EMPATHY AND THE SUBJECTIVE-BEHAVIOURAL EFFECTS OF D1 AND D2 RECEPTOR BLOCKADE IN PATHOLOGICAL GAMBLERS

By

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This study assessed the relationship between Empathy and reinforcing effects of gambling and a psychostimulant drug, in 30 otherwise healthy pathological gamblers (PGs). To explore the roles of dopamine D1 and D2 receptors in these relationships, subjects received either: D2 antagonist, haloperidol (3-mg) or D1-D2 antagonist, fluphenazine (3-mg), in a placebo-controlled, double-blind, counterbalanced design. On separate sessions, subjects played a 15-minute slot machine game and received d-amphetamine (AMPH; 20-mg, oral). Under placebo, Empathy correlated positively with Desire to Gamble (DTG) at all time-points in both groups. Haloperidol negated, whereas fluphenazine enhanced, the correlation between Empathy and pleasurable effects of the slot machine. Haloperidol enhanced, whereas fluphenazine attenuated, the correlation between Empathy and DTG under AMPH. Results suggest post-synaptic D2 receptors may mediate Empathy-related differences in Liking of gambling; D1 receptors may mediate Empathy-related differences in Wanting to gamble under AMPH in PGs. Low statistical power and restricted generalizability were limitations.
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LIST OF ABBREVIATIONS

5-HT  Serotonin
AC    Adenylyl cyclase
ADS   Alcohol Dependence Scale
AMPH  Amphetamine
ARCI-AMP Addiction Research Center Inventory-Amphetamine subscale
BDI   Beck Depression Inventory
cAMP  Cyclic adenosine monophosphate
CNS   Central nervous system
DA    Dopamine
DAST  Drug Abuse Screening Tool
DSM   Diagnostic and Statistical Manual of Mental Disorders
DTG   Desire to gamble
ECG   Electrocardiogram
EIS   Eysenck Impulsiveness Scale
EPI   Eysenck Personality Inventory
FLU   Fluphenazine
fMRI  Functional magnetic resonance imaging
FTND  Flagerström Test for Nicotine Dependence
GABA  γ-Aminobutyric Acid
GBQ   Gambling Beliefs Questionnaire
GDT   Game of Dice Task
GPCR  G-protein coupled receptor
HAL   Haloperidol
HC    Healthy control
NAc   Nucleus accumbens
NE    Norepinephrine
PET   Positron Emission Topography
PFC   Prefrontal cortex
PG    Pathological gambling
PKA   Protein kinase A
POMS  Profile of Moods State
RRT   Rapid Reading Task
SCID  Structured Clinical Interview for the DSM-IV
SOGS  South Oaks Gambling Screen
SST   Stop Signal Task
SUD   Substance Use Dependence
VAS   Visual Analog Scale
VTA   Ventral tegmental area
WAIS  Wechsler Adult Intelligence Scale
WCST  Wisconsin Cart Sort Task
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1. INTRODUCTION

1.1 Study Overview

1.1.1 Importance and Purpose

Pathological gambling (PG)\(^1\) is a chronic and progressive condition, defined as “persistent and recurrent maladaptive gambling behaviour” (American Psychiatric Association 1994). It is the most destructive form of gambling, above at-risk problem gambling, and social gambling, where no long-term problems are incurred. PG affects 1-3% of the Canadian population, a prevalence rate similar to bipolar disorder and schizophrenia (el-Guebaly et al. 2006, Volberg 1994).

PG is one of Canada’s major public health issues as it can have many unintended implications, above the presumed financial loss. Medically, PGs are at increased risk of worsening physical health and developing stress-related conditions such as hypertension, sleep deprivation and cardiovascular disease (Fong 2005). Psychological correlates of PG may include feelings of guilt and shame, deceptive practices, and heightened impulsivity/impaired decision-making. Additionally, the social consequences of PG can range from involvement with the legal system, to lost productivity at work, and strained interpersonal relationships. Due to the increased availability of legalized gambling and its popularity over recent decades, increased attention to the health impacts of gambling behaviour is warranted (Shaffer and Korn 2002).

The etiology of PG is highly heterogeneous. Various neurotransmitters and receptors are thought to be involved (Blaszczynski and Nower 2002). In addition, there are a variety of genetic polymorphisms that further complicate the disorder (Comings et al. 1996, Comings et al. 1997, Lobo et al. 2010). Differences in trait profiles and sensitivity to social-environmental signals for reward and punishment also play a role in predisposition to gambling and PG. Furthermore, PG’s

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\(^1\) Pathological Gambling (PG) was renamed Gambling Disorder in the fifth edition of the Diagnostic Statistical Manual of Mental Disorders (DSM-V). However, this paper will continue to refer to the disorder as PG because the diagnostic tools used in this study were based on the criteria for DSM-IV since there are not currently any validated diagnostic tools for DSM-V.
high comorbidity with substance use disorders (SUDs; particularly alcohol and tobacco dependence), mood disorders and personality disorders make treatment challenging (Cunningham-Williams et al. 1998, Grant and Potenza 2005). Although numerous trials have been conducted and some promising candidate medications exist, to date, there is limited support for the use of any pharmacological agent specific for the treatment of PG (Bartley and Bloch, 2013). Moreover, there is a heterogeneous range of motivations for gambling behaviour – enhancement (i.e., positive reinforcement, linked to excitement and physiological arousal) vs. coping (i.e., negative reinforcement, linked to relief of anxiety, low mood and boredom) (Robbins and Clark 2015). Therefore, it is likely that a medication will have to address the specific dispositional and motivational features of each given patient in order to be effective.

