. These scales are structured with anchor points, which may not fully capture the intricacies of the clinical presentation. Consequently, the reliability of clinical scores is always said to be somewhat subjective. In this context, PET imaging holds the promise of serving as a more delicate indicator of drug effects compared to alterations in clinical rating scales. While this can be particularly valuable in the initial stages of development, the most effective imaging markers also exhibit a clear correlation with clinical outcomes, thereby offering a clinically meaningful endpoint (Wiers et al., 2016a).

Personalised Medicine: PET has the potential to contribute to personalised medicine in addiction treatment. Variability in clinical manifestations is frequently observed in addiction and this diversity extends even within the same sub-class of addiction across different phases of the disorder. While the way individuals manifest the functional consequences of addiction pathophysiology may play a role in this variation, it's highly probable that differences in the underlying biological dysfunction are a significant factor contributing to the diversity in clinical presentations. This underscores the importance of employing imaging techniques to pinpoint patients with a consistent underlying pathophysiological profile. Such an approach can serve as a stratification method for assessing the efficacy of a novel potential drug treatment. Further, in the future it may be possible that through identifying individual differences in clinical presentation, brain function and neurochemistry related to addiction alongside other factors such as pharmacogenomics, clinicians may be able to tailor treatment plans to target specific neural pathways for better outcomes based on an individual's disease phenotype (Ragia and Manolopoulos, 2017).

Simultaneous/combined PET/fMRI

Integration of data from PET target occupancy studies and fMRI methods provides a novel strategy for directly relating information on drug–target interactions directly with a measure of functional effects in the brain. Some studies have adopted this approach with one assessing the extent of binding of an antagonist to its μ-opioid target with modulation of fMRI reward responses to the administration of a palatable food stimulus in healthy volunteers (Rabiner et al., 2011). The study validating the target as a

modulator of satiety responses in humans and the PET data indicated appropriate pharmacological doses range based both on the measures of target interaction and the pharmacodynamic effect. The advent of novel simultaneous PET-fMRI technologies will greatly advance such research and if used in addiction research will represent a profitable technological advance for therapeutic development (Pichler et al., 2010). These systems will undoubtedly pave the way for the most precise molecular-functional-clinical translational bridge that has the capacity to redefine drug development and treatment outcome in patients. Indeed, the tasks and techniques explored throughout this PhD, especially in chapter three and four all aim to provide the basis for exploring multimodal neuroimaging in addiction translational neuropsychopharmacology research in the future.

Aims of thesis

The aims of this thesis are to explore reward system function using fMRI and PET in individuals with addiction. There are both basic neuroscience investigations to explore biomarkers of addiction and translational clinical neuroscience experiments that explore the integration of multimodal imaging methods to investigate the mechanism of action of novel pharmacological interventions.

The three chapters of my thesis will explore the following:

1) Investigating DRD2/3 in addiction: a systematic review and meta-analysis

2) To explore the effects of DRD3 antagonism using fMRI and PET in individuals with alcohol dependence (ADIs)

a) The effect of DRD3 blockade on striatal BOLD activation to monetary rewards/expectation of rewards, using fMRI monetary incentive delay task (MIDT) pre- and post-blockade of the DRD3 among ADIs vs age-gender-matched HC.

b) The relationship between such activation and regional DRD2/3 binding as assessed with PHNO-PET.

3) To validate novel fMRI paradigms in patient with gambling disorder (GD)

a) A novel reward-enriched roulette (RER) fMRI paradigm

b) A novel 5-armed cue-reactivity (5-ACR) fMRI paradigm

Chapter 2: 50 years of highs and lows, imaging dopamine receptor D2 and D3 in addiction: a comprehensive review and meta-analysis

Introduction

Although addictive drugs produce their effects through actions at various targets in the brain's neurotransmitter systems, it is commonly believed that their effects on the activity of dopaminergic mesocorticolimbic pathways are critical for their addictive properties.

The identification of brain correlates associated with mediating rewarding and motivational states were first observed in rodent studies of electrical stimulation in the septal regions of the brains (Olds and Milner, 1954). Subsequently Crow and colleagues demonstrated that this effect was mediated through stimulation of the dopaminergic pathway suggesting this neurotransmitter was critical in operant reinforcement of behaviours (Crow, 1973), a finding later confirmed by self-stimulation paradigms and pharmacological blockade (Wise et al., 1978). The advent of brain dialysis techniques led to the finding that administration of some addictive drugs increased dopamine in the nucleus accumbens (NAc) (Di Chiara and Imperato, 1988) (Pidoplichko et al., 1997) and that blockade of dopamine transmission lead to the diminished rewarding effects of psychostimulants (Koob, 1992). It was soon claimed that all drugs of abuse, either directly or indirectly, increase dopamine release in the NAc (Di Chiara et al., 2004).

Neurochemically, dopamine acts throughout the brain by interaction with dopamine receptors (DR). These are a class of G protein-coupled receptors with five subtypes from two gene families; DRD1 and DRD5 that are stimulatory to G proteins and DRD2, DRD3, DRD4 that are inhibitory. DRD_2 and DRD_3 receptors in the living human brain can be measured using positron emission tomography (PET) (Wagner et al., 1983) and single photon emission computerised tomography (SPECT). For more

than thirty years studies using dopamine PET and SPECT radioligands have investigated the role for the $DRD_{2/3}$ in the neurobiology underlying addiction processes, though in recent years the role of $DRD_{2/3}$ in addiction has witnessed growing critique (Nutt et al., 2015). Early work in psychostimulant (cocaine and methamphetamine) and alcohol dependence supported the notion of reduced $DRD_{2/3}$ binding in cocaine, methamphetamine and alcohol dependent individuals versus matched healthy (non-addicted) controls. More recent studies at the turn of the millenni did not find similar patterns of reduced binding availability in other sub-classes of addiction with a series of negative findings in case-control studies in cannabis-, opiate- and nicotine-dependent subjects (Nutt et al., 2015). Further, molecular neuroimaging in behavioural addictions such as gambling disorder have further questioned the dopaminergic basis of addiction finding no differences in $DRD_{2/3}$ availability between cases and controls. Neuro-biomarker research into gambling disorder in recent years has further propagated our understanding of the brain mechanisms of addiction due to it providing a 'substance-naïve' prototype of an addicted brain (Clark et al., 2019).

Early research with the first available $DRD_{2/3}$ PET radioligands such as [^{11}C] raclopride, [^{11}C]N-methylspiperone, and SPECT ligands such as [^{123}I]iodobenzamide only allowed for receptor quantification in the striatum due to their moderate $DRD_{2/3}$ affinity (yielding a poor signal to noise ratio in extra-striatal regions with a relatively low density of $DRD_{2/3}$). Thus, most imaging studies in addiction have mainly reported receptor binding from this region exclusively which whilst an important region of the reward system does not cover the extent of all regions involved in reward processing which underlie addiction. Recently, the development of a new generation of $DRD_{2/3}$ radioligands with higher receptor affinity has enabled visualisation and quantification of $DRD_{2/3}$ in regions outside the striatum. These include [^{18}F]fallypride (Mukherjee et al., 2002), [^{11}C]FLB-457 (Vilkman et al., 2000), [^{11}C]PHNO (Ginovart et al., 2007, Searle et al., 2013) and [^{11}C]NPA (Narendran et al., 2010). As such, these technological developments in radioligands have provided a more global brain account to investigate putative dopaminergic addiction biomarkers.

Quantification of PET

Data acquired from PET undergoes kinetic modelling to develop numerical readouts of biological targets of interest, in this case receptor availability. Kinetic modelling is a quantitative method used to analyse dynamic PET imaging data obtained from the

administration of radiotracers in living organisms, such as humans or animals. In brief, PET kinetic modelling involves fitting mathematical models to the time-activity curves derived from the dynamic PET data. The time-activity curve represents the concentration of the radiotracer in a specific region of interest over time. Mathematical models are then used to estimate physiological processes, such as blood flow, metabolism, receptor binding, and transport. There are different types of kinetic models, including compartmental models and non-compartmental models. Compartmental models divide the tissue or organ into interconnected compartments, with each representing a specific aspect of tracer kinetics. The fundamental principle behind compartmental modelling is that the movement of the radiotracer in and out of the compartments can be described using a set of differential equations, which can be solved to estimate the kinetic parameters characterising the biological processes of interest. A complete overview of PET kinetic modelling is beyond the scope of this chapter however it is discussed in more detail in the introduction chapter; however, several types of kinetic modelling are described in this paper including the non-displaceable binding potential (BP_{ND}) and the volume of distribution (VT).

Low DRD2/3 binding levels; a biomarker for addiction or a consequence of long-term substance use?

A key question to consider is whether detection of low $DRD_{2/3}$ binding in individuals with addiction represents a (pre-existing) biomarker for addiction or rather is a consequence of long-term substance use. Several theoretical frameworks have been developed to describe how neurochemical and molecular signalling are involved in the development and maintenance of addiction. The reward deficiency theory (Blum et al., 2000b) states that addicted individuals have a hypoactive dopaminergic reward system, translating to a lower responsivity to reward. To support this theory there is evidence that those with severe forms of addiction display reduced amphetamine-induced dopamine release (Wang et al., 2012, Trifilieff and Martinez, 2014). This reduced dopaminergic neurotransmission is also witnessed in individuals with an ultra-high risk of addiction which is defined by having a dense family history of drug and alcohol addiction alongside psycho-social factors known to increase risk of addiction which are discussed as ‘confounders’ in this chapter (Casey et al., 2014, Conway et al., 2006). Thus, the conclusion as to whether this hypodominergic is heritable or epigenetic is up for debate. Nonetheless these endophenotypes may drive an

individual to seek out more hedonic pleasures to fill their apparent void. This theory also extends to the possibility of chronic drug use leading to dopamine receptor downregulation. Early primate work has established an inverse relationship between rates of cocaine self-administration and $DRD_{2/3}$ availability (Nader et al., 2008). This has been proposed as a mechanism leading to a cycle of drug-induced neurotoxicity, mediating addictive behavioural cycles (Koob and Volkow, 2016). These lines of evidence and some human research has begun to support the idea that downregulation of these receptors could be related to drug use. Indeed, assessment of $DRD_{2/3}$ in gambling disorder can also help to establish the proposed causal links between $DRD_{2/3}$ availability and addiction and whether dopamine receptor downregulation could be drug induced or heritable. There are some obvious limitations in the available human PET/SPECT data addressing the question of causality due to the cross-sectional design of such studies, however we will explore the existing data for signs of substance-induced receptor effects.

In this paper we will systematically review PET and SPECT imaging studies examining human brain $DRD_{2/3}$ binding in individuals with addiction to alcohol, cocaine, methamphetamine, opiates, smoking, cannabis, or gambling and compare them to matched control healthy (non-addicted) subjects. For the sake of clarity, we refer to all categories of substance abuse/dependence and gambling disorder as "addictions". $DRD_{2/3}$ availability from both the full striatum and extra-striatal regions will be presented and meta-analysis of both the striatum and substantia nigra (SN) are conducted using data from studies using both antagonist and agonist radioligands wherever possible. Agonist radioligands quantifying SN $DRD_{2/3}$ reflect 100% DRD_3 binding and so are analysed to assess the relative contribution of DRD_2 vs DRD_3 to addiction pathobiology. We will also present and discuss studies that have separated DRD_2 and DRD_3 binding. Furthermore, in order to dissect the possible confounding impacts of impulsivity, socioeconomic status and co-morbid substance use- we will specifically examine studies to assess the degree to which such factors have been controlled for in such studies. Lastly, to address the question of the causal influence of $DRD_{2/3}$ alterations in addictions (when such are detected), we will revisit studies to look for: 1) evidence of a dose-response relationship between $DRD_{2/3}$ binding and the duration/severity of substance abuse, and 2) signs of recovery of $DRD_{2/3}$ binding with prolonged abstinence.

Confounding variables in the $DRD_{2/3}$ and addiction literature

When interpreting results from PET/SPECT studies investigating dopamine receptors in addiction it is important to consider the role of potential confounding factors that may also interact with their distribution and availability. Research into both dopamine and addiction has highlighted the dynamic modulation of the dopaminergic system by factors different from addiction status/severity, including behavioural traits, socioeconomic status, ageing and gender as well as substance use. In particular, the behavioural trait of impulsivity which has been found to be associated with addiction with higher impulsivity scores predicting a greater risk of developing an addiction (Quinn and Harden, 2013). Equally, impulsivity has also been independently associated with $DRD_{2/3}$ availability (Trifilieff and Martinez, 2014) and preclinical work has demonstrated that rats with lower $DRD_{2/3}$ availability are more impulsive (Dalley et al., 2007).

In a seminal experiment for the field of the brain-reward behavioural effects of dopamine, Morgan et al reported that non-human primates occupying higher social ranks exhibited higher levels of $DRD_{2/3}$ binding than those occupying lower social ranks, which was shown to be protective against cocaine self-administration (Morgan et al., 2002). In humans, studies have suggested that social deprivation and low education levels are frequently associated with addiction (Singer, 2012). PET imaging has further qualified an association between striatal $DRD_{2/3}$ levels in healthy non-addicted humans and socioeconomic status (Wiers et al., 2016b).

Another potential confounding variable in investigating the role of dopamine receptors in the pathobiology of a specific sub-class of addiction is the lack of ecological validity in assessing addiction to isolated substances or behaviours. It is well-established that up to 12% of individuals with substance addiction have concurrent alcohol and drug use disorders (Crummy et al., 2020) and in one study addicted individuals reported an average concurrent use of 3.5 substances (Onyeka et al., 2012) with alcohol being the most common (Malcolm et al., 2006). Focusing biomarker investigations solely on a single drug addiction sub-category may therefore result in overlooking possible interactions between different substances on the dopamine system, thereby limiting generalisability to the actual population of individuals with addiction. As such this research could be the reason why drug

development efforts have been limited in outcome due to inaccurate estimations of disease relevant mechanisms.

Methods

Systematic review method

A literature search was carried out using the PubMed/Embase and PSYCHinfo/Medline databases entering the search terms “(DA OR dopamine) AND (receptor OR d2 OR d3 OR drd2 OR drd3 OR d2/3) AND (PET OR SPECT) AND (alcohol OR ethanol OR methamphetamine OR cocaine OR amphetamine OR methylphenidate OR nicotine OR smoking OR heroin OR opiate OR cannabis OR marijuana OR MDMA OR ecstasy OR ketamine OR gambling) from inception date to December 14, 2022. Further papers were identified by searching reference sections in papers returned by the original search. Data demonstrating between group differences as expressed by effect sizes of $DRD_{2/3}$ availability were selected for meta-analysis (method described below) whereas studies that did not explore this directly but had relevant data were included in the qualitative synthesis. The PRISMA flow chart identifies the flow of studies from the search (see fig 3).

Flowchart showing the inclusion of studies for the meta-analysis on dopamine D2/3 receptor availability across addiction.

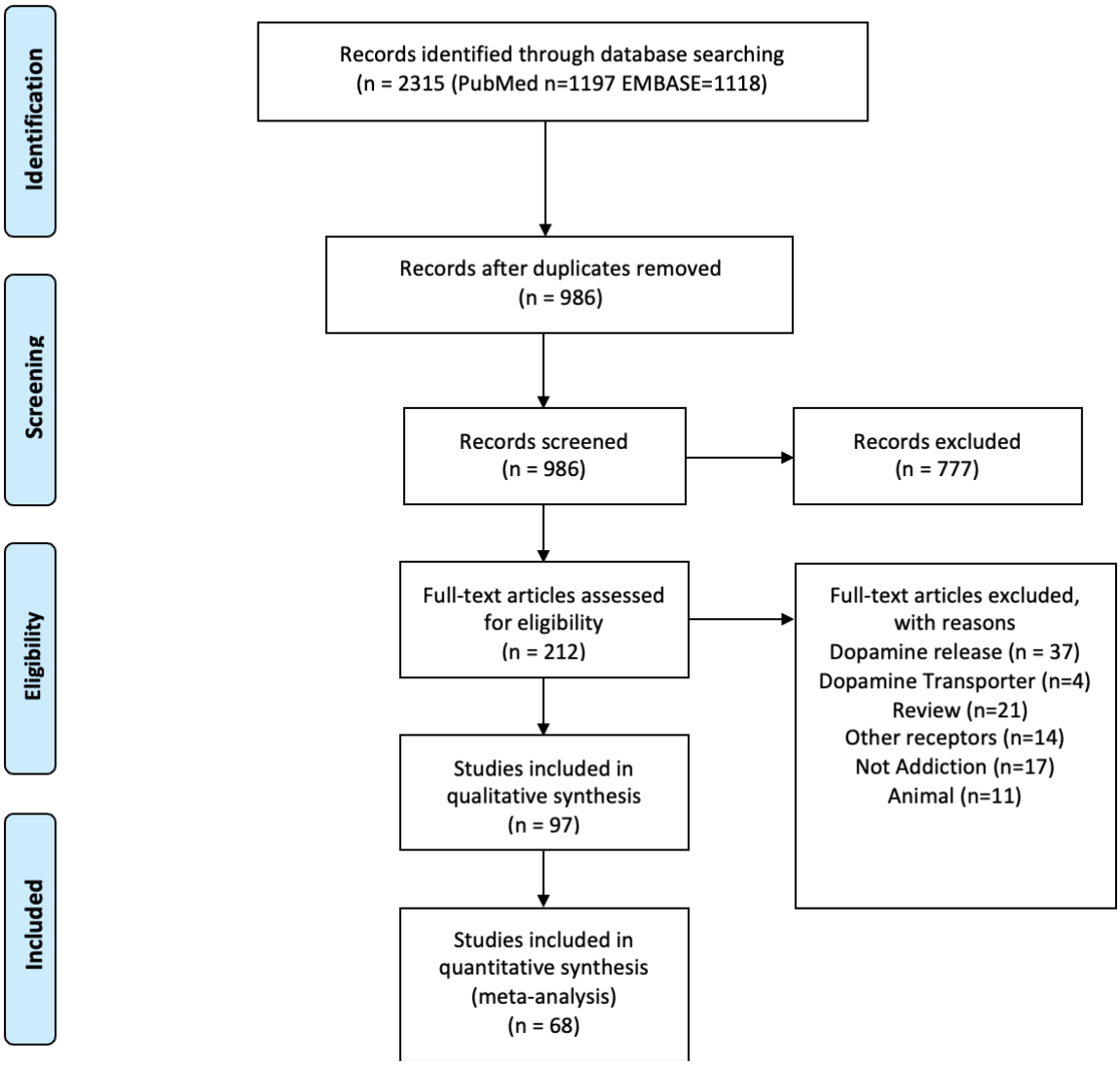


Figure 3: PRISMA flowchart

Fig 3: PRSIMA flow chart to map flow of studies through systematic review and meta-analysis.

Diagnostic criteria

Cases from all the alcohol and heroin studies fulfilled DSM or ICD criteria for dependence. Out of the studies assessing cocaine, in one study only DSM-IV criteria for abuse were required for eligibility in (Volkow et al., 1990) and in one of the methamphetamine studies (eligibility criteria were not clearly described in (Iyo et al., 1993). Cases in two of the six cannabis studies were regular users where requirement for dependence/abuse criteria was not listed. For smoking addiction, most studies refer to cases as “smokers” without reporting criteria for dependence/abuse, and

among smoking studies where the Fagerstrom Test for Nicotine Dependence (FTND) scores were listed, the average group scores for cases were above the 'dependence threshold' of 4 in all apart from one study (Brown et al., 2012). For the gambling studies, all cases fulfilled DSM-IV criteria for pathological gambling or DSM-5 for gambling disorder.

Meta-analysis method

For the meta-analysis, studies were initially separated by radioligands used to assess $DRD_{2/3}$. Those with antagonist radioligands [^{11}C] raclopride, [^{123}I]iodobenzamide and [^{18}F]-N-Methylspiroperidol had enough studies for metanalysis. Of the agonist radioligands only [^{11}C] PHNO for the SN had enough data for inclusion in a meta-analysis. The agonist, [^{11}C]NPA, and high-affinity radioligands including [^{18}F]Fallypride and [^{11}C]FLB 457 were not included in a meta-analysis due to either quantification issues in striatum (i.e. their high affinities results in almost irreversible binding profile (Halldin et al., 1995) with these radioligands or a relative lack of studies using such ligands.

Inclusion and exclusion criteria

All the identified peer-reviewed studies are listed in Table 1-3. Studies that provided information on the average and standard deviation of regional $DRD_{2/3}$ binding in individuals with substance use disorders or behavioural addictions, as compared to age- and gender-matched healthy controls, were included in the meta-analysis if they met the following criteria: a minimum of three separate studies were available for a given addiction, reporting either full or subsets of striatal values, and/or studies using agonist radioligands for the SN. To adhere to standard meta-analysis practices, we excluded studies with sample overlap with another study that had already been included. When studies reported data from overlapping samples, we selected the most recent publication, or the one with the largest sample size.

Data extraction

The primary outcome measure was the difference in the striatal $DRD_{2/3}$ binding between individuals with addiction and matched healthy controls across the several subtypes of addiction described. The following variables were extracted from all the studies: authors, year of publication, subject characteristics of the individuals with addiction and controls (group size, age, sex, diagnosis), imaging characteristics

(method, radioligand), and modelling method. When a value for the total striatum was not presented and only subregional data were reported, data from subregions of the striatum were pooled and weighted according to their relative contribution to the full striatum. A proxy value for the total striatum was calculated using extracted weighted volumes from FSL Harvard-Oxford subcortical atlas (Mazziotta et al., 2001). The relative contributions of the putamen (57.66%), caudate (36.08%) and ventral striatum (6.25%) were combined to create a total pooled striatal value. For studies where only two of the subregions were reported we used this same weighting to produce a proxy measure of total striatal binding by multiplying the sub-regions by its relative weight to provide a total pooled binding availability. Moreover, some of the included studies did not directly report on the average and standard deviation of regional $DRD_{2/3,}$ in this case authors were contacted to obtain these values. In three studies baseline $DRD_{2/3}$ data were only reported graphically, from these studies, the mean and standard deviation of the regions of interested were directly extracted from the figures. When only data from the right and left striatum was reported, data were pooled into an averaged striatum as there is no evidence to suggest interhemispheric differences in dopamine receptor availability. In both alcohol (Guardia et al., 2000) (Volkow et al., 2002b), cocaine (Volkow et al., 1990, Volkow et al., 1993) and methamphetamine (Volkow et al., 2001) addictions, data for some studies were presented for both short and long-term abstinent subjects in which case data were split and inserted in the analysis separately.

Data analysis

Meta-analyses of group differences in mean striatal or SN $DRD_{2/3}$ binding were completed in RevMan (v5.3, Cochrane Collaboration), using study-level differences in (sub)regional binding calculated in Microsoft Excel. The main outcome measure was Hedges' *g*, calculated using a random effects model, where $p < 0.05$ (two-tailed) was considered significant. These analyses also estimated statistical heterogeneity using the I^2 value, where $I^2 < 50\%$ indicates low-to-moderate heterogeneity and $I^2 > 50\%$ indicates moderate-to-high heterogeneity.

Additional analyses were performed to compare within-group variability of whole striatal or substantia nigral $DRD_{2/3}$ binding. Although meta-analyses of mean difference reveal between-group differences in average outcomes, meta-analyses of variance may identify greater interindividual differences between the subjects of one

group than another. Discussed in more detail elsewhere (Howes & Chapman, in review), meta-analysis of variance has already been used to identify patient–control differences in neuroanatomy (Brugger and Howes, 2017) neurobiology (Brugger et al., 2020) and psychotropic treatment response (McCutcheon et al., 2022). We therefore calculated the variability ratio (VR) and coefficient of variability ratio (CVR) – that is, VR adjusted for mean binding – of whole striatal and substantia nigral $DRD_{2/3}$ availability in individuals with addiction versus matched healthy controls, using the Metafor package (Viechtbauer, 2010) within RStudio (v4.2.1, cran.r-project.org). When CVR or VR > 1, outcome variability is greater in the patient (i.e., individual with addiction) than control group, and vice versa. We also used RStudio to complete Egger's tests for small study effects, to comment on potential publication bias.

Confounders

To provide an overview of how different studies have attempted to correct for potential confounding factors, we estimated the evidence level for corrections for each study on the following potential confounders: impulsivity, socioeconomic status and/or IQ/education, alcohol use, and smoking. We assessed each study to determine if a specific confounder was mentioned and partly addressed, or if the case and control groups were matched or statistically corrected for a specific confounding effect. For each potential confounding variable, we attributed a score of 0 if it was not considered, 1 if it was mentioned or partly addressed, and 2 if the groups were matched or statistically corrected for it. We considered IQ and years of education as proxy measures of socioeconomic status for the current review, given the established links between socioeconomic status, education, and cognitive performance reported in previous studies (Piccolo et al., 2016) (von Stumm and Plomin, 2015).

We subsequently calculated a total percentage score of confounding correction for each study and correlated the effect size representing differences in $DRD_{2/3}$ striatal binding between addicted individuals and matched controls with the total percentage score of confounding correction across all addictions and within addiction subtypes. This to enable us to assess how confounders interacted with both effect sizes generally and for between group differences in $DRD_{2/3}$ availability. We performed the analyses using SPSS statistical software (Version 29).

The percentage reporting of confounders as well as of correction for a specific confounder for each addiction subtype were pooled together. Additionally, we divided

all the studies across all addictions into chronological groups of year subgroups (5-9 studies in each) to explore possible developments over time in consideration/correction for confounders. Note: studies using high affinity and agonist radioligands which were excluded from the meta-analysis of the striatum are included in the overview of confounding effects.

Reversibility

Each study was inspected for evidence of signs of reversibility of addiction-induced alterations of the $DRD_{2/3}$. This was defined as the presence of signs of recovery of $DRD_{2/3}$ binding with prolonged abstinence and indicated by a significant inverse association between each study's average abstinence time prior to scanning and total $DRD_{2/3}$ binding availability in the individuals with addiction group.

Individual studies received a score of 0 if the issue of reversibility was not considered, a score of 1 if reversibility was assessed but no signs of reversibility were found and a score of 2 if signs of reversibility were found. Subsequently, a total percentage score of studies from each of these categories were computed for each addiction subtype. These percentages are illustrated in pie graphs below and pie sizes reflect the total number of studies in each addiction subtype.

Reversibility analysis was not included for gambling and smoking addictions since the time of abstinence was not clearly addressed. In addition, we also explored if there was evidence of between-study sign of reversibility. To do this, we correlated the effect size of differences in $DRD_{2/3}$ between addicted individuals and controls with the average number of days since last use of the substance (also tested as logarithm to the number of days or abstinence due to an abnormal distribution for some of the addictions) for each individual addiction.

Dose-Response

Studies were reviewed for evidence of dose-response. Each study received a score of 0 if dose-response was not investigated, of 1 if it was investigated but no significant signs of dose response were found and 2 if significant signs were found giving a total percentage score of studies for each addiction subtype. These percentages were then pooled together and illustrated in pie graphs. Pie sizes reflect the total number of studies for each addiction.

Results

Sixty-three peer-reviewed studies of PET or SPECT assessment of baseline $DRD_{2/3}$ availability in alcohol (n=13), cocaine (n=15), methamphetamine (n=9), cannabis (n=6), heroin (n=5), nicotine (n=11), gambling (n=4) addiction were identified, with a total of 1918 (1566 males and 352 females) cases and 1959 (1527 males and 432 females) controls (Tables 1-3).

Table 1: Striatum Meta-analysis

Publication	Addiction	Dep. status	Ligand	Mean abs. days	Cases (sex, age)	Controls (sex, age)	Striatal baseline %diff (user-ctrl), sign
Hietala, West 1994	Alc.	DSM-III-R	[11C]Raclopride-PET	140	n=9 (9m/0f, 40±6)	n=8 (8m/0f, 39±7)	-18.5% ⇓
Volkow 1996	Alc.	DSM-IV	[11C]Raclopride-PET	52	n=10 (9m/1f, 44±10)	n=17 (15m/2f, 47±16)	-22.2% ⇓
Guardia 2000 (relapsers)	Alc.	DSM-IV	[123I]IBZM-SPECT	8	n=12 (18m/3f, 43±10)	n=9 (4m/5f, 39±8)	+2.8% ⇔
Guardia 2000 (non relapsers)	Alc.	DSM-IV	[123I]IBZM-SPECT	8	n=9	/	-5.1% ⇔
Volkow 2002 (early detox)	Alc.	DSM-IV	[11C]Raclopride-PET	17	n=14 (13m/1f, 39 to 42±-)	n=11 (10m/1f, 40±10)	-14.9% ⇓
Volkow 2002 (late detox)	Alc.	DSM-IV	[11C]Raclopride-PET	68	n=14 (same rescanned)	/	-9.7% ⇓
Martinez 2005	Alc.	DSM-IV	[11C]Raclopride-PET	14	n=15 (13m/2f, 34±6)	n=15 (12m/3f, 35±6)	-18.4% ⇓
Volkow 2007	Alc.	DSM-IV	[11C]Raclopride-PET	79	n=20 (20m/0f, 41±6)	n=20 (20m/0f, 41±6)	-2.3% ⇔
Oberlin 2016	Alc.	DSM-IV	[11C]Raclopride-PET	/	n=10 (7m/3f, 33±7)	n=13 (7m/6f, 34±7)	-0.8% ⇔
Volkow 1990 (short abstinents)	Coc.	DSM-III-R (for abuse)	[[18F]N-Methylspiroperidol	2	n=7 (10m/0f, 27±4)	n=10 (10m/0f, 28±3)	-38%, ⇓
Volkow 1990 (long abstinents)	Coc.	DSM-III-R (for abuse)	[[18F]N-Methylspiroperidol	28	n=3	/	+17.9% ⇔

Volkow 1993 (short abstinents)	Coc.	DSM-III-R	[[18F]N-Methylspiroperidol	0	n=20 (20m/0f, 34±8)	n=20 (20m/0f, 34±10)	-15.6% ⇓
Volkow 1993 (long abstinents)	Coc.	DSM-III-R	[[18F]N-Methylspiroperidol	90	n=7	/	-14.0%. ⇓
Volkow 2005	Coc.	DSM-IV	[11C]Raclopride-PET	14	n=21 (21m/0f, 36±5)	n=15 (15m/0f, 35±7)	-10.7% ⇓
Martinez 2007	Coc.	DSM-IV	[11C]Raclopride-PET	14	n=24 (19m/5f, 39±3)	n=24 (19m/5f, 38±5)	-12.0%. ⇓
Martinez 2009	Coc.	DSM-IV	[11C]Raclopride-PET	14	n=15 (13m/2f, 39±6)	n=15 (13m/2f, 39±5)	-9.6% ⇓
Narendran 2011	Coc.	DSM-IV	[11C]Raclopride-PET	14	n=10 (7m/3f, 44±8)	n=10 (7m/3f, 42±8)	-7.4% ⇓
Martinez 2011	Coc.	DSM-IV	[11C]Raclopride-PET	14	n=25 (22m/3f, 37±7)	n=24 (21m/3f, 36±6)	-6.6% ⇓
Payer 2013	Coc.	DSM-IV	[11C]Raclopride-PET	50	n=15 (13m/2f, 42±9)	n=15 (13m/2f, 42±11)	-11.3% ⇓
Volkow 2014	Coc.	DSM-IV	[11C]Raclopride-PET	3	n=19 (19m/0f, 45±3)	n=19 (19m/0f, 42±4)	-5.8%, ⇓
Gene Wang 2019	Coc.	DSM-IV	[11C]Raclopride-PET	/	n=23 (19m/4f, 44±4)	n=23 (19m/4f, 42±4)	+2.4%. ⇔
Iyo 1993	Meth.	History of MA induced psychosis	11C-N-Methylspiperone-PET	45	n=6 (6m/0f, 28±3)	n=10 (10m/0f, 29±7)	-10.6% ⇔
Volkow 2001 (short abstinents)	Meth.	DSM-IV	[11C]Raclopride-PET	60	n=15 (6m/9f, 32±7)	n=10 (14m/6f, 31±7)	-12.2% ⇓
Volkow 2001 (long abstinents)	Meth.	DSM-IV	[11C]Raclopride-PET	671	n=3	n=10	-11.9% ⇔
Wang 2012	Meth.	DSM-IV	[11C]Raclopride-PET	14	n=16 (13m/3f, 39±5)	n=15 (13m/2f, 37±4)	-9.2%, ⇓
Sevy 2008	Cann.	DSM-IV	[11C]Raclopride-PET	105	n=6 (6m/0f, 20±1)	n=6 (6m/0f, 20±2)	-0.4% ⇔
Stokes 2012	Cann.	Recreational use. Dependence not mentioned	[11C]Raclopride-PET	543	n=10 (6m/4f, 33±8)	n=10 (9m/1f, 37±5)	-3.1% ⇔
Urban 2012	Cann.	DSM-IV	[11C]Raclopride-PET	28	n=16 (15m/1f, 27±6)	n=16 (14m/2f, 28±7)	+3.28% ⇔
Albrecht 2013	Cann.	Chronic use. Dependence not mentioned	[11C]Raclopride-PET	1	n=10 (10m/0f, 25±5)	n=8 (8m/0f, 26±6)	-2.74% ⇔

Volkow 2014	Cann.	DSM-IV	[11C]Raclopride-PET	4	n=24 (12m/12f, 27±7)	n=24 (12m/12f, 28±6)	-0.42% ⟺
Tomasi 2015	Cann.	DSM-IV	[11C]Raclopride-PET	/	n=18 (9m/9f, 27±8)	n=14(9m/5f, 26±5)	+1.31% ⟺
Wang 1997	Her.	DSM-IV	[11C]Raclopride-PET	/	n=11 (11m/0f, 40,7±4)	n=11 (11m/0f, 39,6±4)	-17.85% ↓
Zijlstra 2008	Her.	DSM-IV	[123]IBZM (SPECT)	57	n=12 (12m/0f, 60±10)	n=18 (18m/0f, 40±6)	-5.97% ⟺
Daglish 2008 (study1)	Her.	ICD-10	[11C]Raclopride-PET	1	n=6 (6m/0f, 33±7)	n=5 (5m/0f, 30±7)	+1.96% ⟺
Daglish 2008 (study2)	Her.	ICD-10	[11C]Raclopride-PET	1	n=8 (8m/0f, 35±3)	n=5 (5m/0f, 37±5)	+1.96% ⟺
Martinez 2012	Her.	DSM-IV	[11C]Raclopride-PET	14	n=16 (14m/2f, 39±5)	n=16 (14m/2f, 38±6)	-9.84% ↓
Yuang 2006	Nic.	"Smokers" Fagestrom 5.8±0.9	[123]IBZM (SPECT)	/	n=15 (15m/0f, 33±11)	n=15 (15m/0f, 33±12)	+3.33% ⟺
Scott 2007	Nic.	"Smokers". Fagerstrom 5.0±2.0	[11C]Raclopride-PET	/	n=6 (6m/0f, 25±5)	n=6 (6m/0f, 27±5)	Non diff. ⟺
Takahashi 2008	Nic.	"Smokers". Fagerstrom ?	[11C]Raclopride-PET	/	n=6 (6m/0f, 26±3)	n=6 (6m/0f, 24±3)	+6.49% ⟺
Yuang 2008	Nic.	"Smokers". Fagerstrom 5.6±1.0	[123]IBZM (SPECT)	/	n=11 (11m/0f, 29±9)	n=11 (11m/0f, 27±6)	+10.11% ⟺
Busto 2009	Nic.	"Smokers". Fagerstrom 4.9±1.2	[11C]Raclopride-PET	/	n=9 (8m/1f, 37±13)	n=11 (3m/8f, 28±4)	-4.78% ⟺
Linnet 2012	Gamb.	DSM-IV for PG	[11C]Raclopride-PET	/	n=18 (18m/0f, 34±9)	n=16 (16m/0f, 32±8)	-3.51% ⟺
Clark 2012	Gamb.	DSM-IV for PG	[11C]Raclopride-PET	/	n=9 (9m/0f, 35±9)	n=9 (9m/0f, 37±6)	+2.07% ⟺
Joutsa 2012	Gamb.	DSM-IV for PG	[11C]Raclopride-PET	/	n=12 (12m/0f, 27±2)	n=12 (12m/0f, 30±3)	-4.53% ⟺
Boileau 2013	Gamb.	DSM-IV for PG	[11C]Raclopride-PET	/	n=13 (13m/0f, 33±9)	n=12 (12m/0f, 34±11)	-5.12% ⟺

Table 1. Studies included in the meta-analysis of total Striatum. An empty horizontal arrow indicates the lack of significant differences between cases and controls, vertical arrows indicate the presence of significant differences, with the direction of those differences (increase/decrease) being indicated by the direction of the arrow. Percentage differences have either been retrieved from original papers or calculated from striatal subregions with the formula presented in the study methods.

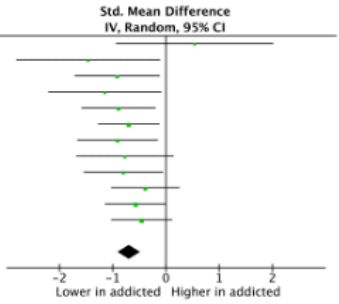
Meta-analysis of total striatal and SN DRD2/3 binding with antagonist and agonist radioligands

Mean striatal $DRD_{2/3}$ binding was significantly lower in cocaine ($p < 0.0001$, Hedges' $g = –0.69$ [–0.90, –0.47], number of studies (n) = 12, heterogeneity (I^2) = 0%), alcohol ($p = 0.0004$, $g = –0.78$ [–1.21, –0.34], n = 9, $I^2 = 57\%$) and methamphetamine ($p = 0.0001$, $g = –0.91$ [–1.38, –0.45], n = 4, $I^2 = 0\%$) addictions than in controls. There were no significant differences in striatal $DRD_{2/3}$ binding in opiate ($p = 0.09$, $g = –0.43$ [–0.93, 0.07], n = 5, $I^2 = 36\%$), smoking ($p = 0.84$, $g = –0.05$ [–0.52, 0.42], n = 6, $I^2 = 49\%$), gambling ($p = 0.29$, $g = –0.21$ [–0.61, 0.18], n = 4, $I^2 = 0\%$) or cannabis ($p = 0.93$, $g = 0.01$ [–0.29, 0.32], n = 6, $I^2 = 0\%$) addictions compared with controls. (Fig 4: $DRD_{2/3}$ antagonist radioligand Meta-analyses).

Cocaine

Study or Subgroup	Addicted Mean	SD	Total	Non-addicted Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Volkow 1990 (LTA)	4.93	1.25	3	4.18	1.19	5	2.1%	0.54 [-0.94, 2.02]	1990
Volkow 1990 (STA)	2.59	0.86	7	4.18	1.19	5	2.5%	-1.46 [-2.81, -0.11]	1990
Volkow 1993 (LTA)	2.65	0.46	20	3.08	0.45	10	7.2%	-0.92 [-1.71, -0.12]	1993
Volkow 1993 (STA)	2.6	0.3	7	3.08	0.45	10	4.1%	-1.15 [-2.21, -0.09]	1993
Volkow 2005	2.59	0.34	21	2.9	0.34	15	9.5%	-0.89 [-1.59, -0.19]	2005
Martinez 2007	0.88	0.11	24	1	0.21	25	13.8%	-0.70 [-1.28, -0.12]	2007
Martinez 2009	2.08	0.28	15	2.3	0.18	15	8.1%	-0.91 [-1.67, -0.15]	2009
Narendran 2011	2.39	0.26	10	2.58	0.21	10	5.5%	-0.77 [-1.69, 0.15]	2011
Martinez 2013	3.06	0.47	15	3.45	0.48	15	8.3%	-0.80 [-1.55, -0.05]	2013
Payer 2013	2.45	0.46	19	2.6	0.27	19	11.2%	-0.39 [-1.03, 0.25]	2013
Volkow 2014a	2.11	0.28	23	2.26	0.24	25	13.8%	-0.57 [-1.15, 0.01]	2014
Gene 2019	2.38	0.18	25	2.45	0.11	23	14.0%	-0.46 [-1.03, 0.12]	2019
Total (95% CI)			**189**			**177**	**100.0%**	**-0.69 [-0.90, -0.47]**	

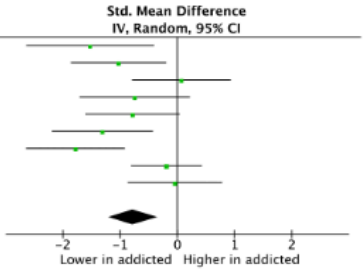
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 7.32$, df = 11 (P = 0.77); $I^2 = 0\%$
Test for overall effect: Z = 6.27 (P < 0.00001)



Alcohol

Study or Subgroup	Addicted Mean	SD	Total	Non-addicted Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Hietala 1994	2.73	0.32	9	3.35	0.45	8	8.5%	-1.52 [-2.64, -0.41]	1994
Volkow 1996	2.1	0.5	10	2.7	0.6	17	11.4%	-1.03 [-1.86, -0.19]	1996
Guardia 2000a	1.83	0.9	12	1.78	0.12	9	11.1%	0.07 [-0.79, 0.93]	2000
Guardia 2000b	1.69	0.11	9	1.78	0.12	9	10.0%	-0.74 [-1.71, 0.22]	2000
Volkow 2002 (LTA)	2.61	0.34	14	2.89	0.35	11	11.5%	-0.79 [-1.61, 0.04]	2002
Volkow 2002 (STA)	2.46	0.29	14	2.89	0.35	11	10.9%	-1.31 [-2.19, -0.43]	2002
Martinez 2005	3.38	0.42	15	4.14	0.41	15	11.1%	-1.78 [-2.65, -0.92]	2005
Volkow 2007	3.02	0.37	20	3.09	0.34	20	14.1%	-0.19 [-0.81, 0.43]	2007
Oberlin 2016	1.77	0.21	10	1.78	0.224	13	11.5%	-0.04 [-0.87, 0.78]	2016
Total (95% CI)			**113**			**113**	**100.0%**	**-0.78 [-1.21, -0.34]**	

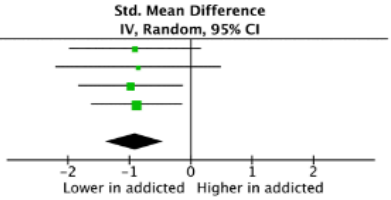
Heterogeneity: $Tau^2 = 0.25$; $Chi^2 = 18.60$, df = 8 (P = 0.02); $I^2 = 57\%$
Test for overall effect: Z = 3.52 (P = 0.0004)



Methamphetamine

Study or Subgroup	Addicted Mean	SD	Total	Non-addicted Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Ilo 1993	3.56	0.55	6	4.12	0.6	10	18.8%	-0.91 [-1.98, 0.17]
Volkow 2001 (LTA)	2.51	0.42	3	2.86	0.37	10	11.9%	-0.86 [-2.21, 0.49]
Volkow 2001 (STA)	2.52	0.31	15	2.86	0.37	10	29.8%	-0.98 [-1.84, -0.13]
Wang 2012	2.85	0.22	16	3.14	0.4	15	39.4%	-0.88 [-1.63, -0.14]
Total (95% CI)			**40**			**45**	**100.0%**	**-0.91 [-1.38, -0.45]**

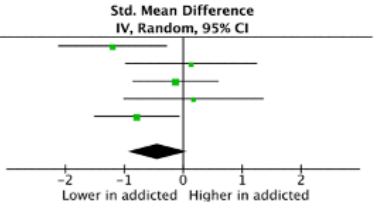
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.04$, df = 3 (P = 1.00); $I^2 = 0\%$
Test for overall effect: Z = 3.84 (P = 0.0001)



Opiate

Study or Subgroup	Addicted Mean	SD	Total	Non-addicted Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Wang 1997	2.44	0.4	11	2.97	0.45	11	19.5%	-1.20 [-2.12, -0.28]	1997
Daglish 2008b	2.345	0.32	8	2.305	0.18	5	14.9%	0.13 [-0.98, 1.25]	2008
Zijlstra 2008	0.63	0.3	12	0.67	0.3	18	25.8%	-0.13 [-0.86, 0.60]	2008
Daglish 2008a	2.35	0.27	6	2.305	0.18	5	13.6%	0.18 [-1.01, 1.37]	2008
Martinez 2012	1.74	0.18	16	1.93	0.28	16	26.2%	-0.79 [-1.51, -0.06]	2012
Total (95% CI)			**53**			**55**	**100.0%**	**-0.43 [-0.93, 0.07]**	

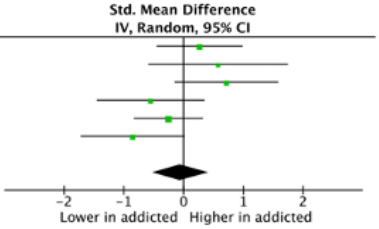
Heterogeneity: $Tau^2 = 0.12$; $Chi^2 = 6.20$, df = 4 (P = 0.18); $I^2 = 36\%$
Test for overall effect: Z = 1.67 (P = 0.09)



Tobacco

Study or Subgroup	Addicted Mean	SD	Total	Non-addicted Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Yuang 2006	1.86	0.15	15	1.8	0.27	15	19.2%	0.27 [-0.45, 0.99]	2006
Takahashi 2008	3.28	0.32	6	3.08	0.32	6	11.0%	0.58 [-0.59, 1.74]	2008
Yuang 2008	0.98	0.33	11	0.8	0.09	11	15.9%	0.72 [-0.15, 1.58]	2008
Busto 2009	2.99	0.2	9	3.14	0.3	11	15.2%	-0.55 [-1.45, 0.35]	2009
Albrecht 2013a	2.17	0.31	21	2.25	0.31	26	22.8%	-0.25 [-0.83, 0.32]	2013
Wiers 2017	2.77	0.33	8	3.03	0.28	18	15.8%	-0.85 [-1.72, 0.02]	2017
Total (95% CI)			**70**			**87**	**100.0%**	**-0.05 [-0.52, 0.42]**	

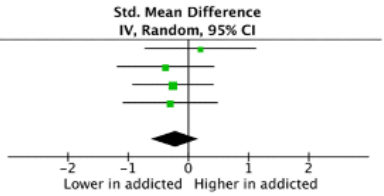
Heterogeneity: $Tau^2 = 0.16$; $Chi^2 = 9.77$, df = 5 (P = 0.08); $I^2 = 49\%$
Test for overall effect: Z = 0.20 (P = 0.84)



Gambling

Study or Subgroup	Addicted Mean	SD	Total	Non-addicted Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Clark 2012	2.46	0.26	9	2.41	0.21	9	18.0%	0.20 [-0.73, 1.13]	2012
Joutsa 2012	2.32	0.27	12	2.43	0.29	12	23.6%	-0.38 [-1.19, 0.43]	2012
Linnet 2012	2.2	0.28	18	2.28	0.33	16	33.7%	-0.26 [-0.93, 0.42]	2012
Boleau 2013	3.39	0.68	13	3.57	0.44	12	24.7%	-0.30 [-1.09, 0.49]	2013
Total (95% CI)			**52**			**49**	**100.0%**	**-0.21 [-0.61, 0.18]**	

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.99$, df = 3 (P = 0.80); $I^2 = 0\%$
Test for overall effect: Z = 1.07 (P = 0.29)



Cannabis

Study or Subgroup	Addicted Mean	SD	Total	Non-addicted Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Sevy 2008	3.41	0.26	6	3.42	0.22	6	7.2%	-0.04 [-1.17, 1.09]	2008
Stokes 2012	2.52	0.28	10	2.6	0.27	10	11.8%	-0.28 [-1.16, 0.60]	2012
Urban 2012	2.83	0.3	16	2.74	0.4	16	19.0%	0.25 [-0.45, 0.94]	2012
Albrecht 2013b	2.52	0.29	10	2.59	0.28	10	11.9%	-0.24 [-1.12, 0.65]	2013
Volkow 2014b	3.1	0.46	24	3.12	0.38	24	28.7%	-0.05 [-0.61, 0.52]	2014
Tomasi 2015	2.64	0.17	18	2.6	0.21	18	21.4%	0.20 [-0.45, 0.86]	2015
Total (95% CI)			**84**			**84**	**100.0%**	**0.01 [-0.29, 0.32]**	

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 1.54$, df = 5 (P = 0.91); $I^2 = 0\%$
Test for overall effect: Z = 0.09 (P = 0.93)

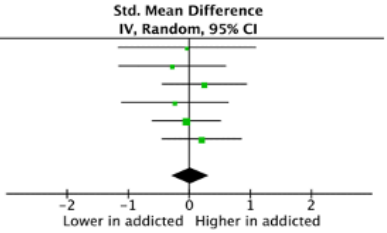


Figure 4: Meta-analysis striatum

Mean SN $DRD_{2/3}$ binding, as assessed with agonist radioligands, was significantly higher in cocaine addiction compared with controls ($p < 0.00001$, $g = 0.92$ [0.56, 1.27], n = 4, $I^2 = 0\%$). (Fig 5. Cocaine $DRD_{2/3}$ SN agonist radioligand meta-analysis)

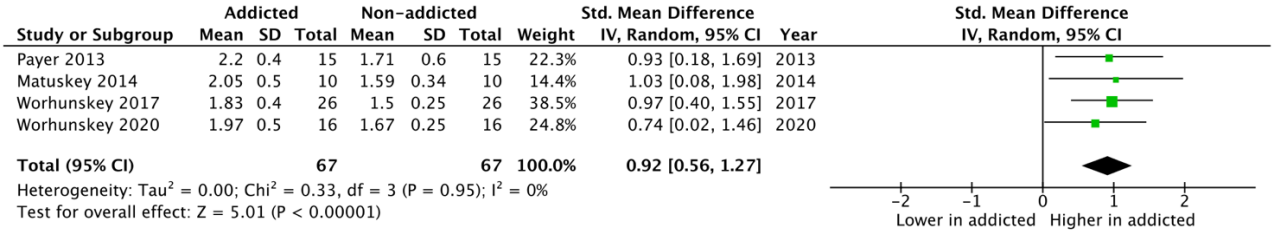


Figure 5: SN Meta-analysis

Meta-analyses of variance revealed no significant difference in the variability of $DRD_{2/3}$ BPND between addicted individuals and controls, for any of the 7 conditions studied.

Publication bias

Egger's tests indicated that there was no significant effect of publication bias in studies of cocaine ($z = 1.32$, $p = 0.19$ for striatum; $z = 0.033$, $p = 0.97$ for substantia nigra), alcohol ($z = –1.60$, $p = 0.11$), methamphetamine ($z = 0.039$, $p = 0.97$), opiate ($z = 0.86$, $p = 0.39$), smoking ($z = 0.50$, $p = 0.62$), cannabis ($z = –0.52$, $p = 0.61$) or gambling ($z = 0.63$, $p = 0.53$) addiction.

Review of striatal and extra-striatal PET imaging studies using agonist radioligands

Table 2 summarises the results of ten addiction studies conducted using agonist radioligands. Most of these studies did not detect statistically significant differences in striatal binding between groups, hereunder the single study using the agonist [^{11}C]NPA (Narendran, 2011). However, a single study of heavy methamphetamine users found an 11% lower [^{11}C]PHNO dorsal striatal binding, likely reflecting mostly DRD_2 binding (Boileau et al., 2012). Similarly, in gambling addiction, a downward trend was reported in the dorsal striatum, with a 10% lower [^{11}C]PHNO, although not in other regions of the striatum (Boileau et al., 2013). In a recent study of

tobacco smokers, [^{11}C]PHNO binding was found to have no significant differences in binding in observed in the ventral striatum (Calakos et al., 2021).

No significant [^{11}C]PHNO binding differences were detected in the thalamus or pallidum between either alcohol (Erritzoe et al., 2014b) or cocaine (Boileau et al., 2012, Matuskey et al., 2014) dependent individuals and controls, although trends were seen for higher binding in the ventral pallidum in methamphetamine users (Boileau et al., 2012), and in the internal pallidum among alcohol-dependent patients.

Interestingly, significantly higher [^{11}C]PHNO binding was reported in the midbrain (ventral tegmental area and/or SN) in five separate populations dependent on stimulants (Matuskey et al., 2014, Boileau et al., 2012, Payer et al., 2014a, Worhunsky et al., 2017, Worhunsky et al., 2021). Additionally, both cocaine- and alcohol-dependent patients (Erritzoe et al., 2014b, Matuskey et al., 2014, Worhunsky et al., 2021) showed significantly elevated [^{11}C]PHNO binding compared to matched controls in the hypothalamus. Importantly, the midbrain and hypothalamus are regions where binding of the DRD3-preferred ligand, [^{11}C]PHNO, reflects DRD_3 rather than DRD_2 binding.

In contrast, no significant [^{11}C]PHNO binding differences were observed in the striatum, pallidum, or midbrain of individuals with gambling disorder and controls (Boileau et al., 2013). Similarly, there were no significant differences in [^{11}C]PHNO binding in the globus pallidus, putamen or caudate of smoking smokers (Calakos et al., 2021).

Table 2: Agonist studies

Publication	Addiction	Depend. status	Ligand	Cases (sex, age)	Controls (sex, age)	ROI Identified	Sign diff in ROI (user-ctrl)
Erritzoe 2014	Alc.	DSM-IV	[11C]-(+)-PHNO	n=16 (16m/0f, 42±10)	n=13 (13m/0f, 42±9)	S. N.- VTA	⟺
						V. Pall.	⟺
						Hypothal.	⬆
						Str.	⟺
						Gl. Pal. internal	⟺
						Gl. Pal. external	⟺
						Thal.	⟺
Chukwueke 2020 (Conf. draft)	Alc.	DSM-V	[11C]-(+)-PHNO	n=10	n=18	Gl. Pal.	⟺
						D. Str.	⟺

						V. Str.	⟺
Narendran 2011	Coc.	DSM-IV	[11C]NPA	n=15 (13m/2f, 39±6)	n=15 (13m/2f, 39±5)	Str.	⟺
Payer 2013	Coc.	DSM-IV	[11C]-(+)-PHNO	n=15 (13m/2f, 42±9)	n=15 (13m/2f, 42±11)	S. N.	⬆
						V. Pall.	⟺
						Gl. Pal.	⟺
						Str.	⟺
Matuskey 2014	Coc.	DSM-IV	[11C]-(+)-PHNO	n=10 (8m/2f, 41±8)	n=10 (8m/2f, 41±6)	S. N.	⬆
						HypoThal.	⬆
						Amy.	⬆
						Str.	⟺
						Gl. Pall.	⟺
Worhunsky 2017	Coc.	DSM-IV	[11C]-(+)-PHNO	n=26(21m/5 f, 46+6)	n=26(21m/5 f, 41+7)	S. N.	⬆
						D. Put.	⬇
						D. Caud.	⟺
						Gl. Pal.	⟺
						HypoThal.	⟺
						V. Pall.	⟺
						V. Str.	⟺
Worhunsky 2020	Coc.	DSM-IV	[11C]-(+)-PHNO	n=16(14m/2 f, 43 +-5)	n=16(12m/4 f, 42 +-6)	D. Put.	⬇
						S. N.	⬆
						Gl. Pall.	⟺
						HypoThal.	⟺
						V. Str.	⟺
Boileau 2012	Meth	DSM-IV	[11C]-(+)-PHNO	n=16 (14m/2f, 28±6)	n=16 (12m/4f, 28±5)	D. Caud. S. N.	⟺ ⬆
						Gl. Pal.	⬆
						V. Pall.	⬆
						D. Str.	⬇
Calakos 2021	Nic.	DSM-V	[11C]-(+)-PHNO	n=22 (15m/7f)	n=20 (13m/7f)	V. Str.	⟺
						Put.	⟺
						Caud.	⟺
						Gl. Pal.	⟺
Boileau 2013	Gamb.	DSM-IV	[11C]-(+)-PHNO	n=13 (13m/0f, 33±9)	n=12 (12m/0f, 34±11)	S. N.	⬆
						D. Str.	⬇
						V. Str.	⟺

						Ant D. Caud.	⇓
						Post. D. Caud.	⟺
						Ant. D. Put.	⟺
						Post. D. Put.	⇑
						Gl. Pal.	⟺

Table 2. Agonist radiotracer studies included in the review grouped per addiction subtype. An empty horizontal arrow indicates the lack of significant differences between cases and controls, vertical arrows indicate the presence of significant differences, with the direction of those differences (increase/decrease) being indicated by the direction of the arrow.