Originally defined as an Impulse Control Disorder, PG was reclassified as a prototypical example of a behavioural addiction under Substance-Related and Addictive Disorders in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM) (Petry et al. 2013, Robbins and Clark 2015). Extensive research has demonstrated that PG and SUDs share phenomenological similarities, biological markers, underlying genetic vulnerabilities and high rates of comorbidity (Bottesi 2013, Petry et al. 2014).

The new classification of PG as a behavioural addiction partly reflects the common circuitry and functional role that DA plays in PG and SUDs (Potenza 2008, Zack and Poulos 2009). For example, a range of genetic, neuroimaging, and behavioural studies demonstrate DA response to addictive stimuli may contribute to PG behaviour, and alterations in DA system reactivity may play a critical role in the clinical presentation of PG, although it remains unclear whether the alterations in DA function reflect an increase, decrease, or some combined pattern of effects, compared to healthy controls (Meyer et al. 2004, Potenza, 2008).

Dopamine (DA) has been implicated in the reinforcement and reward circuitry of the brain (Blum et al. 1995). Here, reinforcement denotes the tendency to repeat a behaviour, and
reward denotes the association between specific stimuli or contexts and positive subjective states (e.g., pleasure, euphoria) that make these stimuli attractive. Such stimuli are said to have incentive salience as they can automatically elicit approach (Robinson and Berridge 2001).

The impact of rewarding stimuli on behaviour and associated risk for addiction, including PG, varies with the features of each subject. Ultimately, the tendency to escalate participation in, and experience consequences of, gambling derives from an interaction between situational and personal variables. Like SUDs, there is a wide degree of individual differences in presentation, etiology and motives that lead to the risk, development, and precipitation of PG. Cloninger et al. (1993) argued that various dimensions of personality are associated with neurotransmitter systems in the central nervous system (CNS), which are determined by a biological constellation of genes and their polymorphic variants. In fact, population studies indicate that approximately 40–50% of inter-individual variability in personality dimensions corresponds to variability in DNA (Bouchard et al. 1990). A large body of research has examined the genetic predisposition for addiction mediated by ‘endophenotypes’: measures of individual neuropsychological, neurophysiological and biochemical functioning that form the link between genes/vulnerability and overt symptoms. Anomalies in endophenotypes are believed to reflect impairments in the underlying neurocognitive processes (Bottesi 2013). For example, impairments in motor inhibition ability and difficulties in delaying gratification and decision making, which are cognitive functions controlled by the prefrontal cortex, have been suggested to underlie problems in behavioural regulation (i.e., impulsive behaviours). In short, endophenotypes are functional manifestations of genetic profiles (e.g., personality traits) that may promote sensitivity to the reinforcing effects of addictive substances or activities, or global deficits in self-regulation.

Studies of the neurobiological substrates of personality traits have largely focused on the most long-standing domains: Neuroticism and Extraversion (DeYoung and Gray 2009, Canli 2004). Subsumed within these domains are the specific dimensions of Impulsivity and Sensation
Seeking, whose role in addictive behaviour, including PG is well documented (Robbins and Clark 2015). Evidence shows that both dimensions have a dopaminergic basis. At the syndrome level, Impulsivity is important for vulnerability, initiation and relapse in impulsive-compulsive disorders (Robbins and Clark 2015). For example, studies of attention deficit hyperactivity disorder illustrate that Impulsivity is associated with reduced DA transmission in the striatum, reduced cerebrospinal fluid levels of the DA metabolite, homovanillic acid (Shaywitz et al. 1977), and reduced urinary excretion (Hanna et al. 1996), while higher homovanillic acid has been associated with better response to medication (Castellanos et al. 1996). Recent Positron Emission Topography (PET) studies have also reported that lower D2/D3 auto-receptor binding in the midbrain was associated with greater questionnaire-measured Impulsivity (Buckholtz et al. 2010), reproducing an earlier finding in the caudate in stimulant-dependent individuals (Lee et al. 2009). Sensation Seeking is associated with polymorphisms at D2-like receptor loci and individual differences in self-reported Sensation Seeking (Munafò et al. 2008). Furthermore, evidence from genetic and PET radioligand displacement studies suggests that individuals higher in Sensation Seeking may exhibit higher endogenous DA levels, greater dopaminergic responses to cues of upcoming reward in striatal regions, as well as increased physiological and subjective responsive stimulants such as amphetamine (AMPH) (Zuckerman 1985).