Review of extra-striatal PET studies using high affinity radioligands

As illustrated in Table 3, in alcohol addiction one study using [^{18}F]fallypride found lower $DRD_{2/3}$ binding amongst cases in brain regions outside the striatum including the thalamus ($p = 0.04$), insular cortex ($p = 0.02$), hippocampus ($p = 0.02$) and temporal cortex ($p = 0.01$) (Rominger, Xiong et al., 2011). However, in a separate study, no significant differences in any of 8 examined cortical regions (Medial Temporal lobe, dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC), medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), temporal cortex (TEMP), parietal cortex(PAR) and occipital cortex (OCC)) were detected with another high affinity radioligand, [^{11}C]FLB-457 (Narendran et al., 2014).

In cocaine addiction, a recent [^{11}C]FLB-457 study by (Narendran et al., 2020) reported lower $DRD_{2/3}$ binding in the medial temporal lobe, DLPFC, OFC, mPFC, ACC, and the TEMP, suggesting an extra striatal down-regulation of $DRD_{2/3}$.

The existing studies using high affinity antagonists to investigate $DRD_{2/3}$ in methamphetamine addiction reported no significant between-group differences in binding in brain regions outside the striatum, including the thalamus, the hippocampus, the insular cortex, the amygdala, the ACC, and the OFC (Moeller et al., 2018, Okita et al., 2015). These non-significant differences were in the direction of lower binding among cases versus controls.

A recent study in nicotine addiction using the high affinity antagonist radioligand [11C]FLB-457 reported lower $DRD_{2/3}$ binding in the DLPFC ($p = 0.016$). Moreover, the reported effect was found to be sex-dependent with male smokers having a lower

DLPFC $DRD_{2/3}$ availability than gender matched controls (Zakiniaeiz et al., 2019). Another follow up study from the same cohort demonstrated that lower DLPFC $DRD_{2/3}$ availability in people who smoke is associated with disruptions in cognitive function that may underlie difficulty with resisting smoking (Zakiniaeiz et al., 2023). Another PET study with smokers identified lower striatal binding in the DLPFC in males but not females (Brown et al., 2012).

Publication	Addiction	Depend. status	Ligand	Cases (sex, age)	Controls (sex, age)	ROI Identified	Sign diff in ROI (user-ctrl)
Heinz 2004	Alc.	DSM-IV	[18F]Desmethoxyfallypride-PET	14	n=11 (11m/0f, 45±7)	n=13 (13m/0f, 43±10)	⇓
Rominger 2011(baseline)	Alc.	DSM IV	[18F]fallypride	n=17 (17m/0f,42±13)	n=14 (14m/0f, 44±12)	Str.	⇓
						Caud. nucelus	⇓
						Ant. Put.	⇔
						Post. Put.	⇔
						Thal.	⇓
						Insular C.	⇓
						Hipp.	⇓
						Amy.	⇔
						V. Str.	⇔
						S. N.	⇔
						Lat. Temp. C	⇓
Roeminger 2011 (first follow up Vs Scan1 7-14 days)	Alc.	DSM IV	[18F]fallypride	/	/	Str.	⇔
						Caud. N.	⇔
						Ant. Put.	⇔
						Post. Put.	⇔
						Thal.	⇔
						Insular Cortex	⇔
						Hipp.	⇔
						Amy.	⇔
						V. Str.	⇔
						S. N.	⇔
						Lat. Temp. C.	⇔
Roeminger 2011 (second follow up Vs Scan 1 - 1 year)	Alc.	DSM IV	[18F]fallypride	/	/	Str.	⇑
						Caud. N	⇑
						Ant. Put.	⇑
						Post. Put.	⇑
						Thal.	⇔
						Insular C.	⇔
						Hipp.	⇔
						Amy.	⇔

						V. Str.	⟺
						S. N.	⟺
						L. Temp. Cort.	⬆
Narendran 2014	Alc.	DSM IV	[11C]FLB-457	n=21 (16m/5f 28±5)	n=21 (16m/5f, 28±4)	MTL, dlPFC, OFC, MPFC, ACC, TEMP, PAR, OCC	⟺
Gleich 2020	Alc.	DSM IV	[18F]fallypride	n= 20 (10m/10f 45 ± 8)	n= 19 (10m/10f 45±8)	Caud.	⬇
						Put.	⬇
						Nacc	⟺
Narendran 2020	Coc.	DSM IV	[11C]FLB-457	n=24 (14m/10f, 28±6)	n=36 (22m/14f, 26±5)	MTL	⬇
						dlPFC	⬇
						OFC	⬇
						MTC	⬇
						ACC	⬇
						TC	⬇
						PC	⟺
						OC	⟺
Ballard 2015	Meth.	DSM IV	[18F]fallypride	n=27 (16m/11f, 33±9)	n=27 (12m/15f, 35±9)	Str.	⬇
Okita 2016 (1)	Meth.	DSM IV	[18F]fallypride	n=19 (10m/9f, 31±8)	n=26 (12m/14f, 29±8)	Amy.	⟺
						Str.	⬇
						Hipp.	⟺
						Gl. Pal.	⟺
						Thal.	⟺
						ACC	⟺
						Ins. C.	⟺
						OFC	⟺
Okita 2016 (2)	Meth.	DSM IV	[18F]fallypride	n=23 (18m/8f, 32±8)	n=17 (9m/8f, 32±8)	ACC	⟺
						Ant. Ins. C	⟺
						Amy.	⟺
						Hipp.	⟺
						Gl. Pal.	⟺
						Thal.	⟺
						Str.	⬇
Moeller 2018	Meth.	DSM IV	[18F]fallypride	n=15 (10m/5f, 37±10)	n=15 (7m/8f, 32±7)	Limbic Str.	⟺
						Ass. Str.	⟺
						Sensorim. Str.	⟺
						Amy.	⟺

						Hipp.	⟺
						Pall.	⟺
						Thal.	⟺
						L. OFC	⟺
						M. OFC	⟺
						ACC	⟺
						Insula	⟺
Fher 2008	Nic.	DSM IV	[18F]fallypride	n=17 (17m/0f, 31±8)	n=21 (21m/0f, 31±5)	Bil. Put.	⬇
						PFC	⟺
						OFC	⟺
						CC	⟺
						TC	⟺
Brown 2012 (men)	Nic.	DSM IV	[18F]fallypride	n=10 (37±13)	n=9 (35±15)	Caud.	⬇
						Put.	⬇
Brown 2012 (women)	Nic.	DSM IV	[18F]fallypride	n=9 (34±12)	n=9 (36±13)	Caud.	⟺
						Put.	⟺
Zakiniaeiz 2019	Nic.	DSM IV	[11C]FLB-457	n= 25 (13m/12f, 29±2)	n= 25 (13m/12f, 29±2)	dlPFC	⬇
Okita 2016 (women)	Nic.	DSM IV	[18F]fallypride	n=7 (37±5)	n=10 (36±5)	SN-VTA	⬆
Okita 2016 (men)	Nic.	DSM IV	[18F]fallypride	n=11 (37±5)	n=9 (36±5)	SN-VTA	⟺

Table 3: High Affinity studies

Table 3. High affinity radiotracer studies included in the review grouped per addiction subtype. An empty horizontal arrow indicates the lack of significant differences between cases and controls, vertical arrows indicate the presence of significant differences, with the direction of those differences (increase/decrease) being indicated by the direction of the arrow.

Reversibility of $DRD_{2/3}$ binding

Figure 6 shows the proportion of studies per addiction subgroup that report and detect on reversibility of the $DRD_{2/3}$ with abstinence (studies using antagonist, agonist and high affinity radioligands were included in this analysis).

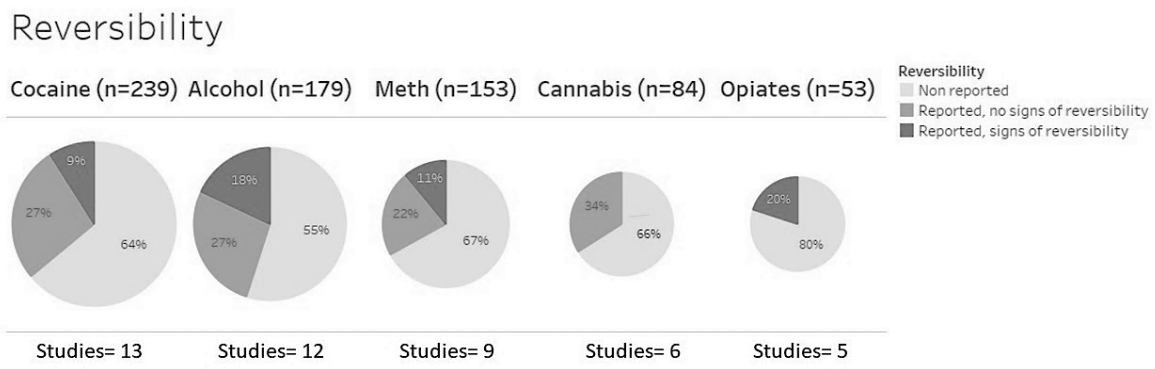


Figure 6: Reversibility analysis

Figure 6. Percentage of studies reporting signs of reversibility across addiction subtypes. Reversibility indicates the presence of signs of recovery of the DRD2/3 binding with prolonged abstinence and indicated by a significant correlation between abstinence time and DRD2/3 binding in the addict group. N indicates the number of total subjects included in the analyses while studies indicate the total number of studies (studies using antagonist radiotracers included in the meta-analysis and studies using high affinity and agonist radiotracers were included). Reversibility analysis was not included for gambling and smoking addictions since abstinence time was not clearly addressed in study reports.

It can be seen from **figure 6** that for all addictions only a minority of studies reported on reversibility. In sum, 36% of cocaine studies looked at reversibility with less than half of these detecting signs of reversibility. Similarly, only 45% of alcohol studies looked for signs of reversibility and of these only a few detected a relationship. In cannabis addiction, the two studies that did look for signs of reversibility reported no relation, whereas in heroin addiction the only study that looked for it found signs of reversibility (in the left and right caudate (Zijlstra, Booij et al. 2008)).

Our reversibility analysis across studies where each study represented one data point, was not suggestive of reversibility of the $DRD_{2/3}$ binding. Specifically, the average between group difference (in %) in striatal binding for each study did not significantly correlate with the study abstinence times (also tested as logarithm to abstinence time), neither for the individual addictions separately nor when all addictions were pooled.

Dose-response of the $DRD_{2/3}$ binding

Figure 7 shows the proportion of studies that reported on dose-response and relationships as well as the proportion of studies that detected such relationships (studies using antagonist, agonist and high affinity radioligands were included in this analysis). Approximately half of the alcohol and cocaine addiction studies examined dose-response relationships, and half of the studies which looked at dose-response found evidence for it. In nicotine addiction, 64% (7/11) of studies looked for a dose-response relationship but only one (Okita et al., 2015) found a significant association. Amongst the methamphetamine studies, approximately half of the studies looked at dose-response but only a quarter of these studies found a relationship – thus a majority (three quarters) of studies assessing the data for this did not detect a relation. In cannabis addiction most studies examined the dataset for a dose-response relation but only two of the studies detected it (Albrecht et al., 2013, Tomasi et al., 2015). In opiate addiction, only 40% (2/5) of the studies looked at the possible presence of dose-response relationships but only one of these (Zijlstra et al., 2008) reported significant results. In gambling addiction, 75% (3/4) of studies reported on dose-response relationships, and 50% (2/4) of them found significant signs of such a relationship (Boileau et al., 2013, Joutsa et al., 2012).

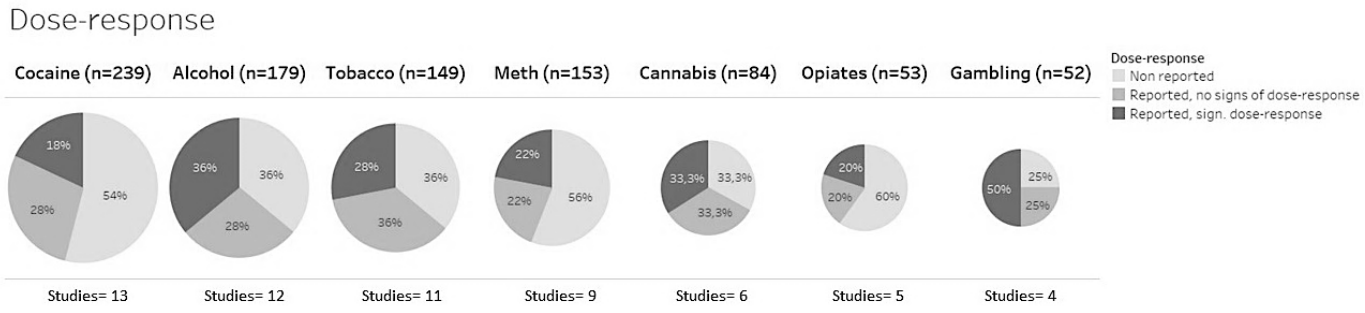


Figure 7: Dose response analysis

Figure 7. Percentage of studies reporting signs of dose-response across addiction subtypes. Dose-response is defined as a detection of significant correlations between DRD2/3 binding and addiction severity or amounts of accumulated substance use. N indicates the number of total subjects included in the analyses while studies indicate

the total number of studies (studies using antagonist radiotracers included in the meta-analysis and studies using high affinity and agonist radiotracers were included).

Confounding factors

Impulsivity

Across all addictions, 18 studies reported on impulsivity. Thirteen of these 18 studies statistically controlled for impulsivity whereas the remaining 5 made no attempt to control or statistically match the groups based on this trait **(Figure 8a/b).**

These studies indicated negative associations between different neuropsychological measures of impulsivity and striatal [^{11}C]fallypride binding in methamphetamine dependent patients (Lee et al., 2009, Ballard et al., 2015). In gambling disorder associations have been found between mood related impulsivity ('Urgency') and striatal [^{11}C]raclopride binding (Clark et al., 2012).

In contrast, positive associations between midbrain binding of the DRD3-preferring agonist ligand, [^{11}C]PHNO, and impulsivity measures were detected in studies of both gamblers (Boileau et al., 2013) and cocaine dependent patients (Payer et al., 2014b). Interestingly, greater impulsive choice was correlated with lower ventral striatum $DRD_{2/3}$ binding across both alcohol dependent subjects and social drinkers as assessed with [^{11}C]raclopride (Oberlin et al., 2015)

There were no associations found with [^{11}C]PHNO binding and impulsivity (Worhunsky et al, 2020) in alcohol dependent patients (Erritzoe et al., 2014b). Equally no such relationships were observed between tobacco smokers and BIS scores with baseline striatal and cortical $DRD_{2/3}$ values as assessed with [^{11}C]raclopride and [^{11}C]FLB-47 respectively (Wiers, 2020; Zakiniaeiz et al, 2020).

Socio-economic status (education/IQ)

Across all addictions, 36 studies reported on socioeconomic status (SES) or on some proxy measures related to SES like education and IQ levels. Twenty-four of these 36 studies statistically controlled or matched the groups for SES/education/IQ levels whereas the remaining 12 made some attempt to control or statistically match the groups. SES/education/IQ levels were predominantly assessed in cannabis, gambling and smoking addictions with fewer studies reporting on it in methamphetamine, cocaine, and alcohol addictions (figure 3b). Twelve out of 28

studies in alcohol and cocaine addiction mentioned SES/education/IQ, and in 5 of these, the individuals with addiction had lower IQ and/or less education than the controls. These group differences were not statistically analysed in the reports (Volkow, Wang et al. 2002, (Volkow et al., 2005, Volkow et al., 2007, Payer et al., 2014b, Volkow et al., 2014a).

In methamphetamine dependence, seven out of nine studies mentioned SES/education/IQ. In four studies the numbers of education years were lower in the patient groups than among healthy controls (Boileau et al., 2012, Wang et al., 2012, Ballard et al., 2015, Okita et al., 2015). Ballard and colleagues in their study concluded that when controlling for participant group, striatal binding was not independently predicted by years of education (Ballard et al., 2015). For the remaining three studies cases and controls were well matched for education (Okita et al., 2016, 2018, London et al., 2020, Moeller et al., 2018).

In opiate addiction, none of the included five studies reported on SES/education/IQ. Nevertheless, in the two groups of patients included in their studies, Daglish and colleagues (Daglish et al., 2008) reported non-significant differences in measures assessing social functioning, vitality, and criminality so these measures were considered as a proxy indicator of education in the present analysis.

In cannabis addiction, five of the six studies reported on SES/education/IQ. All five cannabis studies that reported data on these factors were well matched (although Sevy and colleagues' study only partially) for number of education years (Albrecht et al., 2013, Volkow et al., 2014b, Tomasi et al., 2015), socioeconomic status (Urban et al., 2012) and cognitive ability (but not education years; cannabis users < controls) (Sevy et al., 2008).

In smoking addiction, seven of the 11 studies reported on SES/education/IQ. Six studies that reported data on these variables (Yang et al., 2006, Yang et al., 2008, Brown et al., 2012, Albrecht et al., 2013, Wiers et al., 2017) reported no differences either in education time or IQ between cases and controls. Fehr and colleagues (Fehr et al., 2008), although not reporting significant differences in education, reported a higher verbal IQ in never-smokers compared to smokers so the two groups were not considered as adequetely matched in the current review.

In gambling addiction, three of the four studies reported on SES/education/IQ. All the three studies that reported data on these factors (Clark et al., 2012, Joutsa et

al., 2012, Boileau et al., 2013) found no differences neither in education time nor IQ between cases and controls.

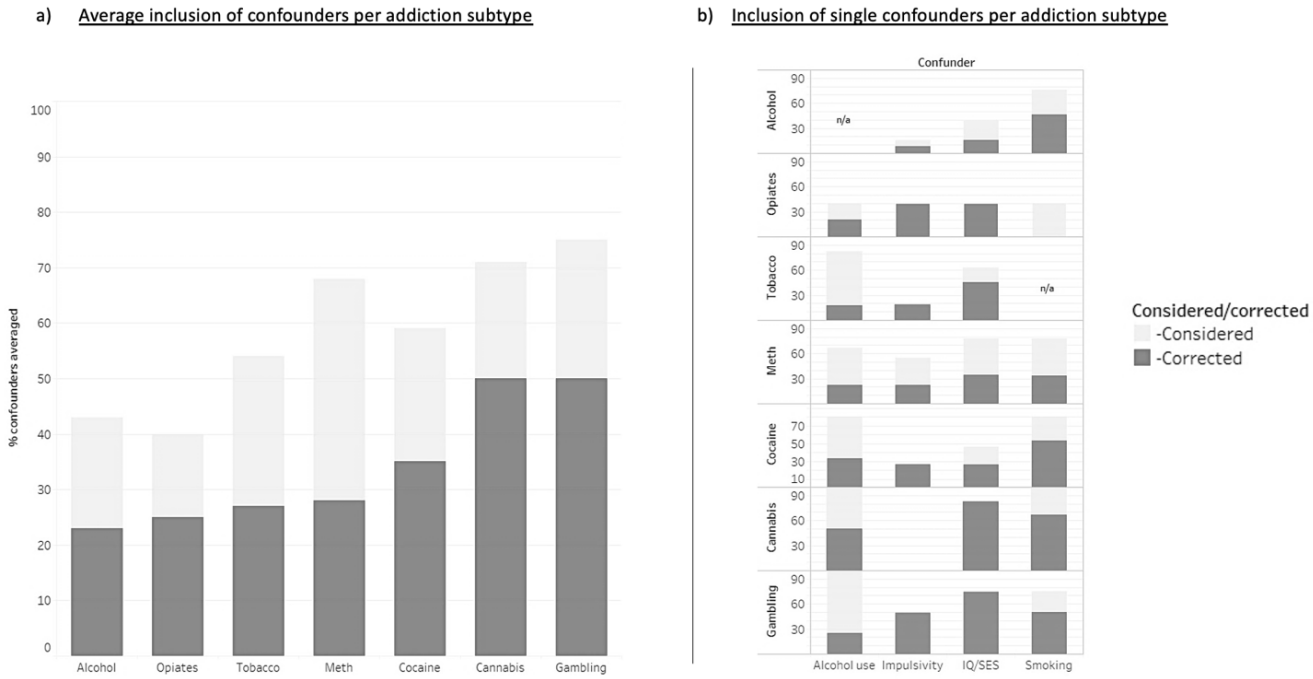
Matching of comorbid alcohol use

All the studies in cannabis and gambling addictions and around half of the studies of cocaine, methamphetamine and smoking addiction accounted for comorbid alcohol use. There was a limited mention of alcohol as a confounding variable for opiate addiction. As can be seen from figure 8b, there is inadequate group correction for alcohol use across cocaine, gambling, methamphetamine and smoking addictions however it is more frequently controlled for in cannabis addiction.

Matching of comorbid smoking use

Across all addictions, 40 studies reported on smoking use, making it the most reported confounder overall. All the studies in cannabis addiction and most studies in stimulant, alcohol, methamphetamine and gambling addictions accounted for comorbid smoking use (figure 8b). Only in opiate addiction this was reported less, with only two out of the five studies taking smoking into account. Most studies statistically

Figure 8a/b: Confounder analysis



controlled or matched the groups for smoking use whereas the remaining ones mentioned and/or partly addressed smoking use without statistically controlling for it.

Figure 8a/b. Figure 8a presents an overview of how the different studies in different addictions have attempted to consider/correct for confounding effects of factors known to be related to $DRD_{2/3}$ binding, Figure 8b zooms in in each addiction subtype, showing how different confounders have been considered/corrected across addictions. "Considered" indicates that a specific confounder was mentioned and/or partly addressed, "Corrected" indicates that case and control groups were matched or statistically corrected for a specific confounding effect, higher % indicates higher consideration/correction for confounders.

Correlational analysis of confounders and effect sizes

Each study included in our meta-analysis with antagonist radioligands was assigned a total score out of eight (/8) to assign the extent to which a study had controlled and statistically accounted for the confounding variables of co-morbid alcohol use, co-morbid smoking, impulsivity and IQ/SES. If studies statistically corrected for any one of these variables, they would get a score of 2, if they were considered but not corrected for a score of 1 was assigned and if there was no correction/consideration of these variables then 0 was assigned. The total sum from the 4 categories (with 8/8 being the highest level of correction indicating all 4 subcategories were sufficiently corrected) was calculated and this was subsequently converted to a percentage confounder correction score with 100% being perfectly controlled for (see methods). The between group difference in striatal $DRD_{2/3}$ binding as assessed through the effect sizes described in the striatal antagonist meta-analysis (figure 4) and percentage confounder correction score were correlated to assess for an association between the strength of confounder correction in studies and differences in striatal dopamine receptor binding. Our analysis indicated a significant moderate inverse correlation between differences in case vs control $DRD_{2/3}$ binding availability across all addiction subtypes and percentage confound score (Spearman's Rho 0.375, $p < 0.01$; figure 9) To extend this we split the group by addiction subtype. Our results similarly showed a significant positive correlation between confounder correction in cocaine addiction (Spearmans Rho 0.547, $p < 0.05$).

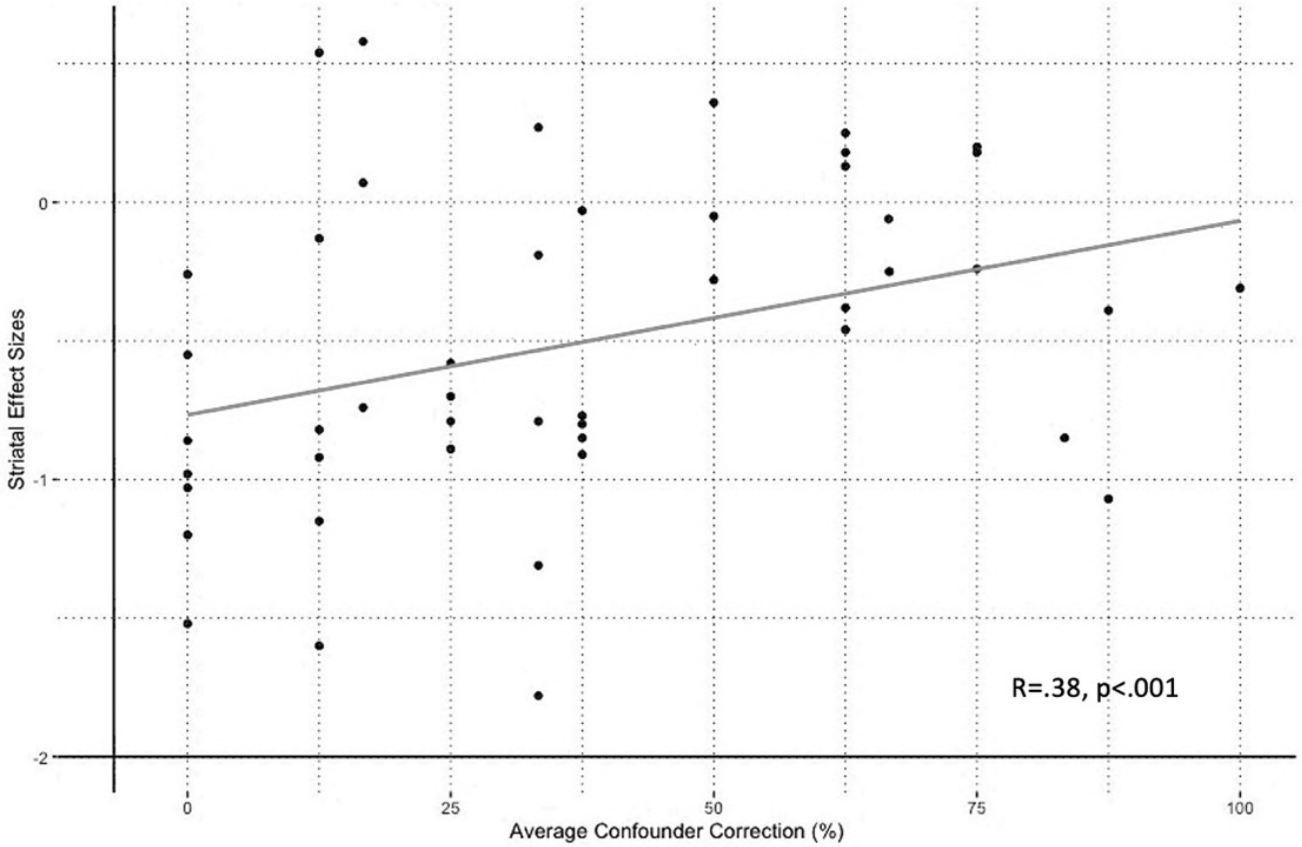


Figure 9: Striatal confounder correlation

Figure 9. Correlation between striatal effect sizes and average correction for confounders. The significant correlation indicates that greater confounder correction is associated with lower effect sizes across addictions, in other words the more you take confounders into account the lower the differences in DRD2/3 levels between cases and controls. Note that effect sizes higher than zero indicate no significant differences between cases and controls, while negative effect sizes indicate significant differences between groups.

Finally, we wanted to assess the extent to which studies controlled or considered aforementioned confounders over time. Overall, it appears that since the inception of PET neuroimaging of the $DRD_{2/3}$ to present day, studies have generally become better at considering and correcting for confounding variables – though there is some variability between addiction subcategories this trend appears throughout showing demonstrable improvements (Fig 10a/b).

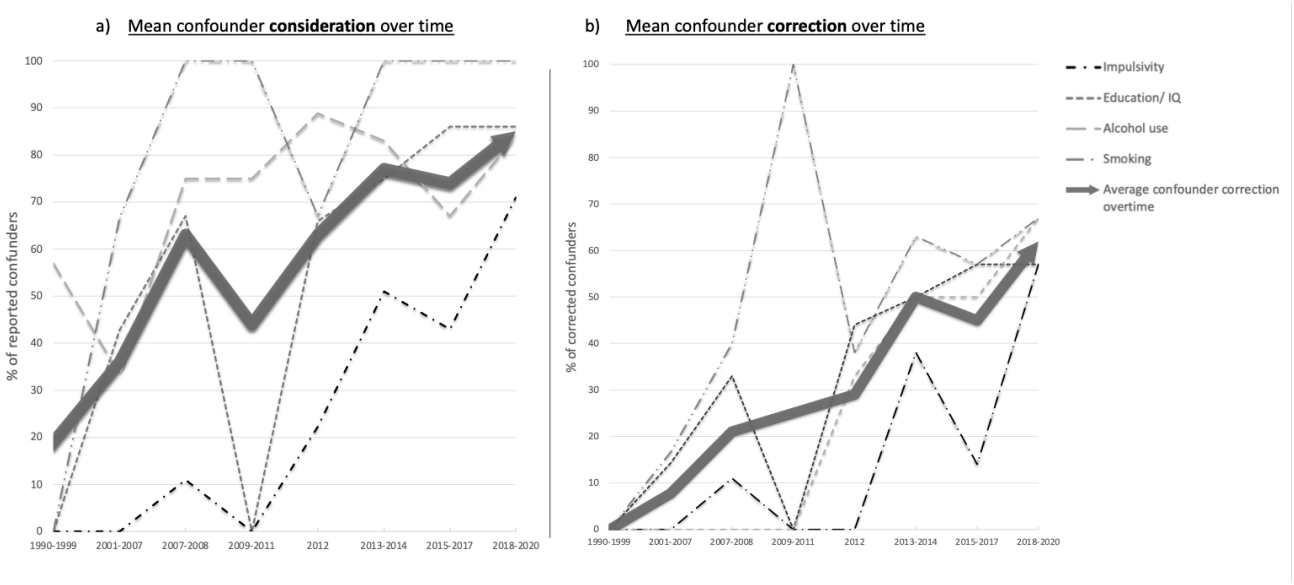


Figure 10a/b: Confounders over time

Figure 10a/b. Figure 10a presents an overview of how the different addiction studies *considered* different confounders over time. Figure 10b presents an overview of how the different studies *controlled* for different confounders over time. Studies across all addictions were divided into chronological groups of year subgroups (5-9 studies in each). “Considered” indicates that a specific confounder was mentioned and/or partly addressed, “Corrected” indicates that case and control groups were matched or statistically corrected for a specific confounding effect, higher % indicates higher consideration/correction for confounders.

Discussion

This review and meta-analysis provide a comprehensive overview and critique of the existing literature concerning the purported roles of $DRD_{2/3}$ receptors in addiction as well as the influence of external confounding variables on results from this receptor subtype that has been investigated for over 50 years for its role in reward, motivation and addiction.

Firstly, PET and SPECT baseline striatal $DRD_{2/3}$ binding availability were found to be significantly reduced in individuals with alcohol, cocaine, and methamphetamine addictions when compared to control groups. However, no significant differences were observed between control groups and those with gambling, smoking, opiate, or cannabis addictions.

Secondly, data obtained from recently developed high affinity antagonist and agonist radioligands suggest that extra-striatal $DRD_{2/3}$ downregulation occurs primarily in the prefrontal cortex, insula, and temporal cortex, specifically in those with stimulant, alcohol, and smoking dependence. The advent of agonist radioligands have been able to determine differences in the expression of DRD2 and DRD3 receptors in individuals with addiction. Meta-analysis of studies using such tools found significant increases in DRD3 receptor availability in the SN in individuals with cocaine addiction compared with healthy controls.

Thirdly, this review examined the potential dose-response relationship (between $DRD_{2/3}$ availability and substance use/addiction severity) and the possibility of recovery of $DRD_{2/3}$ binding with abstinence. Our findings suggest the evidence for signs of dose-response and reversibility in different subcategories of addiction remains inconclusive with varying degrees of reporting and heterogenous results.

Fourthly, early studies lacked sufficient statistical controlling for clinical confounders, such as socioeconomic status, impulsivity, and comorbid alcohol and smoking use. However, these issues have been increasingly addressed over time with greater matching between cases and controls. We found significant variability in the reporting of confounders per addiction subtype and an inadequate reporting is presented throughout the literature limiting any strong conclusions. However, we detected a significant inverse correlation in striatal between-group $DRD_{2/3}$ binding differences between individuals with addiction versus controls and average

confounder correction scores across all subcategories of addiction. The association was such that studies with higher confounding correction scores showed smaller differences in $DRD_{2/3}$ availability between cases and controls which calls into dispute the dopamine theory of addiction, or at least to what extent human $DRD_{2/3}$ PET/SPECT data support this hypothesis. Our findings have significant implications for our understanding of the role of dopamine in addiction. This review and meta-analysis set out to explore, critique and comprehensively assess the molecular evidence for the dopamine hypothesis of addiction. In the following sections we systematically lay out our evidence and pose the question; to what extent has the last half century of dopamine receptor research advanced our understanding of addiction and supported the development of novel and effective therapeutics?

Striatal $DRD_{2/3}$ availability in addiction: case vs control comparisons

To summarise, the present meta-analysis reveals that baseline striatal $DRD_{2/3}$ binding, as assessed using antagonist PET/SPECT radioligands, is consistently lower in individuals with alcohol, cocaine, and methamphetamine dependence when compared to matched non-addicted control subjects, whereas this is not the case among individuals with opiate, cannabis, smoking, and gambling addiction.

Although the meta-analysis did not show significant results across the latter addiction subtypes, it is noteworthy that in the four studies investigating baseline $DRD_{2/3}$ striatal binding in opiate addiction, the first (Wang et al., 1997) and the most recent (Martinez et al., 2012) both detected significant differences between cases and control. On the contrary, Zijlstra and colleagues (Zijlstra et al., 2008) and separate studies by Daglish and colleagues (Daglish et al., 2008) reported no differences in baseline $DRD_{2/3}$ total striatal binding between heroin users and matched database controls. None of the studies in cannabis, gambling and smoking addictions found significant differences in baseline striatal $DRD_{2/3}$ binding between cases and controls. Thus, from the antagonist radioligand studies, it cannot be concluded that $DRD_{2/3}$ striatal binding is lowered in opiate, smoking, cannabis and gambling dependence in the striatum.

For the sake of completeness, it must be noted that in the present meta-analysis striatal subregions from several studies were pooled together to create a total baseline $DRD_{2/3}$ striatal binding value. Some studies (e.g., (Zijlstra et al., 2008, Albrecht et al., 2013)) reported significant differences in $DRD_{2/3}$ binding in some striatal

subregions (e.g., putamen, caudate). These differences, after computing a total striatal value were non-significant, however, as there is evidence to suggest that addictive behaviours such as compulsion are driven by the dorsal striatum (Lipton et al., 2019) a more forensic assessment of striatal subregion-specific dopaminergic alterations is recommended for future studies.

In sum, it does not seem that all addictions are associated with lowered $DRD_{2/3}$ binding. In particular, the absence of low $DRD_{2/3}$ binding in tobacco smoking, opiate and cannabis plus gambling addiction puts the notion of low $DRD_{2/3}$ binding being a general vulnerability marker for addiction into question. There could be several reasons for this including; 1) the participants in these negative studies being only moderately addicted compared with individuals recruited to studies of alcohol and stimulant addiction, 2) having differences in impulsivity, 3) that other neurotransmitter systems other than dopamine are playing a more fundamental role in the maintenance and pathology of these sub-classes of addiction eg. endorphins and serotonin, 4) evidence for the alternative view of $DRD_{2/3}$ binding rather being a consequence of long-term substance use and neurotoxicity rather than pathological biomarkers which is discussed further in the sections below, 5) the addiction categories without detection of altered $DRD_{2/3}$ binding (i.e. cannabis, smoking, gambling) appearing to have been better matched for socio-economic factors. Thus, a poorer socio-economic matching in the “positive finding” addictions - with cases generally scoring lower on socio-economic measures compared to controls - could at least partially have driven the results of reduced $DRD_{2/3}$ binding among these addictions.

These suggestions are supported by the results of our meta-analyses of variance. In none of the seven addictions studied did interindividual variability in $DRD_{2/3}$ availability differ significantly between addicted and control subjects. If CVR (or VR) was significantly <1 for any of the addictions for which a significant case–control difference in $DRD_{2/3}$ binding availability was found, this would reflect lower variability in binding in the addicted group, suggesting that altered $DRD_{2/3}$ availability is a core feature of those addictions. Conversely, a CVR (or VR) significantly >1 would suggest increased biological heterogeneity of $DRD_{2/3}$ availability in addicted versus control subjects. Therefore, the observation that CVR (and VR) were not significantly <1 or >1 for any addiction studied suggests that altered $DRD_{2/3}$ availability is shared by many but not all addicted individuals, where it may be a person-specific signature – but not an integral mechanism – of addiction. However, we must be mindful of important

circumstances in which a true group difference in interindividual variability might go undetected by meta-analysis (Howes & Chapman, in review). First, a CVR (or VR) >1 might result from homogeneity in the control group, perhaps due to healthy user bias. Conversely, exclusion of certain patients – such as those currently using or those who are polydrug users– may erroneously reduce CVR (or VR). Most notably though, a non-significant reduction in CVR (or VR) might be driven by co-existent subgroups of patients: one subgroup may differ significantly from the control group in terms of the mean, with narrow outcome variability, whilst the other (perhaps larger) subgroup does not differ significantly from the control group mean and has much wider outcome variability. It is probable that this condition applies, to some extent, to the studies of methamphetamine addiction where, despite a convincing reduction in $DRD_{2/3}$ availability in addicted versus control subjects (p = 0.0001, g = –0.91), within-group variability in $DRD_{2/3}$ availability was only non-significantly lower in addicts (CVR = 0.85, p = 0.45).

The chicken or the egg: Does the degree of decreased $DRD_{2/3}$ binding detected in several addictions reflect a pre-existing vulnerability/risk or rather a consequence of the behaviour/substance use?

In order to properly address this question, ideally a large-scale longitudinal molecular imaging study that includes children at risk for addiction would be necessary to resolve this. Due to ethical concerns surrounding radiation exposure in healthy children and financial and practical limitations, such a study is not currently feasible. Instead, we rely on the study of associations between receptor binding and different sets of behavioural/symptom data, such as addiction severity or time of abstinence.

In the few cases where the relationship between $DRD_{2/3}$ availability and either severity of substance use/dependence or degree of craving has been investigated, studies have been inconclusive and have reported both significant and non-significant associations. Often cited evidence from human research in support of low $DRD_{2/3}$ availability as being a vulnerability factor for addiction comes from the observation that low $DRD_{2/3}$ levels are related to greater pleasurable feelings after the administration of intravenous methylphenidate in healthy controls (Volkow, Wang et al. 1999, Volkow, Wang et al. 2002). However, this effect was not replicated with intravenous amphetamine as challenge (Laruelle et al., 1995, Abi-Dargham et al., 2003, Martinez

et al., 2003). Discrepancies also exist among other lines of work looking at the role of $DRD_{2/3}$ availability in the risk of developing addiction (Volkow et al., 2006, Alvanzo et al., 2017)

In support of the notion that DRD_2 binding levels are related to the risk of addiction, genetic manipulations that increase the expression of DRD_2 levels have been shown to reduce alcohol and cocaine consumption in rats previously trained to self-administer these substances (Thanos et al., 2004, Thanos et al., 2008), further, low $DRD_{2/3}$ availability in the striatum of non-human primates predicts self-administration of cocaine (Morgan et al., 2002). The latter observation, however, was not confirmed when tested in cocaine dependent human subjects (Martinez et al., 2004). Nevertheless, further research showed that lower $DRD_{2/3}$ binding was associated with higher chances of relapse in cocaine and methamphetamine addiction (Martinez et al., 2011, Wang et al., 2012). The failure of dopamine-depleted stimulant users to stay sober could be a consequence of a deficit in their ability to shift their reward directed behaviour to natural reinforcers due to hypo-functional dopamine neurotransmission. Accordingly, flexible updating of behaviour in set-shifting tasks as well as task switching is associated with increased dopamine neurotransmission through $DRD_{2/3}$ (Klanker et al., 2013).

It should be noted that these results on $DRD_{2/3}$ availability and relapse vulnerability obtained in stimulant addiction might not apply to other sub-categories of addiction (opiate, gambling, cannabis, smoking) where there is no difference in $DRD_{2/3}$ availability between cases and controls. In gambling addiction, there has been fewer PET imaging studies of the dopamine receptor system compared with other addiction subtypes, however some dose response relationships have been observed with lower $DRD_{2/3}$ binding correlating with higher mood-related impulsivity (Clark et al., 2012) and higher SN DRD_3 correlating with increased gambling severity scores (Boileau et al., 2012). Concurrently, a hypo-dopaminergic state may only be predictive of risk of development or relapse in some addictions, with stimulant-use disorder providing the most evidence. This is pharmacologically plausible given that these drugs directly release dopamine. In sum, our analysis and review do not support the notion that a hypo-dopaminergic state is a hallmark state of opiate, smoking or cannabis addiction and other evidence suggests there is a nuanced role for dopamine dysfunction in gambling disorder.

Another important approach when considering the $DRD_{2/3}$ causality matter is to undertake follow-up re-scanning after sustained abstinence or investigate associations between $DRD_{2/3}$ binding and severity of substance use/dependence or time of abstinence. Our analysis of relevant imaging studies reveals that most studies have not reported such data, and when investigated, only a minority of studies have found significant associations. Additionally, a limiting factor for such investigations is a relatively narrow range of abstinence times in most studies.

Rather than relying on correlations with abstinence time between subjects, some studies in alcohol, cocaine and methamphetamine addiction looked at $DRD_{2/3}$ availability both during early and late detoxification. In alcohol addiction, an early study by Volkow, Wang and colleagues (Volkow et al., 2002d) found no signs of significant recovery of $DRD_{2/3}$ receptors during alcohol detoxification. Contrarily, a German study that re-scanned a small number of alcohol dependent individuals after a sustained period of abstinence (Rominger et al., 2012) found that the striatal $DRD_{2/3}$ binding in the 4 cases with sustained abstinence (n=2) or reduced alcohol consumption (n=2) increased by an average of 29% from the first day of abstinence to the re-scan after one year follow-up. This was most pronounced in the two individuals with complete abstinence. It could be speculated that this difference was influenced by the unusually short period of abstinence (max 24 hours) by the time of the initial scan. This explanation, however, seems unlikely since an additional re-scan in 14 out of the 17 included cases after 2 weeks revealed no difference in receptor binding from the initial scan.

In cocaine addiction, an early study using the radioligand [^{18}F]-N-methylpropanediol, tested some subjects less than 1 week after last cocaine use and some subjects more than 1 month after last use and found that, while short abstinent cocaine abusers showed lower striatal $DRD_{2/3}$ binding, individuals who have been detoxified for more than 1 month presented no significant differences compared to controls (Volkow et al., 1990). Nevertheless, a successive study (Volkow, Fowler et al., 1992) which retested 7 subjects after completing detoxification found no significant recovery of striatal $DRD_{2/3}$ receptors with prolonged abstinence. In methamphetamine addiction, a study by Volkow and colleagues (Volkow et al., 2001) compared subjects who had last used methamphetamine within 5 months to subjects who had not used methamphetamine for more than 11 months. No significant difference between the two groups was found, suggesting a lack of recovery after prolonged abstinence. Taken

together, attempts to address whether low $DRD_{2/3}$ receptor binding might be a biomarker for increased risk of becoming addicted to cocaine and alcohol are mixed.

In a more direct attempt to address the reversibility question, we employed an across study approach to explore signs of recovery (e.g. normalisation of the $DRD_{2/3}$ binding during the period of abstinence). However, the average between-group difference (in %) in striatal binding for each study did not significantly correlate with the study abstinence times, neither for the individual addictions separately nor when data from all addictions were pooled. As an example, there was no significant correlation between the average case-control $DRD_{2/3}$ binding difference and the range of abstinence times from cannabis (18 months (Stokes et al., 2012), 15 weeks (Sevy et al., 2008), 4 weeks (Urban et al., 2012), 1-7 days (Volkow et al., 2014b) or 20 hours (Albrecht et al., 2013)). In cocaine addiction, the correlation between group differences in striatal binding and study abstinence times was positively correlated although this did not reach significance.

If low $DRD_{2/3}$ binding predates the development of addiction and thereby constitutes a vulnerability marker, one would assume that this would be seen not only in dependence to substances but also to behavioural addictions such as gambling addiction. An advantage of studying the dopamine system in gamblers is the presumably negligible neurotoxicity due to the lack of drug-induced brain effects. This makes gambling addiction well suited as a 'vulnerability model' for addiction in humans presenting an alternative way of examining cause versus consequence. In contrast to stimulant and alcohol addiction, gambling addiction participants baseline $DRD_{2/3}$ binding levels did not differ from those of non-addicted controls. Thus, the gambling model of addiction does not point to $DRD_{2/3}$ binding being a general vulnerability factor for addiction, though there was some evidence of dysregulated $DRD_{2/3}$ and DRD3 relating to some aspects of the disorder which require further investigation. Based on this data it is therefore difficult to conclusively determine the nature vs nurture effects of drugs, behaviours on this neurobiological marker of interest in addiction.

Alternatively, low $DRD_{2/3}$ binding may reflect a consequence of long-term and/or ongoing substance use. Differences in $DRD_{2/3}$ binding status between addiction subtypes may be explained by the duration and magnitude of the impact on the dopamine system. Thus, it could be speculated that the more brief and transient impact on the dopamine system elicited by tobacco smoking (Morris et al., 2013) compared to stimulant use where long term down regulation is seen (Laruelle et al.,

1997), is a possible explanation of the much less consistent results seen in this addiction subtype. Stimulant–induced down-regulation of $DRD_{2/3}$ binding as assessed with PET imaging is sustained for much longer than the increase in synaptic dopamine levels (Laruelle, 2000) further supporting this as an explanation. Notably, stimulants and alcohol are the only drugs of abuse that reliably release dopamine in the human brain (Nutt et al., 2015).

Considering the two converging lines of evidence, it appears that individuals might be predisposed to addiction by both genetic and environmental factors and chronic substance use can further cause neurotoxic adaptations on neurotransmitter systems that already appear, at least in some cases, dysregulated. The results of the present meta-analysis show that only stimulant and alcohol addiction are associated with reliable differences in striatal $DRD_{2/3}$ binding, and that not all substances of abuse lead to adaptations in this biomarker as suggested by early theories of addiction. The lack of studies in non-addicted individuals with a family history of addiction in addiction subtypes other than cocaine and alcohol also precludes a definitive answer to the causality question in addiction as a whole.

Influence of selected confounders on striatal DRD2/3 availability in addiction

Correlational analysis of effect size with confounder scores

Correlational analysis was performed to assess the association between study confounder correction scores, displayed as percentage out of 100 (with 100% being perfectly corrected for) and the effect sizes of differences in $DRD_{2/3}$ striatal binding between cases and matched controls. Across studies there was a moderate and significant negative correlation (Spearman's Rho 0.375, $p < 0.01$) between higher confounder correction scores (studies better controlled for confounders) with lower differences in striatal $DRD_{2/3}$ binding availability between cases and controls as measured by effect size. These results indicate that studies designed to match for the confounding variables showed on average lower differences in $DRD_{2/3}$ levels when comparing addicted populations versus controls.

To our knowledge, this finding is the first systematic and objective assessment across $DRD_{2/3}$ PET imaging studies in addiction to demonstrate evidence that dopaminergic dysregulation in addiction is unlikely to be as a direct consequence of

'addiction pathobiology' but may represent a wider biopsychosocial marker relevant to addiction plus other factors including behavioural traits and socioeconomic status. Despite individuals with stimulant and alcohol addiction presenting a significant downregulation of $DRD_{2/3}$ binding versus controls, they also present an overall modest correction of confounders across studies. It is thus plausible that these significant results are, at least in part, carried by the effects of confounding factors not fully considered in the studies.

Impulsivity

Studies in addiction show that impulsivity is increased in addicted individuals (for review see (Trifilieff and Martinez, 2014)), and even serves as a predictor of future substance use (Quinn and Harden, 2013) and gambling addiction (Slutske et al., 2005). Among methamphetamine users, a negative relationship has been established between impulsiveness and striatal $DRD_{2/3}$ binding as measured with [^{11}C]fallypride (Lee et al., 2009) and mood related impulsivity ('Urgency') is negatively correlated with [^{11}C]raclopride binding in the striatum among gambling addiction (Clark et al., 2012). Interestingly, siblings of individuals with gambling addiction were found to have elevated impulsivity and an increase in risk taking in the Cambridge Gambling task (Clark et al, 2012). In sum, lower striatal $DRD_{2/3}$ levels have been found to be associated with higher impulsivity in methamphetamine and gambling addiction.

Cannabis users are reported to be less impulsive than people using a range of other substances including alcohol, opiates, cocaine, and ecstasy (Quednow et al., 2007, Bernstein et al., 2015). If impulsiveness not only serves as a vulnerability marker for developing substance dependence but also is related to low $DRD_{2/3}$ levels, then this could in theory explain why cannabis seems different from some other substances regarding the absence of dysregulation of these receptors in cannabis addiction.

To better understand this relationship, it would be beneficial for future studies to routinely incorporate quantitative assessments of impulsivity, in order to disentangle any potential confounding factors. By controlling for impulsivity, any observable between-group differences could potentially be eliminated. Additionally, research has shown that impulsivity is strongly influenced by genetic heritability, so further investigation into family members of individuals with addiction may also be a fruitful avenue to explore.

IQ/education/socioeconomic status

A landmark experiment in non-human primates by Morgan and colleagues (Morgan et al., 2002) showed how $DRD_{2/3}$ binding was not only predictive of self-administration of cocaine but also shown to be modulated by the environment. Once a social hierarchy had been established among the animals, the ones who had obtained the highest status had also developed higher $DRD_{2/3}$ availability and this was found to be protective against cocaine use. A similar relationship has been shown to exist in healthy non-addicted persons where the score of social status was found to be positively correlated to $DRD_{2/3}$ binding in the striatum in two separate studies (Martinez et al., 2010, Wiers et al., 2016b). Similar results have been seen for IQ (Guo et al., 2006) although not consistently (Volkow et al., 1998). However, when the hypothesis was tested among cocaine dependent patients, no significant relationship was found between socioeconomic status (SES) and [^{11}C]raclopride binding (Wiers et al., 2016b). In alcohol addiction, a *negative* association has been reported between years of education and striatal binding of [^{11}C]raclopride in a very small sample of 6 individuals with alcohol addiction (Volkow et al., 2002c). More recently, Matuskey and colleagues also detected a negative association between social status and $DRD_{2/3}$ availability measured with [^{11}C]PHNO in the ventral striatum and in the midbrain (reflecting DRD_3 binding), not only in healthy controls but also among both cocaine dependent patients (Matuskey et al., 2014). The reason for these discrepancies in results obtained from different populations (alcohol versus cocaine versus healthy) with different radioligands (antagonists vs agonists) is not entirely clear and could be linked to the different radioligands. Having these detectable relationships between SES/IQ/education and $DRD_{2/3}$ binding in mind, it should be considered whether the inconsistences in the $DRD_{2/3}$ findings detected between different addictions could reflect pre-existing differences in those parameters.

Studies on cannabis and gambling addiction have shown better reporting and group matching for SES, IQ, and education compared to studies on stimulant or alcohol addiction, where cases often have lower SES, IQ, and education than controls when reported. However, studies on smoking addiction also appear to be well-matched in studies where SES, IQ, and education were reported, though not as extensively as in cannabis and gambling addiction studies. Matching cases and

controls on these parameters have proven to be more challenging for some addiction populations than for others.

It is interesting to note that the addictions with the most successful matching happen to be the ones where there is no difference in $DRD_{2/3}$ binding between cases and controls, and vice versa. Most of the agonist studies in stimulant and alcohol addiction have not reported data on SES, IQ, or education. Moreover, studies that have managed to match cases and controls well on these parameters (e.g., (Wang et al., 2019)for cocaine) have presented no significant differences in baseline striatal $DRD_{2/3}$ binding. It is worth considering the important and overlooked role of environmental factors such as social deprivation on dopaminergic status, given the apparent relationship between SES, IQ, education and $DRD_{2/3}$ binding in well-matched studies of alcohol and stimulant disorders. Notably, in recent years, the National Institute for Drugs of Abuse (NIDA) has begun to reconsider addiction neuroscience through the lens of social deprivation with evidence now emerging that social factors such as academic attainment and living in populous areas being directly related to $DRD_{2/3}$ receptor availability (Calakos et al., 2022). Indeed, from these studies convergent lines of evidence suggest that there is a direct association between socioeconomic status and dopaminergic function in the brain which may moderate or mediate the proposed dysfunction observed in individuals with addiction. Therefore it could be an oversight to control for such variables and instead incorporating the relevance of these factors into the pathoetiology of addiction could provide a more precise and heuristic framework of mechanisms and risk factors underlying addiction vulnerability (Heilig et al., 2021). Future work should aim to assess these factors directly through imaging studies of individuals with varying socioeconomic status to understand its role on dopamine receptor availability and addiction status.

Comorbid smoking and alcohol use

Poly-substance use is common among people who consume illicit drugs and high levels of polydrug dependence have been reported among addicted individuals in diverse treatment and community settings across the globe (Crummy et al., 2020). If it is true that alcohol addiction, as also suggested from our meta-analysis, is associated with low $DRD_{2/3}$ binding levels, then a history of co-morbid alcohol addiction in a group of patients with cocaine or methamphetamine addiction could potentially drive the results. While subjects presenting comorbid alcohol addiction are

usually excluded from the studies, information about alcohol use (e.g., drinks per week) is usually not reported. One study conducted by (Gleich et al., 2021) found a linear decrease in striatal $DRD_{2/3}$ availability from healthy participants, to participants at high risk of developing addiction (not diagnosed with alcohol use disorder)), and to AUD patients although there is other evidence to suggest that binge-drinking is not associated with alterations in striatal dopamine receptor availability (Wai et al., 2019).

Despite the relatively weak evidence - as evidenced by the present meta-analysis - for smoking being related to differences in striatal $DRD_{2/3}$ radioligand binding, smoking status could potentially also be a confounder in these studies. In several studies in alcohol addiction, smoking history is either lacking (Hietala et al., 1994, Volkow et al., 1996, Repo et al., 1999) or not controlled for (Guardia et al., 2000, Heinz et al., 2004, Volkow et al., 2002c, Volkow et al., 2007). However, in one of the antagonist studies (Martinez et al., 2005) where groups were matched for smoking, the between group difference in $DRD_{2/3}$ binding was still robust and similar with what is seen in other studies in alcohol addiction. In contrast, after matching for smoking status, no between group difference was found in in baseline $DRD_{2/3}$ binding in cortical regions (striatum not reported) as assessed with the high affinity antagonist [^{18}F]fallypride (Narendran et al., 2014) or for striatal DRD3 as measured with the agonist [^{11}C]PHNO (Erritzoe et al., 2014b) and DRD3 blocker to quantify the relative contribution of DRD2 and DRD3 in individuals with alcohol addiction. While cocaine addiction studies usually control for smoking, it is not the case in methamphetamine addiction (Wang et al., 2012, Ballard et al., 2015). However, despite matching for smoking status, one study still showed a baseline 18.8% difference in striatal dopamine D2-type receptor availability as measured with [11C]DAA1106 between methamphetamine addicted individuals and controls (London et al., 2020). Thus, studies with matched smoking groups that show changes in $DRD_{2/3}$ binding can be considered to be more robust than those that do not.

As was the case for SES/IQ/education, it also appears to be easier to conduct well-matched studies with regard to co-morbid substance use for some addictions than for others. Compared to methamphetamine and opiate studies, gambling and cannabis addiction studies more efficiently controlled for tobacco smoking (and to a certain extent for alcohol use), thereby minimising their potential confounding effects. Time also seems to play a role; more recent studies have more carefully taken these potential confounders into account when compared to the earlier studies. Since

cannabis and gambling addiction studies have been conducted more recently than most alcohol and cocaine ones, the more careful correction of confounders for these addictions reflects the accumulated knowledge about receptor imaging in addiction and its potential confounders over time leading to better study designs that are well-matched.

Data on use of recreational drugs other than the ones included in the present review are not generally reported in detail but polydrug use is very common in clinical practice. Nevertheless, history of current dependence and/or abuse of substances other than caffeine and nicotine is mentioned as exclusion criteria in most of the reports, allowing the exclusion of extreme cases of poly-substance use. Moreover, most of the included studies also looked at recreational polydrug use through urine toxicology tests. However, this can only provide details for recent substance use (aside from THC) without providing relevant information for drug addiction. The use of clinical batteries for assessing the presence of polydrug use is thus encouraged in future studies. Further, and perhaps consequentially, studies with polydrug users should also be encouraged as to provide an ecologically valid sample of patients. The lack of ecological validity in such addiction biomarker research may be, in part, contributing to the lack of translation of addiction medicines (Nutt et al., 2015)

Agonist radioligands and the role of DRD_3

In our meta-analysis of [^{11}C]PHNO of the SN we found an increase in $DRD_{2/3}$ binding in this midbrain region in stimulant addiction participants when compared with healthy controls. Due to the DRD3 preferring properties of [^{11}C]PHNO and DRD2 vs DRD3 distribution, binding with [^{11}C]PHNO in this region reflects purely DRD3 receptor availability (Tziortzi et al., 2011).

However, in contrast to the diminished $DRD_{2/3}$ binding in the striatum in alcohol and stimulant dependence when measured with antagonist radioligands (predominantly [^{11}C]raclopride), recent studies using agonist radioligands like [^{11}C]PHNO have not confirmed $DRD_{2/3}$ binding differences in this region in cocaine/methamphetamine/alcohol addiction. This discrepancy raises technical questions; could a difference in the fraction of striatal DRD2 in high affinity state ($DRD_{2\text{-}HIGH}$) explain this? Although there is controversy whether $DRD_{2\text{-}HIGH}$ and $DRD_{2\text{-}LOW}$ are detectable in vivo (Seeman, 2012, Skinbjerg et al., 2012), a larger fraction of receptors in the high affinity state among addiction subjects compared with controls could in

theory explain the lack of difference in binding between these two groups when assessed with an agonist in contrast to the lower total $DRD_{2/3}$ binding in (alcohol and stimulant) addiction found with antagonists. However, a study using both the antagonist [11C]raclopride and the agonist [11C]NPA to measure $DRD_{2\text{-}HIGH}$ and $DRD_{2\text{-}LOW}$ reported no differences in $DRD_{2\text{-}HIGH}$ between cocaine addicts and controls (Narendran, 2011), and therefore does not provide support for this explanation.

Alternatively, decreased DRD_2 levels combined with increased DRD_3 levels among addicted individuals could also possibly explain why the group differences could be cancelled out when measuring striatal $DRD_{2/3}$ with a DRD_3 preferring radioligand like [^{11}C]PHNO (Narendran et al., 2011, Boileau et al., 2012). Animal and human studies support this possibility as alcohol and drug addiction may involve increased levels of DRD3 gene expression in the brain. Several studies have shown that blocking DRD3 can reduce relapse-like drinking and alcohol-seeking behaviours in rats and mice (Vengeliene, Leonardi-Essmann et al. 2006; Thanos, Katana et al. 2005). Furthermore, increased expression of DRD3 has been observed in the brains of human cocaine overdose fatalities (Staley and Mash 1996, Segal, Moraes et al. 1997) which further supports the idea of increased DRD3 in addiction and is in line with PET studies which have found increased binding of [^{11}C]PHNO in the SN, which as mentioned previously reflects DRD3 rather than DRD2, which has been observed in certain regions of the brains of individuals with stimulant, alcohol and gambling addiction (Table 2). In contrast, no significant differences were found in the midbrain of alcohol-dependent patients, although higher [^{11}C]PHNO binding was found in the hypothalamus, another region where predominantly DRD3 binding is represented (Erritzoe, 2014) and in cocaine addiction there was mixed findings of DRD3 binding in this region (Table 2).

In the striatum - the main target region for the older antagonist PET studies - where the [^{11}C]PHNO signal reflects both DRD_2 and DRD_3 receptors a single [^{11}C]PHNO PET scan does not allow for the separation of the DRD_2 and the DRD_3 part of the [^{11}C]PHNO signal. However, separating the receptor subtypes is possible using [^{11}C]PHNO before and after a selective DRD_3 blockade (Erritzoe et al., 2014b). This approach was used in patients with alcohol dependence and did not suggest a difference in striatal DRD_3 binding between patients and controls but there are no such studies in other addiction subtypes.

In summary, there is now evidence, as reflected in our meta-analysis for

increased DRD_3 binding in the SN midbrain in cocaine, methamphetamine, gambling but not in alcohol addiction or smoking use, as well as in the hypothalamus in alcohol dependence. It should be noted there is no data indicating that DRD_3 is increased in nicotine addiction in humans. Indeed, the one study that looked at this only observed a downward trend in [^{11}C]PHNO binding in the ventral striatum in smoking addiction (Calakos et al., 2021). Thus, neither among alcohol dependent individuals nor with matched controls was smoking status found to relate to DRD_3 levels (Erritzoe et al., 2014b).

In support of a role of DRD3 in addiction, DRD_3 antagonism in clinical populations has been shown to reduce attentional bias to food cues (Nathan et al., 2012a), reduce appetitive responsiveness to food cues in obesity (Mogg et al., 2012b), reduce craving in nicotine dependence (Mugnaini et al 2013), and acutely ameliorate blunted functional magnetic resonance imaging (fMRI) striatal response in the monetary incentive delay task (MIDT) (Murphy et al., 2017b) and modulate emotional responsivity in polydrug users (Vamvakopoulou et al., 2022b).

Based on these observations – hereunder the evidence from the described PET and fMRI studies - the DRD_3 has been proposed as one of the top 10 highest priority pharmacological targets for addiction drug development (Rasmussen et al., 2019).

High affinity radioligands

Investigations into cortical regions using high-affinity radioligands suggests to some degree there may be a role for dopaminergic receptors in cortical regions in addiction. Clinical studies have consistently found evidence of deficits in PFC functions such as executive function, inhibitory control, attention and goal-directed behaviours in addictions (Goldstein and Volkow, 2011b). Alterations in the fronto-cortical neurocircuitry have been proposed to modulate these clinical characteristics and impairment of executive function and inhibitory control through alterations in dopamine circuits may contribute to subcortically mediated responses (Everitt and Robbins, 2016). The development of high affinity dopamine receptor radioligands (Halldin et al., 1995) has allowed for such quantification of cortical alterations in addiction.

In cocaine addiction, Narendran and colleagues (2020) employed the high-affinity radioligand, [^{11}C]FLB 457, to detect lower baseline $DRD_{2/3}$ receptor availability

in several prefrontal regions, though the same group using the same radioligand had previously found no difference in alcohol addiction (Narendran et al., 2014). In methamphetamine addiction, none of the studies which looked at cortical regions found alterations in $DRD_{2/3}$ binding when compared with matched controls (Table 3). Nevertheless, the non-significant differences were in the expected direction of lower binding among individuals with addiction. Further, a recent study in nicotine addiction found a lower $DRD_{2/3}$ binding in the DLPFC (Zakiniaeiz et al., 2023)

Alterations in the DLPFC, ACC and near prefrontal regions have been demonstrated to be crucial for goal-directed behaviour (Everitt and Robbins, 2016). Accordingly, [^{18}F]fluorodeoxyglucose PET studies showed hypo-activations in the frontal cortex in cocaine addiction (Volkow et al., 1993, Volkow et al., 2002a) which was also confirmed by later fMRI studies (Kaufman et al., 2003, Terraneo et al., 2016). These hypo-activations could lead to limitations of willed control on actions that optimise benefits and reduce harm, together with a tendency to instant gratification and susceptibility to environmental drug cues and contingencies (Peoples, 2002). The generalisability of such findings is questioned due to the findings being reported in only cocaine addiction. Concomitantly, it is not clear whether these cortical changes are a) pathophysiological substrates of such impairments in frontal networks b) irreversible and liable to recovery with abstinence or c) related to a pre-existing vulnerability.

Despite these limitations, more PET research looking at possible dopaminergic alterations in extra-striatal regions is to be encouraged as it might provide a route for new more effective interventions that have cortical targets, including TMS (Ekhtiari et al., 2019) and psychedelic therapy (Zafar et al., 2023a). For example, enhancing dopamine $DRD_{2/3}$ transmission in the PFC might be a successful route for diminishing impulsive and habitual behaviour in addictions. Accordingly, medications increasing PFC dopamine such as methylphenidate, (Moeller et al., 2014) catechol-O-methyltransferase inhibitors, NMDA antagonists (Wedzony et al., 1993, Tunbridge et al., 2004), and DRD3 antagonists (Zafar et al, in prep) have been shown to modulate regions of the brain involved in reward, inhibitory control and relapse in human and animal models of cocaine and alcohol addiction. Further research is needed to ascertain the efficacy of these compounds, and novel ones such as psychedelic therapy in other subtypes of addiction and the neural targets necessitating the clinical effects.

Conclusions

This paper presents a meta-analysis and review of existing literature investigating the roles of $DRD_{2/3}$ receptors in addiction and the influence of confounding factors on receptor availability. The meta-analysis found that baseline striatal $DRD_{2/3}$ binding availability is significantly lower in individuals with alcohol, cocaine and methamphetamine addiction compared to control groups, whereas no significant differences were observed among those with gambling, smoking, opiate, or cannabis addictions. Meta-analysis was also conducted in the SN using agonist radiotracers which reflect purely DRD3 binding, this found significantly higher DRD3 in cocaine addiction. In addition, a review of the existing literature suggests that extra-striatal $DRD_{2/3}$ downregulation primarily occurs in the prefrontal cortex, insula, and temporal cortex, specifically in those with cocaine and smoking dependence though trends for other sub-addictions were observed.

Our results also suggest that certain circumstances, such as a lack of control for potential confounder variables such as comorbid substance use (alcohol and smoking), impulsivity and IQ or socioeconomic status, could have significantly impacted – and in part driven – positive results in studies designed to assess the role of $DRD_{2/3}$ as a causative addiction biomarker. There was significant heterogeneity in the ability of studies to control for such additional variables. Specifically, studies which better controlled for these variables showed smaller or null differences in $DRD_{2/3}$ availability between controls and individuals with addiction and many of these studies are more recent studies, potentially designed on the increase in knowledge of these factors, which call into question the methodology and conclusions of the earlier studies. Our review, meta-analysis and critique concludes that lowered $DRD_{2/3}$ binding may *not* be a universal marker for addiction as not all addiction subtypes are associated with these phenomena. Additionally, altered $DRD_{2/3}$ availability is shared by many but not all addicted individuals, and thus this alteration may be an individual-specific signature, rather than an integral mechanism of addiction. Future research should address the abovementioned pitfalls and produce more well-controlled studies including 1; correcting for confounding variables that have demonstrable independent effects on this receptor subclass and 2; studies that focus on quantifying extra-striatal

regions and those interrogating the relative contributions of DRD2 v DRD3 to foster a more comprehensive understanding of the neurobiology of addiction.

Overall, we believe these findings have substantial implications for our understanding the role of dopamine in addiction and after 50 years of research, it could be fair to assert that specific molecular-level neuroadaptations may not be the putative pathological biomarkers driving conditions such as addiction and the focus of such work has been too narrowly focussed. Back-translational research, that incorporate utilising compounds which have begun to show efficacy, above and beyond current pharmacological interventions, such as psychedelic therapy adjunctive to PET imaging of such receptor systems are encouraged to demonstrate the effects of such interventions on addiction relevant mechanisms. This translational neuropsychopharmacology approach, a step removed from much of the basic neuroscience research described herein, will provide clearer and more important insights into disease and therapeutically relevant biomarkers which will ultimately pave the way for both a better understanding of dopamine in addiction and more importantly the development of efficacious therapeutics.

In sum, despite an abundance of neurobiology research into the dopamine receptor system we have made limited progress in the development of effective treatments in addiction, we encourage future research to describe the mechanistic effects of novel and investigative treatments using PET imaging (alongside other modalities such as fMRI and EEG) to advance the field beyond its current groundhog five decades.

Chapter 3: Dopamine D3 receptor antagonism in alcohol dependence: a multimodal combined fMRI-PET case-control neuroimaging study

Introduction

The global prevalence of all substance use disorders (SUDs) has increased substantially between 1990-2016 with alcohol dependence being the most prevalent (Degenhardt et al., 2018b). The current clinical treatment paradigm for alcohol use disorder (AUD) within the UK is a psychosocial intervention with adjunctive pharmacotherapy but it has limited success with 20% of individuals relapsing within one month and a further 40% within 6 months (Hayes et al., 2020). The effectiveness of treatments for AUD is amongst the lowest of all mental health disorders (Kohn et al., 2004) with only three licensed pharmacotherapies available and only 9% of individuals with AUD receiving any of these treatments (Fairbanks et al., 2020).

The last 40 years have seen the development of a neurobiological understanding of addiction through advances in neuroimaging techniques, providing insight into some of the mechanisms underlying conditions such as AUD (Nutt et al., 2021). Neurochemical neuroimaging investigations using positron emission tomography (PET) have implicated dysregulation in the dopamine neurotransmitter system to be involved in the pathobiology of individuals with AUD with results showing changes in dopamine receptor availability and dopamine release capacity when compared with matched healthy controls. These findings, have also been supported by evidence of impairments in brain function in relation to reward processing (Nutt et al., 2021). These results indicate dopamine dysregulation to be a putative biomarker for AUD and a plausible target for the development of novel pharmacological interventions.

Dopamine is a neurotransmitter that plays a critical role in various aspects of neurobiology, psychopathology and behavior. It is involved in multiple brain functions, including reward processing, motivation, motor control, and cognitive processes (Kienast and Heinz, 2006). Dysregulation of dopamine signaling has been implicated in numerous neurological and psychiatric disorders, such as parkinson's disease,

addiction, and schizophrenia (Foley, 2019). In the brain, dopamine is synthesised in several regions, most notably the substantia nigra (SN) and ventral tegmental area (VTA). These regions project dopaminergic neurons to different target areas, forming distinct dopamine systems. Dopamine exerts its effects by binding to specific dopamine receptors, categorised into D1-like (D1 and D5) and D2-like (D2, D3, and D4) receptor families (Girault and Greengard, 2004). The activation of dopamine receptors initiates intracellular signaling cascades that modulate neuronal excitability, synaptic plasticity, and gene expression. Dopamine is released in response to rewarding stimuli, some drugs of abuse and reinforces behaviour. It is known as the 'motivation' neurotransmitter but is also essential for regulating motor functions, including voluntary movement, coordination, and fine motor control, through its nigrostriatal pathway (Bromberg-Martin et al., 2010).

Recent evidence has highlighted the dopamine D3 receptor (DRD3) as a novel therapeutic target for addiction (Rasmussen et al., 2019). The DRD3 is distributed across the brain, predominantly in reward specific midbrain and limbic regions such as the hypothalamus, SN and the VST; unlike the dopamine D2 receptors (DRD2) which are found primarily in the caudate (CA), putamen (PU) and dorsal striatum (DS) (Rasmussen et al., 2019, Le Foll et al., 2005). These findings support that the DRD3 could be involved in the motivational, cognitive and emotional aspects of reward processing (Le Foll et al., 2005).

In animal models, DRD3 antagonism has demonstrated potential to modify substance use behaviours. For example, DRD3 upregulation has been found following chronic exposure to alcohol (Vengeliene et al., 2006). When given a DRD3 antagonist, rodents show reduced motivation to self-administer drugs (including alcohol) and disrupted cue-induced craving (Vengeliene et al., 2006, Heidbreder and Newman, 2010).

DRD3 has also shown effectiveness in other clinical addicted populations. In obesity, DRD3 antagonism reduced attentional bias to food cues (Nathan et al., 2012b), reduced appetitive responsiveness to food cues (Mogg et al., 2012a) and in nicotine dependent individuals DRD3 antagonsim was seen to reduce cravings (Mugnaini et al., 2013). In another study DRD3 antagonism acutely ameliorated blunted ventral striatal response to the monetary incentive delay task (MIDT) in polydrug users,

although this effect was not seen in users who were dependent on just alcohol (Murphy et al., 2017a). Despite some promising early clinical results regarding the utility of DRD3 antagonism as a pharmacotherapy in addictions, the neurobiological mechanisms supporting these findings have not been conclusively demonstrated in alcohol dependence in humans. A recent human imaging study found increased DRD3 in the hypothalamus in alcohol dependence when assessed using the selective DRD3 radioligand [^{11}C] PHNO-PET (Erritzoe et al., 2014a). Conversely, another study found reduced $DRD_{2/3}$ and no differences in DRD3 availability in early abstinence in individuals with AUD in the SN and sensorimotor striatum (SMST) when compared with matched healthy controls. Though the authors did identify a relationship suggesting that individuals with lower DRD3 levels in the SN exhibited increased demand for alcohol (Chukwueke et al., 2021). This finding highlights the involvement of the DRD3 receptor in the neurobiological mechanisms underlying alcohol-seeking behavior. Further, my meta-analysis findings (chapter 2) found an upregulation of the DRD3 in the SN in cocaine addiction when compared with healthy controls (Zafar et al, in prep, Ch 2). These data suggest the DRD3 is a plausible pharmacological target for individuals with AUD.

PET imaging allows for the quantification of dopamine receptors to understand the molecular basis on which functional brain changes occur. Previous research has demonstrated links between dopamine receptor availability and brain function (Sander et al., 2020) although to date there has been no study to investigate the molecular effects of DRD3 antagonism and its relationship to reward processing in AUD patients.

Individuals with addictive disorders, including AUD, have also shown alterations in brain function as demonstrated by disruptions in striatal and frontal activation as assessed with fMRI during reward processing tasks such as the monetary incentive delay task (MIDT) (Balodis and Potenza, 2015). Findings across studies comparing AUD to HCs have demonstrated functional and structural abnormalities in specific brain regions involved in assigning excessive salience to drug over non-drug-related processes, with concomitant lapses in inhibitory-control, deficits in reward-related decision-making, and a lack of insight into illness (Nutt et al., 2021). fMRI paradigms such as the MIDT (Knutson et al., 2000) can be leveraged to assess the acute effects of pharmacological interventions (Knutson et al., 2004) such as DRD3 antagonism on

reward processing to determine if the intervention can modulate AUD specific dysregulation in reward-processing. This has been evidenced in previous studies which have found that the reward anticipation phase of the MIDT is dysregulated in alcohol dependence (Hägele et al., 2015, Romanczuk-Seiferth et al., 2015, Wrase et al., 2007b, Bjork et al., 2011)

Using multimodal imaging techniques, such as PET and fMRI, offers valuable advantages when investigating the impact of the D3 receptor on addiction behaviors in human subjects. Through the integration of PET and fMRI, complementary sources of information pertaining to the molecular and functional aspects of the role of DRD3 in alcohol addiction can be assessed. PET imaging facilitates the precise assessment of receptor distribution and localisation, providing insights into specific brain regions wherein the DRD3 is most abundant. This information can be further supplemented by fMRI, which enables the identification of brain regions activating to tasks and its corresponding association with D3 receptor-rich areas. Furthermore, the use of task-based fMRI, such as the MIDT, can tap into neuronal processes such as anticipatory reward processing which have been consistently been demonstrated to play a role in addiction neuropathophysiology (Luijten et al., 2017). By combining these imaging modalities, interactions between addiction relevant functional and molecular neural circuits can be comprehensively examined, unraveling the intricate relationships between DRD3 activity and associated brain regions. In addition, the effect of a novel intervention on molecular-functional brain activity can also be assessed to better understand its respective mechanism(s) of action and to examine its usefulness as a therapeutic agent.

Herein, we sought to investigate the effect of DRD3 antagonism on Blood-Oxygen Level Dependent (BOLD) activation to anticipation of monetary rewards using fMRI in the MIDT (pre- vs. post- antagonism of the DRD3) among patients with AUD vs age- and gender-matched HCs and the association with DRD3 availability, as assessed with [11C]PHNO PET. We hypothesised that AUD patients would demonstrate dysregulated frontal and striatal BOLD responsivity to reward anticipation in the MIDT at baseline compared with matched HC subjects. Following DRD3 antagonist administration we hypothesised that AUD patients would show an amelioration in the dysregulated BOLD response to anticipation in the MIDT. Further, we predict that there

will be significant differences in basline DRD3 availability in D3 rich areas of the the reward system with individuals with AUD having higher receptor availability compared to HC. We also hypothesised there will be a correlation between DRD3 availability and BOLD activation to the fMRI MIDT in both the HC and AUD group. Additionally, we investigated the association of individual differences in fMRI BOLD activation to reward processing with several addiction, psychiatric, cognitive and personality questionaires.

Methods

Participants

Participants were recruited from outpatient programmes in Central Northwest London (CNWL) NHS foundation trust and associated addiction services. Patients met DSM-IV criteria for alcohol dependence and had been abstinent from alcohol after undergoing benzodiazepine detoxification for a minimum of four weeks before scanning. For the purpose of clarity, we refer to addiction as 'AUD' across the manuscript to reflect the current DSM 5 diagnosis category, but we state that patients in this study had a DSM-IV diagnosis of which alcohol dependence is the diagnostic label. HCs were recruited from newspaper advertisements and had no history of alcohol abuse or dependence and drank a maximum of 25 units of alcohol per week.

Additional inclusion criteria for all participants were: (1) male; (2) age 24–60 years; (3) no previous or current Axis I disorders, although a history of major depression was allowed for AUD patients; (4) no current or past history of abuse or dependence on drugs other than nicotine, with a negative urine toxicology (a history of use but not dependence on cannabis was allowed but not within the four weeks before scanning); and (5) no significant medical condition or current use of medications. Participants were instructed to refrain from consuming caffeine- or xanthine-containing products on the day of the scans and underwent urine and breathalyser tests to rule out recent drug and alcohol use. Participants were not allowed to smoke for one hour before the MRI scan.

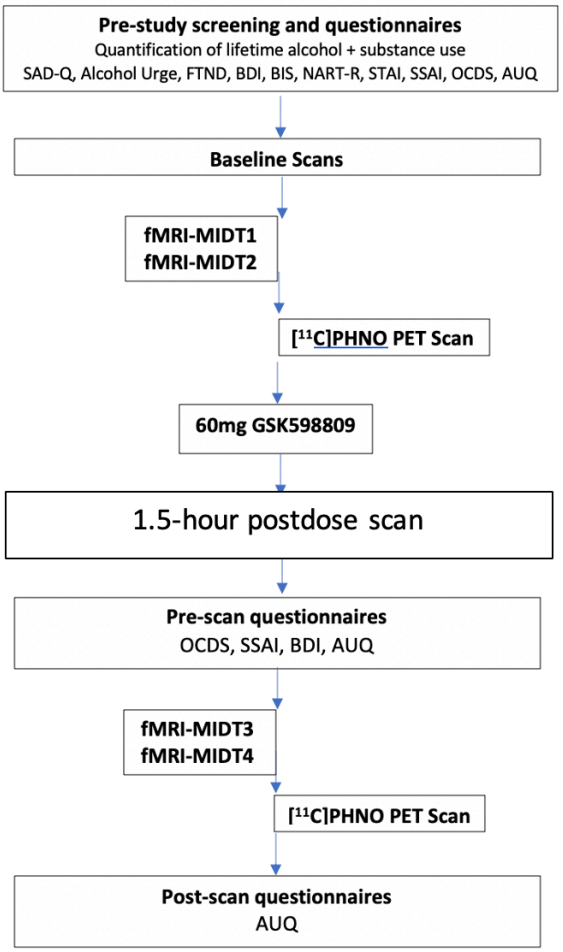
The study was sponsored by Imperial College London, UK, approved by local ethics committees, and all participants provided informed and written consent. Brain scans

were carried out at the GSK Clinical Imaging Centre (now Invicro), Hammersmith Hospital, London, UK.

Sixteen male AUD patients and 13 (age-gender-BMI matched) HC's were included in the study which represents the full sample of participants recruited to the study. The demographic details of participants in the study are described in detail in Table 4 in the results.

Procedures and tasks

This case-control study consisted of two parallel imaging sessions in which all participants received 60mg of DRD3 antagonist GSK598809, produced and manufactured by GlasxoSmithKline. Participants underwent two separate MRI scans: before and 1.5 hours after administration of the study drug. Within each MRI scan, participants undertook two scans of a modified MIDT (described below). Participants also undertook a series of psychiatric, addiction, and personality questionnaires prior to the scan. A schematic of the study timeline can be viewed below.



PET

All participants underwent two [11C]PHNO PET scans before and 3 h after administration of 60 mg of GSK598809. For details regarding preparation of [11C]PHNO, see (32) and for acquisition of PET images, collection of arterial blood samples, derivation of input function, and motion correction, see (33). Full details regarding delineation of ROIs and derivation of PET outcome measures and statistical analysis methods were conducted in previous research (Erritzoe et al., 2014b). Administration of GSK598809 resulted in a significant decrease in cerebellar volume of distribution (VT) (8.3±7.4 and 11.4±9.7%, $p=0.002$ and $p<0.001$ for HC and AUD, respectively). The presence of a specific binding component in the [11C]PHNO signal in the cerebellum led us to use the VT as the primary outcome measure. Regional VT pre- and post-blockade [11C]PHNO were used for analysis and therefore analysed for this for this cohort.

fMRI

Monetary incentive delay task

A modified version of the widely used MIDT was used to probe reward anticipation related brain function. At the start of each trial, a visual cue indicated whether the trial was a potential win ("Win£5"), neutral ("Win£0" or "Lose£0"), or a potential loss ("Lose£5") trial. A variable delay (between 2.0 – 2.5s) followed the cue and then a target stimulus (a blue star) was briefly presented. Participants were instructed to respond by button press prior to the target offset to win, or avoid losing the cued amount. The outcome was presented 0.5s after target offset to inform participants of monetary gains or losses. For example, on "Win£5" trials the outcome was either gain £5 ("Hit! +£5") or gain £0 ("Miss! +£0"), depending on whether the participant successfully responded prior to target offset.

There were 30 trials of each type (i.e., Win£5, Win£0, Lose£0, or Lose£5) presented in two consecutive, fully counterbalanced sessions of 60 trials each. On Win£5 trials, a successful response increased a subject's winnings by £5 and there was no penalty for slower responses. On Lose£5 trials, responding after target offset reduced a subject's winnings by £5. There was no gain or loss in any circumstance for Win£0 and Lose£0 trials. Target durations were adapted on a trial-by-trial basis so that participants successfully "Hit" the target on approximately two-thirds of trials to allow

subjects to win a total of approximately £50 at each of their two fMRI scans. Subjects were not informed of their total winnings until after the second MIDT session. The behavioural endpoints were the average response time for "Hit" trials and accuracy rate over trials for each subject and visit. Trials were marked as "Hit" if the subject responded prior to the target offset, or "Miss" for not responding or responding after target offset. The fMRI endpoint modelled was the BOLD response during the delay between cue and target (referred to as 'Anticipation') due to its relevance as a biomarker for reward processing.

fMRI Acquisition

T2*-weighted, echo-planar images sensitive to BOLD contrast were acquired continuously on a 3T Siemens Trio scanner with a 32-channel head coil. Images were acquired in two fully counterbalanced runs of 274 volumes each, with 46-slices in each volume (TR = 2100 ms, TE = 31 ms, flip angle = 78º, 3.5 x 3.5 x 3.0 mm voxels). To minimize signal dropout in medial temporal, orbitofrontal and ventral striatal regions we used a slice acquisition angled ~30º coronally to the anterior-posterior commissural plane and an in-plane voxel resolution minimised to 3.5 mm. High-resolution T1-weighted anatomical scans (MPRAGE) were acquired with whole-brain coverage for each subject to aid fMRI and PET image co-registration and PET ROI definition (TR = 3000 ms, TE = 3.66 ms, flip angle = 9º, voxel size = 1 mm^3, 208 slices).

fMRI analysis

fMRI data were processed with FSL software (www.fmrib.ox.ac.uk/fsl/). Image analyses were performed using FSL 5.0.4. The functional data were pre-processed using spatial smoothing with a 6mm FWHM (full-width, half-maximum) Gaussian kernel, high-pass temporal filtering (100 s), head-motion correction using MCFLIRT, and non-linear registration to a standard template (MNI152). The anatomical data were skull-stripped using FSL's brain extraction tool (BET) and segmented using FMRIB's automated segmentation tool (FAST), into grey/white matter and cerebro-spinal fluid (CSF).

Severe head motion induces artefacts, and even modest head movement can induce artefacts if the pattern of motion is correlated with the stimulus paradigm. Hence,

individual runs with mean absolute head motion exceeding the in-plane size of a voxel (3.5mm) were excluded. As a result, three subjects had one of their two MIDT runs excluded from the baseline session but they were still included in the overall analysis.

Whole brain voxel-wise data were analysed with FSL's Feat program. There were 3 explanatory variables (EVs): anticipation of win outcomes (Win5-anticipation; EV1), anticipation of loss outcomes (Lose5-anticipation; EV2) and anticipation of neutral outcomes (Neutral-anticipation; EV3). These were modelled as beginning at the onset of the cue and ending at the onset of the target stimulus. Because our primary hypotheses were related to anticipation-related reward processing, the contrast of primary interest was 'Win£5'-anticipation > 'Win£0 and Lose£0 (neutral)'-anticipation. For completeness, we also explored the other anticipation-related contrast Lose£5 > neutral and Win£5 > loss.

Subject level analyses

These were implemented in a general linear model by convolving the onset times of the task conditions with a gamma function model of the hemodynamic response. Motion parameters (standard + temporal derivatives + squared + quadratic) and temporal derivatives were included as regressors-of-no-interest. The FILM pre-whitening procedure was used to account for temporal autocorrelation, and a high-pass filter (100 s cut-off) was used to remove low-frequency noise. Reward anticipation was examined with the Win-anticipation > Neutral-anticipation contrast [-1 1 0], Loss anticipation was examined with the Loss-anticipation > Neutral-anticipation contrast [-1 0 1] and Win > Loss anticipation contrast was examined with [0 -1 1]. Additional contrasts compared the individual task conditions with baseline sections of the time-series: 'win-anticipation, lose-anticipation, neutral-anticipation.' Mid-level analyses were performed in order to generate individual subject level effects. These models compared the pre vs post-dose MIDT scans and gave a measure of the effect of GSK598809 for each subject using FMRIB's local analysis of fixed effects model.

Group level analysis

Group analysis was performed combining both groups and using the mid-level models as inputs to assess the effect of patient group on whole-brain activation related to the drug and task effects. A between-subjects group level model compared AUD patients to HCs, using the individual mid-level drug effects models as inputs. The results of these analyses therefore represent an interaction effect between patient group (contrast conducted at the group-level) and drug condition (contrast conducted at the mid-level), for each of the task conditions/contrasts modelled at the first-level. Group level models used FSL's FLAME-1 algorithm. Cluster-level statistics were used, with a cluster-defining threshold of $Z = 2.3$ and a multiple test corrected cluster-extent threshold of $a = 0.05$.

Additional group-level analysis models were conducted as a validation step in order to visualise the mean task effects across both patient groups, and both drug conditions. These used a simple group-mean model to derive an average of all the first-level analysis models, and used the same threshold.

Region of interest (ROI) analysis

A meta-analytic region of interest approach using data from https://neurosynth.org/ using the term 'monetary-incentive' (uniformity test) was used to determine five common ROIs activated in the MIDT: medial prefrontal cortex (mPFC), midbrain, striatum, amygdala/hippocampus (Amyhipp) and the anterior cingulate cortex (ACC) (Figure 11). Mean win and lose anticipation versus neutral contrasts were extracted from the ROIs in each participant and entered in a mixed-model ANOVA. Additionally, appropriate post-hoc testing was employed using Tukey corrections.

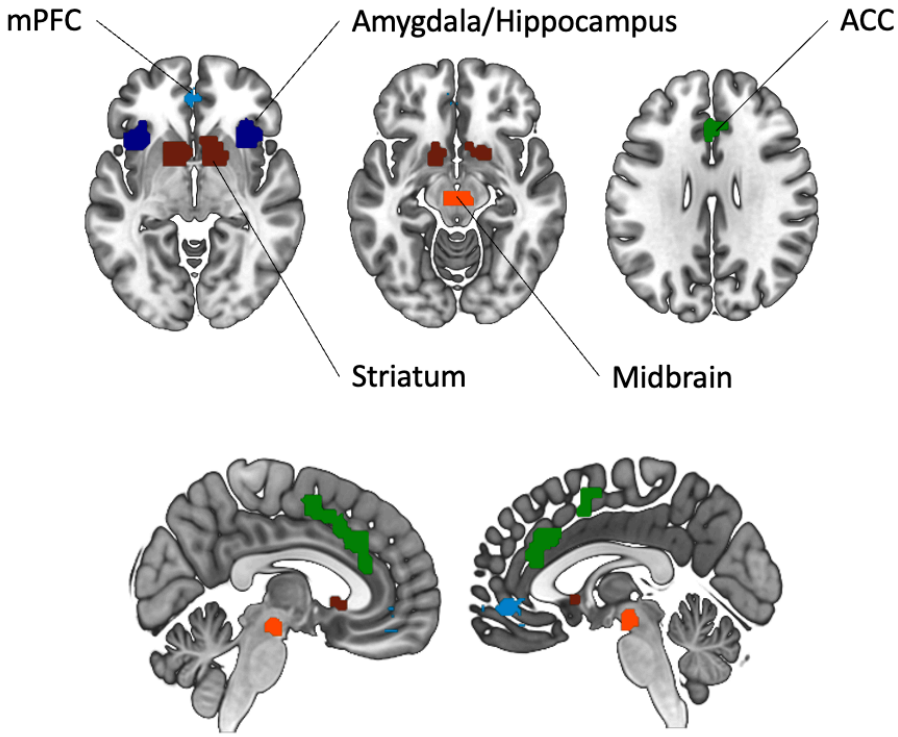


Figure 11: MIDT ROIs

Figure 11. Illustration of ROI's created from functional activation MIDT maps from Neurosynth meta-analytic data in axial and sagittal view. Dark Blue: Amygdala-hippocampus complex (Amyhipp); light blue: Medial Prefrontal Cortex (MPFC); Green: Anterior Cingulate Cortex (ACC); red: Midbrain; maroon: Striatum

Multimodal combined PET-fMRI analysis

Data was collected on VT of DRD3 using [^{11}C]PHNO-PET for three ROIs of interest; the substantia nigra (SN), hypothalamus (Hypo) and the ventral striatum (VST) (Erritzoe et al., 2014b). These were entered into a correlation matrix with the BOLD contrast values for the win-anticipation>neutral-anticipation contrast for the five fMRI ROIs (mPFC, midbrain striatum, amygdala/hippocampus, ACC).

Baseline and postdose values were correlated for both AUD and HC individuals in a 5x3 correlation matrix and r values were calculated. R values were converted to Z scores using Fisher's Z-tranformation to normalise the distribution of the data. The

resulting Z scores were then compared using a method outlined in (Meng et al., 1992), with any results greater than the critical values of Z=1.97 (equivalent to p = 0.05) counted as significant. These tests were used to determine any significant differences in the relationship between baseline DRD3 availability and brain BOLD activation to the MIDT.

Correlational analyses were additionally carried out to investigate the relationships between ROI brain response and psychometric and clinical questionnaires.

Results

Participants and demographics

No group differences were observed for the following: age (p=0.82), body weight (p=0.91), ethnicity, premorbid IQ (NART) score (p=0.70), or composition of current/former/non-smokers. However, AUD patients smoked more cigarettes per day than HCs (p=0.03) and had higher Fagerstrom Nicotine Dependence Scale scores (p<0.01). AUD patients scored higher than HC on all measures of alcohol consumption and dependence (p<0.01) and had been abstinent for 415±254 (range: 39–893) days before scanning. Although participants with current clinical depression or anxiety were excluded, AUD patients reported higher scores on depressive mood and anxiety (all: p<0.01; Table 4).

Demographics, personality, and symptoms	Alcohol-dependent patients (*n*=16)			Healthy controls (*n*=13)			ADPs *vs* HCs
	Mean (median)	**SD**	**Range**	**Mean (median)**	**SD**	**Range**	***p*-value (two-tailed)**
Age	42.4 (45.5)	9.4	24–55	41.5 (39.0)	10.3	27–60	0.822
NART	18.6	10.6	7–37	17.2 (18)	7.3	3–30	0.679
Body weight (kg)	78.5 (75.4)	13.2	56.6–109.0	78.0 (81.6)	8.9	60.5–90.5	0.91
Ethnicity	13 Caucasians 2 Indian 1 Black	NA	NA	11 Caucasians 1 Indian 1 Black	NA	NA	NA
Beck' Depression Inventory score	11.6 (10)	7.7	2–26	1.1 (1)	1.3	0–4	**<0.001**
Spielberger State Anxiety score	38.6 (38)	6.8	28–53	28.8 (30)	5	20–36	**<0.001**
Barret's Impulsivity Scale score	72.3 (71.5)	10.8	57–98	61.9 (62)	11.1	45–81	**0.025**
Smoking status	11 smokers 3 ex-smokers 2 non-smokers	NA	NA	10 smokers 2 ex-smokers 1 non-smoker	NA	NA	NA
Cigarettes per day among smokers	17 (20)	7	4–25	10.9 (12.5)	4.5	1.5–15	**0.027**
Cigarette package years	28.1 (22.7)	29	7.8–123.0	15.0 (15.1)	11.7	1.4–38.3	0.139
Smoking dependence (Fagerstrom score)	5.9 (6.0)	1.6	3–9	3.3 (3.0)	1.5	1–5	**0.001**
Alcohol dependence severity (SADQ score)	45.7 (50)	14.7	14–60	2.2 (1)	2.6	0–7	**<0.001**
Alcohol units per week (up to last detox for ADPs)	348 (338)	131	112–560	9.8 (10.4)	7.6	0–23	**<0.001**
Years of alcohol abuse	26.4 (30.5)	9.5	9–38	NA	NA	NA	NA
Accumulated lifetime alcohol intake (1000 units)	181 (185)	92	35–351	NA	NA	NA	NA
Days since last alcohol	414 (355)	254	39–893	Min 24 h	NA	NA	NA

Table 4: Demographics details comparing HC and AUD patients on clinical and demographic variables.

MIDT fMRI

Effect of task: whole brain analysis

To assess the main whole-brain effects of the MIDT, a total group mean was calculated, i.e., both baseline and GSK598809 conditions in HCs and AUD patients

combined to examine main effects of the task. Analysis of whole-brain activation for win-anticipation > neutral, lose-anticipation > neutral, win-anticipation > lose revealed a mesocorticolimbic activation pattern is presented . This serves to validate this task approach and the acquisition and analysis procedures (figure 12).

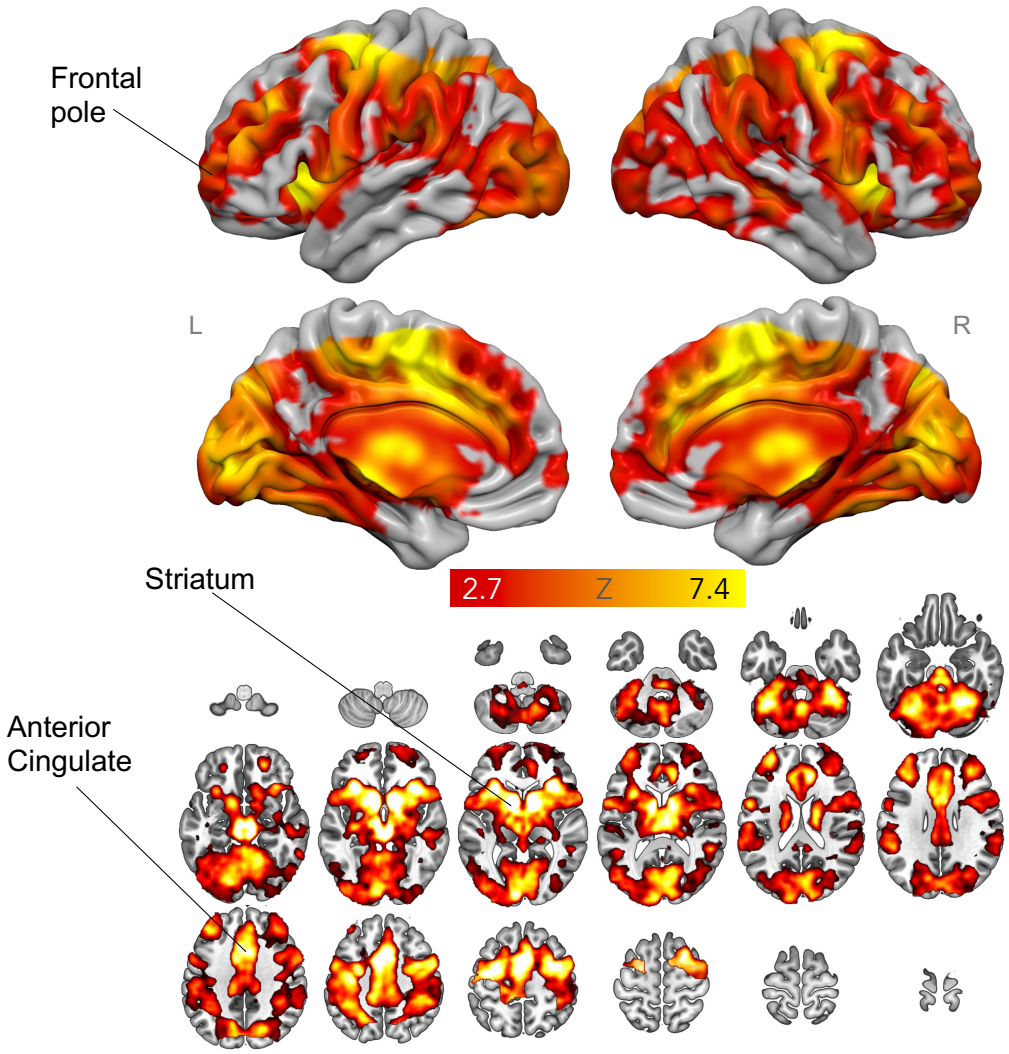


Figure 12: Whole brain MIDT

Fig 12. Whole-brain activation to the MIDT in the win-anticipation > neutral-anticipation in both groups averaged across placebo and drug condition demonstrating the effect of the task with significant activation in the striatum, the anterior cingulate and the frontal pole. Thresholded Z= 2.3 p<0.05, cluster-corrected. The colour bar in the

montage shows the t-value. Significant clusters on 3D cortical surface renders as viewed in saggital and axial slices.

Effects of participant group and drug: whole brain analysis

There were no significant whole-brain differences between participant group (HC v AUD) on the effect of GSK598809 (baseline vs. post-dose), in any active task contrast (win-anticipation > neutral, lose-anticipation > neutral and win-anticipation > lose). Comparing the active task contrasts to baseline (i.e., at rest), we observed a significant participant group * drug interaction in the striatum and the insula during the 'win-anticipation' condition compared to baseline.

In order to further interrogate this complex interaction effect, data were extracted from the set of functional clusters for the win-anticipation > baseline contrast for all subjects, all sessions. The data were then plotted in order to assess the directionality of effects (figure 13). A 2x2 mixed model ANOVA was performed on these data to assess the main effect of paitent group (HC v AUD), drug (baseline v post dose) and interaction effects. The ANOVA indicated no main effect of patient group ($F[1,27]=0.60$, $P=0.45$) or drug ($F[1,27]=1.75$, $P=0.96$), however, a significant interaction effect was found ($F[1,27]=20.66$, $P<0.001$). Post-hoc t-tests confirmed the source of the interaction effect was a relative *decrease* in activation post-dose in the win-anticipation versus baseline contrast in the HC group ($t[27]=3.02$, $P=0.026$, Tukey corrected), but a relative *increase* in the AUD group, ($t[27]=-3.43$, $P=0.01$, Tukey corrected).

For completeness, similar analyses were performed using the same functional clusters with data from the win-anticipation > neutral and lose-anticipation > neutral contrasts. There were no significant main effects of drug or group or any significant interactions found.

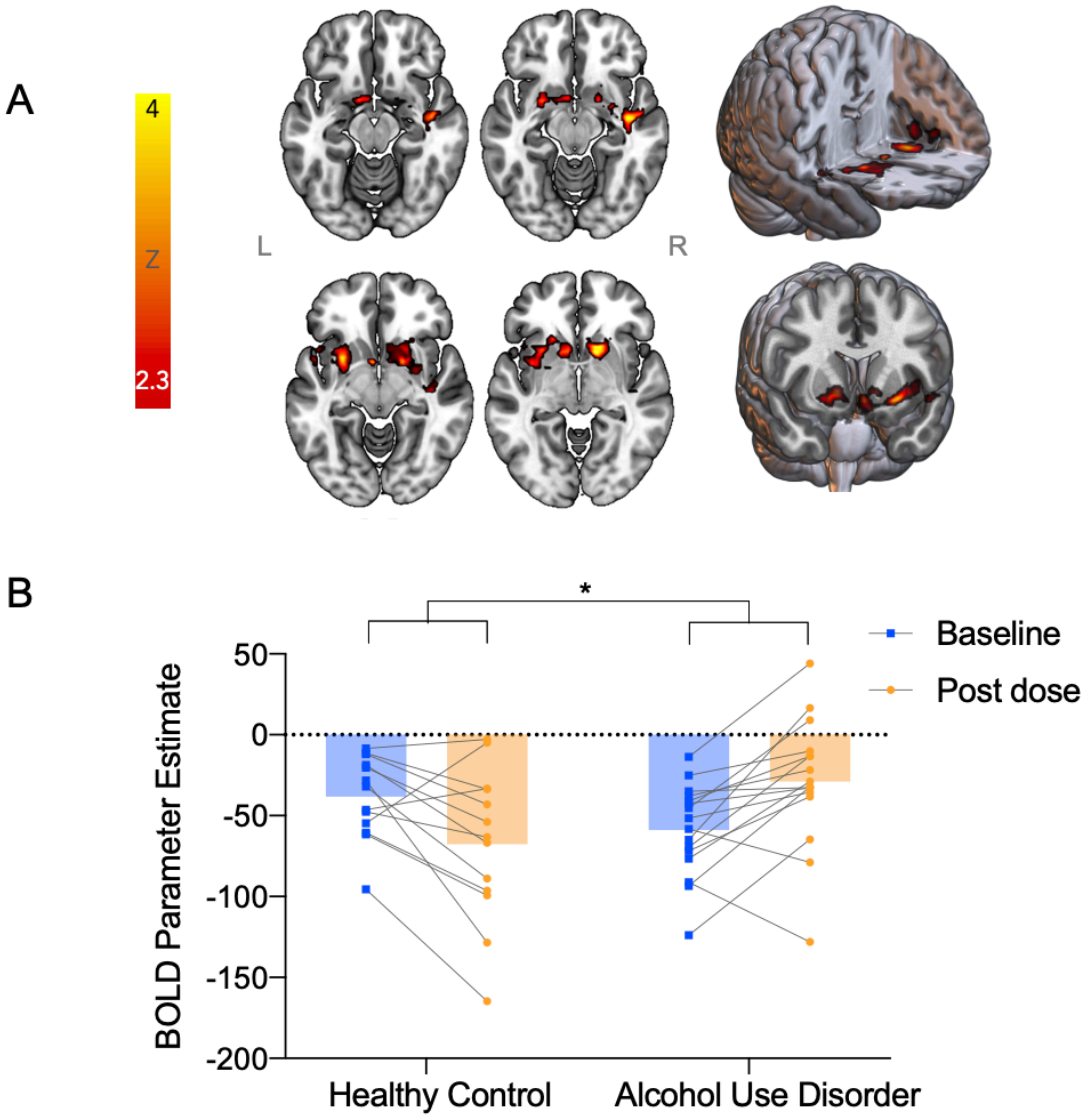


Figure 13: Whole brain HC v AUD

Figure 13. (a) Axial slices and 3D rendered brains showing mean 'win-anticipation' whole-brain activation in AUD vs Control under GSK598809>baseline. Thresholded Z= 2.3 P<0.05 corrected. The colour bar next to the montage shows the Z-values. (b) Bar charts showing data from a whole-brain mask demonstrating main effect of drug, GSK598809 in AUD patients and HCs in the MIDT task in the 'win-anticipation' contrast with baseline.

MID Whole Brain MNI co-ordinates						
Contrast	*Region*	*Cluster size (voxels)*	*MNI coordinates*	*F-value*	*p-Value*	*Direction*
Main effect of the drug	*Striatum-insula*	*2941*	*-16 2 -14*	*20.66*	*0.001*	*GSK>Baseline* AD>HC

ROI analysis

Five *a priori* ROIs were defined based on their involvement in reward processing, these were the mPFC, striatum, midbrain, amygdala/hippocampus and ACC (anterior cingulate cortex). Data were extracted from each of these regions from the three contrasts (win-anticipation > neutral, lose-anticipation > neutral, win-anticipation > lose-anticipation). A 2x2x3x5 ANOVA was conducted to test the main effect of group (HC v AUD), treatment (baseline v post dose), contrast (win-anticipation > neutral, lose-anticipation > neutral, win-anticipation > lose-anticipation) and ROI (mPFC, striatum, midbrain, amy/hipp and ACC).

A significant main effect of treatment ($F[1,27]=4.29$, $P=0.048$), contrast ($F[2,54]=5.87$, $P=0.005$) and ROI ($F[4,108]=15.541$, $P<0.001$) were found. A four-way treatment*group*contrast*ROI interaction effect ($F[8,216]=3.84$, $P<0.001$) was also identified.

Post hoc tests exploring the treatment, contrast, and ROI main effects revealed a general reduction in BOLD activation post treatment ($t[27]=2.07$, $P=0.048$, mean difference (MD): 7.67, standard error (SE): 3.70). The BOLD activation to the win-anticipation>neutral contrast was greater than in the lose-anticipation>neutral contrast ($t[27]=2.07$, $P=0.004$, MD: 10.88, SE: 2.90). The mPFC had significantly less activation than the other four regions (midbrain: $t[27]=-5.68$, $P<0.001$, MD: -15.77, SE: 2.80, striatum: $t[27]=-5.13$, $P<0.001$ MD: -19.23, SE: 3.75, amyhipp: $t[27]=-3.82$, $P=0.006$, MD:-14.56, SE: 3.81, ACC: $t[27]=-4.10$, $P=0.003$, MD: -16.60, SE: 4.04). The striatum had significantly greater activation than the amyhipp complex ($t[27]=3.43$, $P=0.015$, MD: 4.67, SE: 1.36). All p values are Tukey corrected.

To further explore the the treatment*group*contrast*ROI interaction, 15 2x2 ANOVA *post hoc* tests were run to investigate the effect of treatment (baseline v post dose) and group (AUD v HC) in each contrast (win-anticipation > neutral, lose-anticipation > neutral, win-anticipation > lose-anticipation) and each ROI (mPFC, striatum, midbrain, amyhipp and ACC). These tests revealed that the interaction effect was driven by the lose-anticipation > neutral contrast in the mPFC, where a significant group*treatment interaction was identified (F[1,27]=9.64, P=0.004) AUD patients had a significant reduction in BOLD activation in the mPFC to lose-anticipation > neutral trials post dose (t[27]=3.82, P=0.004, MD:38.53, SE:10.08, P Tukey corrected), this effect is outlined in figure 14.

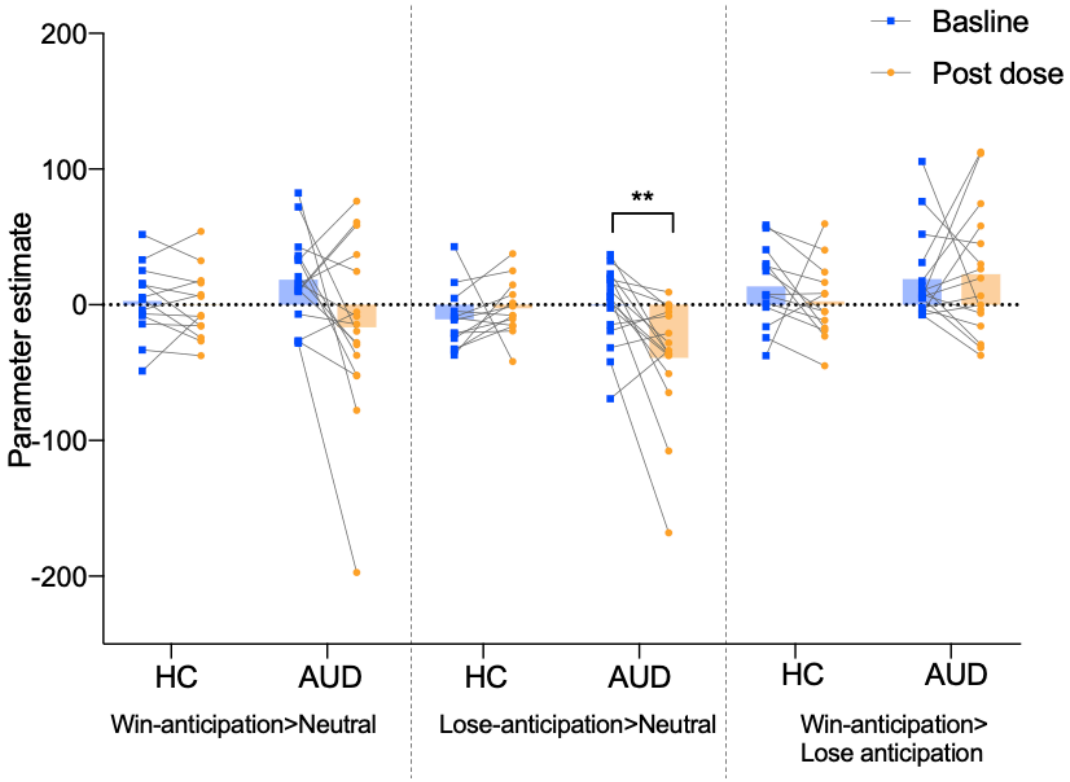


Figure 14: MIDT ROI HC v GD

Figure 14. mPFC ROI response during MID task: Mean win-anticipation > neutral-anticipation, lose anticipation > neutral anticipation, win anticipation > lose anticipation BOLD contrast estimate for baseline and GSK598809 sessions. Dark blue bars represent the baseline session and orange bars represent the GSK598809 section. **p = 0.004

Further, an overall effect of treatment (baseline v post dose) was identified in the ACC (F[1,27]=5.52, P=0.026). Post hoc tests revealed this was driven by the win-anticipation > neutral contrast where there was a significant reduction in activation post dose (t[27]=2.35, P=0.026, MD:14.0, SE:5.97, Tukey corrected). There was no significant difference between the groups (AUD and HC) F[1,27]=3.28, P=0.081, but there tended to be patterns of more deactivation in the AUD group (t[27=-1.81, P=0.081, MD: -16.8, SE: 9.29) but no interaction effect. No other significant effects were identified.

Combined MIDT win-anticipation>neutral fMRI BOLD contrast and [11C]PHNO PET DRD3 VT – ROI analysis

Correlational analyses were performed using data extracted from five ROIs from the fMRI win-anticipation>neutral contrasts and three ROIs using VT DRD3 data as assessed with PET for both AUD and HC subjects pre and post blockade. These values were entered into a correlation matrix to assess associations between brain DRD3 availability and reward processing at baseline in both AUD and HC and to assess the impact of DRD3 antagonism on such associations. These figures were Z transformed to assess for significance as described in the methods. There were no significant associations at baseline for either the HC and AUD groups between DRD3 availability and brain activation to the task, or following DRD3 antagonism (significance indicated by Z critical value >1.97) . The values from this correlation matrix are provided in table 5.

Z Comparison (AUD)	**mPFC (fMRI)**	**Midbrain (fMRI)**	**Striatum (fMRI)**	**Amygdala (fMRI)**	**ACC (fMRI)**
SN (PET)	0.81405121	0.34855458	-0.0465213	0.21397812	0.29756144
Hypothalamus (PET)	-0.1177125	0.32800477	0.29010499	0.303298	0.07912507
VST (PET)	-1.0038719	0.17433406	-0.0097893	-0.0780331	0.03132494

Table 5: fMRI x PET AUD matrix

Table 5: Z comparison scores assessing association between change in PET binding and fMRI activation pre vs post DRD3 antagonism in AUD.

Z Comparison (HC)	**mPFC (fMRI)**	**Midbrain (fMRI)**	**Striatum (fMRI)**	**Amygdala (fMRI)**	**ACC (fMRI)**

SN (PET)	-1.3470305	-0.1313497	-0.7006228	-0.6711762	-1.4272514
Hypothalamus (PET)	-1.485779	1.11499471	0.47384008	0.32476613	-0.0564626
VST (PET)	-1.2464497	-1.1404434	-1.4030289	-1.3186506	-1.2341083

Table 6: fMRI x PET HC matrix

Table 6: Z comparison scores assessing association between change in PET binding and fMRI activation pre vs post DRD3 antagonism in HC.

Exploratory correlation analysis in AUD patients at baseline with MIDT win-anticpation>neutral fMRI BOLD contrast

To test for putative brain-biomarkers of AUD, correlation analyses were performed correlating brain activation in the five ROIs from the AUD group in the win-anticipation > neutral contrast with: impulsivity, substance related scales, anxiety, and personality scales at baseline. There were no significant associations between any of the ROIs and the Barrett Impulsivity Scale (BIS) total score, Severity of Alcohol Dependence Questionnaire (SADQ), and lifetime alcohol use at baseline in the AUD group. Associations between the Spielberger State Anxiety Inventory (SSAI) with win-anticipation > neutral in the all ROIs at baseline was non-significant but demonstrated a trend to show increased anxiety with greater BOLD activation. All results from the correlation can be viewed in table 7. All correlations were strictly exploratory and so no corrections for multiple comparisons were performed.

Table 7: Correlation analysis between fMRI results in the AUD group to the win>neutral contrast at baseline and clinical scores.

Scale	Statistics	mPFC	Midbrain	Striatum	Amyhipp	ACC
BIS	Pearson's r	-0.14	-0.192	-0.073	0.011	-0.052
	p-value	0.604	0.476	0.787	0.968	0.847
BDI	Pearson's r	0.044	0.28	0.173	0.139	0.277
	p-value	0.871	0.294	0.521	0.609	0.3
SSAI	Pearson's r	-0.02	0.341	0.35	0.358	0.436
	p-value	0.942	0.196	0.183	0.173	0.092
STAI	Pearson's r	0.059	0.086	0.216	0.209	0.166
	p-value	0.834	0.761	0.44	0.456	0.554
EPQ-P	Pearson's r	0.139	-0.072	-0.207	-0.133	-0.333
	p-value	0.608	0.79	0.441	0.624	0.208
EPQ-N	Pearson's r	-0.176	-0.29	-0.203	-0.18	-0.097
	p-value	0.515	0.275	0.451	0.504	0.721
EPQ-L	Pearson's r	0.433	0.416	0.414	0.553	0.523
	p-value	0.094	0.109	0.111	0.026	0.038
EPQ-addiction	Pearson's r	-0.033	-0.336	-0.308	-0.263	-0.329
	p-value	0.903	0.203	0.246	0.325	0.214
EPQ-criminality	Pearson's r	-0.155	-0.502	-0.443	-0.452	-0.417
	p-value	0.567	0.047	0.086	0.079	0.108
PADUA	Pearson's r	-0.216	-0.049	-0.109	-0.06	0.038
	p-value	0.458	0.868	0.711	0.84	0.898

Table 7: Clinical correlations AUD

Discussion

This study aimed to investigate the effects of the selective DRD3 antagonist GSK598809 on brain reward networks involved in reward processing in the MIDT in individuals with AUD and HC as assessed with fMRI and PET.

Whole-brain analysis

Whole-brain analysis identified a significant relative increased activation in the 'win-anticipation' condition in the AUD group during the GSK598809 session in regions including the ventral striatum and the insula. These results indicate that GSK598809 increases BOLD responsivity to reward anticipation in reward related regions of the brain in AUD. Conversely, GSK598809 signficantly deactivated the striatal and insula brain regions in the win-anticipation condition in the HCs. This suggests a complex interaction, wherein the HC, who have supposedly 'normal' DRD3 levels, have a reduced striatal response to monetary reward when their DRD3 are blocked with the antagonist. In contrast, the AUD patients, who may have dysregulated DRD3, have an increased or normalised brain response to anticipation of money following administration of the DRD3 antagonist.

The ventral striatum is a key reward-related hub in the basal ganglia and functional dysregulation in this region has been linked to risks for developing addiction and relapse (Leyton and Vezina, 2013a). Our results indicate that GSK598809 increased the brain response to 'win-anticipation' of reward in the ventral striatum and insula in the AUD group compared to baseline, whereas the HC group showed deactivation. Our finding of GSK598809 leading to increased activation in the ventral striatum and insula in AUD during reward processing adds to previous findings of a general restorative or enhancing effect of GSK598809 on reward anticipatory BOLD response in this region in individuals with addiction (Murphy et al., 2017c). The reward enhancing effects of DRD3 blockade have been theoretically linked to the role of the DRD3 as an auto-receptor (Diaz et al., 2000). Some evidence also suggests antagonism of the DRD3 autoreceptor may enhance phasic dopamine signalling (Sokoloff et al., 2006) which leads to increased anticipatory reward responses. While these results were not found in the win-anticipation versus neutral contrast but just in

the ‘win-anticipation versus baseline’, they offer support to previous research and show a restorative effect of DRD3 antagonism in two key regions of the brain known to play a fundamental role in addiction and relapse. Our findings of reduced brain activation to reward anticipation in the control group following DRD3 antagonism further supports the role for DRD3 as a critical pharmacological target involved in reward processing and lends support to its role as an autoreceptor, as antagonism of ‘normalised’ DRD3 levels may lead to deactivation of brain reward responsivity as observed in our data.

ROI analysis

The ROI analysis revealed an overall significant effect of treatment (baseline v post dose) across groups, contrasts, and ROIs, indicating GSK598809 reduced overall BOLD responsivity to reward anticipation generally. Post-hoc exploratory analysis revealed this effect was most pronounced in the ACC of both groups.

A significant group*treatment*contrast*ROI four way interaction was also identified in the ROI analysis. Post-hoc tests revealed this was driven by the mPFC, where GSK598809 decreased BOLD responses to lose anticipation > neutral anticipation in the MIDT of the AUD group specifically.

There were no other differences between groups at baseline or post dose in our ROI analysis. Our findings that are specific to the ACC and the mPFC in the AUD group are unique and to our knowledge have not been described elsewhere previously. These findings offer several intriguing possibilities relevant to the plausible mechanism of action of this compound.

The ACC has previously been identified as a region of the brain sensitive to modulation by DRD3 antagonists in preclinical models, showing changes in dopaminergic and cholinergic neurotransmission (Lacroix et al., 2003). The ACC has been found to be implicated in addiction, being a key hub in addiction related brain networks relevant to decision making, cognition, inhibition, emotion, and motivation and is also a target for neuromodulation (Zhao et al., 2020). Further investigation into the underlying

neurochemical effects of DRD3 antagonism on brain function in this network and its relation to cognition and reward processing in addiction is warranted.

The finding of a significant decrease in mPFC activity following administration of a DRD3 antagonist specifically in AUD patients is intriguing. The mPFC is involved in reward processing, attention, and conflict decision making (Goldstein and Volkow, 2011a). Indeed in the MIDT the mPFC is recruited in receipt of a reward and the subjective valuation of the reward. Our results demonstrate a drug-induced deactivation to anticaptory loss processing which could underlie to neural substrate determining the subjective value of a reward though it is still unclear the extent to which the mPFC is involved in both reward anticipation, outcome as well as win and loss processing (Oldham et al., 2018). A number of studies indicate that mPFC dysfunction from chronic alcohol use leading to impairments in executive domains, episodic memory, and visuospatial capacities (Bernardin et al., 2014). Further, cognitive dysfunction can influence the efficacy of management of treatment and compromise abstinence in this population (Bernardin et al., 2014). Thus, cognitive function is crucial to provide optimal treatment for drug addiction and relieve patients' difficulties in social functioning. Dysfunction in the mPFC would therefore be expected to impair decision making aimed at maximising positive outcome and consequently a relative insensitivity to judging the value of drug and non-drug natural reward alternatives. Our findings could support the notion that DRD3 antagonism is modulating frontal activity involved in maintaining the cognitive dissonance related to reward processing in AUD. In order to corroborate this hypothesis, follow up studies would be required assessing the use of GSK598809 over a longer therapeutic window and follow up MRI scans would be required to assess whether the intervention leads to sustained changes in these domains and the effects these have on brain function. However, there is preclinical evidence to support this theory; DRD3 blockade has been shown to stimulate cholinergic transmission in the frontal cortex, an effect that has pro-cognitive effects which could therefore aid in combatting cognitive dysfunction and impulsivity in addiction (Lacroix et al., 2003, Loiseau and Millan, 2009). Further research should aim to substantiate these findings using molecular imaging techniques to determine the underlying changes in neurochemical signalling mediating changes in BOLD response.

There are two brain mechanisms that we have described in this study. Firstly there appears to be an drug-induced increase in ventral striatal and insula activation to win-anticipation which is interpreted as an amelioration of brain reward processing. Secondly there is a decrease in mPFC activation to lose-anticipation under the drug in AUD. These separate mehcnaisms could both be contributing to the potential anti-addictive effects of DRD3 antagonism and could be related to regional changes in dopamine neurotransmission. According to a proposed model of the role of dopamine in reward processing (Keeler et al., 2014), high levels of synaptic dopamine (e.g. as a result of phasic bursting of dopamine neuron firing) support reward-based learning by modulating plasticity in the direct pathway of the basal ganglia via action at D1 receptors. Conversely, decreases of synaptic dopamine levels (e.g., as a result of phasic inhibition of dopamine neuron firing) support punishment-based learning, by modulating plasticity in the indirect pathway of the basal ganglia via action at D2 receptors. An increase in BOLD signal in the ventral striatum and insula and a reduction in BOLD signal in the mPFC by GSK598809 via DRD3 antagonism could be underlined by differential inhibitory and excitatory dopamine function. Reward based learning facilitated by increased synaptic dopamine levels may describe our findings in the ventral striatum and insula whereas an inhibition of dopaminergic activity in the mPFC in the lose- anticipation > neutral contrast in the AUD group could support the role of DRD3 antagonism facilitating punishment-based learning required in loss anticipatory trials. Further, the results in the mPFC could also be interpreted as DRD3 antagonism reducing brain arousal associated with stress responsivity, a key risk factor in relapse (Koob et al., 2020), although this remains purely hypothetical and was not empirically tested, but could be a useful avenue for further research.

This makes it plausible that DRD3 antagonism could induce region specific activations and deactivations that modulate brain responses differentially based on the valence of reward-related processing. Hence the DRD3 could be a dynamic and non-unidirectional molecular switch to support both reward and punishment-based processing. Consequently, pharmacological modulation of this receptor could restore pathological dopaminergic signalling that plays a role in the maintenance of addiction.

We also found no significant difference in the baseline response to anticipating monetary reward between AUD patients and HCs. This finding is consistent with

findings by other work in AUD using a similar MIDT (Murphy et al., 2017a), who conducted a similar study. The incentive-sensitisation theory of addiction can somewhat explain this result. The incentive-sensitisation theory of addiction posits a hyperactivation of the ventral-striatal reward system in response to drug-predictive cues with no requirement for altered nondrug incentive processing. There is considerable evidence to suggest that the reward circuitry is highly responsive to substance associated cues (Zilverstand et al., 2018) but it is less clear how this system responds to nondrug reward cues such as money. Indeed two studies have found no differences in win>neutral processing at baseline comparing individuals with alcohol dependence with healthy controls (Bjork et al., 2008, Bjork et al., 2011). These studies did have a high degree of comorbid substance users such as the study conducted by our group (Murphy et al., 2017a) which could have reduced the generalisability of the findings to this specific sub-population.

Multimodal fMRI-PET analysis

Our findings of no association between DRD3 availability and reward processing in AUD and HC is curious given the modulation of brain function by DRD3 antagonism as observed here and in other fMRI studies (Murphy et al., 2017c, Vamvakopoulou et al., 2022a). It is plausible that the three PET ROIs chosen for the correlation analysis were not relevant to reward processing in AUD, although our *a priori* assumptions were based on findings of a) the SN having the highest expression of DRD3 b) increased hypothalamus DRD3 availaibility in AUD, and c) the VST being the primary brain region associated with reward processing. It is possible that instead of regional DRD3 availability and consequent antagonism being associated with modulations in brain reward processing that whole-brain DRD3 network architecture as assesed by graph theory may better interrogate the molecular-functional explanatory gap. Due to the small sample size in this study it was not sufficiently powered to conduct such analyses although recent analytical developments using covariance statistics on smaller PET data samples have demonstrated this approach to be useful (Veronese et al., 2019). Further, combined molecular and functional neuroimaging of the salience and reward neworks and mesocorticolimbic circuitry have demonstrated close relationships which may provide greater insight into addiction pathophysiology and the mechanism of novel interventions such as the one being investigated here (McCutcheon et al., 2019).

Advances in multimodal neuroimaging have the potential to pave the way for the development of more effective therapeutics in addiction and future clinical trials should endeavour to incorporate such experimental medicine techniques to optimise drug development efforts (Zafar et al., 2023b).

In summary, the above findings suggest that DRD3 antagonism may play a dual influence on both enhancing reward processing in striatal and limbic reward regions modulating mPFC/ACC function and thereby cognitive processes and punishment-based learning. Further research is required to determine the molecular basis for the functional effects of DRD3 antagonism on BOLD activation in clinical studies.

Strenths and limitations

This study used multi-model imaging to uncover the effects of DRD3 antagonism on reward processing in AUD. A strength of the study was the use of a control group which has generated data to add to our understanding of the neural correlates of reward processing in AUD. Through using whole-brain and *a priori* ROI analysis, we have identified a possible mechanism of reward and punishment based processing and learning mediated via the DRD3 receptor which may hold clinical potential in AUD and other addictions. Another advantage is this is, to the best of our knowledge, the first multimodal (fMRI-PET) neuroimaging and clinical study in patients with AUD exploring the effects of a novel drug. This approach to drug development has scope beyond the field of addiction and in other areas of psychiatry and should be adopted more broadly to profitably enhance neuropsychiatric drug development and reduce the treatment gap in mental health.

There are also several limitations of this study which may have impacted our findings. Firstly, our whole brain analysis was conducted versus baseline as opposed to the anticipation neutral condition and this was a post-hoc exploratory finding. This contrast is inherently noisy as baseline brain activation data is not inherently comparable to task-based condition. The lack of findings versus an anticipation-neutral contrast could be due to the small sample size which is discussed later. Reduced win-anticipation MIDT response in some alcohol dependent patients in other studies when compared with HC (Wrase et al., 2007a, Beck et al., 2009, Romanczuk-Seiferth et al., 2015,

Hägele et al., 2015). In contrast to these studies, we did not find significant blunting in any ROIs in the AUD cohort at baseline compared to HC. This could suggest recovery with long-term abstinence in our cohort, with our cohort having a longer length of abstinence than other studies. However, we did not find a significant association between the length of abstinence and BOLD response in any of the ROIs. Our study also used 60mg of GSK598809. Previous studies have found that 75mg of GSK598809 provide submaximal (72-89%) occupancy of DRD3 and so with a 60 mg dose we may not have achieved full receptor occupancy in order to elicit a complete clinical/therapeutic effect (GSK data primer, ECNP). Further, 75mg (which represents a 25% increased dose to this study) led to a transient reduction in craving in smokers, but there were no differences in effects versus placebo on addiction-salient behavioral tasks which could imply the dosing regimen for this trial may have been too low (Mugnaini et al., 2013). Indeed, studies have demonstrated 175mg (GSK data primer, ECNP) to be well tolerated in individuals and it is plausible that higher doses could have more pronounced and clearer effects on reward function and its association with receptor availabilty as assessed with combined fMRI-PET.

A significant limitation of this study was that it was not placebo-controlled and participants underwent a baseline scan followed by an active drug scan. This means our results may be influenced by order effects and expectancy bias. However, our ROI analysis demonstrated a strong group*treatment*contrast*ROI interaction and post hoc tests revealed this was specifically in the lose-anticipation contrast in the AUD group in the active drug condition. Although not conclusive, this suggests the drug effect is genuine and not simply as the consequence of boredom or fatigue, since the effect was only present in the lose-anticipation>neutral contrast, whereas confounding effects would have been evident across all contrasts. Finally, our sample size of N=29 (16 AUD and 13 HC) was small and the study was not specifically powered for the fMRI outcomes, moreover it was powered for the PET data for which it was adequately powered. As this analysis contained a combination of both fMRI and PET imaging analysis it would be appropriate to interpret these results as a signal of interest to explore further in future larger studies. Further, such signals though not adequately powered form the bedrock of translational neuropsychopharmacology research and are the foundation on which to make decisions concerning future developments of compounds and therefore should not be disregarded.

Conclusion

The overall finding of this study is that DRD3 antagonism can adaptively modulate neural responses to anticipatory reward processing in patients with AUD. Previous work has demonstrated a role of the DRD3 in motivational and cognitive elements of reward processing and antagonism of this receptor has been shown to reduce motivation for drugs of abuse, cue induced craving, and modulate reward and emotional processing in addiction. Our research goes further to find a potential dual mechanism of action of DRD3 antagonism on reward processing in AUD. Further work is required to extend our investigation of the molecular underpinnings of such observations using multimodal neuroimaging techniques including simultaneous PET-fMRI and longitundinal clinical trials to assess for concomittent safety and efficacy. Such techniques will be able to provide the most detailed account of addiction neuropathophysiology and the mechanism of action of novel interventions with the aim of optimising drug development to enable better therapeutics.

Chapter 4: Validation of novel fMRI paradigms in Gambling Disorder

Introduction

Since the mid 1990s, research investigating behavioural addictions has found consistent clinical similarities with SUDs. Due to this, the American Psychiatric Association (APA) redefined the "Substance related disorders" category from the fourth version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association and Association, 1994), as "Substance-related and addictive disorders" category in the DSM-5 (American Psychiatric Association and Association, 2013). This reclassification enabled the inclusion of non-substance related behavioural addictions in the subsection "Non-substance related disorders." Currently, Gambling disorder (GD), originally classified as "compulsive gambling" and as an "Impulse Control Disorder" is the only recognized behavioural addiction in the DSM-5 (Potenza, 2014)

Gambling disorder in the UK

Gambling is defined as an activity involving placing something at risk in the hopes of gaining something greater in value (Potenza et al., 2001). Gambling activities include casino gambling, online sports betting, the lottery, and several activities not classically considered to be gambling, such as investing in stocks or cryptocurrency, that involves some level of monetary risk (Oksanen et al., 2022). According to the DSM-5, gambling becomes a disorder when it is persistent, recurring and leads to substantial distress or functional impairment, typically in one's health, social, relational, or financial domains (American Psychiatric Association and Association, 2013). Prior to the most recent reclassification of GD in the DSM-5, the term 'pathological gambler' was used to define an impulse control disorder, however, it is now considered a behavioural addiction due to the shared biological and behavioural traits between individuals with GD and those with a substance use disorder (SUD) (Potenza, 2008).

According to the Gambling Commission's recent report, 43.5% of UK residents aged 16 or older engaged in gambling in the past year (Commission, 2023). The study

estimates that between 0.3% and 4.3% of these individuals have experienced adverse consequence from gambling, with a statistically significant increase seen in males compared to 2022 (Commission, 2023). This rise in gambling-related issues is concerning for the National Health Services (NHS) and a growing number of referrals suggests an increase in the prevalence and severity of GD.

There are several factors contributing to this rise. One of the most prominent is an increase in marketing done by gambling companies and the rise of online gambling platforms which directly contribute to at-risk gamblers transitioning to GD. Additionally, studies looking at the effects of the COVID-19 pandemic in both the UK and New Zealand indicated a significant escalation in gambling activity among regular online gamblers, reaching up to six times pre-pandemic levels in May 2021 (Bellringer and Garrett, 2021). The pandemic is therefore likely to have exacerbated problematic gambling related behaviours. Widespread economic turmoil, unemployment, impaired social support, and increased stress are all significant factors in modern society and have also previously been linked to increased gambling problems during times of economic unrest (Economou et al., 2019). Whilst the socioeconomic problems related to GD are apparent there is a significant lack of understanding in the mechanisms underlying this disorder. Further, the absence of possible drug induced neurotoxic effects on the brain from chronic substance exposure makes gambling disorder an ideal research target for unravelling core brain alterations associated with addiction.

Over the last 40 years, neuroscientific research has aimed to understand addiction related neuroscientific processes using neuroimaging tools such as fMRI and PET in order to better establish both disease mechanisms and to develop novel medications (Garrison and Potenza, 2014) though to some degree these attempts have not provided definitive conclusions on the neurobiology of GD or led to the development of effective and novel therapeutics (Nutt et al., 2015). Hence, given the rising morbidity and treatment-seeking for GD it is timely that neuroscience-informed investigations seek to 1) identify putative and functionally relevant biomarkers of GD 2) to use these identified targets to develop novel interventions for GD.

In this validation study I aim to use fMRI to investigate two novel tasks; a cue-reactivity paradigm and a reward-enriched roulette task aimed at investigating the brains reward and salience systems and comparing individuals with GD to HCs.Biomarkers of addictive disorders

A biomarker is a naturally occurring biological characteristic by which a particular pathological or physiological process can be identified which is associated with a given disorder. To be clinically relevant, biomarkers need to have diagnostic (help in the differential diagnosis of a specific condition) or prognostic value (help to predict the likely course of a condition or the response to treatment) (Califf, 2018). Neuroimaging has been proposed to be advantageous in defining addiction relevant biomarkers (Verdejo-Garcia et al., 2019). Ultimately the ambition is that this will enable the design of novel, effective treatments by identifying new brain targets that are involved in addiction related processes. Ultimately, once these biomarkers have been validated and novel drugs developed, precision-medicine algorithms can then be developed to predict individual patient relapse risk and likelihood of treatment response (Moujaes et al., 2023). This translational neuroimaging approach to addiction clinical research, which begins with the development of neuroimaging biomarkers, is recommended by a white paper co-authored by over 30 global leading addiction scientists and doctors from the International Society of Addiction Medicine (Verdejo-Garcia et al., 2019).

fMRI biomarkers in addiction

Even though fMRI is a proxy of neural activity, investigating the functional brain effects of specific conditions (“task-based”) such as cue-reactivity or reward processing can give crucial insights into underlying neurological functioning that may be contributing to disease pathology (Volkow et al., 2015). In substance addiction such functional brain imaging tasks have shown potential to predict addiction severity, risk of relapse and treatment outcome (Jasinska et al., 2014) and have also been profitably leveraged to develop novel therapeutics for addiction (Courtney et al., 2016) which implies that similar results can be garnered in GD.

Cue reactivity

Many fMRI studies have used cue-reactivity paradigms to investigate the reward and salience networks in GD and have compared the resulting neural responses to those of healthy subjects or subjects with SUDs (García-Castro et al., 2022). To summarise, there have been several studies which report on the effects of exposure to gambling videos or gambling images in individuals with GD compared with HC. These studies have had generally consistent findings showing upregulation of brain activity in regions including the dPFC, parahippocampal gyrus, amygdala, insula and inferior frontal gyrus (de Greck et al., 2010, Crockford et al., 2005, Goudriaan et al., 2010, Limbrick-Oldfield et al., 2017). Intriguingly, not all studies found this trend with an early study demonstrating reduced activation to gambling cue presentation in pathological gamblers in regions related to impulse control in the frontal cortex (Potenza et al., 2003). Though differences have been observed in relation to gambling videos and pictures the evidence is unclear on how individuals with GD process other salient or natural rewards. In healthy subjects, a meta-analysis (n=87 studies) found a consistent and robust activation of areas involved in the reward circuit (ventral striatum, ventral-medial prefrontal cortex, amygdala, anterior insula and mediodorsal thalamus) in response to primary (food, sex) and secondary (monetary) reward-related cues (Sescousse et al., 2013b).

The neural response to primary and secondary reward-related cues in GDs has been seen to be different with only one study investigating the differential processing of rewards in GDs (Limbrick-Oldfield et al., 2017). In this study no differences in ventral striatal reactivity to food-related cues were found between GDs and HCs (Limbrick-Oldfield et al., 2017), but GDs showed a differential response to monetary versus erotic cues, with a blunted reactivity of the ventral striatum to cues predicting erotic stimuli, and another study found robust reactivity to cues predicting monetary stimuli (Sescousse et al., 2013a) showing the differential sensitivity of GD brain reactivity to naturally rewarding cues. An alternative natural reward cue that is yet to be studied in GD is social cues. Interestingly, loneliness is a risk factor for GD (Castrén et al. 2013), and in healthy individuals loneliness is positively associated with ventral striatal activation to personally meaningful social cues (Inagaki et al. 2016). However, the relationship between social cues and brain processing is not well understood in GD and this could play a role in reward system dysfunction that is observed in GD. Another

natural reward that has not been explored in GD is the role of nature. Indeed increases in nature relatedness (Kettner et al., 2019) and social connectedness (Weiss et al., 2021) have recently been discovered to be key mediators of positive response to psychedelic therapy. Given that psychedelic therapy has been shown to have clinical efficacy in small-medium scale clinical trials in patients with addiction (Zafar et al., 2023b), it would be intriguing to establish whether social and nature processing are core elements of addiction neuropathology and if potentially effective treatments, such as psychedelic therapy, may target these domains.

In sum, cue reactivity paradigms in fMRI studies have been used to investigate GD biomarkers, and although findings are inconsistent for some rewards, they have begun identifying a pattern of neural response: differential activation to natural versus gambling cues, with a blunted response to natural reward cues and a robust response to gambling cues. In our task we wish to establish this further by exploring the differential processing of natural (food, social and nature) cues to (gambling cues) versus (neutral) cues to identify more specific and functionally salient brain biomarkers of GD.

A summary of findings from cue-reactivity tasks in GD v HC can be found in table 8.

Table 8. Differential activation of the reward network in response to gambling versus natural cues and monetary cues.

Cues	Healthy	Gambling
Food	↑	↑
Erotic	↑	↓
Monetary	↑	↑ *

Robust activation	↑
Decreased activation	↓
Significantly increased	↑ *

Table 8: fMRI GD summary

Reward processing

As previously described, HCs produce a consistent and robust activation in the reward circuits in response to monetary reward-related cues (Sescousse et al., 2013b). This is thought to be driven by dopaminergic neurotransmission that underlies the response to monetary processing (Hahn et al., 2021) which was established using both PET and fMRI techniques (Wilson et al., 2018).

Studies have found that using real or hypothetical monetary rewards may differentially influence the brain activity during reward tasks (Xu et al. 2016; Xu et al. 2018) and

stronger brain responses to the value signals during a real choice condition compared to hypothetical choice condition has been demonstrated (Kang et al. 2011). In addition, the total proportion of rewarding trials in a task may influence dopamine neurotransmission as the non-rewarding trials may suppress dopamine release (Zald et al. 2004). Other studies have previously used the monetary incentive delay task to measure reward processing, (a full review of the MIDT can be found in chapter 3) however in this task we wanted to design an experiment that would be more salient to gambling and sensitive enough for PET techniques to measure dopamine release (in a future study) and so our task is optimised for these aims. In our current task design, called the reward-enriched roulette (RER) task, we have enriched our trial with 30/45 trials being hits and a fixed total winning amount of £30 given to each participant. This is in order to stimulate the reward function and supposed dopaminergic signalling that underlies these processes in the brain.

Early preclinical work has placed dopamine as the central neurotransmitter in addiction (Olds and Milner 1954; Wise and Bozarth 1987; Stein 1964) and suggested that it had roles in reward, motivation and incentive behaviour (Robinson and Berridge 1993). As discussed in previous chapters dysregulation of the dopamine system is evident in some addictions using Positron Emission Tomography (PET) (Zafar et al, in preparation, Ch 2). In brief, PET measures of the brain's striatal dopamine receptor D2/3 ($DRD_{2/3}$) levels as well as the dopamine release capacity, were found to be reduced in a range of substance use disorders (Ashok et al. 2017; Martinez et al. 2007). Such observations have provided key support for the prominent "reward deficiency syndrome (RDS) theory" in addiction, proposing that a hypo-dopaminergic state constitutes a marker of addiction (Blum et al. 2000). Importantly, if such changes to the dopamine neurotransmitter release are central to addiction, similar changes would be expected to be apparent across all addictions, including GD. However, in GD $DRD_{2/3}$ levels are unchanged (Zafar et al. inpreparation, Chapter 2) and in fact striatal dopamine release is increased following amphetamine administration (Boileau et al. 2014) which may be a by-product of amphetamine stimulation as opposed to endogenous behaviourally-relevant dopamine release.

Over the years there has been some development in translationally salient, behavioural tasks that release dopamine in the human brain as described in (table 9)

however to date none of these tasks have been done at the same time as acquiring the functional imaging data due to limitations in the development of novel technologies

Study	Tracer	Patients	Task/Stimulus	Findings
(Koepp, 1998)	[11C]-raclopride	8 HC	Video Game	VS RAC Binding reduced in video game. Correlation between performance level and RAC displacement
(Aalto et al., 2005)	[11C]FLB 457	12 HC	Verbal working memory, sustained attention task	During the performance of both tasks, reduced D_2 receptor availability was observed in the left ventral anterior cingulate. verbal WM task reduced D_2 receptor availability in the ventrolateral frontal cortex bilaterally and in the left medial temporal structures (amygdala, hippocampus), suggesting that dopamine release in these regions might have a specific role in WM
(Christian et al., 2006)	[F18]fallypride	8 HC	Spatial attention task	Activation in thalamus; correlated with task performance
(Badgaiyan et al., 2008)	[11C]-raclopride	6 HC	Modified serial reaction time task	Release of dopamine in dorsomedial aspect of posterior putamen and anterior caudate during task
(Badgaiyan et al., 2009)	[F18]fallypride	8 HC	Emotional words	Activation in ligand displacement rate in left amygdala, left MTL, and left inferior frontal gyrus
(Lataster et al., 2011)	[F-18]fallypride	12 HC	Arithmetic stress task (MIST)	Activation throughout PFC; in vmPFC correlated with subjective stress
(Vrieze et al., 2013)	[F-18]fallypride	10 HC	Monetary reward task	Activation in mOFC, vmPFC, dACC, correlated with reduced reward learning
(Badgaiyan and Wack, 2011)	[11C]-raclopride	10 HC	Modified Eriksen's flanker test (congruent and incongruent)	Activation of dorsal aspect of body of left caudate during task. As well as left dorsal putamen, riht dorsal putamen and right dorsal body of caudate
(Ray et al., 2012)	[11C]FLB 457	14 PD: 7 PG+ 7 PG-	Gambling task	DBP_{ND} in midbrain was lower in PG+ compared to PG-; DBP_{ND} correlated with impulsivity
(Nagano-Saito et al., 2013)	[F-18]fallypride	11 HC	Arithmetic stress task (MIST)	Decreased BP_{ND} in small cluster in the dmPFC; DBP_{ND} correlated with HR
(Milella et al., 2016)	[F-18]fallypride	12 CD	Cocaine cues	Decreased BP_{ND} in mOFC and striatum; DBP_{ND} correlated with subjective craving
(Bäckman et al., 2017)	[11C]-raclopride, bolus plus infusion	23 HC	N back transfer test following working memory updating training intervention	Increased striatal DA activity during the n-back transfer test
(Cox et al., 2017)	[11C]-raclopride	6 HC recreational cocaine users	Cocaine cue task	Cocaine cues decrease raclopride BPnd in dorsal striatum (Putamen and caudate) (contrast vs neutral cues)

that can measure both simultaneously.

Table 9: Describing human behavioural neurotransmitter release PET neuroimaging studies (CD = cocaine use disorder, PD= parkinsons disease, PG= pathological gambling)

Table 9: Behavioural PET release studies

The fMRI signal acquired in the brain's reward circuits is several steps removed from the dopaminergic reward neuron activity, such that inferences about changes in dopaminergic activity from fMRI can only be made with caution. However, the recent invention of combined PET/MRI scanners, has opened new ways to scrutinize some of these inconsistencies and translational challenges. In addition to the inherent benefits of temporal synchronisation and spatial alignment, this hybrid technique has several advantages, including streamlined logistics, shorter imaging session times, higher throughput, and better image quality (Lalush 2017). Simultaneous fMRI-PET will allow for the study of both molecular neurotransmitter function with PET and the downstream implications on neural brain activity and connectivity as assessed with fMRI, thereby constituting a powerful new analytical approach that bridges the molecular-functional-clinical translational bridge.

Though this study is aimed at validating the tasks with fMRI only without conducting any PET scans, in future studies we aim to conduct the RER in a simultaneous fMRI-PET scan in patients with addiction, potentially pre and post an interventional treatment such as psychedelic therapy, to assess if the treatment restores molecular and functional deficits associated with the condition.

Research question and hypotheses

The primary objective(s) of this study is (i) to develop novel fMRI tasks that can be used in translational neuropsychopharmacology studies to investigate the neural effects of psychedelic therapy in addiction and (ii) to develop a behavioral model of 'in-vivo amphetamine-challenge' to investigate molecular-functional reward system deficits in addiction and its response to novel treatments and (iii) to investigate reward and salience network deficits in GDs vs HCs The fMRI protocol will allow us:

- To evaluate the fMRI BOLD activation in the reward and salience system during the 5-ACR and RER tasks.
- To compare neural activity assessed with fMRI during the 5-ACR and RER tasks in GDs vs HCs
- To relate brain activity in the 5-ACR and RER with clinical scales assessing gambling addiction severity (MAGS, GRCS, PGSI, GUQ) and impulsivity (BIS):
    - MAGS (Massachusetts Gambling Screen) (Shaffer et al. 1994)

- GRCS (Gambling-Related Cognitions Scale, 23 items) (Raylu and Oei 2004)
- PGSI (Problem Gambling Severity Index, 9 items) (Ferris and Wynne 2001)
- GUQ – (Gambling Urge Questionnaire 7 items)
- BIS (Barratt Impulsiveness Scale (Patton et al. 1995))

Hypotheses

5-ACR task:

1. Higher activation in the reward and salience networks during gambling cues compared to neutral cues in GDs compared to HCs.
2. Lower activation in the reward and salience networks during natural reward (Food, Nature, Social) cues in GDs compared to HCs.
3. Positive correlations between gambling severity and impulsivity scores and anterior insula and ventral striatum activation in the GDs in response to gambling video cues.

RER task:

1. Pronounced activations in reward-related brain circuits, e.g. the ventral striatum during the Hit task condition.
2. Increased activation to Hit and miss tasks in GD vs. HC
3. Positive correlations between GD clinical severity and ventral striatum activation to the Hit condition

Methods

Participants

In total, 18 GDs and 21 HCs participated in the study. The GDs were recruited through the National Problem Gambling Clinic (NPGC) in the UK, which served as the Patient Identification Centre (PIC), and the HCs were recruited through advertisements. All participants were male and right-handed due to a large proportion of individuals on the

NPGC's waiting lists being male, as well as this reflecting the greater clinical prevalence of males with GD (Wong et al., 2013).

All participants conducted a semi-structured interview with a psychiatrist covering the exclusion of all co-morbid psychiatric disorders apart from GD for the GD individuals, with the exception of nicotine addiction which was allowed for both GD and HC subjects. Individuals with GD are more than twice as likely to have psychiatric comorbidities compared to the general population, leading to complex clinical presentations (Sundqvist and Rosendahl, 2019). In our study we will be investigating individuals with a primary diagnosis of gambling addiction however we acknowledge this may not be necessarily reflective of the clinical population but is a pre-requisite to validate and develop GD specific brain imaging paradigms.

All GD patients met DSM-5 diagnostic criteria (APA, 2013) and were permitted to be included in the study if they indicated having a clinical history of depression or anxiety. Additional inclusion criteria were: age 20-65 years, healthy (based on a medical evaluation including medical history, physical examination and psychiatric evaluation). Additional exclusion criteria included: positive pre-study drug/alcohol result on testing, use of psychotropic medication that may interact with fMRI tasks, medical conditions such as epilepsy or severe migraine, history of claustrophobia, presence of a cardiac pacemaker or any of the other contra-indications for MRI listed in the pre-MRI screening questionnaire, history of a neurological diagnosis or clinically significant head injury that may influence the outcome of the scan results, and unwillingness or inability to follow the procedure outlined in the protocol.

Ethics statement

Participants gave written and informed consent to be part of the study, which was approved by Brent Research Ethics Committee (REC) and the NHS Health Research Authority (HRA). The study was conducted in accordance with the recommendations for research on human subjects adopted by the 18th World Medical Assembly, in Helsinki, 1964 and later revisions.

Psychometric Measurements

A range of psychometric questionnaires were self-administered to assess clinical psychopathology levels, cognition, personality and addiction related criteria. These covered scales relevant to the common psychiatric disorders, addiction and impulsivity scales, personality questionnaires and ancillary scales to measure sociability and nature relatedness.

The Massachusetts Gambling Screen (MAGS) is a screening tool designed to assess gambling behaviors and identify individuals at risk of gambling-related problems. Based on DSM-IV criteria, it consists of questions about gambling frequency, money spent, and consequences. Scores categorize individuals into low, moderate, or high-risk groups. While useful for initial screening, a comprehensive evaluation by a professional is needed for a definitive diagnosis.

The Gambling-Related Cognitions Scale (GRCS) is a 23-item tool designed to measure cognitive distortions related to gambling. These distortions are beliefs or thoughts that can influence a person's gambling behavior, potentially leading to problematic gambling. The scale assesses various cognitive aspects such as illusion of control, predictive control, interpretative bias, and gambling-related expectancies. It helps in understanding the cognitive processes underlying gambling behaviors.

The PGSI, or Problem Gambling Severity Index, is a brief screening tool used to assess the severity of gambling-related problems in individuals. It typically consists of nine items that measure various aspects of gambling behavior, such as chasing losses, borrowing money, and feeling guilty about gambling. Scores from the PGSI can categorize individuals into different levels of risk: non-problem, low-risk, moderate-risk, or problem gambling. It helps identify those who may benefit from further assessment or intervention related to their gambling behaviors.

The Gambling Urge Questionnaire (GUQ) is a tool designed to assess the intensity and frequency of urges to gamble in individuals. It typically consists of a series of questions that measure various aspects of gambling urges, such as the strength of urges, resistance to urges, and the frequency of specific gambling-related thoughts or feelings. The GUQ helps researchers and clinicians understand the nature and

intensity of gambling urges experienced by individuals, which can be valuable for assessing and treating gambling-related problems.

The BIS, or Barratt Impulsiveness Scale, is a widely used self-report questionnaire designed to measure impulsivity in individuals. Developed by psychologist Marco Barratt, the scale assesses various aspects of impulsiveness, such as attentional, motor, and non-planning impulsiveness. It consists of a series of statements to which individuals respond based on their own behaviors and feelings. The BIS helps identify tendencies towards impulsive behavior, which can be relevant in various contexts, including clinical assessments and research on behaviors such as gambling, substance abuse, and other impulsive behaviors.

For the purpose of our analysis, we examined all the variables to observe differences in clinical scores between GD and HC groups however we restricted our brain-fMRI task-questionnaire analysis to pre-specified *a priori* hypotheses as listed previously.

Tasks

Two fMRI tasks were employed in this study, the 5-arm cue reactivity (5-ACR) task and the reward-enriched roulette task (RER).

5-armed cue reactivity task (5-ACR)

The five-armed cue reactivity (5-ACR) task is an event-related design that includes five different types of video cues: gambling, social, nature, food, and neutral. The gambling videos show gambling-related activities including scratch cards, various casino games and machines, poker chips, playing cards, roulette and casino fronts. The social videos show scenes where two or more people interact with each other, but never with a sexual component. The nature videos show natural landscapes and scenery. The food videos show prepared meals and the process of cooking. The neutral videos show random objects like a chair or a pen. Each video lasts five seconds and is followed by a three second interval during which the participant rates the content on a five-point Likert scale by pressing the button pad. There were 15 trials for each of the five video cues, totalling 75 trials and following a pseudorandom order. Total task duration is 12 minutes, plus a 10 second buffer period at the end.

A schematic of the task, including pilot unpublished analysis comparing pictures to videos on brain activation and primary and secondary objectives are described in figure 15.

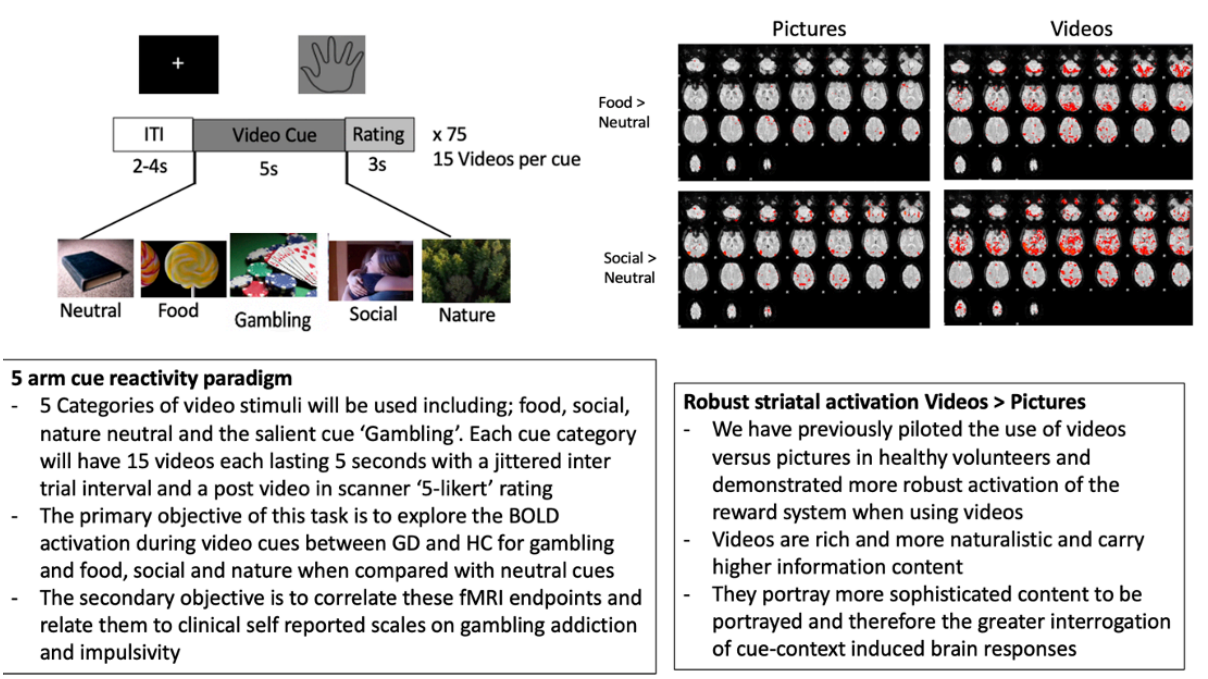


Figure 15: Schematic of 5-ACR

Figure 15: Schematic of the 5-ACR design, primary and secondary objectives and photo/video brain activity data

Reward-enriched roulette task

The RER task is an event-related design task with 45 money trials and 15 neutral trials in a pseudorandom order. During money trials, participants choose between a black or red chip during the gambling period, then an animated roulette wheel spins and the outcome shows either “Hit! Win £1” or “Miss” on the screen. Within the money trials, 30 are pre-determined to be hits and 15 are misses. During the neutral trials, participants choose between a white square or triangle during the ‘gambling’ period, and then a colour wheel spins during the anticipation period, finally the outcome period shows meaningless symbols. The contrasts between the fMRI BOLD signals from the Hit, Miss and Neutral trials is used to analyse brain activation. Total task duration is 17 minutes and 30 seconds plus a 10 second buffer at the end. A schematic of the RER can be seen below in figure 16.

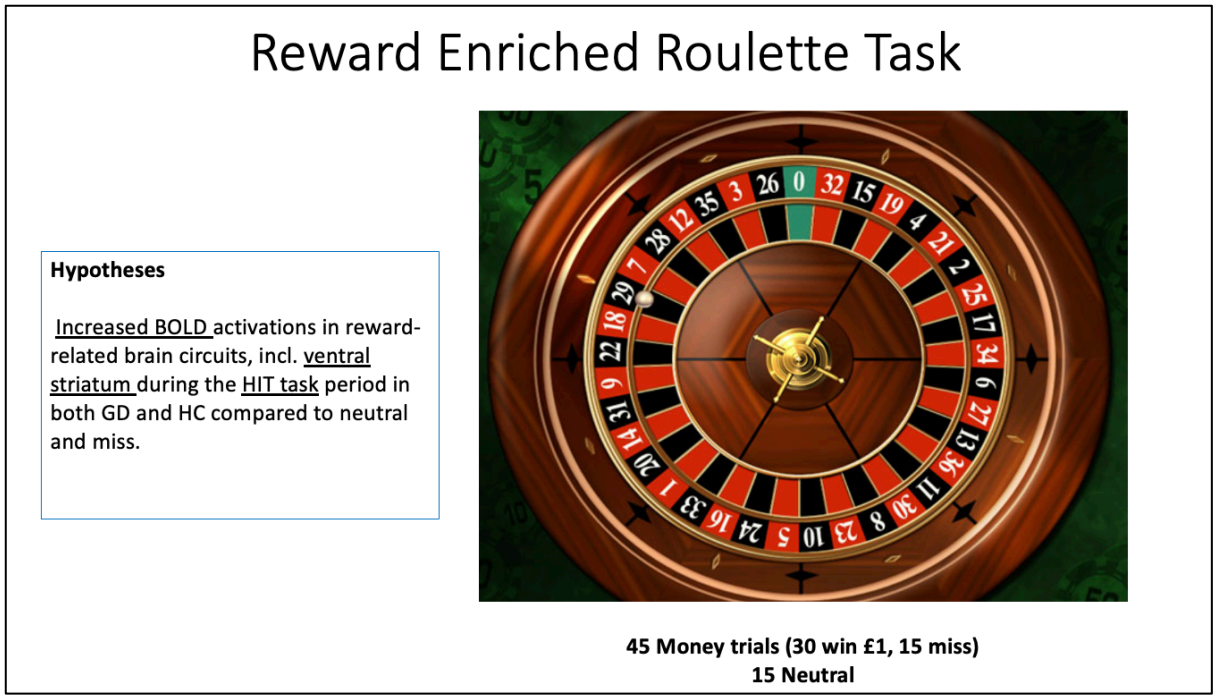


Figure 16: Schematic of RER

Figure 16: Schematic to show the RER task including a hypothesis of effects.

Procedures and design

Participants were first contacted via telephone and administered the telephone screening case-report form (CRF), which included an initial psychiatric assessment, background medical history, substance use assessments and magnetic resonance imaging (MRI) pre-screening questionnaire. The participant information sheet (PIS) was then sent to the participant for review. Visit 1 took place at St. Charles clinic. It included a physical examination, alcohol breath test, urine drug screen and the administration of the national adult reading test (NART) to assess premorbid intelligence, where a higher score due to more errors is associated with lower intelligence (Nelson, 1982), as well as self-reported questionnaires. Visit 2 took place at the Invicro imaging facility at Hammersmith hospital. It included assessments of sleep, food and nicotine intake, gambling evisual analog scale (VAS), alcohol breath

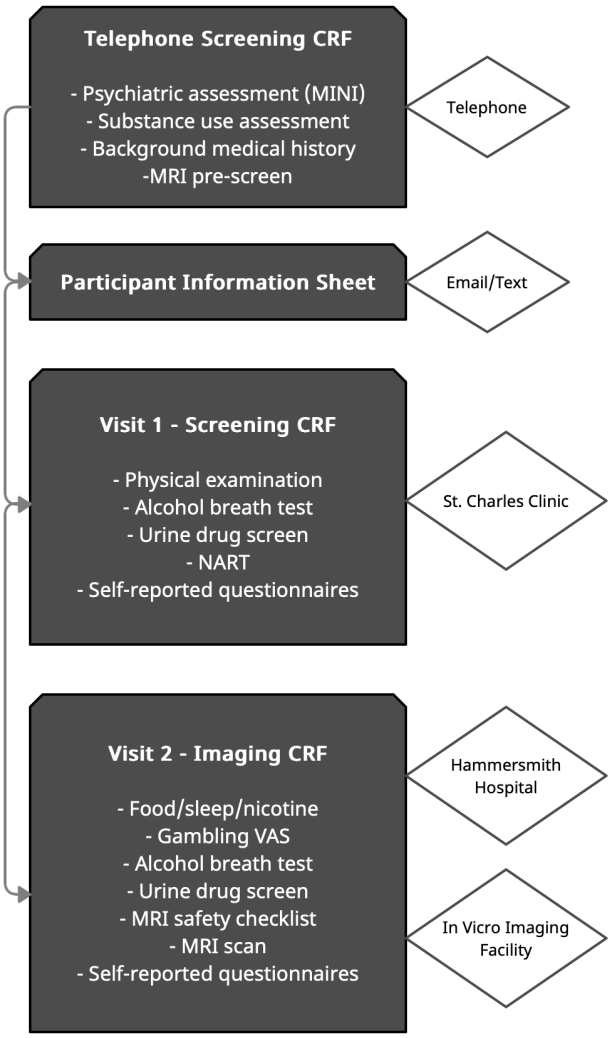


Figure 17: GD study flow

test and urine drug screen, administration of the MRI safety checklist, the MRI scan, and self-reported questionnaires.

FMRI acquisition and pre-processing preceded 1st and 2nd level analyses, group level whole brain analyses followed, then ROI analyses and finally correlational analyses between clinical scores and ROI data was conducted.

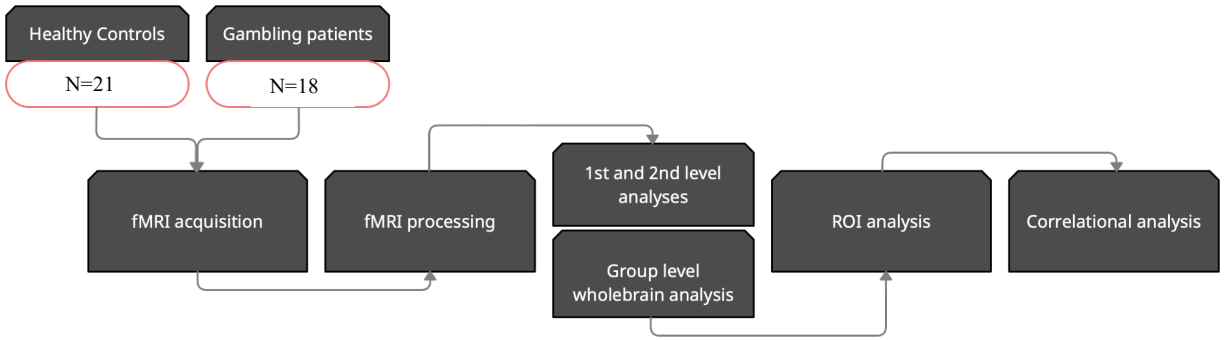


Figure 18: GD study fMRI analysis pipeline

fMRI

fMRI tasks

All participants undertook a 60-minute fMRI scan in which both fMRI tasks were performed. Prior to the fMRI tasks scores were collected to assess for scores related to hunger, sleep and time of last sleep. There were no significant differences between the groups indicating these variables, known to effect fMRI brain activity were controlled for between the groups.

Acquisition

T2*-weighted, gradient echo, Echo-Planar Imaging (EPI) images sensitive to BOLD contrast were acquired continuously on a 3 Tesla General Electric Signa PET/MR scanner with a 32-channel head coil. Settings used for this functional sequence were: TR = 2000, TE = 30, flip angle = 80°, 3mm x 3mm in-plane resolution (64 x 64 matrix), slice thickness = 3.6 mm, 36 axial slices. 365 volumes were acquired to cover the 12 minute 10 second task duration. In addition a T1 weighted IR-SPGR sequence was acquired to provide an anatomical image (TI = 400 ms, TE = minimum, flip angle = 11°, 256 x 256 matrix, 1 mm isotropic voxels, sagittal slices).

Preprocessing

FMRI data were processed with FSL software (www.fmrib.ox.ac.uk/fsl/). Image analyses were performed using FSL 5.0.4. The functional data were pre-processed using spatial smoothing with a 6mm FWHM (full-width, half-maximum) Gaussian kernel, high-pass temporal filtering (100 s), head-motion correction using MCFLIRT, and non-linear registration to a standard template (MNI152). The anatomical data were skull-stripped using FSL's brain extraction tool (BET) and segmented using FMRIB's automated segmentation tool (FAST), into grey/white matter and cerebro-spinal fluid (CSF). The criteria for excluding data based on head motion was set at mean absolute head motion exceeding 3mm.

Subject-level analysis

Whole brain voxel-wise data were analysed with FSL's FEAT (FSL Expert Analysis Tool) program.

5-ACR

There were five explanatory variables (EVs): food (EV1), nature (EV2), social (EV3), gambling (EV4) and neutral (EV5). These were modelled as beginning at the onset and ending of the video cue. The videos were randomised with a jittered inter-trial interval between each followed by an in-scan five-point Likert scale rating. Subject-level analyses were implemented in a general linear model by convolving the stimulus-related onsets with a gamma function model of the hemodynamic response. Motion parameters (standard + temporal derivatives + squared + quadratic) and temporal derivatives of the stimulus onset were included as regressors-of-no-interest. The FILM pre-whitening procedure was used to account for temporal autocorrelation, and a high-pass filter (100 s cut-off) was used to remove low-frequency noise. Cue reactivity was examined for contrasts of the five stimulus conditions relative to baseline (inter-trial intervals), Food [1 0 0 0 0], Nature [0 1 0 0 0], Social [0 0 1 0 0], Gambling [0 0 0 1 0] and Neutral [0 0 0 0 1] and used to generate individual subject level effects.

RER

There were three explanatory variables (EVs): Hit (EV1), Miss (EV2), Neutral (EV3). These were modelled as beginning at the onset of each task type and at the end when the outcome was announced. The trial type was randomised with a jittered inter-trial interval. Subject-level analyses were implemented in a general linear model by

convolving the stimulus-related onsets with a gamma function model of the hemodynamic response. Motion parameters (standard + temporal derivatives + squared + quadratic) and temporal derivatives of the stimulus onset were included as regressors-of-no-interest. The FILM pre-whitening procedure was used to account for temporal autocorrelation, and a high-pass filter (100 s cut-off) was used to remove low-frequency noise. Task activity was examined for contrasts of the three conditions relative to baseline (inter-trial intervals), Hit [1 0 0], Miss [0 1 0], Neutral [0 0 1] and used to generate individual subject level effects.

Group-level analysis

A between-groups model compared HCs to GDs, using the individual subject level effects as inputs. Group level models used the FSL FLAME-1 algorithm. Cluster-level statistics were used, with a cluster-defining threshold of Z=2.3, and a multiple test corrected cluster-extent threshold of $\alpha = 0.05$. In addition, validation models of mean task effects across both subject groups were calculated, in order to visualise mean effects of the tasks. These models used the same FLAME-1 model and thresholding as the between-groups models.

ROI analysis

For the 5-ACR, eight ROI's were derived from meta-analytic data from www.neurosyth.com using the term 'reward'. These covered the anterior cingulate cortex (ACC), amygdala, anterior insula, dorsal striatum, midbrain, mPFC, thalamus and ventral striatum. See figure 19.

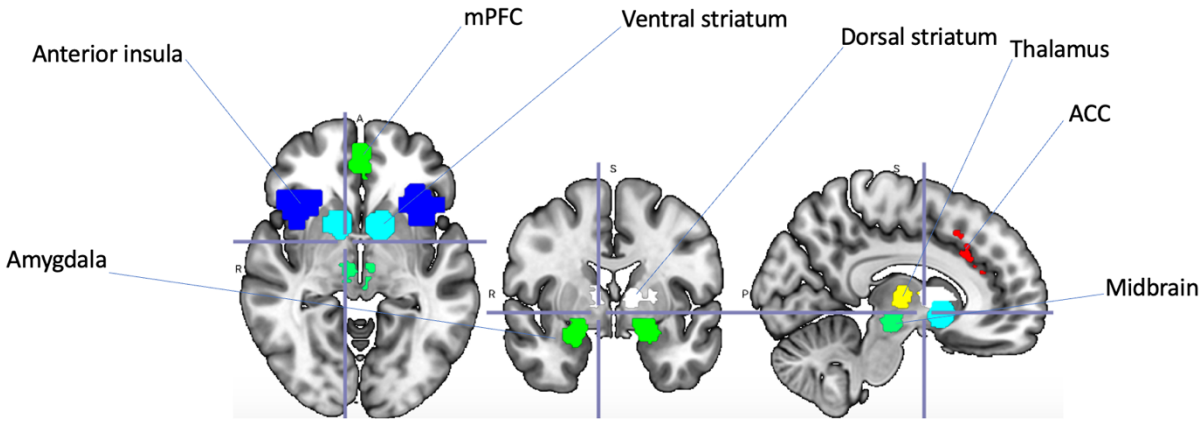


Figure 19: 5-ACR ROIs

Figure 19: showing eight ROIs from the ‘reward’ term from neurosynth for the 5-ACR

For the RER, five ROIs were derived from meta-analytic data using the term ‘gambling’ from www.neurosyth.com covering the ACC, anterior insula, dorsal striatum, mPFC and ventral striatum. See figure 20.

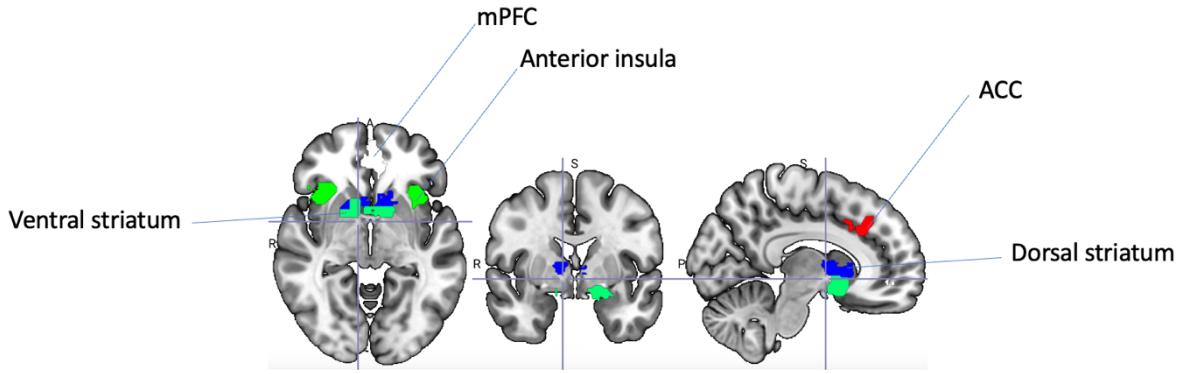


Figure 20: RER ROIs

Figure 20: showing five ROIs from the ‘gambling’ term from neurosynth for the RER

Statistical Analysis

Unpaired two sample t-tests were used to analyse between-group differences in demographic and clinical characteristics. Whole brain analysis was done using cluster-level statistics, with a cluster-defining threshold of Z=2.3, and a multiple test corrected cluster-extent threshold of $\alpha = 0.05$. For the ROI data, a mixed model analysis of variance (ANOVA) assessed the effect of two independent variables: Group (HC or GD) and Video cue (Food, Nature, Social, Gambling or Neutral) or Task condition (Hit, Miss, Neutral), on the ROI dependant variables: ROI parameter estimates for the eight described ROIs for the 5-ACR and the five ROIs for the RER which corresponds to the relative voxel activation for each ROI mask. Bivariate Pearson or Spearman correlations corrected for multiple comparisons were run in order to look at relationships between brain and clinical variables of interest. All tests were calibrated at a 95% confidence interval (significance α =.05) and run on Jamovi 2.3.28.

Results

Demographic and clinical characteristics

Thirty-nine right-handed males aged 21-62 years volunteered to participate in the study, including 18 with GD and 21 HCs (summary demographics in **Table 10**). There were no significant differences between patients, age, weight, ethnicity and the number of nicotine smokers although there was a significant difference in participant height with HCs being significantly taller than GD (p=0.0003), BMI, with HCs having significantly lower BMI than GDs (p=0.032), education with GDs having lower number of years of education post 16 (p=0.0001) and with NART errors with GDs having a greater number compared to HCs (p=0.019).

Table 10. Demographic and neuropsychological characteristics of participants. Significant differences between groups reported via *t*-test. *p<0.05, **p<0.001.

	GD (n=18)	**HC (n=21)**	**Test statistics (*t*-test)**
Demographic Data			
Age (years)	29.71 (10.25)	28.08 (7.63)	t=1.606, p=0.1169
Height (cm)	174.91 (6.57)	184.04 (7.24)	t=4.052, p=0.0003**
Weight (kg)	85.13 (17.83)	81.79 (10.78)	t=0.7066, p=0.4844
BMI	27.7 (4.34)	24.1 (3.76)	t= 2.277, p= 0.032*
Education (years post 16)	3.47 (1.66)	6.70 (1.63)	t= 5.96, p=0.0001**
Ethnicity	White Caucasian (15) Caribbean (x1) Asian (x2)	White Caucasian (18) Asian (x2) Hispanic (x1)	NA
NART Errors	13.92 (4.92)	9.95 (3.30)	t= 2.46, p= 0.019*
Participants with nicotine addiction	2	2	NA

Table 10: GD study demographics

Body Mass Index; BMI. National Adult Reading Test; NART. Gambling disorder: GD, Healthy controls; HC.

Questionnaire and clinical data

Clinical characteristics showed GDs had significantly higher mean MAGS scores ($p<0.001$), PGSI ($p<0.001$), total mean GRCS ($p<0.001$) scores than HCs. GDs also had elevated BDI ($p<0.001$), UPPS-P negative urgency ($p<0.001$), GRCS inability to stop ($p<0.001$), UPPS-P emotion based rash reactions ($p<0.001$), STAI ($p<0.001$), SSAI ($p<0.001$), UCLA ($p<0.001$), GRCS interpretive bias ($p<0.001$), SPQ-B ($p<0.001$), BIS non-planning ($p<0.001$), and significantly lower SCS scores ($p<0.003$) when compared with HCs. There were no significant differences between groups in any of the other psychometric tests administered.

5-arm video cue reactivity task

Task effects

As a validation step, we wanted to see if the task engaged areas of the brain related to the cue shown in the scanner. An analysis of mean task effects across both groups was conducted to visualise these task effects. Due to excessive head motion one GD participant had to be removed from the analysis (using a predefined limit of 3mm maximum movement in any direction).

For the contrast of food video cues > neutral cues, strong activations were found in the insula, occipital cortex and robust bilateral amygdala activation was demonstrated. (see figure 21).

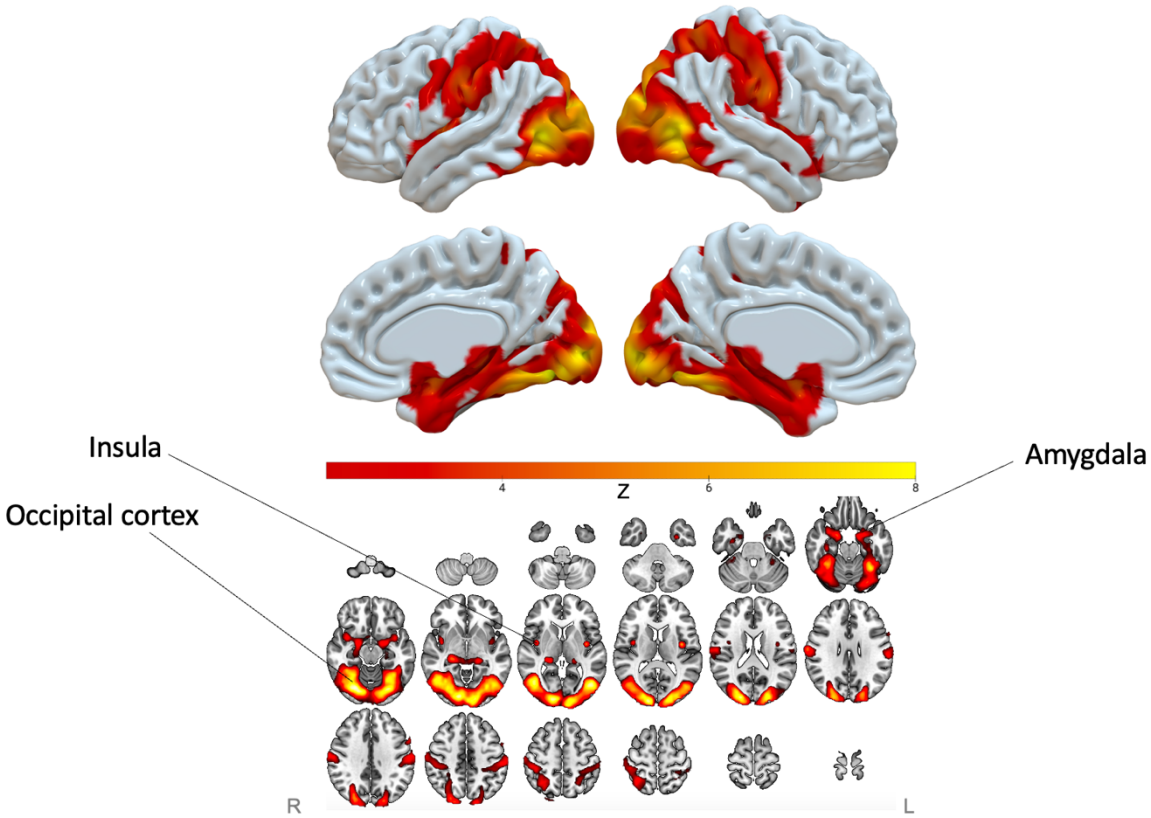


Figure 21: Whole brain Food>Neutral

Figure 21: Group average showing task effects of all participants. Red/yellow shows areas activated to the food>neutral video cues. Results are cluster corrected and thresholded to Z=2.3, P<0.05, N=38.

In the social video cues > neutral cues, strong activations were found in the mPFC, the primary visual cortex and the hippocampus. A significant cluster can also be seen in the posterior cingulate cortex and the occipital fusiform gyrus. (see figure 22).

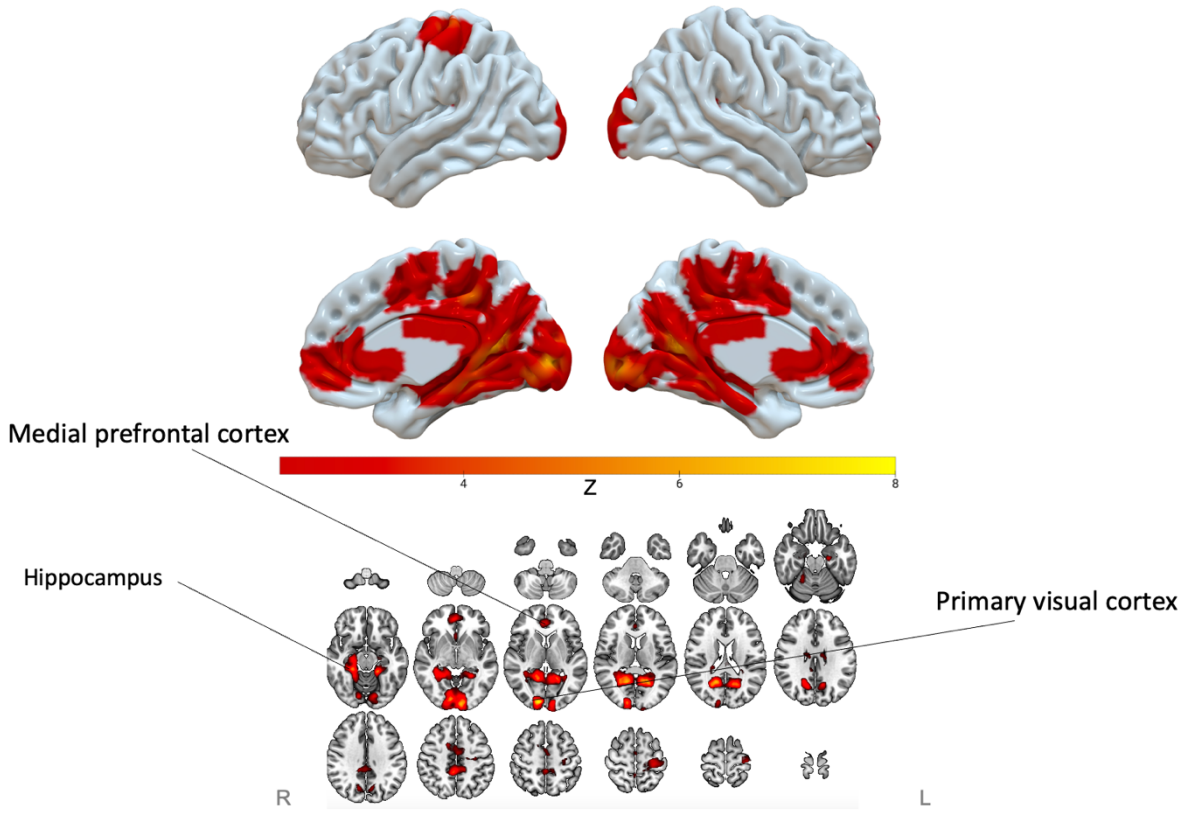


Figure 22: Whole brain Social>Neutral

Figure 22: Group average showing task effects of all participants. Red/yellow shows areas activated to the social>neutral video cues. Results are cluster corrected and thresholded to Z=2.3, P<0.05, N=38.

The nature video cues > neutral cues across all individuals demonstrated significant activations in the hippocampus and amygdala, visual area V5 and the medial prefrontal cortex (figure 23).

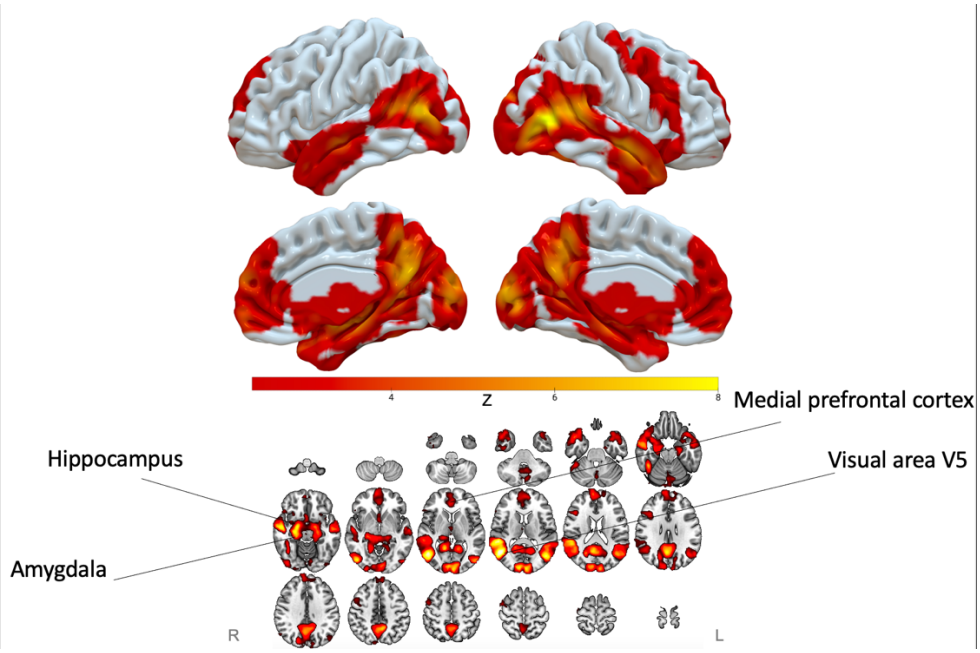


Figure 23: Whole brain Nature>Neutral

Figure 23: Group average showing task effects of all participants. Red/yellow shows areas activated to the nature>neutral video cues. Results are cluster corrected and thresholded to Z=2.3, P<0.05, N=38.

In the gambling>neutral video cue task for all individuals there was significant frontal pole activation, a large cluster over the posterior cingulate gyrus and activation in the occipital fusiform gyrus (figure 24).

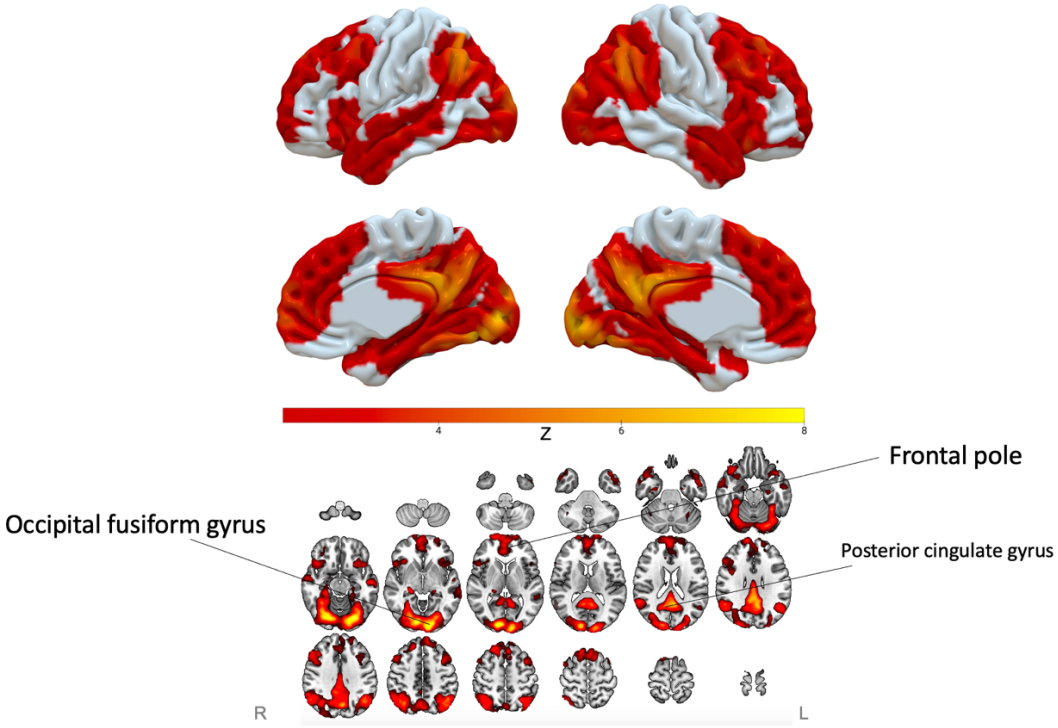


Figure 24: Whole brain Gambling>Neutral

Figure 24: Group average showing task effects of all participants. Red/yellow shows areas activated to the Gambling>neutral video cues. Results are cluster corrected and thresholded to Z=2.3, P<0.05, N=38.

Task by participant group effects

The second objective of this validation experiment was to establish any group differences between GD and HC in brain activation to these video cues. Group level analysis was conducted where the contrasts were compared between GD (N=17) and HC (N-21). The results showed significantly greater activation in the GD (gambling>neutral) > HC (gambling > neutral) contrast in the caudate and putamen, and the thalamus. There was a large cluster of activation covering these limbic regions with no cortical activity demonstrated as seen in figure 25.

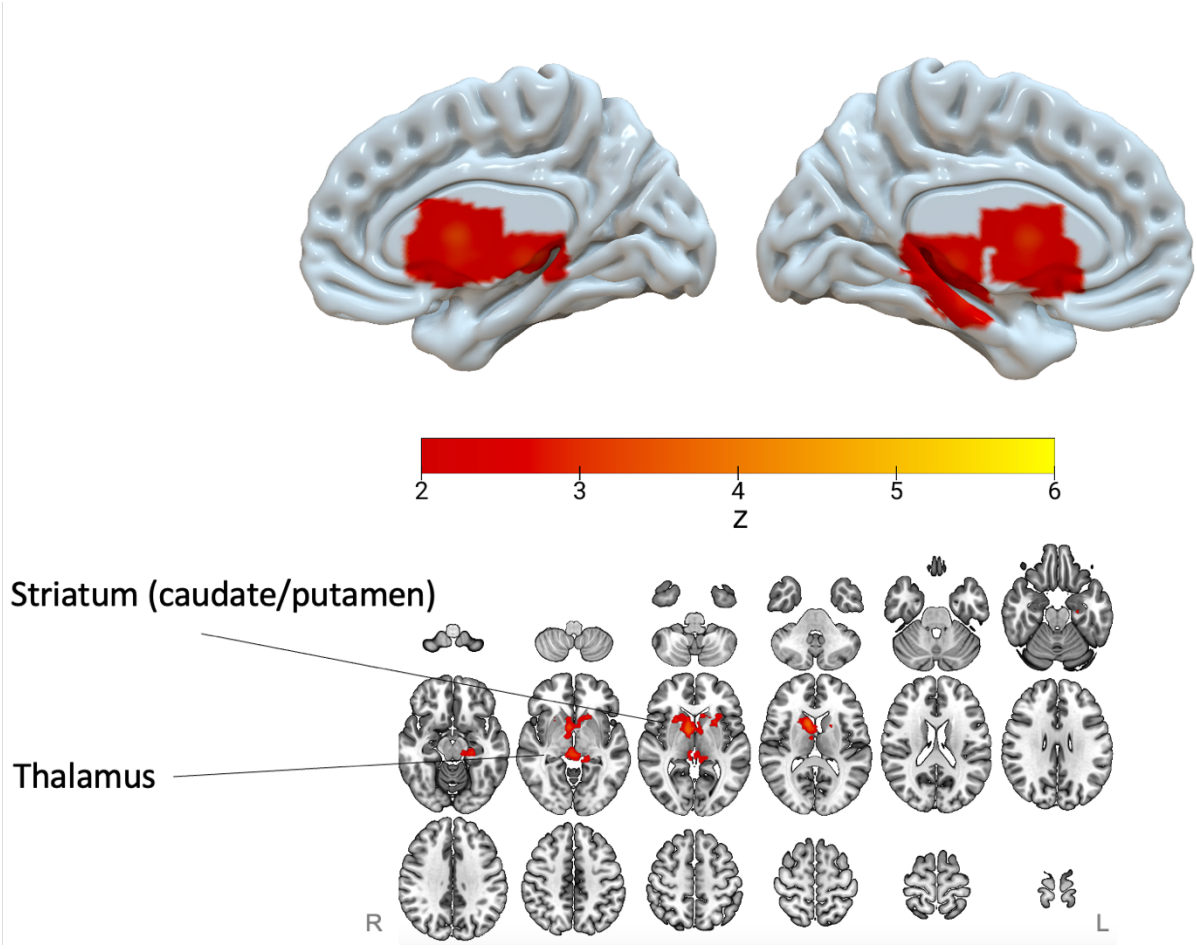


Figure 25: Whole brain Gambling>Neutral GD>HC

Figure 25: Between group contrast (GD > HC) showing greater activation of the striatum and thalamus in GD subjects to gambling > neutral cues than HCs. Results are cluster corrected and thresholded to Z=2.3, P<0.05, N=17 GD and N=21 HC.

Another whole brain between group effect was seen in the nature > neutral contrasts for the GD > HC comparison where GD individuals showed significantly increased activation in the left insula and the left superior temporal gyrus compared with HCs (Figure 26).

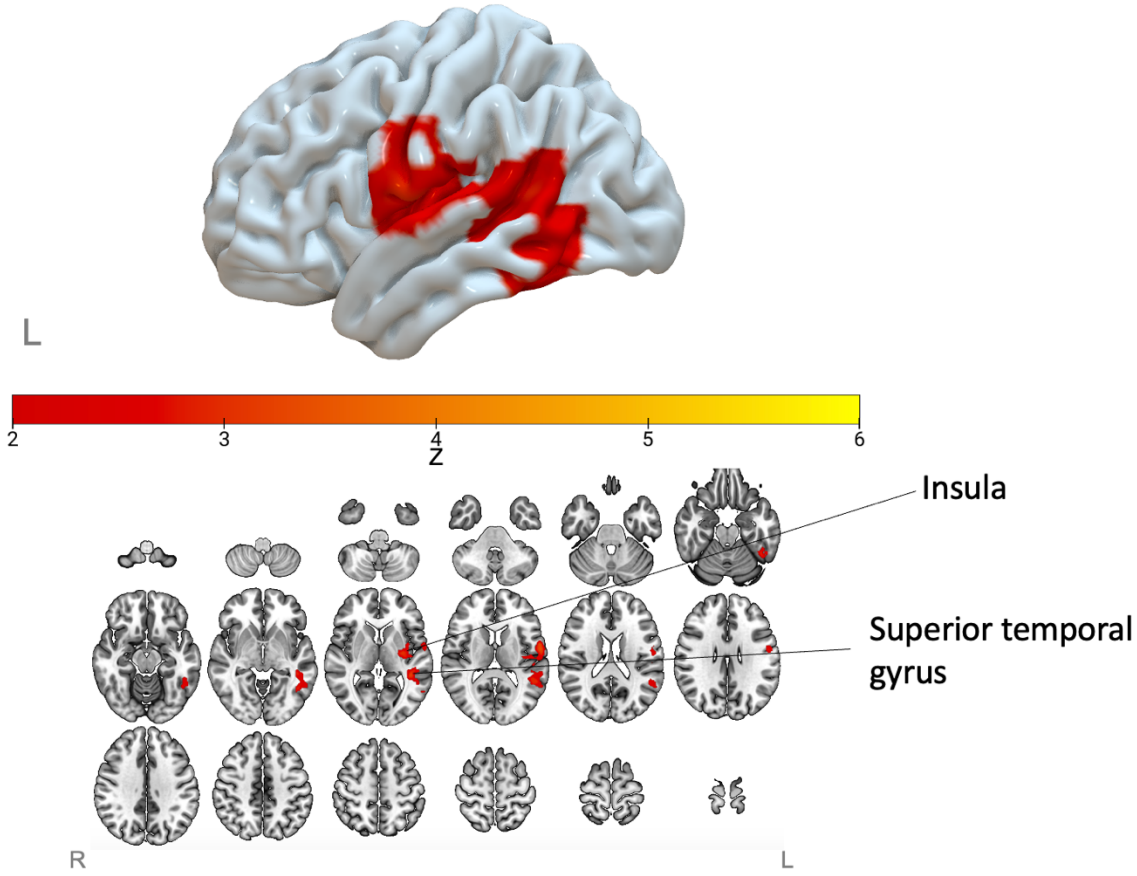


Figure 26: Whole brain Nature>Neutral GD>HC

Figure 26: Between group contrast (GD > HC) showing greater activation of the insula and superior temporal gyrus in GD subjects to nature > neutral cues than HCs. Results are cluster corrected and thresholded to Z=2.3, P<0.05, N=17 GD and N=21 HC.

No other between group effects were observed in the whole brain activation analysis for any of the other contrasts explored. These were food>neutral, social>neutral, gambling>food, gambling>nature, gambling>social, food>gambling, nature>gambling and social>gambling.

A summary of the MNI coordinates and cluster size and location are described below

Contrast	*Region*	*Cluster size (voxels)*	*MNI coordinates*	*p-Value*	*Direction*
Food>Neutral	*Insula/Amygdala*	*35797*	*12 -94 2*	*0.001*	*group*
Nature>Neutral	*Amygdala/Hippocampus*	*15764*	*-8 -96 -2*	*0.005*	*group*
Nature>Neutral	*mPFC*	*1082*	*2 50 -4*	*0.001*	*group*
Social>Neutral	*Hippocampus/Striatum*	*35025*	*54 -66 8*	*0.001*	*group*
Social>Neutral	*mPFC*	*3848*	*2 62 24*	*0.001*	*group*
Gambling>Neutral	*Frontal pole*	*33553*	*-22 -82 -16*	*0.001*	*group*
Gambling>Neutral	*Hippocampus/Amygdala*	*15497*	*-4 20 66*	*0.001*	*group*
Gambling>Neutral	*Occipital fusiform gyrus*	*3325*	*-46 18 -8*	*0.001*	*group*
Gambling>Neutral	*Posterior cingulate gyrus*	*1290*	*-46 12 48*	*0.002*	*group*
Gambling>Neutral	*Striatum*	*1523*	*4 4 8*	*0.001*	*GD>HC*
Gambling>Neutral	*Thalamus*	*744*	*-6 -38 2*	*0.037*	*GD>HC*
Nature>Neutral	*Insula*	*739*	*-60 -4 6*	*0.0404*	*GD>HC*
Nature>Neutral	*Superior temporal gyrus*	*1169*	*-60 -54 12*	*0.00339*	*GD>HC*

ROI analysis 5-ACR

In the present investigation, regions of interest (ROIs) were *a priori* defined as pivotal nodes within the reward circuits, as stipulated in the methodology, to observe the outcomes of the 5-arm cue-reactivity task. Extraction of temporal time series data from each distinct ROI within each task was conducted, followed by mixed-model analysis of variance (ANOVA) and unpaired t-tests. Corrections for multiple comparisons were applied using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995) .

There were several statistically significant main and interaction effects, elucidating the interplay between cue type, ROI, and participant group. A main effect was found for cue type ($F(4,144) = 2.47$, $p = 0.047$), reflecting the influence of discrete cue types on the observed neural responses. Likewise, a substantial main effect was observed concerning the ROI ($F(7,252) = 22.90$, $p = 0.001$), demonstrating the distinct roles of different ROIs in modulating the neural substrates.

Further, a main effect of participant group was found ($F(1,36) = 4.19$, $p = 0.048$), showing differential neural activations between the GD and HC cohorts. Notably, a salient interaction effect emerged between ROI and participant group ($F(7,242) = 2.79$, $p = 0.008$), which shows the modulatory influence of ROI-specific patterns in the context of divergent participant groups. Similarly, an interaction of notable significance was identified between Cue type and participant group ($F(28,1008) = 8.54$, $p = 0.001$), signifying the interplay between distinct cue categories and participant cohorts in shaping neural responses.

Post hoc analyses were performed to assess distinctions between the brain responses to specific cues in specific ROIs in the GD and HC groups respectively. Particularly, heightened activity in the anterior insula in response to food cues was found in the GD ($t(36) = 3.48$, $p = 0.001$). Analogously, distinct patterns emerged for nature cues, with the anterior cingulate cortex (ACC) exhibiting heightened activity ($t(35) = 2.87$, $p = 0.007$), alongside the anterior insula ($t(35) = 3.17$, $p = 0.003$) and midbrain ($t(35) = 3.07$, $p = 0.004$) in the GD group when compared to HC. Furthermore, during exposure to gambling cues, the ACC has increased activity ($t(35) = 2.51$, $p = 0.017$), accompanied by the anterior insula ($t(35) = 4.03$, $p = 0.0003$), midbrain ($t(35) = 2.4$, $p = 0.022$), and ventral striatum ($t(35) = 2.87$, $p = 0.006$) in the GD group thereby highlighting the disparate neural responses of GD and HC groups to this relevant cue stimuli. A summary of these findings are displayed in figure 27.

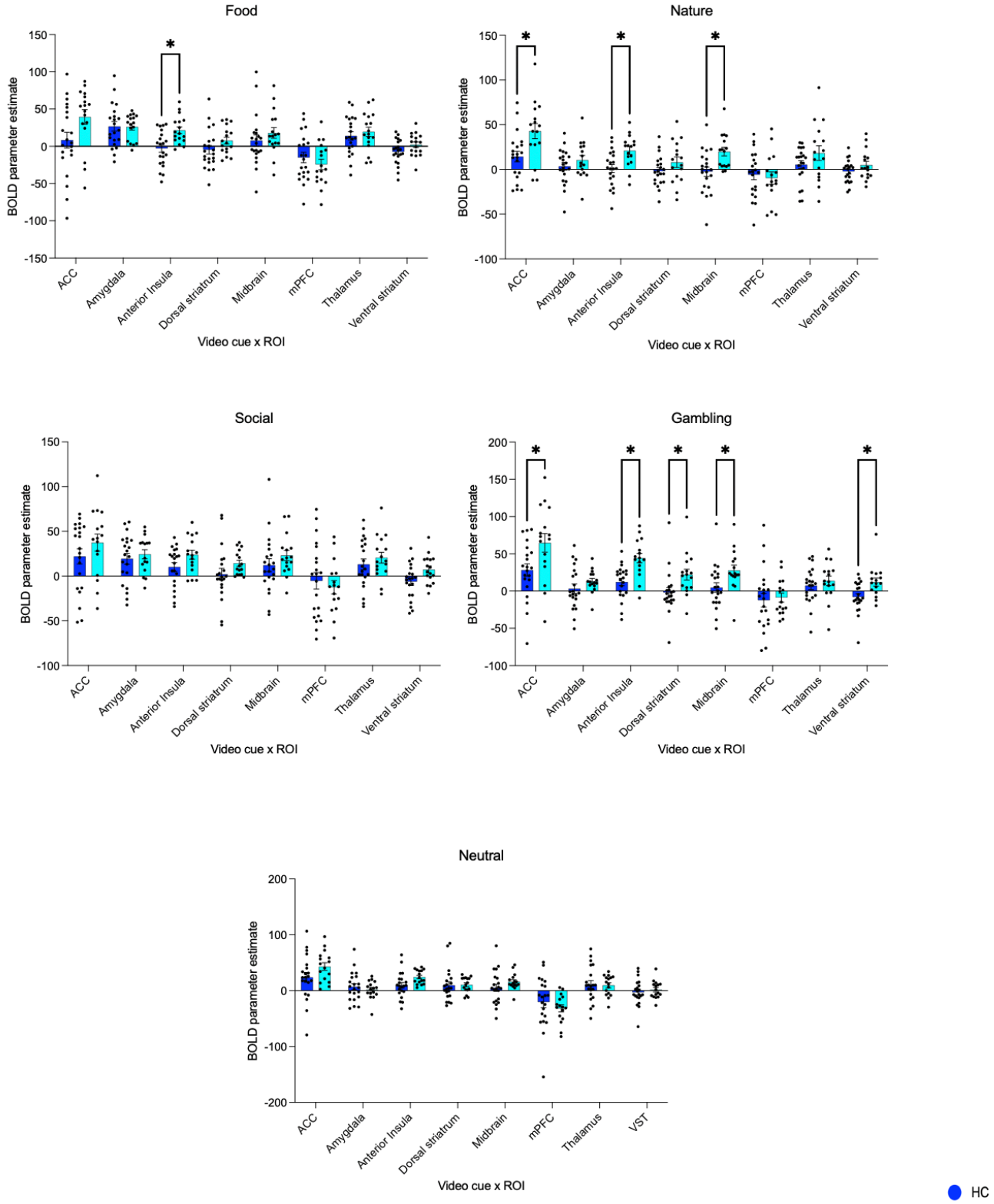


Figure 27: 5-ACR ROI T-tests

Figure 27: *Post hoc* multiple comparison corrected t-tests between GD and HC comparing ROI activation to the 5-ACR. Benjamini-Hochberg multiple comparison correction applied.

Tables highlighting results of these unpaired T-tests are shown below:

Table 11: Post hoc unpaired t-test of Food cues

ROI	P value	Mean of HC	Mean of GD	Difference	SE of difference	t ratio	df
ACC	0.041441	8.363	39.46	-31.10	14.71	2.115	36.0
Amygdala	0.930391	26.81	26.10	0.7071	8.038	0.08797	36.0
Anterior Insula	**0.001328***	**-2.914**	**21.63**	**-24.55**	**7.052**	**3.481**	**36.0**
Dorsal striatrum	0.112751	-4.658	7.740	-12.40	7.626	1.626	36.0
Midbrain	0.342912	7.720	17.93	-10.21	10.63	0.9611	36.0
mPFC	0.351254	-15.07	-24.44	9.366	9.917	0.9444	36.0
Thalamus	0.516046	14.02	19.53	-5.501	8.387	0.6559	36.0
Ventral striatum	0.080638	-7.347	2.071	-9.418	5.239	1.798	36.0

Table 11: 5-ACR Food T-tests

*denotes significant between group differences as assessed with Benjamini-Hochberg multiple comparison procedure.

Table 12: Post hoc unpaired t-test of Nature cues

ROI	P value	Mean of HC	Mean of GD	Difference	SE of difference	t ratio	df
ACC	**0.006864***	**14.38**	**42.85**	**-28.48**	**9.911**	**2.873**	**35.00**
Amygdala	0.310992	3.510	10.42	-6.912	6.724	1.028	35.00
Anterior Insula	**0.003162***	**0.9694**	**21.08**	**-20.11**	**6.344**	**3.170**	**35.00**

Dorsal striatrum	0.145393	-1.873	8.051	-9.924	6.664	1.489	35.00
Midbrain	**0.004188***	**-2.465**	**20.00**	**-22.46**	**7.331**	**3.064**	**35.00**
mPFC	0.665933	-5.898	-9.721	3.823	8.779	0.4354	35.00
Thalamus	0.147589	5.375	18.30	-12.92	8.725	1.481	35.00
Ventral striatum	0.141769	-2.307	4.939	-7.246	4.820	1.503	35.00

Table 12: 5-ACR Nature T-tests

*denotes significant between group differences as assessed with Benjamini-Hochberg multiple comparison procedure

Table 13: Post hoc unpaired t-test of Social cues

ROI	P value	Mean of HC	Mean of GD	Difference	SE of difference	t ratio	df
ACC	0.237387	21.99	37.38	-15.39	12.80	1.202	35.00
Amygdala	0.549063	19.35	24.33	-4.986	8.240	0.6050	35.00
Anterior Insula	0.064459	10.16	23.88	-13.72	7.184	1.909	35.00
Dorsal striatum	0.137415	2.054	14.31	-12.25	8.060	1.520	35.00
Midbrain	0.264836	12.16	23.08	-10.92	9.632	1.133	35.00
mPFC	0.562534	-5.311	-12.59	7.275	12.44	0.5846	35.00
Thalamus	0.394283	13.13	20.61	-7.480	8.673	0.8625	35.00
Ventral striatum	0.026525	-6.233	7.316	-13.55	5.857	2.313	36.00

Table 13: 5-ACR Social T-tests

Table 14: Post hoc unpaired t-test of Gambling cues

ROI	P value	Mean of HC	Mean of GD	Difference	SE of difference	t ratio	df
ACC	**0.016730***	**28.25**	**64.74**	**-36.49**	**14.52**	**2.513**	**35.00**
Amygdala	0.226014	3.312	13.06	-9.744	7.906	1.232	35.00
Anterior Insula	**0.000286***	**12.19**	**44.22**	**-32.03**	**7.948**	**4.030**	**35.00**
Dorsal striatrum	**0.017814***	**-1.860**	**22.06**	**-23.92**	**9.619**	**2.487**	**35.00**
Midbrain	**0.022046***	**4.831**	**27.82**	**-22.99**	**9.593**	**2.396**	**35.00**
mPFC	0.770562	-12.22	-8.771	-3.451	11.74	0.2939	35.00
Thalamus	0.451381	7.396	13.75	-6.354	8.343	0.7616	35.00
Ventral striatum	**0.006896***	**-7.908**	**12.14**	**-20.04**	**6.981**	**2.871**	**35.00**

Table 14: 5-ACR Gambling T-tests

*denotes significant between group differences as assessed with Benjamini-Hochberg multiple comparison procedure.

Table 15: Post hoc unpaired t-test of Neutral cues

ROI	P value	Mean of HC	Mean of GD	Difference	SE of difference	t ratio	df
ACC	0.108007	23.84	43.24	-19.40	11.76	1.649	35.00
Amygdala	0.489509	6.933	1.713	5.220	7.473	0.6985	35.00
Anterior Insula	0.026103	9.656	24.35	-14.70	6.327	2.323	35.00
Dorsal striatrum	0.905673	9.622	10.57	-0.9532	7.986	0.1194	35.00
Midbrain	0.199264	5.249	15.90	-10.65	8.136	1.308	35.00
mPFC	0.374028	-20.32	-31.86	11.54	12.82	0.9005	35.00
Thalamus	0.849188	11.27	9.571	1.702	8.886	0.1916	35.00

Ventral striatum	0.519956	-2.678	1.692	-4.369	6.723	0.6500	35.00

Table 15: 5-ACR Neutral T-tests

Correlation analysis 5-ACR

Correlational analyses were performed to look at the correlations between GD brain activation in the GD group to gambling cues in the 5-arm cue reactivity test as per a *priori* hypotheses. Clinical scores from the BIS, PGSI, GRCS, MAGS and GUQ questionnaires were correlated with time series data extracted from the anterior insula and VST. Normality tests were performed on the data to determine whether Pearson's or Spearman's correlation were the most appropriate test to be used and one-tailed positive correlations were performed as per our *a priori* hypothesis. Descriptive statistics and normality tests for these data are shown below in table 16.

	Anterior insula GD	**VST GD**	**BIS**	**PGSI**	**GRCS**	**MAGS**	**GUQ**
N	17	17	16	16	16	15	16
Missing	0	0	1	1	1	2	1
Mean	42.9	11.7	22.9	19.3	15.4	3.16	16.4
Median	42.2	10.2	22.7	19.5	16.8	3.31	16.5
Standard deviation	25.2	21.0	2.22	4.40	5.49	1.25	7.81
Minimum	-9.27	-19.6	20.0	10	5.00	0.570	6
Maximum	87.7	76.3	27.3	27	23.7	5.45	29
Shapiro-Wilk W	0.982	0.855	0.945	0.952	0.947	0.973	0.930
Shapiro-Wilk p	0.975	0.013	0.416	0.529	0.443	0.906	0.248

Table 16: GD correlations

Table 16: Descriptive statistics and normality data for correlation analysis in GD subjects for the 5-ACR.

Bivariate Pearson's correlations in the GD group was applied to data extracted from the anterior insula to gambling cues revealed no significant correlation between this ROI and the BIS (r= -0.117, p= 0.665), GRCS (r= - 0.195, p= 0.468), MAGS (r= 0.247, p=0.375), PGSI, (r=0.049, p=0.858), and GUQ (r=0.179, p=0.507). Similarly, VST activation to gambling video cues in the GD group was correlated with the same clinical variables. Spearman's rho was applied due to the data being non-normally distributed. No significant correlations were detected between the VST ROI and BIS (r= -0.242, p= 0.816), GRCS (r= - 0.075, p= 0.391), MAGS (r=- 0.206, p=0.770), PGSI (r=-0.476, p=0.969) and GUQ (r=-0.131, p=0.686).

Reward enriched roulette task

The second fMRI task to validate was the reward-enriched roulette task. This task was designed to activate the reward system and to be used in future simultaneous fMRI-PET experiments to explore the relationship between reward processing and neurotransmitter release.

For this experiment we trialled the paradigm on 37 participants (both groups). Two individuals from the GD group were excluded from the task due to excessive head motion (using a predefined limit of 3mm maximum movement in any direction) thus there was a total of 16 GD and 21 HC analysed for this task. All results are cluster corrected and thresholded at Z=2.3, P=0.05.

A full group analysis was initially conducted on the Hit>neutral and Miss>neutral contrasts to establish if the task activated areas involved with both win and loss reward processing.

For the mean task effects analysis (N=37) in the Hit > neutral contrast significant activations were observed in several regions involved in reward processing including the striatum (caudate, putamen, ventral striatum and nucleus accumbens) as well as the insula and the medial prefrontal cortex (figure 28).

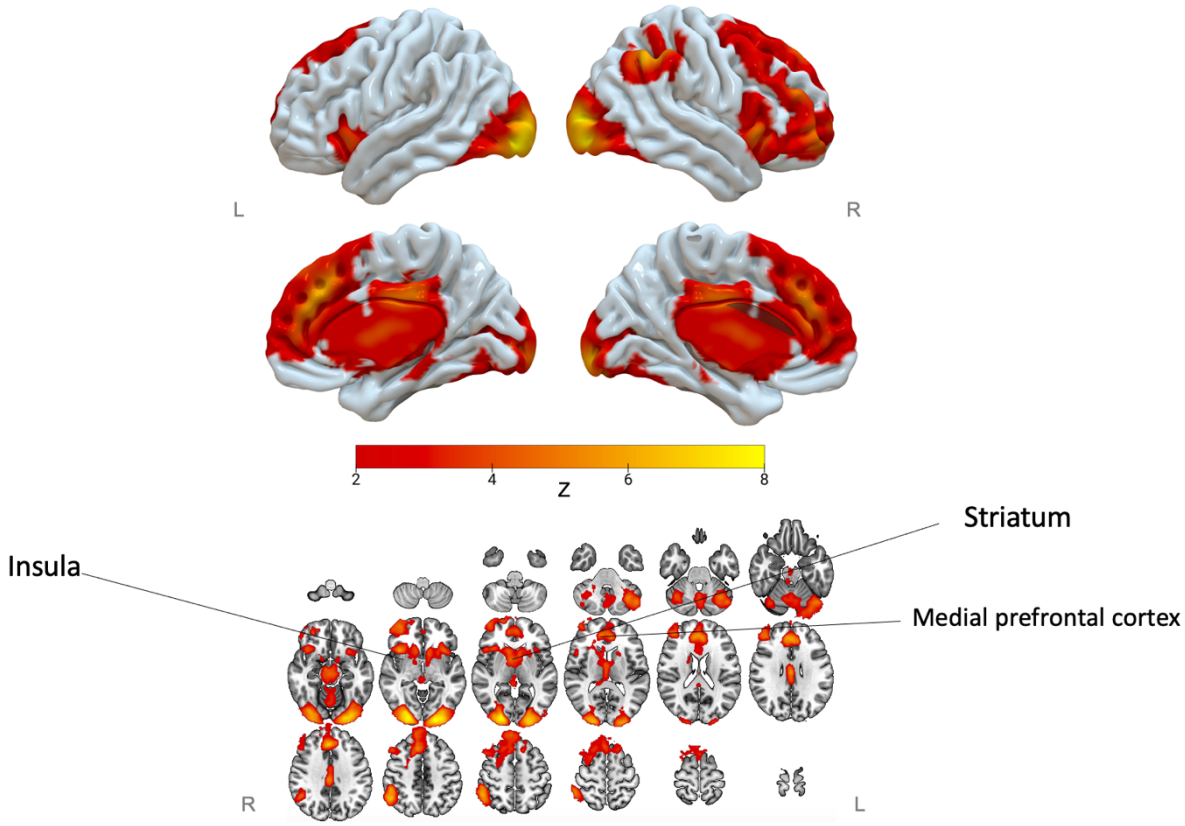


Figure 28: Whole brain Hit>Neutral

Figure 28: Group average showing task effects of all participants. Red/yellow shows areas activated to the hit>neutral roulette cues. Results are cluster corrected and thresholded to Z=2.3, P<0.05, N=37.

For the Miss > neutral contrasts for all participants, significant clusters of activation were found in the anterior cingulate cortex and the superior frontal gyrus (figure 29).

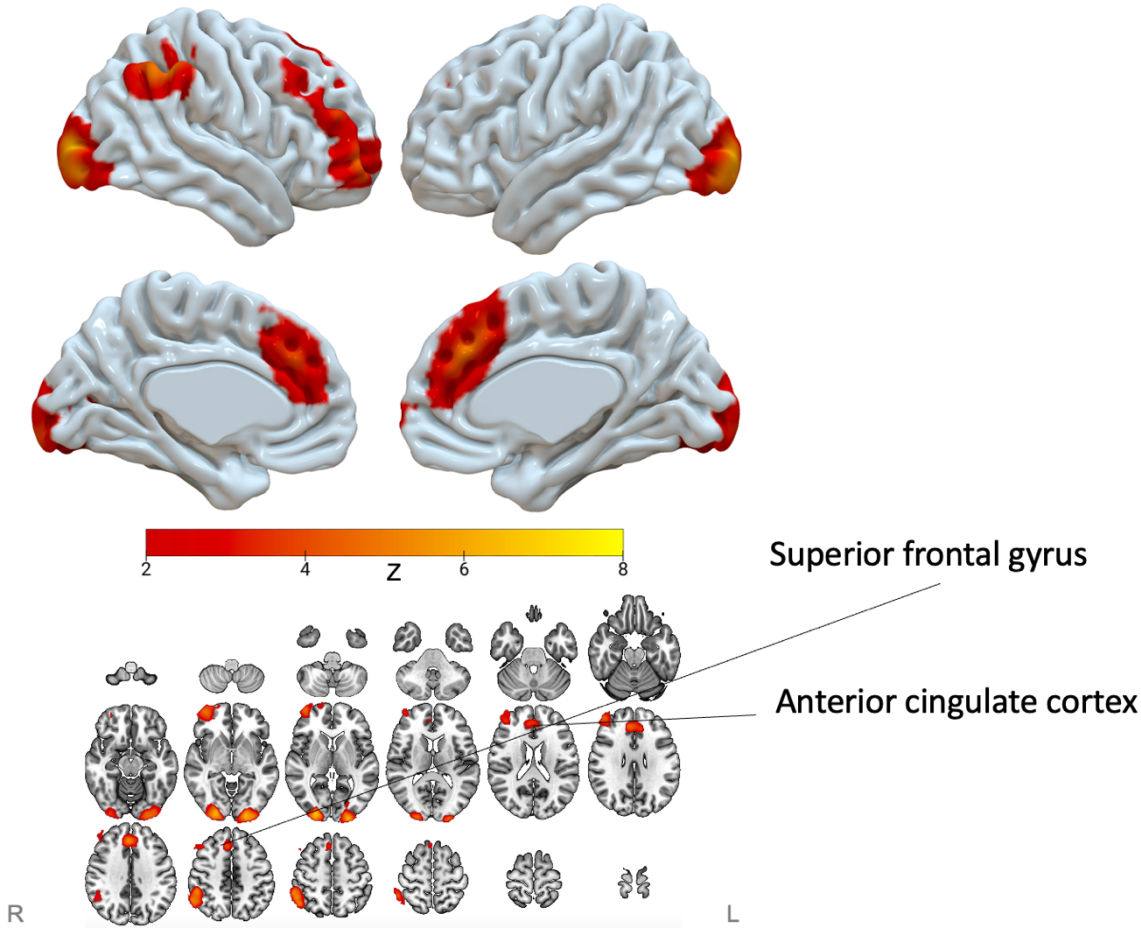


Figure 29: Whole brain Miss>Neutral

Figure 29: Group average showing task effects of all participants. Red/yellow shows areas activated to the miss>neutral roulette cues. Results are cluster corrected and thresholded to Z=2.3, P<0.05, N=37.

Task by participant group effects

For this task we conducted a between-groups comparison to understand the differential processing between GD and HC to reward in this task.

In the roulette Hit > miss contrast comparing GD to HC there was a significant increase in activation in the right lateral occipital cortex, the left insula and the left frontal gyrus. This lateralisation can be viewed in Fig 30.

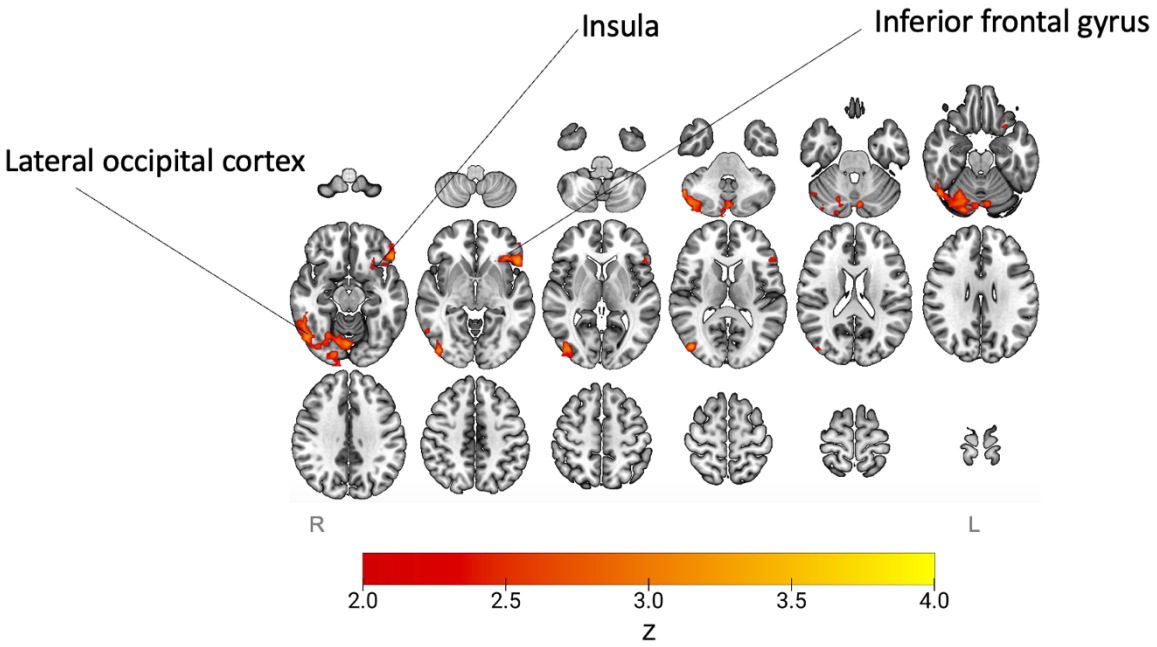


Figure 30: RER Hit>Miss GD>HC

Figure 30: Between group contrast (GD > HC) showing greater activation of the left inferior frontal gyrus, left insula and the lateral occipital cortex in GD subjects compared with HC in the Hit > miss contrast. Results are cluster corrected and thresholded to Z=2.3, P<0.05, N=16 GD and N=21 HC.

In the miss > neutral contrast for the HC > GD condition there was a significant deactivation of the left inferior frontal gyrus and the left insula (as seen in fig 31). There were no other whole brain effects between groups for any other contrast explored.

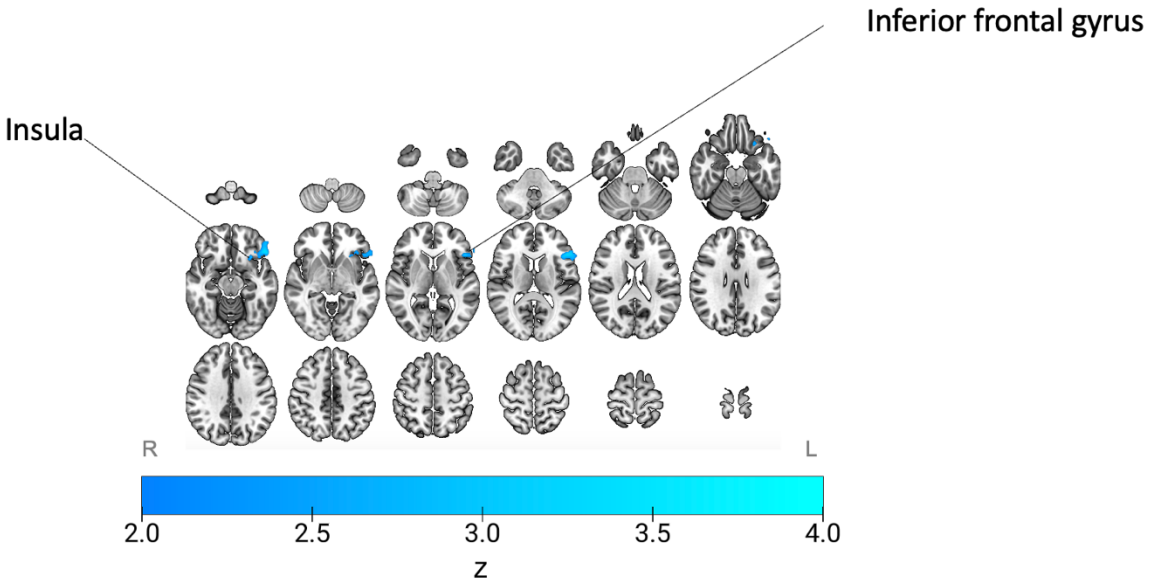


Figure 31: Whole brain Miss>Neutral HC>GD

Figure 31: Between group contrast (GD > HC) showing greater decreased activation of the left inferior frontal gyrus and the left insula HC subjects compared with GD in the Miss > neutral contrast. Results are cluster corrected and thresholded to Z=2.3, P<0.05, N=16 GD and N=21 HC.

A summary of the MNI coordinates and cluster locations and sizes are described below

Contrast	*Region*	*Cluster size (voxels)*	*MNI coordinates*	*p-Value*	*Direction*
Hit>Neutral	*Striatum*	*26674*	*-22 -98 -4*	*0.001*	*group*
Hit>Neutral	*Insula*	*4317*	*25 -94 0*	*0.001*	*group*
Hit>Neutral	*mPFC*	*2029*	*52 -44 58*	*0.001*	*group*
Miss>Neutral	*Superior frontal gyrus*	*1842*	*42 58 0*	*0.001*	*group*
Miss>Neutral	*Anterior cingulate cortex*	*1633*	*-22 -98 -6*	*0.001*	*group*
Hit>Miss	*Inferior frontal gyrus/Insula*	*875*	*-50 20 -6*	*0.0232*	*GD > HC*
Miss>Neutral	*Inferior frontal gyrus/Insula*	*1009*	*-48 20 -8*	*0.0123*	*HC > GD*

ROI analysis

For the roulette task there was a significant main effect of condition ($F(2,70) = 14.33$, $p = 0.01$) but no main effect of ROI. There was a significant interaction effect between task condition and ROI ($F(8,280) = 8.92$, $p=0.01$) however no other interaction effects were observed including no significant differences between patient groups, or interactions involving the between-subjects factor.

Post hoc tests showed significant effects in the comparison of Hit compared to Miss ($t(35) = 4.19$, $p = 0.01$) and Hit compared to Neutral ($t(35) = 5.38$, $p = 0.01$) with Hit demonstrating greater activation across all subjects.

There were no significant between group effects in ROI activation to any of the task conditions as shown in figure 32.

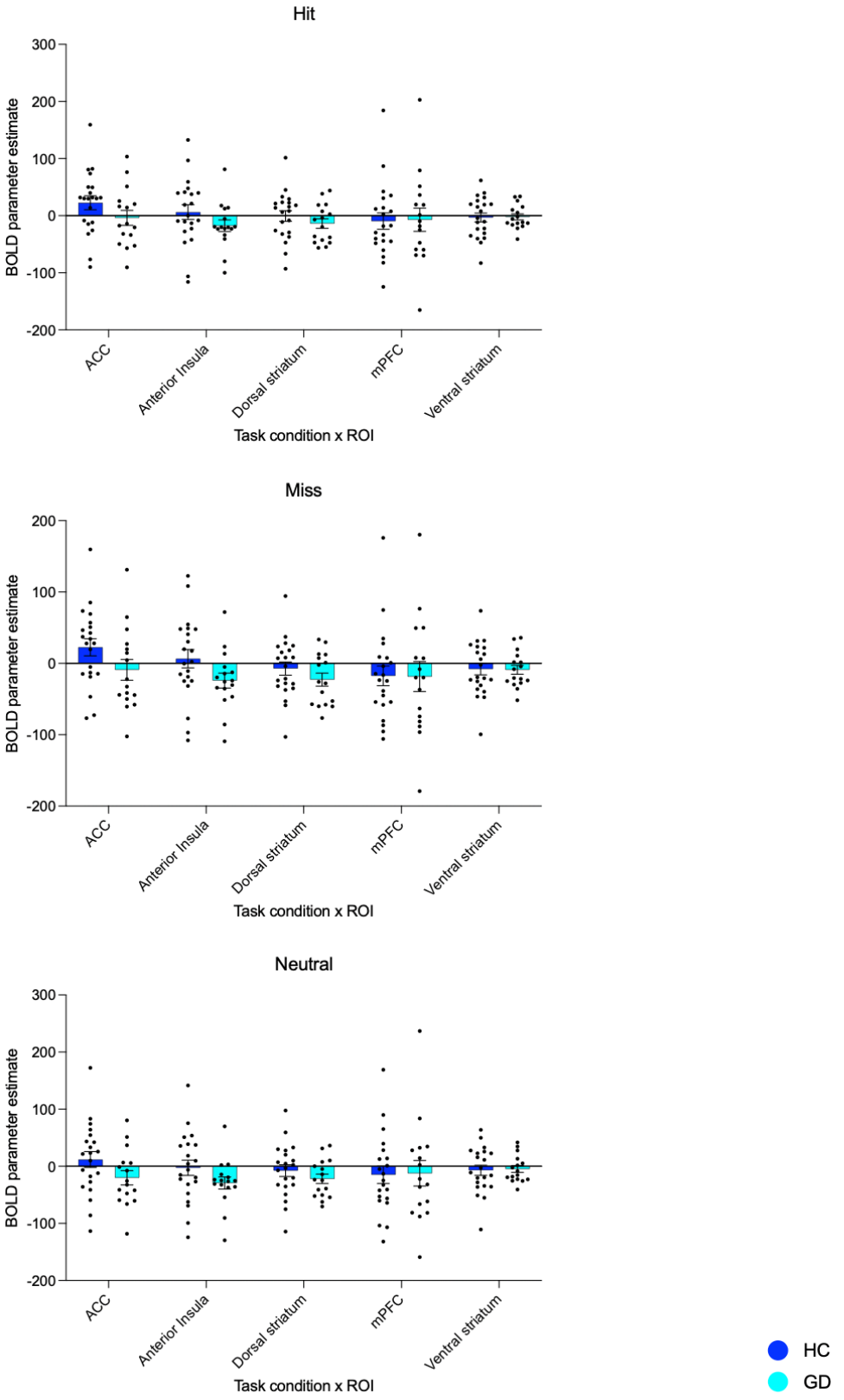


Figure 32: RER ROI T-tests

Figure 32: *Post hoc* multiple comparison corrected t-tests between GD and HC comparing ROI activation to the 5-ACR. Bejamini-Hochberg multiple comparison correction applied.

Table 17: Post hoc unpaired t-test of Hit cues

ROI	P value	Mean of HC	Mean of GD	Difference	SE of difference	t ratio	df
ACC	0.149489	22.67	-3.733	26.40	17.91	1.474	35.00
Anterior Insula	0.173172	6.315	-17.61	23.93	17.21	1.390	35.00
Dorsal striatrum	0.309421	-0.5475	-13.85	13.30	12.90	1.031	35.00
mPFC	0.919519	-9.528	-7.077	-2.451	24.08	0.1018	35.00
Ventral striatum	0.896603	-3.300	-1.986	-1.314	10.04	0.1309	35.00

Table 17: RER Hit T-tests

Table 18: Post hoc unpaired t-test of Miss cues

ROI	P value	Mean of HC	Mean of GD	Difference	SE of difference	t ratio	df
ACC	0.103088	22.34	-9.092	31.43	18.78	1.674	35.00
Anterior Insula	0.087664	6.326	-24.07	30.40	17.30	1.757	35.00
Dorsal striatrum	0.253687	-7.548	-22.77	15.22	13.12	1.161	35.00
mPFC	0.972347	-17.66	-18.50	0.8455	24.22	0.03491	35.00
Ventral striatum	0.936665	-8.148	-9.026	0.8779	10.97	0.08004	35.00

Table 18: RER Miss T-tests

Table 19: Post hoc unpaired t-test of neutral cues

ROI	P value	Mean of HC	Mean of GD	Difference	SE of difference	t ratio	df
ACC	0.106673	11.81	-20.08	31.89	19.26	1.656	35.00
Anterior Insula	0.146066	-2.763	-29.33	26.57	17.87	1.487	35.00
Dorsal striatrum	0.308794	-7.671	-21.95	14.28	13.82	1.033	35.00
mPFC	0.917138	-15.00	-12.25	-2.748	26.23	0.1048	35.00
Ventral striatum	0.840907	-7.136	-4.823	-2.313	11.44	0.2022	35.00

Table 19: RER Neutral T-tests

Roulette task correlation analysis

Correlational analyses were performed to look at the correlations between brain activation to the reward enriched roulette task and clinical scores in the GD group, as per the *a priori* hypotheses. Clinical scores from the BIS, PGSI, GRCS, MAGS and GUQ questionnaires were correlated with data extracted from the VST to Hit trials. Normality tests were performed on the data to determine whether Pearson's or Spearman's correlation were the most appropriate test to be used and one tailed positive correlations were performed as per our *a priori* hypothesis.

Descriptive statistics and normality tests for these data are shown below.

	Hit VST	**BIS**	**PGSI**	**GRCS**	**MAGS**	**GUQ**
N	16	16	16	16	15	15
Missing	0	0	0	0	1	1
Mean	-1.99	22.9	19.3	15.4	3.16	17.0
Median	-8.63	22.7	19.5	16.8	3.31	18

	Hit VST	BIS	PGSI	GRCS	MAGS	GUQ
Standard deviation	21.0	2.22	4.40	5.49	1.25	7.75
Minimum	-41.0	20.0	10	5.00	0.570	6
Maximum	33.8	27.3	27	23.7	5.45	29
Shapiro-Wilk W	0.943	0.945	0.952	0.947	0.973	0.939
Shapiro-Wilk p	0.394	0.416	0.529	0.443	0.906	0.367

Table 20: RER correlations

Table 20: Descriptive statistics and normality tests for RER HIT VST and clinical scales for correlation analysis.

Bivariate Pearson's correlations in GDs was applied to time series data extracted from the VST to the Hit contrast using one tailed correlations which revealed no significant correlation between this ROI and the BIS (r= -0.225, p= 0.779), GRCS (r= -0.242, p= 0.817), MAGS (r= -0.349, p=0.899), PGSI (r=-0.216, p=0.789), and GUQ (r=-0.073, p=0.602). In the HC group we also assessed the relationship between the VST ROI Hit time series data and the GUQ in N=12 HC. The results found no significant correlation between HIT VST and GUQ (r=-0.286, p=0.816).

Discussion

The aim of the study was to validate two novel fMRI paradigms (5-ACR and RER) exploring the brain's reward and salience system function in individuals with GD compared with HCs. In the following sections I will summarise my key findings initially and thereafter present them in context of the relevant literature.

Clinical and demographic differences between GD and HC

I found considerable differences in demographic and clinical scales used to assess psychopathology when comparing individuals with GD to HC. These included clinical

scales covering, depression, anxiety, gambling addiction, impulsivity and schizotypal personality and well as psychometric scales assessing social connectedness and demographic measures including educational attainment, height and BMI.

Educational attainment. Educational attainment has been linked with gambling severity (Williams et al., 2021), suggesting lower educational attainment may be an associated factor for developing GD. Our findings support this by identifying lower educational attainment in the GD population when compared to HCs as assessed by significantly increased NART scores and significantly reduced number of years of education post the age of 16.

Depression. The significantly higher BDI scores in GDs are consistent with previous findings suggesting that GDs are more likely to suffer from depression (Rogier et al., 2019, Sharman et al., 2021) and that gambling severity positively correlates with depression symptomatology (Schluter et al., 2019, Huțul and Karner-Huțuleac, 2022). Although there were significantly higher BDI scores, as we controlled for depression in the pre-screening the mean BDI score in GD was 12 which is under the clinical threshold.

Anxiety. There has been an increasing body of research linking anxiety with gambling severity (Huțul and Karner-Huțuleac, 2022, Cardwell et al., 2022). My findings support this with both the SSAI and STAI in GD demonstrating significantly higher scores in GD compared to HC.

Impulsivity. The relationship between impulsivity and gambling addiction has been well documented and received considerable attention in the field of addictive behaviours. (Blaszczynski and Nower, 2002, Dowling et al., 2015) have also demonstrated that engaging in chronic gambling can lead to heightened impulsivity. The phenomenon of "gambling-induced impulsivity" posits that repeated exposure to the immediate rewards and losses associated with gambling may dysregulate the neural circuitry underlying impulse control (van Holst et al., 2010). Our findings of increased facets of impulsivity in GD vs HC support these earlier findings. Such findings underscore the bidirectional nature of the impulsivity-gambling addiction relationship.

Social connectedness scale. Several studies have offered insights into the relationship between gambling addiction and social connectedness. A study by (Williams et al., 2013) found that individuals with GD reported lower levels of social connectedness compared to those without. Another study identified a negative association between social connectedness and problem gambling severity, suggesting that higher levels of social connectedness may act as a protective factor against the development of gambling addiction (Petry and Weiss, 2009). Our findings support these finding of lower social connectedness being a core facet of GD.

Cue-reactivity in GD (5-ACR)

For the 5-ACR there were significant brain activations to all video cue contrasts when compared with neutral cues in both HC and GD cohorts in regions of the frontal, salience, and reward networks of the brain demonstrating the task is effective in engaging the brain systems we wished to explore. Significantly greater activation was found in the GD vs HC cohorts in core reward regions of the basal ganglia (striatum, insula and thalamus) in response to gambling video cues in the 5-ACR. These results were confirmed by ROI analyses, which corroborated significantly increased activation in five out of eight key ROIs in reward regions in the gambling video cue vs neutral contrast in GDs compared to HCs. Intriguingly three ROIs related to nature processing and one related to food processing also seemed to be significantly elevated in the GD group. There were no significant correlations between these ROI activation patterns and clinical scores that were assessed.

Over the last couple of decades, the incentive salience theory of addiction or incentive sensitisation (a process by which classical conditioning can elicit rewarding responses to drug-related cues in the absence of the drug; (Robinson and Berridge, 2008)) has been used to explain the differential activity of reward system function in the context of addictive disorders. In the context of fMRI cue-reactivity, the theory puts forward an increase in ventral striatal and reward system activation to salient/addiction relevant cues in patients with a given addiction when compared to other cues or when compared to healthy controls (Zilverstand et al., 2018). This is purported to be because the salience system progressively amplifies the response to salient cues over time in addiction as neural pathways canalise over the repetitive behavioural/reward

processing driven by dopamine signalling events (Berridge and Robinson, 2016). This theory has been supported by a number of fMRI studies showing greater ventral striatal activity in response to gambling cues in GD (García-Castro et al., 2022). In our study we found a statistically significant increase in activation to gambling cues in GD vs HC in both whole-brain and ROI analysis in core regions of the reward circuitry including the ACC, the anterior insula, the dorsal striatum, the midbrain, and the ventral striatum. These findings support much previous gambling cue-reactivity work which has also shown greater brain activity in the ACC and insula to gambling cues in GD (Limbrick-Oldfield et al., 2017, García-Castro et al., 2022).

Our results did not demonstrate reductions in reward system activation to the naturally rewarding cues, however the incentive sensitisation theory predicts an increased response in reward-related circuitry to addiction-related cues, but is agnostic on the response to natural rewards (Leyton and Vezina, 2013b). Intriguingly, we also found relative increases in the responses to food in the anterior insula in the GD group though these findings have not been shown before representing a novel result, as well as a first finding of increased nature related processing. Though some functional imaging studies have shown increased neural activation to natural rewards in active treatment gamblers, including food, (Sescousse et al., 2013a, Noori et al., 2016, Limbrick-Oldfield et al., 2017). There is also clinical evidence of co-morbid disordered eating with gambling disorder (Lemón et al., 2021) and those with combined diagnosis generally have been seen to have worse psychopathologies. Further, many gamblers also consume food whilst gambling and there could be a potential cross cue-reactivity associating food cues to gambling memories. As these are new findings, we recommend further research is needed to explore this cross-cue hyperactivation.

Our finding lends support to the hypothesised aberrant salience response, and its potential role as a biomarker of GD although our results differed to some other studies in that we did not find associations between clinical measures, such as craving levels and gambling severity, and the brain response. There could be several reasons for this. Our GD cohort were recruited from treatment waiting lists where they were motivated to undertake treatment. Research has found that the length of abstinence in gambling relates to reduced craving (Tavares et al., 2005) and as many of our participants had described not gambling for a while or had started a preparing for

treatment it could be plausible that craving scores were not the most useful metric to describe GD related pathology. Further, our gambling severity measure (PGSI) did not correlate with neural activity to gambling video cues. The PGSI score emphasizes gambling harms (primarily financial consequences) across the last 12 months and the lack of relationship between this score and neural response was also detected in another study (Limbrick-Oldfield et al., 2017) perhaps demonstrating that this scale may not be the best to use for patients recruited from treatment waiting lists. Further it may be that there is a complex non-linear relationship between clinical measures and brain activity that may account for the inter-study differences in brain-behaviour or clinical outcome (Perez Velazquez, 2005).

Our present study found generally greater insula activity in GDs compared to HCs, and significantly greater activity in GDs compared to HCs in response to gambling cues. These findings suggest that increased insula activation in GDs may lead to a heightened detection of gambling-related stimuli at a perceptual level, lending support to the conception of addiction as a disorder of contracted conscious awareness (Metzner, 1994). The insula is involved in multimodal sensory input processing and the detection of behaviourally relevant stimuli and the concurrent coordinated response to those (Droutman et al., 2015b). Further, meta-analyses of fMRI cue reactivity demonstrate increased insula recruitment in patients with substance-use disorders (Tang et al., 2012) and particularly in more complex polymodal cue reactivity tasks which is what we describe here (Yalachkov et al., 2012). Research has also implicated a role for the insula in the pathophysiology of GD through its role in distorted cognitive processing. It has been found that damage to the insula in problem gamblers led to reductions in motivation to gamble and reduced engagement of brain circuitry to support this following a near-miss (Clark et al., 2014). These finding support a key role of the insula in supporting distorted cognitive processing of near misses and identify reductions in insula reactivity as a target for novel interventions (Clark et al., 2014). Finally, our results involving the co-activation/deactivation of the inferior frontal gyrus and insula in response to win and loss processing in the RER reflect the role of this critical brain network through which the ventral attentional network can ‘alert’ the brain to salient stimuli and as such dyfunction of which could be key in mediating addictive behaviours (Cazzoli et al., 2021)

In sum, my findings confirm a key role of the insula in modulating the underlying mechanisms of the salience network in GD, and warrant further research into the modulation of insula activity in relation to gambling related cognitive distortions and its role in treatment outcome for individuals with GD.

Reward processing task (RER)

For the RER we observed significant whole-brain activation for hit and miss trials compared with neutral. These findings (collapsed across both HC and GD) demonstrated strong activation of the ventral striatum, nucleus accumbens and some frontal regions. There was also significantly greater activation in the left insula, left inferior frontal gyrus and visual processing areas in GDs in the Hit>Miss contrast. Interestingly we saw a greater deactivation in miss processing in the HCs > GD in the same regions. There were no significant correlations between these ROI activation patterns and clinical scores that were assessed.

Previous research in gamblers with gambling related tasks such as blackjack have found increases in brain activation in GDs vs occasional gamblers in the right inferior frontal gyrus, the superior temporal gyrus and the thalamus (Miedl et al., 2010). A slot machine task also saw increases in ventral striatal responsivity in GDs vs HC (Worhunsky et al., 2014) and a gain-loss gambling task also demonstrated increased activation in the caudate nucleus in GD s HC (Gelskov et al., 2016) which is in agreement with our findings of increased left insula, left inferior frontal gyrus and bilateral occipital cortex processing in GD in Hit>Miss tasks. We also saw a significantly greater deactivation in HCs vs GD in the insula and inferior frontal gyrus in miss > neutral processing which reflects the failure of GD subjects to undertake adaptive loss processing. Similar observations have also been seen previously with amplified striatal responses to near-miss outcomes in pathological gamblers (Sescousse et al., 2016) which likely contribute to persisting gambling behaviours. Dysfunction in miss/loss processing twinned with hyperactivation to wins are known to motivate individuals to continue gambling (Bibby, 2016).

Another key objective of this task, aside from to understand reward processing in GD vs HC was to develop a combined fMRI-PET task to link rewards system processing

to neurotransmitter release function in addiction generally. There is evidence for blunted dopamine release in cocaine, opiate, and alcohol dependence but not cannabis dependence as assessed with the antagonist radioligand [11C]raclopride (Nutt et al., 2015). A recent review demonstrated that, behaviourally, blunted striatal dopamine transmission could reflect increased impulsivity and altered cost/benefit computations that are associated with addiction. The data suggest that blunted dopamine neurotransmission is a biomarker for increased risk of developing addiction and treatment-resistance, which is associated with impulsive behaviour and reduced motivation and is linked to increased drug consumption (Trifilieff et al., 2017).

Understanding the neurotransmitter events that underlie these activation patterns is necessary to develop a molecular-functional understanding of reward system dysfunction in addiction, though one study found that miss-processing was not related to dopaminergic regulation (Sescousse et al., 2016). Further, a meta-analysis has reported no significant differences in striatal $DRD_{2/3}$ receptor availability in GD relative to healthy controls (Zafar et al, in prep, chapter 2), though to date no study has assessed the role of dopaminergic neurotransmission as related to reward win/loss processing in HCs or GDs using simultaneous fMRI-PET. We found no correlation between GD roulette activation and craving or clinical scores which as mentioned previously may reflect the stage of recovery that the individuals with GD were in.

Several novel agonist radiotracers including [11C]PHNO have been found to be sensitive to endogenous dopamine (Ginovart et al., 2006). Using this radiotracer in a multimodal fMRI-PET study with behaviourally salient, dopamine-enhancing tasks such as the RER task, that probe addiction-related brain processes, could theoretically release dopamine in the living human brain. Interestingly and in direct contrast to substance use disorders, in GD a DA-releasing amphetamine challenge found significantly greater [11C]PHNO displacement (reflecting DA release) in the dorsal striatum, as well as positive correlations with gambling severity in VS (Boileau et al., 2014). These results are consistent with a hyperdopaminergic state in GD though no research to date has specifically looked at the link between behaviourally salient tasks and GD dopamine neurotransmission which arguably provides a more ecologically valid interpretation of GD molecular-function pathology. We encourage, and intend to conduct, future research specifically bridging these two imaging modalities.

Strengths and Limitations

Our tasks demonstrate an increased degree of ecologically salient validity with gambling specific games as well as videos of cues which are salient and reflect real world events, indeed realistic task designs have been shown to be one of the factors that may influence the BOLD response to nonspecific rewarding stimuli in the reward system (van Holst et al. 2010) though there is less control over low-level visual features when comparing videos to pictures.

Even though an effort was made to match demographics between patients and controls, significant variation in demographic characteristics including height, IQ, BMI and some other clinical characteristics suggests this could have been better matched. Previous research has shown that individuals with GD have a significantly higher BMI compared to HCs and this is associated with decreased brain volume and greater cognitive decline (Michaud et al., 2018; Ward et al., 2005). However, as the GD group was only classified as overweight, not clinically obese, the impact may be less significant.

Another limitation is that this study only investigated men, and even though the reported prevalence of GD is higher in men, studies should also look at women suffering from GD. This study's inclusion of only male participants, driven by the gender disparity in GD, may restrict the generalisability of findings to females with GD. As such, caution should be applied during study design when considering the application of these methods in females with GD though research is encouraged to look at neurophysiological differences across sexes in GD. A recent review has indeed highlighted neuroimaging differences in SUD research and it is possible, though currently unknown if this extends to behavioural addictions too (Cornish and Prasad, 2021). Another potential limitation is sample size, though exploratory and validation fMRI studies commonly use 20 v 20 and this sample size is consistent with empirically derived estimates to allow sufficient power to detect moderate-sized effects in fMRI studies (Murphy and Garavan, 2004).

Another recent article has highlighted the importance of using imaging measures in addiction research instead of just using drug-use outcomes (Goldstein, 2022), however, to date clinical effectiveness of imaging-based biomarkers for addictive and psychiatric disorders has been limited. A study looking at a wealth of neuroimaging measures in a very large sample of depressed patients and matched controls, found that the classification accuracy of the identified imaging markers was close to chance (53-55%) (Winter et al., 2022), even though depression is one of the better investigated psychiatric disorders in neuroimaging research. Further, despite three decades of research, psychiatric neuroimaging has not led to a single biomarker of clinical utility.

We propose that simple unimodal investigations may be inadequate for complex disorders such as GD. Different analytical approaches such as univariate inferential statistical models with multivariate machine learning or artificial intelligence models could more accurately reflect the variability of these disorders at population level (Nour et al., 2022). Further, using a multimodal approach to imaging by combining fMRI with other techniques such as PET and electroencephalography (EEG), might also be a way of getting greater granularity on the neurobiological processes underlying disorders such as GD.

Finally, there is a limited use of human neuroimaging in addiction drug development which has likely contributed largely to ineffective medicines to treat addiction disorder. Translational addiction neuropsychopharmacology research, which has been conducted previously and pioneered in centres like ours (McGonigle et al., 2017), provides the only way to objectively assess the effects of novel addiction interventions in the living human brain. Such data may pave the way for precision psychiatry efforts which aim to objectively diagnose, stratify and provide prognosis for patients.

Conclusion

In sum, the present study found the hypothesised differential neural response to gambling versus natural cues in GDs vs HCs in both video cue reactivity and the reward enriched roulette task demonstrating GD specific neural adaptations. The results suggest neural hypersensitivity to gambling-related stimuli may be occurring in

GDs which lends support to the incentive salience theory of addiction. Further, the present study revealed along with other core reward-related regions a significant role of the insula in GD neuropathology. This finding confirms the key role of the insula in GDs' responses to gambling-related stimuli and warrants a more granular investigation into its role as a modulator of the cognitive and environmental processing and associated behaviours observed in GDs. Indeed, the insula could also be a valid and valuable target for the development of novel and effective therapeutics in GD.

Chapter 5: General discussion

In this final chapter I will briefly outline the aims of the thesis and the findings from the three experimental chapters which address these aims. After laying these out I will explore the implications of the results for understanding the role of the reward system in substance use and behavioural addictions and how these insights may improve the development of novel therapeutics. I will finish by outlining future research areas from this body of work with specific reference to the use of the described neuroimaging techniques that have been validated and explored in this thesis in clinical trials of psychedelic therapy for addiction.

Briefly, the aims of this thesis were:

1) Investigating the $DRD_{2/3}$ in addiction: a comprehensive systematic review and meta-analysis
2) To explore the effects of DRD3 antagonism on reward system function using fMRI and PET in individuals with alcohol dependence
3) To validate two novel fMRI biomarker paradigms in individuals with gambling disorder:
    a) A novel reward-enriched roulette (RER) fMRI paradigm
    b) A novel 5-armed cue-reactivity (5-ACR) fMRI paradigm

Summary of main results

In chapter 2 I conducted a systematic review and meta-analysis of all available PET/SPECT neuroimaging studies comparing $DRD_{2/3}$ availability in individuals with substance use and behavioural addictions versus healthy controls. For more than 30 years the role of the $DRD_{2/3}$ in the neurobiology underlying addiction processes has been investigated in humans using PET/SPECT neuroimaging, though in recent years the function of $DRD_{2/3}$ as a generalised addiction biomarker has seen growing critique (Nutt et al., 2015). For the purpose of this investigation, I wanted to explore the differential availability of the $DRD_{2/3}$ in individuals with every sub-category of addiction versus healthy controls utilising different radioligands that could quantify both subcortical and cortical receptor availability. Additionally, I conducted analyses in studies using selective DRD3 agonist radioligands that can quantify the specific

contribution of this receptor subtype to $DRD_{2/3}$ related addiction neuropathology. Other key objectives of my critical analysis were to explore the role of confounding variables such as impulsivity, co-morbid substance use and social status on $DRD_{2/3}$ availability from the studies that had been conducted. I also wanted to establish whether there was an association between $DRD_{2/3}$ availability with regards to a dose-response relationship between drug use and effects on receptor availability and whether there was a reversal of any $DRD_{2/3}$ downregulation with abstinence.

Our main findings indicate baseline striatal $DRD_{2/3}$ binding availability was reduced in individuals with addictions to alcohol, cocaine, and methamphetamine compared with control cohorts. Conversely, discernible distinctions were not evident when comparing control groups to those with gamblingg, tobacco, opiate, or cannabis dependencies. To my knowledge, this meta-analysis is the first to combine analyses of the $DRD_{2/3}$ across both substance use and behavioural addictions, hence providing the most comprehensive account of this receptor systems role in addiction to date. These findings confirm previous research that catalysed a more critical assessment of the dopamine receptor theory of addiction (Nutt et al., 2015) and I believe these findings have significant implications for our understanding of dopamine more broadly, highlighting its dynamic role in modulation of social, behavioural, psychological and physiological processes. These results build on a body of evidence that addiction is not due to a singular molecular dysfunction and supports the concept of addiction as a multiple neurotransmitter disorder (Wiers et al., 2016a) that sits within a broader biopsychosocial framework (Skewes and Gonzalez, 2013).

I also discovered patterns of extra striatal $DRD_{2/3}$ dysfunction amongst certain addiction subtypes as assessed with high-affinity antagonist and agonist radioligands which demonstrated downregulation of extra-striatal $DRD_{2/3}$ occurring in the prefrontal cortex, insula, and temporal cortex particularly in stimulant, alcohol and tobacco addiction. Additionally, a separate meta-analytic examination of DRD_3 availability within the substantia nigra found significantly higher binding in cocaine addiction to healthy controls. These findings validate the need to develop and explore novel PET radioligands in addiction as they could offer valuable insights to discover novel targets. My results provide the first direct molecular neuroscience evidence that targeting the DRD_3 in cocaine addiction could be a plausible and potentially clinically effective

intervention which furthers the call from the National Institute on Drug Abuse's (NIDA) on targeting this receptor system for opiate addiction (Rasmussen et al., 2019), and relates to the empirical research in chapter 3 where this receptor was targeted for alcohol dependence.

The potential of a dose-response relationship between $DRD_{2/3}$ availability and the severity of substance use or addiction, as well as potential reversibility of $DRD_{2/3}$ binding following periods of abstinence was also investigated to establish the extent to which drug use played a role in addiction-related neuropathology or if individuals with addiction had genetically inherited dysregulation in dopamine receptor function. My findings cast uncertainty upon the presence of definitive evidence supporting dose-response dynamics and the reversibility of $DRD_{2/3}$ binding in the different subcategories of addiction explored. The literature displayed a mosaic of methodologies to report these variables and heterogeneous outcomes, contributing to this inconclusiveness. Given the inconsistency in results across addiction it is likely that there will continue to be no resolute answer to the 'nature vs nurture' conundrum of addiction aetiology. More contemporary models describe a combination between inherited biological traits and epigenetic modification leading to neural adaptations that underly the cognitive and behavioural processes involved in the risk of developing addiction, relapse and recovery (Green et al., 2021).

My final analysis revealed that early PET investigations exhibited notable shortcomings in statistical control, particularly concerning clinical confounders such as socioeconomic status, impulsivity, and concurrent use of alcohol and tobacco. Nevertheless, efforts to address these deficiencies have grown over time, with enhanced matching between case and control groups. Despite this, there persists substantial variability in the reporting of confounding variables across different addiction subtypes, with an overall inadequacy in their documentation. The main finding from this analysis identified a substantial inverse correlation between inter-group differences in striatal $DRD_{2/3}$ binding among individuals with addiction and controls and scores assigned to studies reflecting the quality of correction for confounding factors across all addiction subcategories. This association indicated that studies incorporating more robust confounder correction methods tended to exhibit diminished differences in $DRD_{2/3}$ availability between cases and controls. This

observation raises questions regarding the dopamine theory of addiction, or at the very least, the extent to which human $DRD_{2/3}$ PET/SPECT data bolster this theory. My findings bear notable implications for the prevailing understanding of dopamine's role in addiction and call for greater molecular neurobiology research into the role of other neurotransmitter systems including the opiate (Belzeaux et al., 2018), serotonin (Müller and Homberg, 2015), endocannabinoid (Parsons and Hurd, 2015) and GABAergic systems (Nutt et al., 2013) which to date have received comparatively limited attention.

For chapter 3 I used fMRI and PET to investigate the effects of DRD3 antagonism on BOLD response to the MIDT in AUD and healthy controls. My findings suggest that in the AUD group, GSK598809 heightened the brain's response to the anticipation of rewards in the ventral striatum and insula when compared to baseline, whereas the HC group exhibited deactivation in these regions. This discovery of GSK598809 increasing activation in the ventral striatum and insula during reward processing in individuals with AUD builds upon earlier research indicating a broader beneficial impact of GSK598809 on enhancing the BOLD response related to reward anticipation in this particular brain region (Murphy et al., 2017c). This result confirms that D3 antagonism works on a core feature of reward system dysfunction in addiction and is evidence to suggest it may have efficacy as a clinical tool. Another key finding was a specific reduction in mPFC activity to loss reward processing. This is a novel finding not reported elsewhere in the literature that proposes DRD3 antagonism as being a modulator of punishment or stress-related frontal brain activity in individuals with AUD which is a known functional target for addiction broadly (Koob, 2021). The fMRI results proposed a pharmacologically induced bidirectional effect on brain function suggesting that DRD3 antagonism could modulate both reward and punishment-based processing. Another aim of our study was to combine the fMRI data with PET imaging data in the same subjects where data on DRD3 receptor density was available (which, as discussed in chapter 2, is a biomarker of interest in addiction neuroreceptor research). Previous research has identified increased hypothalamus DRD3 in individuals with AUD vs HC (Erritzoe et al., 2014b) and hence it was theorised that DRD3 availability could be contributing to aberrant reward processing. I did not find an association between baseline DRD3 availability and reward processing in AUD nor an association between DRD3 availability post-dose and subsequent brain activation.

Despite no association between the imaging modalities, this is the first study to explore the acute molecular-functional-clinical effects of administering an investigational novel drug in individuals with alcohol addiction. Such methodologies are at the forefront of experimental medicine and hold the promise of delivering the most comprehensive insights into the neuropathophysiological aspects of addiction and the underlying mechanisms of novel interventions. This multi-systems approach seeks to refine the drug development process, ultimately facilitating the creation of more effective therapeutic approaches of the future.

For the final experimental chapter, I set out to validate two novel fMRI paradigms in GD. These were the 5-ACR and RER which aimed to explore cue-reactivity and reward processing and to determine if there were any differences in brain activation between HCs and GDs. The objective of this study was to develop functional assays of brain function that had greater ecological validity than previous studies that have explored brain reward-related function in GD. We also specifically designed these tasks for future testing in multimodal neuroimaging and clinical trials of psychedelic therapy in GD as well as other addictions to explore the mechanism of action of this novel and promising intervention that is currently being explored as a treatment for addiction (Zafar et al., 2023b). In both tasks across both groups, I demonstrated that the tasks activate the brains reward and salience networks which validates the tasks working reliably in activating core addiction-relevant brain neurocircuitry. I also found specific GD related brain activation establishing these paradigms to be plausible functional biomarkers of GD which have the potential to be theragnostic tools if incorporated into clinical trial research. Specifically, in the 5-ACR there was significantly greater neural cue-reactivity to gambling>neutral cues in GDs compared to HCs though no hypoactivation to natural rewards were discovered. Significantly greater activations to the GD video cue were present across five of the eight reward related ROIs explored, showing widespread engagement across the mesocorticolimbic pathway. Intriguingly we saw increased food cue processing and a novel finding of increased nature cue processing in GDs vs HCs which could be associated with reported hyper-dopaminergic function in GDs (van Holst et al., 2018). In the RER we saw similar GD specific neural responses to Hit>Miss trials and differences in Miss>neutral processing. The brain regions activated specifically in GD to the RER included the inferior frontal gyrus and the insula. Across both tasks a role

for the insula was discovered. Increasingly, a greater number of studies are demonstrating the involvement of the insula in GD related cognitive distortions (Clark et al., 2014) and reward and cue processing (Limbrick-Oldfield et al., 2017). My findings extend this and suggest that the insula may be a core neuroanatomical biomarker in GD and an important target for novel therapeutics. Indeed, more recent research extends the role of the insula to other emerging behavioural addictions such as internet gaming (Turel et al., 2021) highlighting this region as a possible hallmark biomarker candidate for behavioural addiction and addiction related brain processing that goes beyond drug-induced neural adaptations which may be involved in more core dopaminergic reward regions such as the ventral striatum.

Strengths and limitations

Addiction is a complex and debilitating neuropsychiatric disorder and developing effective treatments for addiction is crucial. This PhD has explored the pivotal role of fMRI and PET in advancing our understanding of addiction and the development of novel therapeutic strategies. I have described how these neuroimaging techniques have contributed to elucidating the neurobiological mechanisms of addiction, identifying potential drug targets, and assessing mechanism of action of drugs. Furthermore, I have contributed to recent advancements in addiction research facilitated by fMRI and PET, including the study of neural circuits, neurotransmitter systems, and the potential for precision medicine approaches.

In chapter two I conducted the first and largest meta-analysis and systematic review of $DRD_{2/3}$ across both substance and behavioural addictions and the first meta-analysis of the DRD3 in cocaine addiction. The obvious advantage of meta-analysis is that it reflects the totality of the evidence base to reach definitive conclusions about the role of specific markers of interest in given disorders. Previous research has never combined all subcategories of substance use disorders with behavioural addictions in one meta-analysis and instead has focused solely on representing results related to stimulant addiction specifically (Ashok et al., 2017). This may have contributed to the false assertion of this specific biomarker being integral to all sub-categories categories of addiction. My results do not support that and build on a challenge to the dopamine theory of addiction published nearly a decade ago that attempted to add greater

nuance to the human dopamine neurobiology and addiction story (Nutt et al., 2015). These results do not go as far as to discredit the entire dopamine theory of addiction as that does not reflect our findings. Instead, I have described data that demonstrates the dynamic and interactive effects between confounders, addiction and brain function. Addiction related brain dysfunction, some of which have been reported here, should now be integrated into frameworks such as the US National Institutes of Mental Health Research Domain Criteria (RDoC) which seeks to stimulate research into biologically validated neuropsychological dimensions across mental illness symptoms and diagnoses. A recent expert consensus paper describes the use of RDoC constructs including five from the Positive Valence System (reward valuation, expectancy, action selection, reward learning, habit); one from the Cognitive Control System (response selection/inhibition); and one expert-initiated construct (compulsivity) to test transdiagnostic concepts in addiction research, which could lead to direct implications for assessment, diagnosis, staging of disorder, and treatment. Integrating such PET neuroreceptor data alongside measurements of these neuropsychological dimensions could advantageously improve the precision of addiction biomarkers in the future. A final reflection on the basic molecular neuroscience research reviewed here reminds me of a pertinent quote by an eminent clinical psychiatrist, neuroscientist and ex-head of the National Institute of Mental Mealth (NIMH) Prof Thomas Insel who said: “I spent 13 years at NIMH really pushing on the neuroscience of mental disorders and when I look back on that I realize that while I think I succeeded at getting lots of really cool papers published by cool scientists at fairly large costs—I think $20 billion—I don’t think we moved the needle in reducing suicide, reducing hospitalisations, improving recovery for the tens of millions of people who have mental illness.” This statement has been somewhat of a guiding and grounding principle on which I have sought to critique research and optimise my own research. In the case of the studies described in chapter 2, PET neuroimaging has been used which is the costliest of all imaging modalities and the cost of the studies listed in the systematic review reflect tens if not hundreds of millions of pounds of costs. For the conclusion of the results of my work to be ‘inconclusive’, this echoes some of the problems experienced by the NIMH and demonstrates how the future of neuroimaging must incorporate a translational element that goes beyond simply describing brain pathology associated with disorder. Future work should incorporate effective interventions and back-translate these findings to

understand core disorder processes. This bench to bed to bench feedback loop will mutually serve both interests of pathophysiology and treatment.

fMRI provides a reliable way to identify the acute effects of drugs on brain functional processes that may be important to disorders such as addiction. In chapter 3 we compared the differences in activation to reward processing in both HCs and AUD individuals both at baseline and post DRD3 antagonism. This combined between and within group design offers the greatest level of interrogation on both the effect of the drug on brain systems but also the comparative effects of the drug between HCs and AUD which provides disorder specific mechanism of action. Further, by using a baseline and drug condition on the same participant, inter-participant variability is reduced, which increases the power and reliability of results (Aguirre et al., 1998). When designing within participant pharmacological fMRI studies, order effects also need to be considered as this can bias the results (Bourke and Wall, 2015). We overcame this by conducting two MIDT tasks at baseline and two whilst under the drug and using the average of these to get a reliable measure of task relevant brain activity in both conditions. Another major advantage and a significant advancement to the field was the inclusion of PET neuroreceptor data to compliment the fMRI results. Increasingly multimodal neuroimaging studies have demonstrated that combining imaging modalities could improve the performance of individualised prediction in psychiatry (Ooi et al., 2022). Further, the predictive potential of multimodal neuroimaging which represents multi-dimensional brain information (e.g. molecular/functional/electrophysiological) is significantly higher than the performance of unimodal brain imaging (Sui et al., 2020). Hence this study is a methodological advancement in the field of translational addiction neuropsychopharmacology research and future research should continue to aim to combine across imaging modalities, which was an expressed ambition when developing the fMRI RER task described in chapter 4. There were a few limitations with our current study and one of them was the patient cohort that we selected. These patients were abstinent alcohol dependent individuals with a mean of 414 ($\underline{+}$254) days since their last drink. This could account for the lack of association between DRD3 availability and functional brain effects as previous research has described the restoration of dysfunctional $DRD_{2/3}$ in patients with AUD with up to one year of abstinence (Rominger et al., 2012). There is also evidence that the MIDT ‘normalises’ over time with abstinence and recovery

(Balodis et al., 2016). Hence future tasks should employ longitudinal study designs to investigate the enduring effects of novel interventions on changes in brain receptor availability and function and its association with clinical outcome as well as pay consideration to the specific course of the addiction recovery stage a patient is in when making inferences on brain data.

Neuroimaging biomarkers for behavioural addictions such as GD have not yet been developed and have received much less attention from the addiction neuroscience community than substance addiction biomarkers. My results showed powerful case versus control differences in brain activation in core reward-related regions to tasks which confirm neuropathological brain processing in GD. There were several advantages to our study including a case vs control design which allowed for quantification of GD specific biomarkers. Power in our studies was increased by using studies with videos which are known to elicit stronger brain responses than still images (Schultz and Pilz, 2009). The inclusion of GDs with a history of depression and anxiety demonstrates representativeness of our sample to the clinical population (Falk et al., 2013). Though the sample was representative we recruited from treatment waiting lists rather than active gamblers which reiterates a similar limitation to that described in chapter three in that abstinence can lead to differences in response which may explain the lack of clinical correlation scores with brain effects. Another limitation to the study design was the lack of personalisation of the task. We used mainly video cues related to casino gambling and slot machines and used only the roulette task to probe reward function. According to the recent UK government Gambling Related Harms Evidence Review 2023, harmful gambling involved high participation in online gambling (including online slots), casino and bingo games, electronic gambling machines in bookmakers, sports and other event betting, betting exchanges, and dog racing. Though we still saw significant between-group effects to our gambling cues future research should aim to develop online gambling app stimuli, that are reflective of modern-day online betting and are suitable for fMRI scanning. Cue-reactivity fMRI has also shown utility in being able to predict addiction severity, risk of relapse and treatment outcome (Jasinska et al., 2014) and has been leveraged to develop novel therapeutics in substance addictions (Courtney et al., 2016) but to date, there have been no published literature assessing the modulation of GD brain processing in response to medicines that are being used to treat patients with addiction or INDs.

This may be because no medicine has yet been approved by the United States (US) Food and Drug Administration (FDA) for the treatment of any behavioural addiction (Ginley et al., 2019) which may have contributed to the limited translational research within the GD space. Hence, the development and validation of both these tasks aim to provide a neuroimaging platform on which novel interventions that will arise in the future can be tested to develop novel and therapeutically effective interventions for individuals with GD.

Future research

In this final section, I will discuss how advancements in human neuropsychopharmacology research can be leveraged to optimise the development of psychedelic therapies for addiction and how they lend themselves to personalised medicine and precision psychiatry efforts.

Currently several clinical trials and studies are underway to explore the therapeutic use of psilocybin, MDMA, ketamine, DMT and ibogaine in the treatment of substance addictions. I am also leading a future Imperial college research project to investigate the effect of psychedelic therapy in gambling addiction, which represents a world first. A combination of historical, real world and early stage (phase I/II) clinical trial data have shown generally positive and promising effects of psychedelics in treating substance addictions in relation to safety, feasibility, tolerability and efficacy (Zafar et al., 2023b). Despite these early and encouraging signals there has been no translational neuropsychopharmacology research to explore the mechanism of action of psychedelic therapy in addiction.

The availability of advanced functional and molecular neuroimaging techniques, some of which have been described in this thesis allows us to directly test the hypotheses that psychedelics may *re-broaden hijacked reward and salience system processing in addiction.*

This could be explored with fMRI:
a) Under the acute influence of psychedelics to see the immediate neuropsychopharmacological effects on domains related to reward processing, cue-reactivity, and its relevance to craving and/or anxiety.

b) In a pre- vs. post-psychedelic therapy within-subject design to assess the sub-acute and longer-term changes in brain function in response to fMRI tasks exploring cognitive flexibility, impulsivity, and inhibitory control and its relation to psychological and behavioural scales and clinical outcomes associated with addiction such as cognitive function and relapse.
I further recommend reward, social and music processing and cue-reactivity to be incorporated into this type of study design to see the longer-term impacts psychedelics have in these central domains linked to addiction.

To assess the effects of psychedelic therapy on molecular dysfunction I could explore:

a) Imaging of dopamine, opioid and GABA receptor availability pre – vs. post psychedelic therapy in addiction populations to assess changes in neuroreceptor availability and its association with clinical outcome and facets of impulsivity (selection of neuroreceptor to investigate must be dependent on evidence for dysfunction in the specific disorder which has been described)
b) Multimodal FMRI-PET imaging to assess changes in dopaminergic modulation in relation to monetary reward tasks in addiction populations pre- vs. post-psychedelic therapy. This would be the first 'molecular-functional-clinical' mechanistic pathway for its role in addiction. These results would allow for the identification of molecular and functional stratification and prognostic biomarkers and would greatly advance the field of psychedelic addiction drug development. Such multimodal imaging platforms are advised in all fields of psychiatric and neurological drug development and represent the most advanced area of experimental translational neuroscience research
c) PET neurotransmitter release studies of novel dopamine, opioids and serotonin radiotracers to assess the changes in neurotransmitter tone following psychedelic therapy. There is evidence that dysregulation in neurotransmitter tone may be a more pleiotropic biomarker of addiction due to its presence in addiction populations in the absence on neuroreceptor abnormalities. Selection of radiotracer should be

dependent on the addiction sub-population being investigated and based on previous evidence of dysregulation in the specific system.

Techniques examining neuroreceptor differences in addiction have pinpointed specific biomarkers for different addiction sub-classes, such as dopamine receptors in alcohol and stimulant disorders, opioid receptors in cocaine and opioid dependence, and GABAA in various addictions compared to controls. New radiotracers enhance the specificity of identifying receptor subclasses, aiding drug development through PET imaging and highlighting its potential in psychedelic drug research. While preclinical studies indicate psilocybin's potential in altering molecular receptors and influencing alcohol-related and other addiction like behaviors in animals , human research on its impact remains unexplored. Further, recent research as previously discussed indicates that reduced striatal dopamine/endorphin transmission may signify heightened impulsivity and altered decision-making linked to addiction. This diminished dopamine/endorphin function appears to be a marker for addiction susceptibility, treatment resistance, and increased drug consumption due to impulsive behavior and decreased motivation. If psychedelic therapy is effective in treating addiction, they should enhance such neurotransmitter levels, thereby improving brain function and normalising neurotransmitter imbalances. Thus based on these remaining questions the potential therapeutic efficacy of psilocybin in addressing addiction's molecular dysfunctions requires further investigation in human studies.

These findings would help us to identify for the first time the brain mechanisms mediating the relationship between psychedelic therapy and clinical, behavioural and neurocognitive outcomes and how they relate to relapse vulnerability in individuals with addiction

Conclusion

In this doctoral thesis, my work encompasses a series of studies aimed at the validation and exploration of biomarkers related to substance use and behavioural addictions. Leveraging advanced human neuroimaging methodologies, including task-based fMRI, I have explored the brain's reward mechanisms and its response to psychopharmacological interventions. Complimentary to this approach, my research delves into the the molecular neurobiology of addiction, employing PET neuroimaging

techniques to critically examine the role of dopamine receptors in addiction neuropathology, as well as the impact of modulating these receptors on brain function.

A noteworthy contribution of my work is the unveiling of a fundamental insight: dopamine receptor availability does not serve as a universally applicable biomarker of addiction. My investigations underscore that the availability of $DRD_{2/3}$ receptors is subject to a multitude of external factors extending beyond the diagnosis of addiction. This revelation challenges the conventional paradigm of the dopamine receptor theory of addiction, suggesting that it may be an antiquated construct.

Furthermore, my research also included the development, validation and assessment of a functionally relevant, ecologically valid biomarker for gambling addiction. This marker is intended for deployment in future multimodal neuroimaging studies designed to evaluate the clinical and neural effects of psychedelic therapy in the context of addiction treatment.

The culmination of my research underscores a pivotal realisation: unimodal neuroimaging modalities may be insufficient in their capacity to unravel the complex nature of neuropsychiatric disorders, including addiction. My PhD coincided with a propitious moment in the field of addiction neuroscience, where progress has enabled the formulation of multimodal neuroimaging that bridge the domains of molecular and clinical neuroscience. The convergence of these diverse methodologies, in tandem with the emergence of promising therapeutic modalities such as psychedelic therapies—known for their safety and efficacy in addiction patients—holds the potential to chart a more promising course for the development of enhanced and more efficacious treatment approaches through translational neuropsychopharmacology approaches.

Throughout the past four years of my doctoral pursuit, my unwavering commitment has been to render my research output clinically relevant, with the objective of contributing to the amelioration of patients' health and well-being. I hold a sanguine outlook that the skills, techniques and methodologies that have been acquired and honed during these formative years will contribute to developing interventions. It is my deeply held scientific belief that we continue to ask ‘why?’ even when we have

answers. It is through such enquiry that tangible benefits for individuals grappling with addiction and other complex neuropsychiatric disorders will be realised.

References:

(Some authors have not been mentioned in full due to specific bibliography formatting in EndNote 20).

2018. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet (London, England),* 392**,** 1789-1858.

AALTO, S., BRÜCK, A., LAINE, M., NÅGREN, K. & RINNE, J. O. 2005. Frontal and temporal dopamine release during working memory and attention tasks in healthy humans: a positron emission tomography study using the high-affinity dopamine D2 receptor ligand [11C] FLB 457. *Journal of Neuroscience,* 25**,** 2471-2477.

ABI-DARGHAM, A., KEGELES, L. S., MARTINEZ, D., INNIS, R. B. & LARUELLE, M. 2003. Dopamine mediation of positive reinforcing effects of amphetamine in stimulant naive healthy volunteers: results from a large cohort. *Eur Neuropsychopharmacol,* 13**,** 459-68.

ALBRECHT, D. S., SKOSNIK, P. D., VOLLMER, J. M., BRUMBAUGH, M. S., PERRY, K. M., MOCK, B. H., ZHENG, Q. H., FEDERICI, L. A., PATTON, E. A., HERRING, C. M. & YODER, K. K. 2013. Striatal D(2)/D(3) receptor availability is inversely correlated with cannabis consumption in chronic marijuana users. *Drug Alcohol Depend,* 128**,** 52-7.

ALVANZO, A. A., WAND, G. S., KUWABARA, H., WONG, D. F., XU, X. & MCCAUL, M. E. 2017. Family history of alcoholism is related to increased D(2) /D(3) receptor binding potential: a marker of resilience or risk? *Addict Biol,* 22**,** 218-228.

AMERICAN PSYCHIATRIC ASSOCIATION, A. & ASSOCIATION, A. P. 1994. *Diagnostic and statistical manual of mental disorders: DSM-IV*, American psychiatric association Washington, DC.

AMERICAN PSYCHIATRIC ASSOCIATION, D. & ASSOCIATION, A. P. 2013. *Diagnostic and statistical manual of mental disorders: DSM-5*, American psychiatric association Washington, DC.

ASHOK, A. H., MIZUNO, Y., VOLKOW, N. D. & HOWES, O. D. 2017. Association of Stimulant Use With Dopaminergic Alterations in Users of Cocaine, Amphetamine, or Methamphetamine: A Systematic Review and Meta-analysis. *JAMA Psychiatry,* 74**,** 511-519.

BÄCKMAN, L., WARIS, O., JOHANSSON, J., ANDERSSON, M., RINNE, J. O., ALAKURTTI, K., SOVERI, A., LAINE, M. & NYBERG, L. 2017. Increased dopamine release after working-memory updating training: Neurochemical correlates of transfer. *Scientific reports,* 7**,** 7160.

BADGAIYAN, R. D., FISCHMAN, A. J. & ALPERT, N. M. 2008. Explicit motor memory activates the striatal dopamine system. *Neuroreport,* 19**,** 409-412.

BADGAIYAN, R. D., FISCHMAN, A. J. & ALPERT, N. M. 2009. Dopamine release during human emotional processing. *Neuroimage,* 47**,** 2041-2045.

BADGAIYAN, R. D. & WACK, D. 2011. Evidence of dopaminergic processing of executive inhibition. *PLoS One,* 6**,** e28075.

BALER, R. D. & VOLKOW, N. D. 2006. Drug addiction: the neurobiology of disrupted self-control. *Trends Mol Med,* 12**,** 559-66.

BALLARD, M. E., MANDELKERN, M. A., MONTEROSSO, J. R., HSU, E., ROBERTSON, C. L., ISHIBASHI, K., DEAN, A. C. & LONDON, E. D. 2015. Low Dopamine D2/D3 Receptor Availability is Associated with Steep Discounting of Delayed Rewards in Methamphetamine Dependence. *Int J Neuropsychopharmacol,* 18**,** pyu119.

BALODIS, I. M., KOBER, H., WORHUNSKY, P. D., STEVENS, M. C., PEARLSON, G. D., CARROLL, K. M. & POTENZA, M. N. 2016. Neurofunctional Reward Processing Changes in Cocaine Dependence During Recovery. *Neuropsychopharmacology,* 41**,** 2112-2121.

BALODIS, I. M. & POTENZA, M. N. 2015. Anticipatory reward processing in addicted populations: a focus on the monetary incentive delay task. *Biological psychiatry,* 77**,** 434-444.

BECK, A., SCHLAGENHAUF, F., WÜSTENBERG, T., HEIN, J., KIENAST, T., KAHNT, T., SCHMACK, K., HÄGELE, C., KNUTSON, B., HEINZ, A. & WRASE, J. 2009. Ventral Striatal Activation During Reward Anticipation Correlates with Impulsivity in Alcoholics. *Biological Psychiatry,* 66**,** 734-742.

BELLRINGER, M. E. & GARRETT, N. 2021. Risk Factors for Increased Online Gambling during COVID-19 Lockdowns in New Zealand: A Longitudinal Study. *Int J Environ Res Public Health,* 18.

BELZEAUX, R., LALANNE, L., KIEFFER, B. L. & LUTZ, P.-E. 2018. Focusing on the opioid system for addiction biomarker discovery. *Trends in molecular medicine,* 24**,** 206-220.

BENJAMINI, Y. & HOCHBERG, Y. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological),* 57**,** 289-300.

BERNARDIN, F., MAHEUT-BOSSER, A. & PAILLE, F. 2014. Cognitive impairments in alcohol-dependent subjects. *Frontiers in Psychiatry,* 5**,** 78.

BERNSTEIN, M. H., MCSHEFFREY, S. N., VAN DEN BERG, J. J., VELA, J. E., STEIN, L. A., ROBERTS, M. B., MARTIN, R. A. & CLARKE, J. G. 2015. The association between impulsivity and alcohol/drug use among prison inmates. *Addict Behav,* 42**,** 140-3.

BERRIDGE, K. C. & ROBINSON, T. E. 2016. Liking, wanting, and the incentive-sensitization theory of addiction. *Am Psychol,* 71**,** 670-679.

BIBBY, P. A. 2016. Loss-Chasing, Alexithymia, and Impulsivity in a Gambling Task: Alexithymia as a Precursor to Loss-Chasing Behavior When Gambling. *Front Psychol,* 7**,** 3.

BJORK, J. M., SMITH, A. R., CHEN, G. & HOMMER, D. W. 2011. Mesolimbic recruitment by nondrug rewards in detoxified alcoholics: Effort anticipation, reward anticipation, and reward delivery. *Human Brain Mapping,* 33**,** 2174-2188.

BJORK, J. M., SMITH, A. R. & HOMMER, D. W. 2008. Striatal sensitivity to reward deliveries and omissions in substance dependent patients. *NeuroImage,* 42**,** 1609-1621.

BLASZCZYNSKI, A. & NOWER, L. 2002. A pathways model of problem and pathological gambling. *Addiction,* 97**,** 487-99.

BLUM, K., BRAVERMAN, E. R., HOLDER, J. M., LUBAR, J. F., MONASTRA, V. J., MILLER, D., LUBAR, J. O., CHEN, T. J. & COMINGS, D. E. 2000a. Reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors. *J Psychoactive Drugs,* 32 Suppl**,** i-iv, 1-112.

BLUM, K., BRAVERMAN, E. R., HOLDER, J. M., LUBAR, J. F., MONASTRA, V. J., MILLER, D., LUBAR, J. O., CHEN, T. J. H. & COMINGS, D. E. 2000b. The Reward Deficiency Syndrome: A Biogenetic Model for the Diagnosis and Treatment of Impulsive, Addictive and Compulsive Behaviors. *Journal of Psychoactive Drugs,* 32**,** 1-112.

BLUM, K., GONDRÉ-LEWIS, M., STEINBERG, B., ELMAN, I., BARON, D., MODESTINO, E. J., BADGAIYAN, R. D. & GOLD, M. S. 2018. Our evolved unique pleasure circuit makes humans different from apes: Reconsideration of data derived from animal studies. *J Syst Integr Neurosci,* 4.

BOILEAU, I., PAYER, D., CHUGANI, B., LOBO, D., BEHZADI, A., RUSJAN, P. M., HOULE, S., WILSON, A. A., WARSH, J., KISH, S. J. & ZACK, M. 2013. The D2/3 dopamine receptor in pathological gambling: a positron emission tomography study with [11C]-(+)-propyl-hexahydro-naphtho-oxazin and [11C]raclopride. *Addiction,* 108**,** 953-63.

BOILEAU, I., PAYER, D., CHUGANI, B., LOBO, D. S., HOULE, S., WILSON, A. A., WARSH, J., KISH, S. J. & ZACK, M. 2014. In vivo evidence for greater amphetamine-induced dopamine release in pathological gambling: a positron emission tomography study with [(11)C]-(+)-PHNO. *Mol Psychiatry,* 19**,** 1305-13.

BOILEAU, I., PAYER, D., HOULE, S., BEHZADI, A., RUSJAN, P. M., TONG, J., WILKINS, D., SELBY, P., GEORGE, T. P., ZACK, M., FURUKAWA, Y., MCCLUSKEY, T., WILSON, A. A. & KISH, S. J. 2012. Higher binding of the dopamine D3 receptor-preferring ligand [11C]-(+)-propyl-hexahydro-naphtho-oxazin in methamphetamine polydrug users: a positron emission tomography study. *J Neurosci,* 32**,** 1353-9.

BORSOOK, D., BECERRA, L. & FAVA, M. 2013. Use of functional imaging across clinical phases in CNS drug development. *Translational psychiatry,* 3**,** e282-e282.

BOWDEN-JONES, H., HOOK, R. W., GRANT, J. E., IOANNIDIS, K., CORAZZA, O., FINEBERG, N. A., SINGER, B. F., ROBERTS, A., BETHLEHEM, R. & DYMOND, S. 2022. Gambling disorder in the UK: key research priorities and the urgent need for independent research funding. *The Lancet Psychiatry,* 9**,** 321-329.

BROMBERG-MARTIN, E. S., MATSUMOTO, M. & HIKOSAKA, O. 2010. Dopamine in motivational control: rewarding, aversive, and alerting. *Neuron,* 68**,** 815-834.

BROWN, A. K., MANDELKERN, M. A., FARAHI, J., ROBERTSON, C., GHAHREMANI, D. G., SUMEREL, B., MOALLEM, N. & LONDON, E. D. 2012. Sex differences in striatal dopamine D2/D3 receptor availability in smokers and non-smokers. *Int J Neuropsychopharmacol,* 15**,** 989-94.

BRUGGER, S. P., ANGELESCU, I., ABI-DARGHAM, A., MIZRAHI, R., SHAHREZAEI, V. & HOWES, O. D. 2020. Heterogeneity of Striatal Dopamine Function in Schizophrenia: Meta-analysis of Variance. *Biological Psychiatry,* 87**,** 215-224.

BRUGGER, S. P. & HOWES, O. D. 2017. Heterogeneity and Homogeneity of Regional Brain Structure in Schizophrenia. *JAMA Psychiatry,* 74**,** 1104.

BUCHANAN, J. & YOUNG, L. 2000. The war on drugs–a war on drug users.

BUROCK, M. A., BUCKNER, R. L., WOLDORFF, M. G., ROSEN, B. R. & DALE, A. M. 1998. Randomized event-related experimental designs allow for extremely rapid presentation rates using functional MRI. *Neuroreport,* 9**,** 3735-9.

BUXTON, R. B. 2013. The physics of functional magnetic resonance imaging (fMRI). *Rep Prog Phys,* 76**,** 096601.
CALAKOS, K. C., HILLMER, A. T., ANGARITA, G. A., BALDASSARRI, S. R., NAJAFZADEH, S., EMERY, P. R., MATUSKEY, D., HUANG, Y. & COSGROVE, K. P. 2021. Recently Abstinent Smokers Exhibit Mood-Associated Dopamine Dysfunction in the Ventral Striatum Compared to Nonsmokers: A [11C]-(+)-PHNO PET Study. *Nicotine & Tobacco Research,* 24**,** 745-752.
CALAKOS, K. C., RUSOWICZ, A., PITTMAN, B., GALLEZOT, J. D., POTENZA, M. N., COSGROVE, K. P. & MATUSKEY, D. 2022. Relationships between dopamine D2/3 receptor availability and social-environmental factors in humans. *Neurosci Lett,* 771**,** 136463.
CALIFF, R. M. 2018. Biomarker definitions and their applications. *Exp Biol Med (Maywood),* 243**,** 213-221.
CANNELLA, N., UBALDI, M., MASI, A., BRAMUCCI, M., ROBERTO, M., BIFONE, A. & CICCOCIOPPO, R. 2019. Building better strategies to develop new medications in Alcohol Use Disorder: Learning from past success and failure to shape a brighter future. *Neurosci Biobehav Rev,* 103**,** 384-398.
CARDWELL, E., HOFF, R. A., GARAKANI, A., KRISHNAN-SARIN, S., POTENZA, M. N. & ZHAI, Z. W. 2022. An exploratory study of anxiety-motivated gambling in adolescents: Associations with minority status and gambling, health and functioning measures. *Journal of Psychiatric Research,* 151**,** 445-453.
CARPENTER, J. E., LAPRAD, D., DAYO, Y., DEGROTE, S. & WILLIAMSON, K. 2018. An Overview of Pharmacotherapy Options for Alcohol Use Disorder. *Fed Pract,* 35**,** 48-58.
CASEY, K. F., BENKELFAT, C., CHERKASOVA, M. V., BAKER, G. B., DAGHER, A. & LEYTON, M. 2014. Reduced dopamine response to amphetamine in subjects at ultra-high risk for addiction. *Biol Psychiatry,* 76**,** 23-30.
CAZZOLI, D., KAUFMANN, B. C., PALADINI, R. E., MÜRI, R. M., NEF, T. & NYFFELER, T. 2021. Anterior insula and inferior frontal gyrus: where ventral and dorsal visual attention systems meet. *Brain Commun,* 3**,** fcaa220.
CHRISTIAN, B. T., LEHRER, D. S., SHI, B., NARAYANAN, T. K., STROHMEYER, P. S., BUCHSBAUM, M. S. & MANTIL, J. C. 2006. Measuring dopamine neuromodulation in the thalamus: using [F-18] fallypride PET to study dopamine release during a spatial attention task. *Neuroimage,* 31**,** 139-152.
CHUKWUEKE, C. C., NONA, C. N., MCPHEE, M. D., MANSOURI, E., RUBIN-KAHANA, D. S., MARTINEZ, D., BOILEAU, I., HENDERSHOT, C. S. & LE FOLL, B. 2021. Exploring regulation and function of dopamine D3 receptors in alcohol use disorder. A PET [11C]-(+)-PHNO study. *Neuropsychopharmacology,* 46**,** 2112-2120.
CLARK, L., BOILEAU, I. & ZACK, M. 2019. Neuroimaging of reward mechanisms in Gambling disorder: an integrative review. *Mol Psychiatry,* 24**,** 674-693.
CLARK, L., STOKES, P. R., WU, K., MICHALCZUK, R., BENECKE, A., WATSON, B. J., EGERTON, A., PICCINI, P., NUTT, D. J., BOWDEN-JONES, H. & LINGFORD-HUGHES, A. R. 2012. Striatal dopamine D(2)/D(3) receptor binding in pathological gambling is correlated with mood-related impulsivity. *Neuroimage,* 63**,** 40-6.
CLARK, L., STUDER, B., BRUSS, J., TRANEL, D. & BECHARA, A. 2014. Damage to insula abolishes cognitive distortions during simulated gambling. *Proc Natl Acad Sci U S A,* 111**,** 6098-103.

COLASANTI, A., SEARLE, G. E., LONG, C. J., HILL, S. P., REILEY, R. R., QUELCH, D., ERRITZOE, D., TZIORTZI, A. C., REED, L. J., LINGFORD-HUGHES, A. R., WALDMAN, A. D., SCHRUERS, K. R., MATTHEWS, P. M., GUNN, R. N., NUTT, D. J. & RABINER, E. A. 2012. Endogenous opioid release in the human brain reward system induced by acute amphetamine administration. *Biol Psychiatry,* 72**,** 371-7.

COMMISSION, G. 2023. *Statistics on participation and problem gambling for the year to March 2023*, UK Government

CONWAY, K. P., COMPTON, W., STINSON, F. S. & GRANT, B. F. 2006. Lifetime comorbidity of DSM-IV mood and anxiety disorders and specific drug use disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *J Clin Psychiatry,* 67**,** 247-57.

COOLS, A. R. & VAN ROSSUM, J. M. 1976. Excitation-mediating and inhibition-mediating dopamine-receptors: a new concept towards a better understanding of electrophysiological, biochemical, pharmacological, functional and clinical data. *Psychopharmacologia,* 45**,** 243-54.

CORNISH, J. L. & PRASAD, A. A. 2021. Sex Differences in Substance Use Disorders: A Neurobiological Perspective. *Front Glob Womens Health,* 2**,** 778514.

COURTNEY, K. E., SCHACHT, J. P., HUTCHISON, K., ROCHE, D. J. & RAY, L. A. 2016. Neural substrates of cue reactivity: association with treatment outcomes and relapse. *Addict Biol,* 21**,** 3-22.

COX, S. M., YAU, Y., LARCHER, K., DURAND, F., KOLIVAKIS, T., DELANEY, J. S., DAGHER, A., BENKELFAT, C. & LEYTON, M. 2017. Cocaine cue-induced dopamine release in recreational cocaine users. *Scientific reports,* 7**,** 46665.

CROCKFORD, D. N., GOODYEAR, B., EDWARDS, J., QUICKFALL, J. & EL-GUEBALY, N. 2005. Cue-induced brain activity in pathological gamblers. *Biol Psychiatry,* 58**,** 787-95.

CROCQ, M. A. 2007. Historical and cultural aspects of man's relationship with addictive drugs. *Dialogues Clin Neurosci,* 9**,** 355-61.

CROW, T. J. 1973. Catecholamine-containing neurones and electrical self-stimulation: 2. A theoretical interpretation and some psychiatric implications. *Psychological Medicine,* 3**,** 66-73.

CRUMMY, E. A., O'NEAL, T. J., BASKIN, B. M. & FERGUSON, S. M. 2020. One Is Not Enough: Understanding and Modeling Polysubstance Use. *Front Neurosci,* 14**,** 569.

DAGLISH, M. R., WILLIAMS, T. M., WILSON, S. J., TAYLOR, L. G., EAP, C. B., AUGSBURGER, M., GIROUD, C., BROOKS, D. J., MYLES, J. S., GRASBY, P., LINGFORD-HUGHES, A. R. & NUTT, D. J. 2008. Brain dopamine response in human opioid addiction. *Br J Psychiatry,* 193**,** 65-72.

DALLEY, J. W., FRYER, T. D., BRICHARD, L., ROBINSON, E. S., THEOBALD, D. E., LAANE, K., PENA, Y., MURPHY, E. R., SHAH, Y., PROBST, K., ABAKUMOVA, I., AIGBIRHIO, F. I., RICHARDS, H. K., HONG, Y., BARON, J. C., EVERITT, B. J. & ROBBINS, T. W. 2007. Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *Science,* 315**,** 1267-70.

DAMIER, P., HIRSCH, E. C., AGID, Y. & GRAYBIEL, A. M. 1999. The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain,* 122 (Pt 8)**,** 1437-48.

DE GRECK, M., ENZI, B., PRÖSCH, U., GANTMAN, A., TEMPELMANN, C. & NORTHOFF, G. 2010. Decreased neuronal activity in reward circuitry of pathological gamblers during processing of personal relevant stimuli. *Hum Brain Mapp,* 31**,** 1802-12.

DEGENHARDT, L., CHARLSON, F., FERRARI, A., SANTOMAURO, D., ERSKINE, H., MANTILLA-HERRARA, A., WHITEFORD, H., LEUNG, J., NAGHAVI, M. & GRISWOLD, M. 2018a. The global burden of disease attributable to alcohol and drug use in 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Psychiatry,* 5**,** 987-1012.

DEGENHARDT, L., CHARLSON, F., FERRARI, A., SANTOMAURO, D., ERSKINE, H., MANTILLA-HERRARA, A., WHITEFORD, H., LEUNG, J., NAGHAVI, M., GRISWOLD, M., REHM, J., HALL, W., SARTORIUS, B., SCOTT, J., VOLLSET, S. E., KNUDSEN, A. K., HARO, J. M., PATTON, G., KOPEC, J., MALTA, D. C., TOPOR-MADRY, R., MCGRATH, J., HAAGSMA, J., ALLEBECK, P., PHILLIPS, M., SALOMON, J., HAY, S., FOREMAN, K., LIM, S., MOKDAD, A., SMITH, M., GAKIDOU, E., MURRAY, C. & VOS, T. 2018b. The global burden of disease attributable to alcohol and drug use in 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Psychiatry,* 5**,** 987-1012.

DI CHIARA, G., BASSAREO, V., FENU, S., DE LUCA, M. A., SPINA, L., CADONI, C., ACQUAS, E., CARBONI, E., VALENTINI, V. & LECCA, D. 2004. Dopamine and drug addiction: the nucleus accumbens shell connection. *Neuropharmacology,* 47 Suppl 1**,** 227-41.

DI CHIARA, G. & IMPERATO, A. 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci U S A,* 85**,** 5274-8.

DIAZ, J., PILON, C., FOLL, B. L., GROS, C., TRILLER, A., SCHWARTZ, J.-C. & SOKOLOFF, P. 2000. Dopamine D3 Receptors Expressed by All Mesencephalic Dopamine Neurons. *Journal of Neuroscience,* 20**,** 8677-8684.

DOWLING, N. A., COWLISHAW, S., JACKSON, A. C., MERKOURIS, S. S., FRANCIS, K. L. & CHRISTENSEN, D. R. 2015. Prevalence of psychiatric co-morbidity in treatment-seeking problem gamblers: A systematic review and meta-analysis. *Aust N Z J Psychiatry,* 49**,** 519-39.

DREVETS, W. C., GAUTIER, C., PRICE, J. C., KUPFER, D. J., KINAHAN, P. E., GRACE, A. A., PRICE, J. L. & MATHIS, C. A. 2001. Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. *Biol Psychiatry,* 49**,** 81-96.

DROUTMAN, V., BECHARA, A. & READ, S. J. 2015a. Roles of the Different Sub-Regions of the Insular Cortex in Various Phases of the Decision-Making Process. *Frontiers in Behavioral Neuroscience,* 9.

DROUTMAN, V., READ, S. J. & BECHARA, A. 2015b. Revisiting the role of the insula in addiction. *Trends Cogn Sci,* 19**,** 414-20.

ECONOMOU, M., SOULIOTIS, K., MALLIORI, M., PEPPOU, L. E., KONTOANGELOS, K., LAZARATOU, H., ANAGNOSTOPOULOS, D., GOLNA, C., DIMITRIADIS, G., PAPADIMITRIOU, G. & PAPAGEORGIOU, C. 2019. Problem Gambling in Greece: Prevalence and Risk Factors During the Financial Crisis. *J Gambl Stud,* 35**,** 1193-1210.

EGERTON, A., MEHTA, M. A., MONTGOMERY, A. J., LAPPIN, J. M., HOWES, O. D., REEVES, S. J., CUNNINGHAM, V. J. & GRASBY, P. M. 2009. The

dopaminergic basis of human behaviors: A review of molecular imaging studies. *Neurosci Biobehav Rev,* 33**,** 1109-32.
EKHTIARI, H., TAVAKOLI, H., ADDOLORATO, G., BAEKEN, C., BONCI, A., CAMPANELLA, S., CASTELO-BRANCO, L., CHALLET-BOUJU, G., CLARK, V. P., CLAUS, E., DANNON, P. N., DEL FELICE, A., DEN UYL, T., DIANA, M., DI GIANNANTONIO, M., FEDOTA, J. R., FITZGERALD, P., GALLIMBERTI, L., GRALL-BRONNEC, M., HERREMANS, S. C., HERRMANN, M. J., JAMIL, A., KHEDR, E., KOUIMTSIDIS, C., KOZAK, K., KRUPITSKY, E., LAMM, C., LECHNER, W. V., MADEO, G., MALMIR, N., MARTINOTTI, G., MCDONALD, W. M., MONTEMITRO, C., NAKAMURA-PALACIOS, E. M., NASEHI, M., NOËL, X., NOSRATABADI, M., PAULUS, M., PETTORRUSO, M., PRADHAN, B., PRAHARAJ, S. K., RAFFERTY, H., SAHLEM, G., SALMERON, B. J., SAUVAGET, A., SCHLUTER, R. S., SERGIOU, C., SHAHBABAIE, A., SHEFFER, C., SPAGNOLO, P. A., STEELE, V. R., YUAN, T. F., VAN DONGEN, J. D. M., VAN WAES, V., VENKATASUBRAMANIAN, G., VERDEJO-GARCÍA, A., VERVEER, I., WELSH, J. W., WESLEY, M. J., WITKIEWITZ, K., YAVARI, F., ZARRINDAST, M. R., ZAWERTAILO, L., ZHANG, X., CHA, Y. H., GEORGE, T. P., FROHLICH, F., GOUDRIAAN, A. E., FECTEAU, S., DAUGHTERS, S. B., STEIN, E. A., FREGNI, F., NITSCHE, M. A., ZANGEN, A., BIKSON, M. & HANLON, C. A. 2019. Transcranial electrical and magnetic stimulation (tES and TMS) for addiction medicine: A consensus paper on the present state of the science and the road ahead. *Neurosci Biobehav Rev,* 104**,** 118-140.
ELLIOTT, R. & DEAKIN, B. 2005. Role of the orbitofrontal cortex in reinforcement processing and inhibitory control: evidence from functional magnetic resonance imaging studies in healthy human subjects. *Int Rev Neurobiol,* 65**,** 89-116.
ERRITZOE, D. 2014. In vivo imaging of cerebral dopamine D3 receptors in alcoholism. *Neuropsychopharmacology,* 39.
ERRITZOE, D., ASHOK, A. H., SEARLE, G. E., COLASANTI, A., TURTON, S., LEWIS, Y., HUIBAN, M., MOZ, S., PASSCHIER, J., SALEEM, A., BEAVER, J., LINGFORD-HUGHES, A., NUTT, D. J., HOWES, O. D., GUNN, R. N., KNUDSEN, G. M. & RABINER, E. A. 2020. Serotonin release measured in the human brain: a PET study with [(11)C]CIMBI-36 and d-amphetamine challenge. *Neuropsychopharmacology,* 45**,** 804-810.
ERRITZOE, D., TZIORTZI, A., BARGIELA, D., COLASANTI, A., SEARLE, G. E., GUNN, R. N., BEAVER, J. D., WALDMAN, A., NUTT, D. J., BANI, M., MERLO-PICH, E., RABINER, E. A. & LINGFORD-HUGHES, A. 2014a. In Vivo Imaging of Cerebral Dopamine D3 Receptors in Alcoholism. *Neuropsychopharmacology,* 39**,** 1703-1712.
ERRITZOE, D., TZIORTZI, A., BARGIELA, D., COLASANTI, A., SEARLE, G. E., GUNN, R. N., BEAVER, J. D., WALDMAN, A., NUTT, D. J., BANI, M., MERLO-PICH, E., RABINER, E. A. & LINGFORD-HUGHES, A. 2014b. In vivo imaging of cerebral dopamine D3 receptors in alcoholism. *Neuropsychopharmacology,* 39**,** 1703-12.
EVERITT, B. J. & ROBBINS, T. W. 2016. Drug Addiction: Updating Actions to Habits to Compulsions Ten Years On. *Annu Rev Psychol,* 67**,** 23-50.
FAIRBANKS, J., UMBREIT, A., KOLLA, B. P., KARPYAK, V. M., SCHNEEKLOTH, T. D., LOUKIANOVA, L. L. & SINHA, S. 2020. Evidence-Based

Pharmacotherapies for Alcohol Use Disorder: Clinical Pearls. *Mayo Clinic Proceedings,* 95**,** 1964-1977.
FALK, E. B., HYDE, L. W., MITCHELL, C., FAUL, J., GONZALEZ, R., HEITZEG, M. M., KEATING, D. P., LANGA, K. M., MARTZ, M. E., MASLOWSKY, J., MORRISON, F. J., NOLL, D. C., PATRICK, M. E., PFEFFER, F. T., REUTER-LORENZ, P. A., THOMASON, M. E., DAVIS-KEAN, P., MONK, C. S. & SCHULENBERG, J. 2013. What is a representative brain? Neuroscience meets population science. *Proc Natl Acad Sci U S A,* 110**,** 17615-22.
FEHR, C., YAKUSHEV, I., HOHMANN, N., BUCHHOLZ, H. G., LANDVOGT, C., DECKERS, H., EBERHARDT, A., KLAGER, M., SMOLKA, M. N., SCHEURICH, A., DIELENTHEIS, T., SCHMIDT, L. G., ROSCH, F., BARTENSTEIN, P., GRUNDER, G. & SCHRECKENBERGER, M. 2008. Association of low striatal dopamine d2 receptor availability with nicotine dependence similar to that seen with other drugs of abuse. *Am J Psychiatry,* 165**,** 507-14.
FOLEY, P. B. 2019. Dopamine in psychiatry: a historical perspective. *Journal of Neural Transmission,* 126**,** 473-479.
FRISTON, K. J., HOLMES, A. P., POLINE, J. B., GRASBY, P. J., WILLIAMS, S. C., FRACKOWIAK, R. S. & TURNER, R. 1995. Analysis of fMRI time-series revisited. *Neuroimage,* 2**,** 45-53.
GANDHI, K. D., MANSUKHANI, M. P., KARPYAK, V. M., SCHNEEKLOTH, T. D., WANG, Z. & KOLLA, B. P. 2020. The Impact of Varenicline on Alcohol Consumption in Subjects With Alcohol Use Disorders: Systematic Review and Meta-Analyses. *J Clin Psychiatry,* 81.
GARCÍA-CASTRO, J., CANCELA, A. & CÁRDABA, M. A. M. 2022. Neural cue-reactivity in pathological gambling as evidence for behavioral addiction: a systematic review. *Curr Psychol***,** 1-12.
GARRISON, K. A. & POTENZA, M. N. 2014. Neuroimaging and biomarkers in addiction treatment. *Curr Psychiatry Rep,* 16**,** 513.
GELSKOV, S. V., MADSEN, K. H., RAMSØY, T. Z. & SIEBNER, H. R. 2016. Aberrant neural signatures of decision-making: Pathological gamblers display cortico-striatal hypersensitivity to extreme gambles. *Neuroimage,* 128**,** 342-352.
GINOVART, N., GALINEAU, L., WILLEIT, M., MIZRAHI, R., BLOOMFIELD, P. M., SEEMAN, P., HOULE, S., KAPUR, S. & WILSON, A. A. 2006. Binding characteristics and sensitivity to endogenous dopamine of [11C]-(+)-PHNO, a new agonist radiotracer for imaging the high-affinity state of D2 receptors in vivo using positron emission tomography. *J Neurochem,* 97**,** 1089-103.
GINOVART, N., WILLEIT, M., RUSJAN, P., GRAFF, A., BLOOMFIELD, P. M., HOULE, S., KAPUR, S. & WILSON, A. A. 2007. Positron emission tomography quantification of [11C]-(+)-PHNO binding in the human brain. *J Cereb Blood Flow Metab,* 27**,** 857-71.
GIRAULT, J.-A. & GREENGARD, P. 2004. The neurobiology of dopamine signaling. *Archives of Neurology,* 61**,** 641-644.
GLEICH, T., SPITTA, G., BUTLER, O., ZACHARIAS, K., AYDIN, S., SEBOLD, M., GARBUSOW, M., RAPP, M., SCHUBERT, F., BUCHERT, R., HEINZ, A. & GALLINAT, J. 2021. Dopamine D2/3 receptor availability in alcohol use disorder and individuals at high risk: Towards a dimensional approach. *Addiction Biology,* 26.

GOLDSTEIN, R. Z. & VOLKOW, N. D. 2011a. Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. *Nat Rev Neurosci,* 12**,** 652-69.
GOLDSTEIN, R. Z. & VOLKOW, N. D. 2011b. Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. *Nature reviews. Neuroscience,* 12**,** 652-669.
GORELICK, D. A., ZANGEN, A. & GEORGE, M. S. 2014. Transcranial magnetic stimulation in the treatment of substance addiction. *Annals of the New York Academy of Sciences,* 1327**,** 79-93.
GOUDRIAAN, A. E., DE RUITER, M. B., VAN DEN BRINK, W., OOSTERLAAN, J. & VELTMAN, D. J. 2010. Brain activation patterns associated with cue reactivity and craving in abstinent problem gamblers, heavy smokers and healthy controls: an fMRI study. *Addict Biol,* 15**,** 491-503.
GREEN, K. E., BLUE, J. R. & NATAL, S. N. 2021. An integrated model of nature and nurture factors that contribute to addiction and recovery. *Substance Use & Misuse,* 56**,** 1095-1107.
GUARDIA, J., CATAFAU, A. M., BATLLE, F., MARTIN, J. C., SEGURA, L., GONZALVO, B., PRAT, G., CARRIO, I. & CASAS, M. 2000. Striatal dopaminergic D(2) receptor density measured by [(123)I]iodobenzamide SPECT in the prediction of treatment outcome of alcohol-dependent patients. *Am J Psychiatry,* 157**,** 127-9.
GUO, J. F., KUANG YANG, Y., TSING CHIU, N., LIEH YEH, T., SEE CHEN, P., LEE, I. H. & LIN CHU, C. 2006. The correlation between striatal dopamine D2/D3 receptor availability and verbal intelligence quotient in healthy volunteers. *Psychol Med,* 36**,** 547-54.
HÄGELE, C., SCHLAGENHAUF, F., RAPP, M., STERZER, P., BECK, A., BERMPOHL, F., STOY, M., STRÖHLE, A., WITTCHEN, H.-U., DOLAN, R. J. & HEINZ, A. 2015. Dimensional psychiatry: reward dysfunction and depressive mood across psychiatric disorders. *Psychopharmacology,* 232**,** 331-341.
HAHN, A., REED, M. B., PICHLER, V., MICHENTHALER, P., RISCHKA, L., GODBERSEN, G. M., WADSAK, W., HACKER, M. & LANZENBERGER, R. 2021. Functional dynamics of dopamine synthesis during monetary reward and punishment processing. *J Cereb Blood Flow Metab,* 41**,** 2973-2985.
HALLDIN, C., FARDE, L., HÖGBERG, T., MOHELL, N., HALL, H., SUHARA, T., KARLSSON, P., NAKASHIMA, Y. & SWAHN, C. G. 1995. Carbon-11-FLB 457: a radioligand for extrastriatal D2 dopamine receptors. *J Nucl Med,* 36**,** 1275-81.
HAYES, A., HERLINGER, K., PATERSON, L. & LINGFORD-HUGHES, A. 2020. The neurobiology of substance use and addiction: evidence from neuroimaging and relevance to treatment. *BJPsych Advances,* 26**,** 367-378.
HEIDBREDER, C. A. & NEWMAN, A. H. 2010. Current perspectives on selective dopamine D(3) receptor antagonists as pharmacotherapeutics for addictions and related disorders. *Annals of the New York Academy of Sciences,* 1187**,** 4-34.
HEILIG, M., MACKILLOP, J., MARTINEZ, D., REHM, J., LEGGIO, L. & VANDERSCHUREN, L. 2021. Addiction as a brain disease revised: why it still matters, and the need for consilience. *Neuropsychopharmacology,* 46**,** 1715-1723.

HEINZ, A., SIESSMEIER, T., WRASE, J., HERMANN, D., KLEIN, S., GRÜSSER-SINOPOLI, S. M., FLOR, H., BRAUS, D. F., BUCHHOLZ, H. G., GRÜNDER, G., SCHRECKENBERGER, M., SMOLKA, M. N., RÖSCH, F., MANN, K. & BARTENSTEIN, P. 2004. Correlation Between Dopamine D2 Receptors in the Ventral Striatum and Central Processing of Alcohol Cues and Craving. *American Journal of Psychiatry,* 161**,** 1783-1789.

HIETALA, J., WEST, C., SYVÄLAHTI, E., NÅGREN, K., LEHIKOINEN, P., SONNINEN, P. & RUOTSALAINEN, U. 1994. Striatal D2 dopamine receptor binding characteristics in vivo in patients with alcohol dependence. *Psychopharmacology (Berl),* 116**,** 285-90.

HOWES, O. D., KAMBEITZ, J., KIM, E., STAHL, D., SLIFSTEIN, M., ABI-DARGHAM, A. & KAPUR, S. 2012. The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch Gen Psychiatry,* 69**,** 776-86.

HUȚUL, T.-D. & KARNER-HUȚULEAC, A. 2022. The Level of Gambling Prediction Using Depression and Anxiety in the Romanian Population. *Journal of Gambling Studies,* 38**,** 653-662.

INNIS, R. B., CUNNINGHAM, V. J., DELFORGE, J., FUJITA, M., GJEDDE, A., GUNN, R. N., HOLDEN, J., HOULE, S., HUANG, S.-C. & ICHISE, M. 2007. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *Journal of Cerebral Blood Flow & Metabolism,* 27**,** 1533-1539.

INSEL, T., CUTHBERT, B., GARVEY, M., HEINSSEN, R., PINE, D. S., QUINN, K., SANISLOW, C. & WANG, P. 2010. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *Am J Psychiatry,* 167**,** 748-51.

IYO, M., NISHIO, M., ITOH, T., FUKUDA, H., SUZUKI, K., YAMASAKI, T., FUKUI, S. & TATENO, Y. 1993. Dopamine D2 and serotonin S2 receptors in susceptibility to methamphetamine psychosis detected by positron emission tomography. *Psychiatry Res,* 50**,** 217-31.

JASINSKA, A. J., STEIN, E. A., KAISER, J., NAUMER, M. J. & YALACHKOV, Y. 2014. Factors modulating neural reactivity to drug cues in addiction: a survey of human neuroimaging studies. *Neurosci Biobehav Rev,* 38**,** 1-16.

JOUTSA, J., JOHANSSON, J., NIEMELA, S., OLLIKAINEN, A., HIRVONEN, M. M., PIEPPONEN, P., ARPONEN, E., ALHO, H., VOON, V., RINNE, J. O., HIETALA, J. & KAASINEN, V. 2012. Mesolimbic dopamine release is linked to symptom severity in pathological gambling. *Neuroimage,* 60**,** 1992-9.

KAUFMAN, J. N., ROSS, T. J., STEIN, E. A. & GARAVAN, H. 2003. Cingulate hypoactivity in cocaine users during a GO-NOGO task as revealed by event-related functional magnetic resonance imaging. *J Neurosci,* 23**,** 7839-43.

KEELER, J. F., PRETSELL, D. O. & ROBBINS, T. W. 2014. Functional implications of dopamine D1 vs. D2 receptors: A 'prepare and select' model of the striatal direct vs. indirect pathways. *Neuroscience,* 282**,** 156-175.

KELLEY, A. E. & BERRIDGE, K. C. 2002. The neuroscience of natural rewards: relevance to addictive drugs. *J Neurosci,* 22**,** 3306-11.

KETTNER, H., GANDY, S., HAIJEN, E. & CARHART-HARRIS, R. L. 2019. From Egoism to Ecoism: Psychedelics Increase Nature Relatedness in a State-Mediated and Context-Dependent Manner. *Int J Environ Res Public Health,* 16.

KIENAST, T. & HEINZ, A. 2006. Dopamine and the diseased brain. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders),* 5**,** 109-131.
KLANKER, M., FEENSTRA, M. & DENYS, D. 2013. Dopaminergic control of cognitive flexibility in humans and animals. *Front Neurosci,* 7**,** 201.
KNUTSON, B., BJORK, J. M., FONG, G. W., HOMMER, D., MATTAY, V. S. & WEINBERGER, D. R. 2004. Amphetamine modulates human incentive processing. *Neuron,* 43**,** 261-9.
KNUTSON, B., FONG, G., KAISER, E., ADAMS, C. & HOMMER, D. 2000. Anticipation of monetary rewards activates nucleus accumbens. *Neuroimage,* 11.
KOEPP, M. J. 1998. Evidence for striatal dopamine release during a video game. *Nature,* 393.
KOHN, R., SAXENA, S., LEVAV, I. & SARACENO, B. 2004. The treatment gap in mental health care. *Bulletin of the World Health Organization,* 82**,** 858-866.
KOOB, G. F. 1992. Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol Sci,* 13**,** 177-84.
KOOB, G. F. 2021. Drug Addiction: Hyperkatifeia/Negative Reinforcement as a Framework for Medications Development. *Pharmacol Rev,* 73**,** 163-201.
KOOB, G. F., POWELL, P. & WHITE, A. 2020. Addiction as a Coping Response: Hyperkatifeia, Deaths of Despair, and COVID-19. *American Journal of Psychiatry,* 177**,** 1031-1037.
KOOB, G. F. & VOLKOW, N. D. 2016. Neurobiology of addiction: a neurocircuitry analysis. *Lancet Psychiatry,* 3**,** 760-773.
KRYSTAL, A. D., PIZZAGALLI, D. A., MATHEW, S. J., SANACORA, G., KEEFE, R., SONG, A., CALABRESE, J., GODDARD, A., GOODMAN, W., LISANBY, S. H., SMOSKI, M., WEINER, R., IOSIFESCU, D., NURNBERGER, J., SZABO, S., MURROUGH, J., SHEKHAR, A. & POTTER, W. 2019. The first implementation of the NIMH FAST-FAIL approach to psychiatric drug development. *Nature Reviews Drug Discovery,* 18**,** 82-84.
KWAKO, L. E., BICKEL, W. K. & GOLDMAN, D. 2018. Addiction Biomarkers: Dimensional Approaches to Understanding Addiction. *Trends Mol Med,* 24**,** 121-128.
KWAKO, L. E., MOMENAN, R., LITTEN, R. Z., KOOB, G. F. & GOLDMAN, D. 2016. Addictions Neuroclinical Assessment: A Neuroscience-Based Framework for Addictive Disorders. *Biol Psychiatry,* 80**,** 179-89.
LACROIX, L. P., HOWS, M. E. P., SHAH, A. J., HAGAN, J. J. & HEIDBREDER, C. A. 2003. Selective Antagonism at Dopamine D3 Receptors Enhances Monoaminergic and Cholinergic Neurotransmission in the Rat Anterior Cingulate Cortex. *Neuropsychopharmacology,* 28**,** 839-849.
LAMMERTSMA, A. A. & HUME, S. P. 1996. Simplified reference tissue model for PET receptor studies. *Neuroimage,* 4**,** 153-158.
LARUELLE, M. 2000. Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. *J Cereb Blood Flow Metab,* 20**,** 423-51.
LARUELLE, M., ABI-DARGHAM, A., VAN DYCK, C. H., ROSENBLATT, W., ZEA-PONCE, Y., ZOGHBI, S. S., BALDWIN, R. M., CHARNEY, D. S., HOFFER, P. B., KUNG, H. F. & ET AL. 1995. SPECT imaging of striatal dopamine release after amphetamine challenge. *J Nucl Med,* 36**,** 1182-90.

LARUELLE, M., IYER, R. N., AL-TIKRITI, M. S., ZEA-PONCE, Y., MALISON, R., ZOGHBI, S. S., BALDWIN, R. M., KUNG, H. F., CHARNEY, D. S., HOFFER, P. B., INNIS, R. B. & BRADBERRY, C. W. 1997. Microdialysis and SPECT measurements of amphetamine-induced dopamine release in nonhuman primates. *Synapse,* 25**,** 1-14.

LATASTER, J., COLLIP, D., CECCARINI, J., HAAS, D., BOOIJ, L., VAN OS, J., PRUESSNER, J., VAN LAERE, K. & MYIN-GERMEYS, I. 2011. Psychosocial stress is associated with in vivo dopamine release in human ventromedial prefrontal cortex: a positron emission tomography study using [18F] fallypride. *Neuroimage,* 58**,** 1081-1089.

LE FOLL, B., GOLDBERG, S. R. & SOKOLOFF, P. 2005. The dopamine D3 receptor and drug dependence: effects on reward or beyond? *Neuropharmacology,* 49**,** 525-541.

LEE, B., LONDON, E. D., POLDRACK, R. A., FARAHI, J., NACCA, A., MONTEROSSO, J. R., MUMFORD, J. A., BOKARIUS, A. V., DAHLBOM, M., MUKHERJEE, J., BILDER, R. M., BRODY, A. L. & MANDELKERN, M. A. 2009. Striatal dopamine d2/d3 receptor availability is reduced in methamphetamine dependence and is linked to impulsivity. *J Neurosci,* 29**,** 14734-40.

LEMÓN, L., FERNÁNDEZ-ARANDA, F., JIMÉNEZ-MURCIA, S. & HÅKANSSON, A. 2021. Eating disorder in gambling disorder: A group with increased psychopathology. *J Behav Addict,* 10**,** 540-545.

LEYTON, M. & VEZINA, P. 2013a. Striatal ups and downs: their roles in vulnerability to addictions in humans. *Neuroscience and Biobehavioral Reviews,* 37**,** 1999-2014.

LEYTON, M. & VEZINA, P. 2013b. Striatal ups and downs: their roles in vulnerability to addictions in humans. *Neurosci Biobehav Rev,* 37**,** 1999-2014.

LIMBRICK-OLDFIELD, E. H., MICK, I., COCKS, R. E., MCGONIGLE, J., SHARMAN, S. P., GOLDSTONE, A. P., STOKES, P. R., WALDMAN, A., ERRITZOE, D., BOWDEN-JONES, H., NUTT, D., LINGFORD-HUGHES, A. & CLARK, L. 2017. Neural substrates of cue reactivity and craving in gambling disorder. *Transl Psychiatry,* 7**,** e992.

LIPTON, D. M., GONZALES, B. J. & CITRI, A. 2019. Dorsal Striatal Circuits for Habits, Compulsions and Addictions. *Front Syst Neurosci,* 13**,** 28.

LOISEAU, F. & MILLAN, M. J. 2009. Blockade of dopamine D(3) receptors in frontal cortex, but not in sub-cortical structures, enhances social recognition in rats: similar actions of D(1) receptor agonists, but not of D(2) antagonists. *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology,* 19**,** 23-33.

LONDON, E. D., OKITA, K., KINNEY, K. R., DEAN, A. C., MCCLINTICK, M. N., RIZOR, E. J., JOHNSON, M. C., MAHMOUDIE, T., BRODY, A. L., NURMI, E. L., SEAMAN, L. C., FARAHI, J., GINDER, N. & MANDELKERN, M. A. 2020. No significant elevation of translocator protein binding in the brains of recently abstinent methamphetamine users. *Drug Alcohol Depend,* 213**,** 108104.

LOTH, E., CHARMAN, T., MASON, L., TILLMANN, J., JONES, E. J. H., WOOLDRIDGE, C., AHMAD, J., AUYEUNG, B., BROGNA, C., AMBROSINO, S., BANASCHEWSKI, T., BARON-COHEN, S., BAUMEISTER, S., BECKMANN, C., BRAMMER, M., BRANDEIS, D., BÖLTE, S., BOURGERON, T., BOURS, C., DE BRUIJN, Y., CHAKRABARTI, B., CRAWLEY, D., CORNELISSEN, I., ACQUA, F. D., DUMAS, G., DURSTON, S., ECKER, C.,

FAULKNER, J., FROUIN, V., GARCES, P., GOYARD, D., HAYWARD, H., HAM, L. M., HIPP, J., HOLT, R. J., JOHNSON, M. H., ISAKSSON, J., KUNDU, P., LAI, M. C., D'ARDHUY X, L., LOMBARDO, M. V., LYTHGOE, D. J., MANDL, R., MEYER-LINDENBERG, A., MOESSNANG, C., MUELLER, N., O'DWYER, L., OLDEHINKEL, M., ORANJE, B., PANDINA, G., PERSICO, A. M., RUIGROK, A. N. V., RUGGERI, B., SABET, J., SACCO, R., CÁCERES, A. S. J., SIMONOFF, E., TORO, R., TOST, H., WALDMAN, J., WILLIAMS, S. C. R., ZWIERS, M. P., SPOOREN, W., MURPHY, D. G. M. & BUITELAAR, J. K. 2017. The EU-AIMS Longitudinal European Autism Project (LEAP): design and methodologies to identify and validate stratification biomarkers for autism spectrum disorders. *Mol Autism,* 8**,** 24.

LUIJTEN, M., SCHELLEKENS, A. F., KÜHN, S., MACHIELSE, M. W. & SESCOUSSE, G. 2017. Disruption of Reward Processing in Addiction : An Image-Based Meta-analysis of Functional Magnetic Resonance Imaging Studies. *JAMA Psychiatry,* 74**,** 387-398.

MALCOLM, B. P., HESSELBROCK, M. N. & SEGAL, B. 2006. Multiple substance dependence and course of alcoholism among Alaska native men and women. *Subst Use Misuse,* 41**,** 729-41.

MANJÓN, J. V. 2017. MRI Preprocessing. *In:* MARTÍ-BONMATÍ, L. & ALBERICH-BAYARRI, A. (eds.) *Imaging Biomarkers: Development and Clinical Integration.* Cham: Springer International Publishing.

MARTINEZ, D., BROFT, A., FOLTIN, R. W., SLIFSTEIN, M., HWANG, D. R., HUANG, Y., PEREZ, A., FRANKLE, W. G., COOPER, T., KLEBER, H. D., FISCHMAN, M. W. & LARUELLE, M. 2004. Cocaine dependence and d2 receptor availability in the functional subdivisions of the striatum: relationship with cocaine-seeking behavior. *Neuropsychopharmacology,* 29**,** 1190-202.

MARTINEZ, D., CARPENTER, K. M., LIU, F., SLIFSTEIN, M., BROFT, A., FRIEDMAN, A. C., KUMAR, D., VAN HEERTUM, R., KLEBER, H. D. & NUNES, E. 2011. Imaging dopamine transmission in cocaine dependence: link between neurochemistry and response to treatment. *Am J Psychiatry,* 168**,** 634-41.

MARTINEZ, D., GIL, R., SLIFSTEIN, M., HWANG, D. R., HUANG, Y., PEREZ, A., KEGELES, L., TALBOT, P., EVANS, S., KRYSTAL, J., LARUELLE, M. & ABI-DARGHAM, A. 2005. Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. *Biol Psychiatry,* 58**,** 779-86.

MARTINEZ, D., ORLOWSKA, D., NARENDRAN, R., SLIFSTEIN, M., LIU, F., KUMAR, D., BROFT, A., VAN HEERTUM, R. & KLEBER, H. D. 2010. Dopamine type 2/3 receptor availability in the striatum and social status in human volunteers. *Biol Psychiatry,* 67**,** 275-8.

MARTINEZ, D., SACCONE, P. A., LIU, F., SLIFSTEIN, M., ORLOWSKA, D., GRASSETTI, A., COOK, S., BROFT, A., VAN HEERTUM, R. & COMER, S. D. 2012. Deficits in dopamine D(2) receptors and presynaptic dopamine in heroin dependence: commonalities and differences with other types of addiction. *Biol Psychiatry,* 71**,** 192-8.

MARTINEZ, D., SLIFSTEIN, M., BROFT, A., MAWLAWI, O., HWANG, D. R., HUANG, Y., COOPER, T., KEGELES, L., ZARAHN, E., ABI-DARGHAM, A., HABER, S. N. & LARUELLE, M. 2003. Imaging human mesolimbic dopamine transmission with positron emission tomography. Part II: amphetamine-induced dopamine release in the functional subdivisions of the striatum. *J Cereb Blood Flow Metab,* 23**,** 285-300.

MATUSKEY, D., GALLEZOT, J. D., PITTMAN, B., WILLIAMS, W., WANYIRI, J., GAISER, E., LEE, D. E., HANNESTAD, J., LIM, K., ZHENG, M. Q., LIN, S. F., LABAREE, D., POTENZA, M. N., CARSON, R. E., MALISON, R. T. & DING, Y. S. 2014. Dopamine D(3) receptor alterations in cocaine-dependent humans imaged with [(1)(1)C](+)PHNO. *Drug Alcohol Depend,* 139**,** 100-5.

MAZZIOTTA, J., TOGA, A., EVANS, A., FOX, P., LANCASTER, J., ZILLES, K., WOODS, R., PAUS, T., SIMPSON, G., PIKE, B., HOLMES, C., COLLINS, L., THOMPSON, P., MACDONALD, D., IACOBONI, M., SCHORMANN, T., AMUNTS, K., PALOMERO-GALLAGHER, N., GEYER, S., PARSONS, L., NARR, K., KABANI, N., LE GOUALHER, G., BOOMSMA, D., CANNON, T., KAWASHIMA, R. & MAZOYER, B. 2001. A probabilistic atlas and reference system for the human brain: International Consortium for Brain Mapping (ICBM). *Philos Trans R Soc Lond B Biol Sci,* 356**,** 1293-322.

MCCUTCHEON, R. A., NOUR, M. M., DAHOUN, T., JAUHAR, S., PEPPER, F., EXPERT, P., VERONESE, M., ADAMS, R. A., TURKHEIMER, F., MEHTA, M. A. & HOWES, O. D. 2019. Mesolimbic Dopamine Function Is Related to Salience Network Connectivity: An Integrative Positron Emission Tomography and Magnetic Resonance Study. *Biological Psychiatry,* 85**,** 368-378.

MCCUTCHEON, R. A., PILLINGER, T., EFTHIMIOU, O., MASLEJ, M., MULSANT, B. H., YOUNG, A. H., CIPRIANI, A. & OLIVER 2022. Reappraising the variability of effects of antipsychotic medication in schizophrenia: a meta-analysis. *World Psychiatry,* 21**,** 287-294.

MCGONIGLE, J., MURPHY, A., PATERSON, L. M., REED, L. J., NESTOR, L., NASH, J., ELLIOTT, R., ERSCHE, K. D., FLECHAIS, R. S., NEWBOULD, R., ORBAN, C., SMITH, D. G., TAYLOR, E. M., WALDMAN, A. D., ROBBINS, T. W., DEAKIN, J. W., NUTT, D. J., LINGFORD-HUGHES, A. R. & SUCKLING, J. 2017. The ICCAM platform study: An experimental medicine platform for evaluating new drugs for relapse prevention in addiction. Part B: fMRI description. *J Psychopharmacol,* 31**,** 3-16.

MEINHARDT, M. W., PFARR, S., FOUQUET, G., ROHLEDER, C., MEINHARDT, M. L., BARROSO-FLORES, J., HOFFMANN, R., JEANBLANC, J., PAUL, E., WAGNER, K., HANSSON, A. C., KÖHR, G., MEIER, N., VON BOHLEN UND HALBACH, O., BELL, R. L., ENDEPOLS, H., NEUMAIER, B., SCHÖNIG, K., BARTSCH, D., NAASSILA, M., SPANAGEL, R. & SOMMER, W. H. 2021. Psilocybin targets a common molecular mechanism for cognitive impairment and increased craving in alcoholism. *Sci Adv,* 7**,** eabh2399.

MENG, X.-L., ROSENTHAL, R. & RUBIN, D. B. 1992. Comparing correlated correlation coefficients. *Psychological bulletin,* 111**,** 172.

METZNER, R. 1994. Addiction and transcendence as altered states of consciousness. *Journal of Transpersonal Psychology,* 26**,** 1-17.

MIEDL, S. F., FEHR, T., MEYER, G. & HERRMANN, M. 2010. Neurobiological correlates of problem gambling in a quasi-realistic blackjack scenario as revealed by fMRI. *Psychiatry Res,* 181**,** 165-73.

MILELLA, M. S., FOTROS, A., GRAVEL, P., CASEY, K. F., LARCHER, K., VERHAEGHE, J. A., COX, S. M., READER, A. J., DAGHER, A. & BENKELFAT, C. 2016. Cocaine cue–induced dopamine release in the human prefrontal cortex. *Journal of Psychiatry and Neuroscience,* 41**,** 322-330.

MOELLER, S. J., HONORIO, J., TOMASI, D., PARVAZ, M. A., WOICIK, P. A., VOLKOW, N. D. & GOLDSTEIN, R. Z. 2014. Methylphenidate enhances

executive function and optimizes prefrontal function in both health and cocaine addiction. *Cereb Cortex,* 24**,** 643-53.
MOELLER, S. J., OKITA, K., ROBERTSON, C. L., BALLARD, M. E., KONOVA, A. B., GOLDSTEIN, R. Z., MANDELKERN, M. A. & LONDON, E. D. 2018. Low Striatal Dopamine D2-type Receptor Availability is Linked to Simulated Drug Choice in Methamphetamine Users. *Neuropsychopharmacology,* 43**,** 751-760.
MOGG, K., BRADLEY, B. P., O'NEILL, B., BANI, M., MERLO-PICH, E., KOCH, A., BULLMORE, E. T. & NATHAN, P. J. 2012a. Effect of dopamine D_3 receptor antagonism on approach responses to food cues in overweight and obese individuals. *Behavioural Pharmacology,* 23**,** 603-608.
MOGG, K., BRADLEY, B. P., O'NEILL, B., BANI, M., MERLO-PICH, E., KOCH, A., BULLMORE, E. T. & NATHAN, P. J. 2012b. Effect of dopamine D_3 receptor antagonism on approach responses to food cues in overweight and obese individuals. *Behav Pharmacol,* 23**,** 603-8.
MORGAN, D., GRANT, K. A., GAGE, H. D., MACH, R. H., KAPLAN, J. R., PRIOLEAU, O., NADER, S. H., BUCHHEIMER, N., EHRENKAUFER, R. L. & NADER, M. A. 2002. Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. *Nat Neurosci,* 5**,** 169-74.
MORGAN, P., VAN DER GRAAF, P. H., ARROWSMITH, J., FELTNER, D. E., DRUMMOND, K. S., WEGNER, C. D. & STREET, S. D. 2012. Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discov Today,* 17**,** 419-24.
MORRIS, E. D., ENDRES, C. J., SCHMIDT, K. C., CHRISTIAN, B. T., MUZIC, R. F. & FISHER, R. E. 2004. Kinetic modeling in positron emission tomography. *Emission tomography: The fundamentals of PET and SPECT,* 46**,** 499-540.
MORRIS, E. D., KIM, S. J., SULLIVAN, J. M., WANG, S., NORMANDIN, M. D., CONSTANTINESCU, C. C. & COSGROVE, K. P. 2013. Creating dynamic images of short-lived dopamine fluctuations with lp-ntPET: dopamine movies of cigarette smoking. *J Vis Exp*.
MOUJAES, F., PRELLER, K. H., JI, J. L., MURRAY, J. D., BERKOVITCH, L., VOLLENWEIDER, F. X. & ANTICEVIC, A. 2023. Toward Mapping Neurobehavioral Heterogeneity of Psychedelic Neurobiology in Humans. *Biol Psychiatry,* 93**,** 1061-1070.
MUGNAINI, M., IAVARONE, L., CAVALLINI, P., GRIFFANTE, C., OLIOSI, B., SAVOIA, C., BEAVER, J., RABINER, E. A., MICHELI, F., HEIDBREDER, C., ANDORN, A., MERLO PICH, E. & BANI, M. 2013. Occupancy of brain dopamine D3 receptors and drug craving: a translational approach. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology,* 38**,** 302-312.
MUKHERJEE, J., CHRISTIAN, B. T., DUNIGAN, K. A., SHI, B., NARAYANAN, T. K., SATTER, M. & MANTIL, J. 2002. Brain imaging of 18F-fallypride in normal volunteers: blood analysis, distribution, test-retest studies, and preliminary assessment of sensitivity to aging effects on dopamine D-2/D-3 receptors. *Synapse,* 46**,** 170-88.
MÜLLER, C. P. & HOMBERG, J. R. 2015. The role of serotonin in drug use and addiction. *Behavioural brain research,* 277**,** 146-192.
MURPHY, A., NESTOR, L. J., MCGONIGLE, J., PATERSON, L., BOYAPATI, V., ERSCHE, K. D., FLECHAIS, R., KUCHIBATLA, S., METASTASIO, A., ORBAN, C., PASSETTI, F., REED, L., SMITH, D., SUCKLING, J., TAYLOR, E., ROBBINS, T. W., LINGFORD-HUGHES, A., NUTT, D. J., DEAKIN, J. F. &

ELLIOTT, R. 2017a. Acute D3 Antagonist GSK598809 Selectively Enhances Neural Response During Monetary Reward Anticipation in Drug and Alcohol Dependence. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology,* 42**,** 1049-1057.

MURPHY, A., NESTOR, L. J., MCGONIGLE, J., PATERSON, L., BOYAPATI, V., ERSCHE, K. D., FLECHAIS, R., KUCHIBATLA, S., METASTASIO, A., ORBAN, C., PASSETTI, F., REED, L., SMITH, D., SUCKLING, J., TAYLOR, E., ROBBINS, T. W., LINGFORD-HUGHES, A., NUTT, D. J., DEAKIN, J. F. W. & ELLIOTT, R. 2017b. Acute D3 Antagonist GSK598809 Selectively Enhances Neural Response During Monetary Reward Anticipation in Drug and Alcohol Dependence. *Neuropsychopharmacology,* 42**,** 1049-1057.

MURPHY, A., NESTOR, L. J., MCGONIGLE, J., PATERSON, L., BOYAPATI, V., ERSCHE, K. D., FLECHAIS, R., KUCHIBATLA, S., METASTASIO, A., ORBAN, C., PASSETTI, F., REED, L., SMITH, D., SUCKLING, J., TAYLOR, E., ROBBINS, T. W., LINGFORD-HUGHES, A., NUTT, D. J., DEAKIN, J. F. W., ELLIOTT, R. & PLATFORM, I. 2017c. Acute D3 Antagonist GSK598809 Selectively Enhances Neural Response During Monetary Reward Anticipation in Drug and Alcohol Dependence. *Neuropsychopharmacology,* 42**,** 1049-1057.

NADER, M. A., CZOTY, P. W., GOULD, R. W. & RIDDICK, N. V. 2008. Review. Positron emission tomography imaging studies of dopamine receptors in primate models of addiction. *Philos Trans R Soc Lond B Biol Sci,* 363**,** 3223-32.

NAGANO-SAITO, A., DAGHER, A., BOOIJ, L., GRAVEL, P., WELFELD, K., CASEY, K. F., LEYTON, M. & BENKELFAT, C. 2013. Stress-induced dopamine release in human medial prefrontal cortex--18F-fallypride/PET study in healthy volunteers. *Synapse,* 67**,** 821-30.

NARENDRAN, R. 2011. Imaging of dopamine D2/3 agonist binding in cocaine dependence: a [11C] NPA positron emission tomography study. *Synapse,* 65.

NARENDRAN, R., MARTINEZ, D., MASON, N. S., LOPRESTI, B. J., HIMES, M. L., CHEN, C. M., MAY, M. A., PRICE, J. C., MATHIS, C. A. & FRANKLE, W. G. 2011. Imaging of dopamine D2/3 agonist binding in cocaine dependence: a [11C]NPA positron emission tomography study. *Synapse,* 65**,** 1344-9.

NARENDRAN, R., MASON, N. S., HIMES, M. L. & FRANKLE, W. G. 2020. Imaging Cortical Dopamine Transmission in Cocaine Dependence: A [(11)C]FLB 457-Amphetamine Positron Emission Tomography Study. *Biol Psychiatry,* 88**,** 788-796.

NARENDRAN, R., MASON, N. S., LAYMON, C. M., LOPRESTI, B. J., VELASQUEZ, N. D., MAY, M. A., KENDRO, S., MARTINEZ, D., MATHIS, C. A. & FRANKLE, W. G. 2010. A comparative evaluation of the dopamine D(2/3) agonist radiotracer [11C](-)-N-propyl-norapomorphine and antagonist [11C]raclopride to measure amphetamine-induced dopamine release in the human striatum. *J Pharmacol Exp Ther,* 333**,** 533-9.

NARENDRAN, R., MASON, N. S., PARIS, J., HIMES, M. L., DOUAIHY, A. B. & FRANKLE, W. G. 2014. Decreased prefrontal cortical dopamine transmission in alcoholism. *Am J Psychiatry,* 171**,** 881-8.

NATHAN, P. J., O'NEILL, B. V., MOGG, K., BRADLEY, B. P., BEAVER, J., BANI, M., MERLO-PICH, E., FLETCHER, P. C., SWIRSKI, B., KOCH, A., DODDS, C. M. & BULLMORE, E. T. 2012a. The effects of the dopamine D3 receptor antagonist GSK598809 on attentional bias to palatable food cues in

overweight and obese subjects. *International Journal of Neuropsychopharmacology,* 15**,** 149-161.
NATHAN, P. J., O'NEILL, B. V., MOGG, K., BRADLEY, B. P., BEAVER, J., BANI, M., MERLO-PICH, E., FLETCHER, P. C., SWIRSKI, B., KOCH, A., DODDS, C. M. & BULLMORE, E. T. 2012b. The effects of the dopamine D_3 receptor antagonist GSK598809 on attentional bias to palatable food cues in overweight and obese subjects. *The International Journal of Neuropsychopharmacology,* 15**,** 149-161.
NATHAN, P. J., PHAN, K. L., HARMER, C. J., MEHTA, M. A. & BULLMORE, E. T. 2014. Increasing pharmacological knowledge about human neurological and psychiatric disorders through functional neuroimaging and its application in drug discovery. *Curr Opin Pharmacol,* 14**,** 54-61.
NESTLER, E. J. 2005. Is there a common molecular pathway for addiction? *Nat Neurosci,* 8**,** 1445-9.
NESTOR, L. J., MURPHY, A., MCGONIGLE, J., ORBAN, C., REED, L., TAYLOR, E., FLECHAIS, R., PATERSON, L. M., SMITH, D. & BULLMORE, E. T. 2017. Acute naltrexone does not remediate fronto-striatal disturbances in alcoholic and alcoholic polysubstance-dependent populations during a monetary incentive delay task. *Addiction biology,* 22**,** 1576-1589.
NOORI, H. R., COSA LINAN, A. & SPANAGEL, R. 2016. Largely overlapping neuronal substrates of reactivity to drug, gambling, food and sexual cues: A comprehensive meta-analysis. *Eur Neuropsychopharmacol,* 26**,** 1419-1430.
NUTT, D., HAYES, A., FONVILLE, L., ZAFAR, R., PALMER, E. O. C., PATERSON, L. & LINGFORD-HUGHES, A. 2021. Alcohol and the Brain. *Nutrients,* 13**,** 3938.
NUTT, D. J., KING, L. A. & PHILLIPS, L. D. 2010. Drug harms in the UK: a multicriteria decision analysis. *The Lancet,* 376**,** 1558-1565.
NUTT, D. J., LINGFORD-HUGHES, A., ERRITZOE, D. & STOKES, P. R. 2015. The dopamine theory of addiction: 40 years of highs and lows. *Nat Rev Neurosci,* 16**,** 305-12.
NUTT, P. D. J., NESTOR, D. L. J., NUTT, D. & NESTOR, L. 2013. 5253The GABA system and addiction. *Addiction.* Oxford University Press.
OBERLIN, B. G., ALBRECHT, D. S., HERRING, C. M., WALTERS, J. W., HILE, K. L., KAREKEN, D. A. & YODER, K. K. 2015. Monetary discounting and ventral striatal dopamine receptor availability in nontreatment-seeking alcoholics and social drinkers. *Psychopharmacology,* 232**,** 2207-2216.
OKITA, K., GHAHREMANI, D. G., PAYER, D. E., ROBERTSON, C. L., DEAN, A. C., MANDELKERN, M. A. & LONDON, E. D. 2016. Emotion dysregulation and amygdala dopamine D2-type receptor availability in methamphetamine users. *Drug and Alcohol Dependence,* 161**,** 163-170.
OKITA, K., GHAHREMANI, D. G., PAYER, D. E., ROBERTSON, C. L., MANDELKERN, M. A. & LONDON, E. D. 2015. Relationship of Alexithymia Ratings to Dopamine D2-type Receptors in Anterior Cingulate and Insula of Healthy Control Subjects but Not Methamphetamine-Dependent Individuals. *International Journal of Neuropsychopharmacology,* 19.
OKSANEN, A., HAGFORS, H., VUORINEN, I. & SAVOLAINEN, I. 2022. Longitudinal perspective on cryptocurrency trading and increased gambling problems: a 3 wave national survey study. *Public Health,* 213**,** 85-90.
OLDHAM, S., MURAWSKI, C., FORNITO, A., YOUSSEF, G., YÜCEL, M. & LORENZETTI, V. 2018. The anticipation and outcome phases of reward and

loss processing: A neuroimaging meta-analysis of the monetary incentive delay task. *Hum Brain Mapp,* 39**,** 3398-3418.
OLDS, J. & MILNER, P. 1954. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of Comparative and Physiological Psychology,* 47**,** 419-427.
ONYEKA, I. N., UOSUKAINEN, H., KORHONEN, M. J., BEYNON, C., BELL, J. S., RONKAINEN, K., FOHR, J., TIIHONEN, J. & KAUHANEN, J. 2012. Sociodemographic characteristics and drug abuse patterns of treatment-seeking illicit drug abusers in Finland, 1997-2008: the Huuti study. *J Addict Dis,* 31**,** 350-62.
OOI, L. Q. R., CHEN, J., ZHANG, S., KONG, R., TAM, A., LI, J., DHAMALA, E., ZHOU, J. H., HOLMES, A. J. & YEO, B. T. T. 2022. Comparison of individualized behavioral predictions across anatomical, diffusion and functional connectivity MRI. *Neuroimage,* 263**,** 119636.
PARSONS, L. H. & HURD, Y. L. 2015. Endocannabinoid signalling in reward and addiction. *Nature Reviews Neuroscience,* 16**,** 579-594.
PATERSON, L. M., FLECHAIS, R. S., MURPHY, A., REED, L. J., ABBOTT, S., BOYAPATI, V., ELLIOTT, R., ERRITZOE, D., ERSCHE, K. D., FALUYI, Y., FARAVELLI, L., FERNANDEZ-EGEA, E., KALK, N. J., KUCHIBATLA, S. S., MCGONIGLE, J., METASTASIO, A., MICK, I., NESTOR, L., ORBAN, C., PASSETTI, F., RABINER, E. A., SMITH, D. G., SUCKLING, J., TAIT, R., TAYLOR, E. M., WALDMAN, A. D., ROBBINS, T. W., DEAKIN, J. F., NUTT, D. J. & LINGFORD-HUGHES, A. R. 2015. The Imperial College Cambridge Manchester (ICCAM) platform study: An experimental medicine platform for evaluating new drugs for relapse prevention in addiction. Part A: Study description. *J Psychopharmacol,* 29**,** 943-60.
PAYER, D. E., BEHZADI, A., KISH, S. J., HOULE, S., WILSON, A. A., RUSJAN, P. M., TONG, J., SELBY, P., GEORGE, T. P., MCCLUSKEY, T. & BOILEAU, I. 2014a. Heightened D3 Dopamine Receptor Levels in Cocaine Dependence and Contributions to the Addiction Behavioral Phenotype: A Positron Emission Tomography Study with [11C]-(+)-PHNO. *Neuropsychopharmacology,* 39**,** 311-318.
PAYER, D. E., BEHZADI, A., KISH, S. J., HOULE, S., WILSON, A. A., RUSJAN, P. M., TONG, J., SELBY, P., GEORGE, T. P., MCCLUSKEY, T. & BOILEAU, I. 2014b. Heightened D3 dopamine receptor levels in cocaine dependence and contributions to the addiction behavioral phenotype: a positron emission tomography study with [11C]-+-PHNO. *Neuropsychopharmacology,* 39**,** 311-8.
PEOPLES, L. L. 2002. Neuroscience. Will, anterior cingulate cortex, and addiction. *Science,* 296**,** 1623-4.
PEREZ VELAZQUEZ, J. L. 2005. Brain, behaviour and mathematics: Are we using the right approaches? *Physica D: Nonlinear Phenomena,* 212**,** 161-182.
PETRY, N. M. & WEISS, L. 2009. Social support is associated with gambling treatment outcomes in pathological gamblers. *Am J Addict,* 18**,** 402-8.
PETTIGREW, J. 2011. Iconography in Bradshaw rock art: breaking the circularity. *Clin Exp Optom,* 94**,** 403-17.
PICCOLO, L. D. R., ARTECHE, A. X., FONSECA, R. P., GRASSI-OLIVEIRA, R. & SALLES, J. F. 2016. Influence of family socioeconomic status on IQ, language, memory and executive functions of Brazilian children. *Psicologia: Reflexão e Crítica,* 29.

PICHLER, B. J., KOLB, A., NÄGELE, T. & SCHLEMMER, H. P. 2010. PET/MRI: paving the way for the next generation of clinical multimodality imaging applications. *J Nucl Med,* 51**,** 333-6.
PIDOPLICHKO, V. I., DEBIASI, M., WILLIAMS, J. T. & DANI, J. A. 1997. Nicotine activates and desensitizes midbrain dopamine neurons. *Nature,* 390**,** 401-404.
PIKE, V. W. 2016. Considerations in the Development of Reversibly Binding PET Radioligands for Brain Imaging. *Curr Med Chem,* 23**,** 1818-69.
POISSON, C. L., ENGEL, L. & SAUNDERS, B. T. 2021. Dopamine Circuit Mechanisms of Addiction-Like Behaviors. *Front Neural Circuits,* 15**,** 752420.
POTENZA, M. N. 2008. Review. The neurobiology of pathological gambling and drug addiction: an overview and new findings. *Philos Trans R Soc Lond B Biol Sci,* 363**,** 3181-9.
POTENZA, M. N. 2014. The neural bases of cognitive processes in gambling disorder. *Trends Cogn Sci,* 18**,** 429-38.
POTENZA, M. N., KOSTEN, T. R. & ROUNSAVILLE, B. J. 2001. Pathological gambling. *Jama,* 286**,** 141-4.
POTENZA, M. N., STEINBERG, M. A., SKUDLARSKI, P., FULBRIGHT, R. K., LACADIE, C. M., WILBER, M. K., ROUNSAVILLE, B. J., GORE, J. C. & WEXLER, B. E. 2003. Gambling Urges in Pathological Gambling: A Functional Magnetic Resonance Imaging Study. *Archives of General Psychiatry,* 60**,** 828-836.
QUEDNOW, B. B., KUHN, K. U., HOPPE, C., WESTHEIDE, J., MAIER, W., DAUM, I. & WAGNER, M. 2007. Elevated impulsivity and impaired decision-making cognition in heavy users of MDMA ("Ecstasy"). *Psychopharmacology (Berl),* 189**,** 517-30.
QUELCH, D. R., MICK, I., MCGONIGLE, J., RAMOS, A. C., FLECHAIS, R. S. A., BOLSTRIDGE, M., RABINER, E., WALL, M. B., NEWBOULD, R. D., STEINIGER-BRACH, B., VAN DEN BERG, F., BOYCE, M., ØSTERGAARD NILAUSEN, D., BREUNING SLUTH, L., MEULIEN, D., VON DER GOLTZ, C., NUTT, D. & LINGFORD-HUGHES, A. 2017. Nalmefene Reduces Reward Anticipation in Alcohol Dependence: An Experimental Functional Magnetic Resonance Imaging Study. *Biol Psychiatry,* 81**,** 941-948.
QUINN, P. D. & HARDEN, K. P. 2013. Differential changes in impulsivity and sensation seeking and the escalation of substance use from adolescence to early adulthood. *Dev Psychopathol,* 25**,** 223-39.
RABINER, E. A., BEAVER, J., MAKWANA, A., SEARLE, G., LONG, C., NATHAN, P. J., NEWBOULD, R. D., HOWARD, J., MILLER, S. R., BUSH, M. A., HILL, S., REILEY, R., PASSCHIER, J., GUNN, R. N., MATTHEWS, P. M. & BULLMORE, E. T. 2011. Pharmacological differentiation of opioid receptor antagonists by molecular and functional imaging of target occupancy and food reward-related brain activation in humans. *Mol Psychiatry,* 16**,** 826-35, 785.
RAGIA, G. & MANOLOPOULOS, V. G. 2017. Personalized Medicine of Alcohol Addiction: Pharmacogenomics and Beyond. *Curr Pharm Biotechnol,* 18**,** 221-230.
RASMUSSEN, K., WHITE, D. A. & ACRI, J. B. 2019. NIDA's medication development priorities in response to the Opioid Crisis: ten most wanted. *Neuropsychopharmacology,* 44**,** 657-659.
RAY, N. J., MIYASAKI, J. M., ZUROWSKI, M., KO, J. H., CHO, S. S., PELLECCHIA, G., ANTONELLI, F., HOULE, S., LANG, A. E. & STRAFELLA, A. P. 2012.

Extrastriatal dopaminergic abnormalities of DA homeostasis in Parkinson's patients with medication-induced pathological gambling: a [11C] FLB-457 and PET study. *Neurobiol Dis,* 48**,** 519-25.
REPO, E., KUIKKA, J. T., BERGSTROM, K. A., KARHU, J., HILTUNEN, J. & TIIHONEN, J. 1999. Dopamine transporter and D2-receptor density in late-onset alcoholism. *Psychopharmacology (Berl),* 147**,** 314-8.
ROBINSON, T. E. & BERRIDGE, K. C. 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev,* 18**,** 247-91.
ROBINSON, T. E. & BERRIDGE, K. C. 2008. Review. The incentive sensitization theory of addiction: some current issues. *Philos Trans R Soc Lond B Biol Sci,* 363**,** 3137-46.
ROGIER, G., PICCI, G. & VELOTTI, P. 2019. Struggling with Happiness: A Pathway Leading Depression to Gambling Disorder. *J Gambl Stud,* 35**,** 293-305.
ROMANCZUK-SEIFERTH, N., KOEHLER, S., DREESEN, C., WÜSTENBERG, T. & HEINZ, A. 2015. Pathological gambling and alcohol dependence: neural disturbances in reward and loss avoidance processing. *Addiction Biology,* 20**,** 557-569.
ROMINGER, A., CUMMING, P., XIONG, G., KOLLER, G., BÖNING, G., WULFF, M., ZWERGAL, A., FÖRSTER, S., REILHAC, A., MUNK, O., SOYKA, M., WÄNGLER, B., BARTENSTEIN, P., LA FOUGÈRE, C. & POGARELL, O. 2012. [18F]Fallypride PET measurement of striatal and extrastriatal dopamine D 2/3 receptor availability in recently abstinent alcoholics. *Addict Biol,* 17**,** 490-503.
SADRAEE, A., PAULUS, M. & EKHTIARI, H. 2021. fMRI as an outcome measure in clinical trials: A systematic review in clinicaltrials.gov. *Brain Behav,* 11**,** e02089.
SANDER, C. Y., HANSEN, H. D. & WEY, H. Y. 2020. Advances in simultaneous PET/MR for imaging neuroreceptor function. *J Cereb Blood Flow Metab,* 40**,** 1148-1166.
SCHLUTER, M. G., KIM, H. S., POOLE, J. C., HODGINS, D. C., MCGRATH, D. S., DOBSON, K. S. & TAVERES, H. 2019. Gambling-related cognitive distortions mediate the relationship between depression and disordered gambling severity. *Addict Behav,* 90**,** 318-323.
SCHULTZ, W. 1998. Predictive reward signal of dopamine neurons. *J Neurophysiol,* 80**,** 1-27.
SEARLE, G. E., BEAVER, J. D., TZIORTZI, A., COMLEY, R. A., BANI, M., GHIBELLINI, G., MERLO-PICH, E., RABINER, E. A., LARUELLE, M. & GUNN, R. N. 2013. Mathematical modelling of [(1)(1)C]-(+)-PHNO human competition studies. *Neuroimage,* 68**,** 119-32.
SEEMAN, P. 2012. Dopamine agonist radioligand binds to both D2High and D2Low receptors, explaining why alterations in D2High are not detected in human brain scans. *Synapse,* 66**,** 88-93.
SESCOUSSE, G., BARBALAT, G., DOMENECH, P. & DREHER, J. C. 2013a. Imbalance in the sensitivity to different types of rewards in pathological gambling. *Brain,* 136**,** 2527-38.
SESCOUSSE, G., CALDÚ, X., SEGURA, B. & DREHER, J. C. 2013b. Processing of primary and secondary rewards: a quantitative meta-analysis and review of human functional neuroimaging studies. *Neurosci Biobehav Rev,* 37**,** 681-96.

SESCOUSSE, G., JANSSEN, L. K., HASHEMI, M. M., TIMMER, M. H. M., GEURTS, D. E. M., TER HUURNE, N. P., CLARK, L. & COOLS, R. 2016. Amplified Striatal Responses to Near-Miss Outcomes in Pathological Gamblers. *Neuropsychopharmacology,* 41**,** 2614-2623.
SEVY, S., SMITH, G. S., MA, Y., DHAWAN, V., CHALY, T., KINGSLEY, P. B., KUMRA, S., ABDELMESSIH, S. & EIDELBERG, D. 2008. Cerebral glucose metabolism and D2/D3 receptor availability in young adults with cannabis dependence measured with positron emission tomography. *Psychopharmacology (Berl),* 197**,** 549-56.
SHARMAN, S., ROBERTS, A., BOWDEN-JONES, H. & STRANG, J. 2021. Gambling in COVID-19 Lockdown in the UK: Depression, Stress, and Anxiety. *Front Psychiatry,* 12**,** 621497.
SINGER, M. 2012. Anthropology and addiction: an historical review. *Addiction,* 107**,** 1747-1755.
SKEWES, M. C. & GONZALEZ, V. M. 2013. The biopsychosocial model of addiction. *Principles of addiction,* 1**,** 61-70.
SKINBJERG, M., SIBLEY, D. R., JAVITCH, J. A. & ABI-DARGHAM, A. 2012. Imaging the high-affinity state of the dopamine D2 receptor in vivo: fact or fiction? *Biochem Pharmacol,* 83**,** 193-8.
SLUTSKE, W. S., CASPI, A., MOFFITT, T. E. & POULTON, R. 2005. Personality and problem gambling: a prospective study of a birth cohort of young adults. *Arch Gen Psychiatry,* 62**,** 769-75.
SOKOLOFF, P., DIAZ, J., FOLL, B. L., GUILLIN, O., LERICHE, L., BEZARD, E. & GROSS, C. 2006. The Dopamine D3 Receptor: A Therapeutic Target for the Treatment of Neuropsychiatric Disorders. *CNS & Neurological Disorders - Drug Targets- CNS & Neurological Disorders),* 5**,** 25-43.
STOKES, P. R., EGERTON, A., WATSON, B., REID, A., LAPPIN, J., HOWES, O. D., NUTT, D. J. & LINGFORD-HUGHES, A. R. 2012. History of cannabis use is not associated with alterations in striatal dopamine D2/D3 receptor availability. *J Psychopharmacol,* 26**,** 144-9.
SUI, J., JIANG, R., BUSTILLO, J. & CALHOUN, V. 2020. Neuroimaging-based individualized prediction of cognition and behavior for mental disorders and health: methods and promises. *Biological psychiatry,* 88**,** 818-828.
TANG, D. W., FELLOWS, L. K., SMALL, D. M. & DAGHER, A. 2012. Food and drug cues activate similar brain regions: a meta-analysis of functional MRI studies. *Physiol Behav,* 106**,** 317-24.
TAVARES, H., ZILBERMAN, M. L., HODGINS, D. C. & EL-GUEBALY, N. 2005. Comparison of craving between pathological gamblers and alcoholics. *Alcohol Clin Exp Res,* 29**,** 1427-31.
TERRANEO, A., LEGGIO, L., SALADINI, M., ERMANI, M., BONCI, A. & GALLIMBERTI, L. 2016. Transcranial magnetic stimulation of dorsolateral prefrontal cortex reduces cocaine use: A pilot study. *Eur Neuropsychopharmacol,* 26**,** 37-44.
THANOS, P. K., MICHAELIDES, M., UMEGAKI, H. & VOLKOW, N. D. 2008. D2R DNA transfer into the nucleus accumbens attenuates cocaine self-administration in rats. *Synapse,* 62**,** 481-6.
THANOS, P. K., TAINTOR, N. B., RIVERA, S. N., UMEGAKI, H., IKARI, H., ROTH, G., INGRAM, D. K., HITZEMANN, R., FOWLER, J. S., GATLEY, S. J., WANG, G. J. & VOLKOW, N. D. 2004. DRD2 gene transfer into the nucleus

accumbens core of the alcohol preferring and nonpreferring rats attenuates alcohol drinking. *Alcohol Clin Exp Res,* 28**,** 720-8.
TOMASI, D., WANG, G.-J. & VOLKOW, N. D. 2015. Balanced modulation of striatal activation from D2/D3receptors in caudate and ventral striatum: Disruption in cannabis abusers. *Human Brain Mapping,* 36**,** 3154-3166.
TRIFILIEFF, P., DUCROCQ, F., VAN DER VELDT, S. & MARTINEZ, D. 2017. Blunted Dopamine Transmission in Addiction: Potential Mechanisms and Implications for Behavior. *Semin Nucl Med,* 47**,** 64-74.
TRIFILIEFF, P. & MARTINEZ, D. 2014. Imaging addiction: D2 receptors and dopamine signaling in the striatum as biomarkers for impulsivity. *Neuropharmacology,* 76 Pt B**,** 498-509.
TUNBRIDGE, E. M., BANNERMAN, D. M., SHARP, T. & HARRISON, P. J. 2004. Catechol-o-methyltransferase inhibition improves set-shifting performance and elevates stimulated dopamine release in the rat prefrontal cortex. *J Neurosci,* 24**,** 5331-5.
TUREL, O., HE, Q., WEI, L. & BECHARA, A. 2021. The role of the insula in internet gaming disorder. *Addict Biol,* 26**,** e12894.
TZIORTZI, A. C., SEARLE, G. E., TZIMOPOULOU, S., SALINAS, C., BEAVER, J. D., JENKINSON, M., LARUELLE, M., RABINER, E. A. & GUNN, R. N. 2011. Imaging dopamine receptors in humans with [11C]-(+)-PHNO: dissection of D3 signal and anatomy. *Neuroimage,* 54**,** 264-77.
URBAN, N. B., SLIFSTEIN, M., THOMPSON, J. L., XU, X., GIRGIS, R. R., RAHEJA, S., HANEY, M. & ABI-DARGHAM, A. 2012. Dopamine release in chronic cannabis users: a [11c]raclopride positron emission tomography study. *Biol Psychiatry,* 71**,** 677-83.
VAMVAKOPOULOU, I. A., FONVILLE, L., HAYES, A., MCGONIGLE, J., ELLIOTT, R., ERSCHE, K. D., FLECHAIS, R., ORBAN, C., MURPHY, A., SMITH, D. G., SUCKLING, J., TAYLOR, E. M., DEAKIN, B., ROBBINS, T. W., NUTT, D. J., LINGFORD-HUGHES, A. R. & PATERSON, L. M. 2022a. Selective D3 receptor antagonism modulates neural response during negative emotional processing in substance dependence. *Frontiers in Psychiatry,* 13**,** 998844.
VAMVAKOPOULOU, I. A., FONVILLE, L., HAYES, A., MCGONIGLE, J., ELLIOTT, R., ERSCHE, K. D., FLECHAIS, R., ORBAN, C., MURPHY, A., SMITH, D. G., SUCKLING, J., TAYLOR, E. M., DEAKIN, B., ROBBINS, T. W., NUTT, D. J., LINGFORD-HUGHES, A. R. & PATERSON, L. M. 2022b. Selective D3 receptor antagonism modulates neural response during negative emotional processing in substance dependence. *Front Psychiatry,* 13**,** 998844.
VAN HOLST, R. J., SESCOUSSE, G., JANSSEN, L. K., JANSSEN, M., BERRY, A. S., JAGUST, W. J. & COOLS, R. 2018. Increased Striatal Dopamine Synthesis Capacity in Gambling Addiction. *Biol Psychiatry,* 83**,** 1036-1043.
VAN HOLST, R. J., VAN DEN BRINK, W., VELTMAN, D. J. & GOUDRIAAN, A. E. 2010. Why gamblers fail to win: a review of cognitive and neuroimaging findings in pathological gambling. *Neurosci Biobehav Rev,* 34**,** 87-107.
VENGELIENE, V., LEONARDI-ESSMANN, F., PERREAU-LENZ, S., GEBICKE-HAERTER, P., DRESCHER, K., GROSS, G. & SPANAGEL, R. 2006. The dopamine D3 receptor plays an essential role in alcohol-seeking and relapse. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology,* 20**,** 2223-2233.
VERDEJO-GARCIA, A., LORENZETTI, V., MANNING, V., PIERCY, H., BRUNO, R., HESTER, R., PENNINGTON, D., TOLOMEO, S., ARUNOGIRI, S., BATES,

M. E., BOWDEN-JONES, H., CAMPANELLA, S., DAUGHTERS, S. B., KOUIMTSIDIS, C., LUBMAN, D. I., MEYERHOFF, D. J., RALPH, A., REZAPOUR, T., TAVAKOLI, H., ZARE-BIDOKY, M., ZILVERSTAND, A., STEELE, D., MOELLER, S. J., PAULUS, M., BALDACCHINO, A. & EKHTIARI, H. 2019. A Roadmap for Integrating Neuroscience Into Addiction Treatment: A Consensus of the Neuroscience Interest Group of the International Society of Addiction Medicine. *Front Psychiatry,* 10**,** 877.

VERONESE, M., MORO, L., ARCOLIN, M., DIPASQUALE, O., RIZZO, G., EXPERT, P., KHAN, W., FISHER, P. M., SVARER, C., BERTOLDO, A., HOWES, O. & TURKHEIMER, F. E. 2019. Covariance statistics and network analysis of brain PET imaging studies. *Scientific Reports,* 9**,** 2496.

VIECHTBAUER, W. 2010. Conducting Meta-Analyses in R with the metafor Package. *Journal of Statistical Software,* 36**,** 1 - 48.

VIJAYRAGHAVAN, S., WANG, M., BIRNBAUM, S. G., WILLIAMS, G. V. & ARNSTEN, A. F. 2007. Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nat Neurosci,* 10**,** 376-84.

VILKMAN, H., KAJANDER, J., NAGREN, K., OIKONEN, V., SYVALAHTI, E. & HIETALA, J. 2000. Measurement of extrastriatal D2-like receptor binding with [11C]FLB 457--a test-retest analysis. *Eur J Nucl Med,* 27**,** 1666-73.

VOLKOW, N. D. 2007. Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. *J. Neurosci.,* 27.

VOLKOW, N. D., CHANG, L., WANG, G. J., FOWLER, J. S., DING, Y. S., SEDLER, M., LOGAN, J., FRANCESCHI, D., GATLEY, J., HITZEMANN, R., GIFFORD, A., WONG, C. & PAPPAS, N. 2001. Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. *Am J Psychiatry,* 158**,** 2015-21.

VOLKOW, N. D., FOWLER, J. S., WANG, G. J. & GOLDSTEIN, R. Z. 2002a. Role of dopamine, the frontal cortex and memory circuits in drug addiction: insight from imaging studies. *Neurobiol Learn Mem,* 78**,** 610-24.

VOLKOW, N. D., FOWLER, J. S., WANG, G. J., HITZEMANN, R., LOGAN, J., SCHLYER, D. J., DEWEY, S. L. & WOLF, A. P. 1993. Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse,* 14**,** 169-77.

VOLKOW, N. D., FOWLER, J. S., WOLF, A. P., SCHLYER, D., SHIUE, C. Y., ALPERT, R., DEWEY, S. L., LOGAN, J., BENDRIEM, B., CHRISTMAN, D. & ET AL. 1990. Effects of chronic cocaine abuse on postsynaptic dopamine receptors. *Am J Psychiatry,* 147**,** 719-24.

VOLKOW, N. D., GUR, R. C., WANG, G. J., FOWLER, J. S., MOBERG, P. J., DING, Y. S., HITZEMANN, R., SMITH, G. & LOGAN, J. 1998. Association between decline in brain dopamine activity with age and cognitive and motor impairment in healthy individuals. *Am J Psychiatry,* 155**,** 344-9.

VOLKOW, N. D. & MORALES, M. 2015. The brain on drugs: from reward to addiction. *Cell,* 162**,** 712-725.

VOLKOW, N. D., TOMASI, D., WANG, G. J., LOGAN, J., ALEXOFF, D. L., JAYNE, M., FOWLER, J. S., WONG, C., YIN, P. & DU, C. 2014a. Stimulant-induced dopamine increases are markedly blunted in active cocaine abusers. *Mol Psychiatry,* 19**,** 1037-43.

VOLKOW, N. D., WANG, G.-J., MAYNARD, L., FOWLER, J. S., JAYNE, B., TELANG, F., LOGAN, J., DING, Y.-S., GATLEY, S. J., HITZEMANN, R., WONG, C. & PAPPAS, N. 2002b. Effects of alcohol detoxification on

dopamine D2 receptors in alcoholics: a preliminary study. *Psychiatry Research: Neuroimaging,* 116**,** 163-172.
VOLKOW, N. D., WANG, G. J., BEGLEITER, H., PORJESZ, B., FOWLER, J. S., TELANG, F., WONG, C., MA, Y., LOGAN, J., GOLDSTEIN, R., ALEXOFF, D. & THANOS, P. K. 2006. High levels of dopamine D2 receptors in unaffected members of alcoholic families: possible protective factors. *Arch Gen Psychiatry,* 63**,** 999-1008.
VOLKOW, N. D., WANG, G. J., FOWLER, J. S., LOGAN, J., GATLEY, S. J., HITZEMANN, R., CHEN, A. D., DEWEY, S. L. & PAPPAS, N. 1997. Decreased striatal dopaminergic responsiveness in detoxified cocaine-dependent subjects. *Nature,* 386**,** 830-3.
VOLKOW, N. D., WANG, G. J., FOWLER, J. S., LOGAN, J., HITZEMANN, R., DING, Y. S., PAPPAS, N., SHEA, C. & PISCANI, K. 1996. Decreases in dopamine receptors but not in dopamine transporters in alcoholics. *Alcohol Clin Exp Res,* 20**,** 1594-8.
VOLKOW, N. D., WANG, G. J., FOWLER, J. S., THANOS, P. P., LOGAN, J., GATLEY, S. J., GIFFORD, A., DING, Y. S., WONG, C. & PAPPAS, N. 2002c. Brain DA D2 receptors predict reinforcing effects of stimulants in humans: replication study. *Synapse,* 46**,** 79-82.
VOLKOW, N. D., WANG, G. J., MA, Y., FOWLER, J. S., WONG, C., DING, Y. S., HITZEMANN, R., SWANSON, J. M. & KALIVAS, P. 2005. Activation of orbital and medial prefrontal cortex by methylphenidate in cocaine-addicted subjects but not in controls: relevance to addiction. *J Neurosci,* 25**,** 3932-9.
VOLKOW, N. D., WANG, G. J., MAYNARD, L., FOWLER, J. S., JAYNE, B., TELANG, F., LOGAN, J., DING, Y. S., GATLEY, S. J., HITZEMANN, R., WONG, C. & PAPPAS, N. 2002d. Effects of alcohol detoxification on dopamine D2 receptors in alcoholics: a preliminary study. *Psychiatry Res,* 116**,** 163-72.
VOLKOW, N. D., WANG, G. J., TELANG, F., FOWLER, J. S., ALEXOFF, D., LOGAN, J., JAYNE, M., WONG, C. & TOMASI, D. 2014b. Decreased dopamine brain reactivity in marijuana abusers is associated with negative emotionality and addiction severity. *Proc Natl Acad Sci U S A,* 111**,** E3149-56.
VOLKOW, N. D., WANG, G. J., TELANG, F., FOWLER, J. S., LOGAN, J., JAYNE, M., MA, Y., PRADHAN, K. & WONG, C. 2007. Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. *J Neurosci,* 27**,** 12700-6.
VON STUMM, S. & PLOMIN, R. 2015. Socioeconomic status and the growth of intelligence from infancy through adolescence. *Intelligence,* 48**,** 30-36.
VRIEZE, E., CECCARINI, J., PIZZAGALLI, D. A., BORMANS, G., VANDENBULCKE, M., DEMYTTENAERE, K., VAN LAERE, K. & CLAES, S. 2013. Measuring extrastriatal dopamine release during a reward learning task. *Hum Brain Mapp,* 34**,** 575-86.
WAGNER, H. N., JR., BURNS, H. D., DANNALS, R. F., WONG, D. F., LANGSTROM, B., DUELFER, T., FROST, J. J., RAVERT, H. T., LINKS, J. M., ROSENBLOOM, S. B., LUKAS, S. E., KRAMER, A. V. & KUHAR, M. J. 1983. Imaging dopamine receptors in the human brain by positron tomography. *Science,* 221**,** 1264-6.
WAI, J. M., GRASSETTI, A., SLIFSTEIN, M., MATUSKEY, D., NABULSI, N., ROPCHAN, J., LABAREE, D., HUANG, Y. & MARTINEZ, D. 2019. Binge

alcohol use is not associated with alterations in striatal dopamine receptor binding or dopamine release. *Drug Alcohol Depend,* 205**,** 107627.
WALL, M. B. 2019. Reliability starts with the experimental tools employed. *Cortex,* 113**,** 352-354.
WANG, G.-J., WIERS, C. E., SHUMAY, E., TOMASI, D., YUAN, K., WONG, C. T., LOGAN, J., FOWLER, J. S. & VOLKOW, N. D. 2019. Expectation effects on brain dopamine responses to methylphenidate in cocaine use disorder. *Translational Psychiatry,* 9.
WANG, G. J., SMITH, L., VOLKOW, N. D., TELANG, F., LOGAN, J., TOMASI, D., WONG, C. T., HOFFMAN, W., JAYNE, M., ALIA-KLEIN, N., THANOS, P. & FOWLER, J. S. 2012. Decreased dopamine activity predicts relapse in methamphetamine abusers. *Mol Psychiatry,* 17**,** 918-25.
WANG, G. J., VOLKOW, N. D., FOWLER, J. S., LOGAN, J., ABUMRAD, N. N., HITZEMANN, R. J., PAPPAS, N. S. & PASCANI, K. 1997. Dopamine D2 receptor availability in opiate-dependent subjects before and after naloxone-precipitated withdrawal. *Neuropsychopharmacology,* 16**,** 174-82.
WEDZONY, K., KLIMEK, V. & GOŁEMBIOWSKA, K. 1993. MK-801 elevates the extracellular concentration of dopamine in the rat prefrontal cortex and increases the density of striatal dopamine D1 receptors. *Brain Res,* 622**,** 325-9.
WEISS, B., NYGART, V., POMMERENCKE, L. M., CARHART-HARRIS, R. L. & ERRITZOE, D. 2021. Examining Psychedelic-Induced Changes in Social Functioning and Connectedness in a Naturalistic Online Sample Using the Five-Factor Model of Personality. *Front Psychol,* 12**,** 749788.
WIERS, C. E., CABRERA, E., SKARDA, E., VOLKOW, N. D. & WANG, G.-J. 2016a. PET imaging for addiction medicine: From neural mechanisms to clinical considerations. *Progress in brain research,* 224**,** 175-201.
WIERS, C. E., CABRERA, E. A., TOMASI, D., WONG, C. T., DEMIRAL, Ş. B., KIM, S. W., WANG, G.-J. & VOLKOW, N. D. 2017. Striatal Dopamine D2/D3 Receptor Availability Varies Across Smoking Status. *Neuropsychopharmacology,* 42**,** 2325-2332.
WIERS, C. E., SHOKRI-KOJORI, E., CABRERA, E., CUNNINGHAM, S., WONG, C., TOMASI, D., WANG, G. J. & VOLKOW, N. D. 2016b. Socioeconomic status is associated with striatal dopamine D2/D3 receptors in healthy volunteers but not in cocaine abusers. *Neurosci Lett,* 617**,** 27-31.
WILLIAMS, R. J., LEE, C. K. & BACK, K. J. 2013. The prevalence and nature of gambling and problem gambling in South Korea. *Soc Psychiatry Psychiatr Epidemiol,* 48**,** 821-34.
WILLIAMS, R. J., LEONARD, C. A., BELANGER, Y. D., CHRISTENSEN, D. R., EL-GUEBALY, N., HODGINS, D. C., MCGRATH, D. S., NICOLL, F., SMITH, G. J. & STEVENS, R. M. G. 2021. Predictors of gambling and problem gambling in Canada. *Canadian Journal of Public Health,* 112**,** 521-529.
WILSON, R. P., COLIZZI, M., BOSSONG, M. G., ALLEN, P., KEMPTON, M., ABE, N., BARROS-LOSCERTALES, A. R., BAYER, J., BECK, A., BJORK, J., BOECKER, R., BUSTAMANTE, J. C., CHOI, J. S., DELMONTE, S., DILLON, D., FIGEE, M., GARAVAN, H., HAGELE, C., HERMANS, E. J., CONSORTIUM, I., IKEDA, Y., KAPPEL, V., KAUFMANN, C., LAMM, C., LAMMERTZ, S. E., LI, Y., MURPHY, A., NESTOR, L., PECINA, M., PFABIGAN, D., PIZZAGALLI, D., RADEMACHER, L., ADMON, R., SOMMER, T., STARK, R., SUZUKI, H., VAN AMELSVOORT, T., VAN HELL,

E., VINK, M., VOTINOV, M., WOTRUBA, D., BHATTACHARYYA, S. & MTAC 2018. The Neural Substrate of Reward Anticipation in Health: A Meta-Analysis of fMRI Findings in the Monetary Incentive Delay Task. *Neuropsychology Review,* 28**,** 496-506.
WISE, R. A. & BOZARTH, M. A. 1987. A psychomotor stimulant theory of addiction. *Psychological Review,* 94**,** 469-492.
WISE, R. A., SPINDLER, J., DEWIT, H. & GERBERG, G. J. 1978. Neuroleptic-Induced "Anhedonia" in Rats: Pimozide Blocks Reward Quality of Food. *Science,* 201**,** 262-264.
WONG, E. H., YOCCA, F., SMITH, M. A. & LEE, C. M. 2010. Challenges and opportunities for drug discovery in psychiatric disorders: the drug hunters' perspective. *Int J Neuropsychopharmacol,* 13**,** 1269-84.
WONG, G., ZANE, N., SAW, A. & CHAN, A. K. 2013. Examining gender differences for gambling engagement and gambling problems among emerging adults. *J Gambl Stud,* 29**,** 171-89.
WORHUNSKY, P. D., ANGARITA, G. A., ZHAI, Z. W., MATUSKEY, D., GALLEZOT, J.-D., MALISON, R. T., CARSON, R. E. & POTENZA, M. N. 2021. Multimodal investigation of dopamine D2/D3 receptors, default mode network suppression, and cognitive control in cocaine-use disorder. *Neuropsychopharmacology,* 46**,** 316-324.
WORHUNSKY, P. D., MALISON, R. T., ROGERS, R. D. & POTENZA, M. N. 2014. Altered neural correlates of reward and loss processing during simulated slot-machine fMRI in pathological gambling and cocaine dependence. *Drug Alcohol Depend,* 145**,** 77-86.
WORHUNSKY, P. D., MATUSKEY, D., GALLEZOT, J. D., GAISER, E. C., NABULSI, N., ANGARITA, G. A., CALHOUN, V. D., MALISON, R. T., POTENZA, M. N. & CARSON, R. E. 2017. Regional and source-based patterns of [(11)C]-(+)-PHNO binding potential reveal concurrent alterations in dopamine D(2) and D(3) receptor availability in cocaine-use disorder. *Neuroimage,* 148**,** 343-351.
WRASE, J., KAHNT, T., SCHLAGENHAUF, F., BECK, A., COHEN, M. X., KNUTSON, B. & HEINZ, A. 2007a. Different neural systems adjust motor behavior in response to reward and punishment. *NeuroImage,* 36**,** 1253-1262.
WRASE, J., SCHLAGENHAUF, F., KIENAST, T., WÜSTENBERG, T., BERMPOHL, F., KAHNT, T., BECK, A., STRÖHLE, A., JUCKEL, G., KNUTSON, B. & HEINZ, A. 2007b. Dysfunction of reward processing correlates with alcohol craving in detoxified alcoholics. *NeuroImage,* 35**,** 787-794.
YALACHKOV, Y., KAISER, J. & NAUMER, M. J. 2012. Functional neuroimaging studies in addiction: multisensory drug stimuli and neural cue reactivity. *Neurosci Biobehav Rev,* 36**,** 825-35.
YANG, Y. K., YAO, W. J., MCEVOY, J. P., CHU, C. L., LEE, I. H., CHEN, P. S., YEH, T. L. & CHIU, N. T. 2006. Striatal dopamine D2/D3 receptor availability in male smokers. *Psychiatry Res,* 146**,** 87-90.
YANG, Y. K., YAO, W. J., YEH, T. L., LEE, I. H., CHEN, P. S., LU, R. B. & CHIU, N. T. 2008. Decreased dopamine transporter availability in male smokers -- a dual isotope SPECT study. *Prog Neuropsychopharmacol Biol Psychiatry,* 32**,** 274-9.
ZAFAR, R., SIEGEL, M., HARDING, R., BARBA, T., AGNORELLI, C., SUSEELAN, S., ROSEMAN, L., WALL, M., NUTT, D. J. & ERRITZOE, D. 2023a.

Psychedelic therapy in the treatment of addiction: the past, present and future. *Frontiers in Psychiatry,* 14.
ZAFAR, R., SIEGEL, M., HARDING, R., BARBA, T., AGNORELLI, C., SUSEELAN, S., ROSEMAN, L., WALL, M., NUTT, D. J. & ERRITZOE, D. 2023b. Psychedelic therapy in the treatment of addiction: the past, present and future. *Front Psychiatry,* 14**,** 1183740.
ZAKINIAEIZ, Y., GUEORGUIEVA, R., PELTIER, M. R., ROBERTS, W., VERPLAETSE, T. L., BURKE, C., MORRIS, E. D., MCKEE, S. A. & COSGROVE, K. P. 2023. Lower Dopamine D2/3 Receptor Availability is Associated With Worse Verbal Learning and Memory in People Who Smoke Cigarettes. *Nicotine Tob Res,* 25**,** 1047-1051.
ZAKINIAEIZ, Y., HILLMER, A. T., MATUSKEY, D., NABULSI, N., ROPCHAN, J., MAZURE, C. M., PICCIOTTO, M. R., HUANG, Y., MCKEE, S. A., MORRIS, E. D. & COSGROVE, K. P. 2019. Sex differences in amphetamine-induced dopamine release in the dorsolateral prefrontal cortex of tobacco smokers. *Neuropsychopharmacology,* 44**,** 2205-2211.
ZHAO, Y., SALLIE, S. N., CUI, H., ZENG, N., DU, J., YUAN, T., LI, D., DE RIDDER, D. & ZHANG, C. 2020. Anterior Cingulate Cortex in Addiction: New Insights for Neuromodulation. *Neuromodulation: Journal of the International Neuromodulation Society*.
ZIJLSTRA, F., BOOIJ, J., VAN DEN BRINK, W. & FRANKEN, I. H. 2008. Striatal dopamine D2 receptor binding and dopamine release during cue-elicited craving in recently abstinent opiate-dependent males. *Eur Neuropsychopharmacol,* 18**,** 262-70.
ZILVERSTAND, A., HUANG, A. S., ALIA-KLEIN, N. & GOLDSTEIN, R. Z. 2018. Neuroimaging Impaired Response Inhibition and Salience Attribution in Human Drug Addiction: A Systematic Review. *Neuron,* 98**,** 886-903.

Appendices

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