Impulsivity and Sensation Seeking influence how an individual will respond to stimuli that are directly experienced – pleasure, pain, excitement, etc. This is evidently important to understanding the subjective effects of drugs. In the case of gambling, there is no pharmacological agent that directly increases or decreases neurotransmission. Reward and reinforcement are mediated by external signals in the environment. As such, trait sensitivity to environmental signals may be especially important to the incentive salience and subjective effects of gambling. One trait that has received considerable attention in research on disorders with a social dimension (e.g., autism, schizophrenia) is Empathy.
In contrast to the psychiatric literature, to date, relatively few studies appear to have examined the role of Empathy in addictive behaviours. Empathy does not have a universally accepted definition, although empathic traits can be reliably assessed through self-reported measures (Bernhardt and Singer 2012). Operationally, Empathy is a trait that quantifies the capacity to be sensitive to signals that convey another individual’s emotions, or their subjective response to an action. One way to understand how Empathy might relate to addiction and PG is to consider Psychopathy, a sub-domain of Impulsivity. Psychopathy is characterized by a lack in the ability to process or experience vicarious pain, including the emotional impact of their behaviour on other people (i.e., callous indifference) and has been linked with DA release and reward anticipation-related neural activity in the nucleus accumbens (NAc) in response to pharmacological and monetary reinforcers, respectively in healthy individuals (Buckholtz et al. 2010). From a clinical perspective, Empathy may be understood as the polar opposite of Psychopathy. PET scan data show that Psychopathy is associated with low pre-synaptic D2 (Buckholtz et al. 2010) and data from bromocriptine challenge show that Psychopathy is associated with low post-synaptic D2 receptor levels (Gerra et al. 2003). If we were to extrapolate this receptor pattern to Empathy, we might infer that highly empathic individuals have high levels of D2 receptors – expressed pre- and/or post-synaptically.

From a motivational standpoint, individuals with high Empathy are sensitive to the pain of others (Singer et al. 2004), and must therefore be able to appreciate how pain feels personally as well as vicariously. Pain sensitivity in highly empathic individuals may increase susceptibility to addictive behaviour as a means of coping with aversive feelings (Coenena et al. 2011).

Thus, gaining a better understanding of the relationship between Empathy, reinforcement and reward in PG may help to clarify the complex role of DA in PG etiology and symptom profile. This in turn may facilitate development of personalized pharmacotherapies for PG.
1.1.2 Study Objectives

The main objectives of this study are:

a) To assess the relationship between Empathy and the reinforcing and rewarding properties of a slot machine game and the prototypic drug of abuse and DA releaser, d-amphetamine (AMPH) in PGs;

b) To clarify the roles of D1 and D2 in mediating the observed relationships.

Reward will be assessed by pleasurable effects, and the stimulant-like effects of the slot machine and dose of AMPH. Reinforcement will be assessed by self-reported motivation to gamble after the slot machine and dose of AMPH.

1.2 Background and Rationale

1.2.1 Neurochemistry of PG

Several neurotransmitter systems have been implicated in the development and progression of PG (Blaszczyński and Nower 2002), each playing a role in a specific aspect of the disorder. The majority of studies focus on the role of serotonin (5-hydroxytryptamine; 5-HT) in behavioural initiation and cessation, norepinephrine (NE) in arousal and excitement, and DA in reward and reinforcement (Comings et al. 2001, Potenza 2008).

1.2.1.1 Serotonin

Implicated in the control of a variety of physiological and behavioural functions including regulation of mood, learning and impulse control, 5-HT is believed to play a critical role in behavioural inhibition in general and in PG specifically (Miszkiel et al. 2011, Pallanti et al. 2010). Studies of PG subjects have found disturbances in 5-HT metabolism, with reduced activity of the primary 5-HT metabolizing enzyme, monoamine oxidase (Blanco et al. 1996);
decreased levels of 5-HT and its precursor, 5-hydroxytryptophan; as well as decreased levels of its main metabolite, 5-hydroxyindoleacetic acid, and elevated Impulsivity (Nordin and Sjödin 2006). PG subjects also demonstrate hyper-reactivity to 5-HT challenge and hypo-reactivity to 5-HT transporter inhibitors, where a greater degree of dysregulation is correlated with increased symptom severity (Pallanti et al. 2006). The literature also suggests several 5-HT receptor polymorphisms may be interlaced in PG. A C/C variant of 5-HT2A at T102C has been linked with the development of a PG phenotype (Wilson et al. 2013), while increased levels of 5-HT1B receptors have been linked with PG severity (Potenza et al. 2013). The use of selective serotonin reuptake inhibitors (SSRIs) as potential treatment of PG further supports the involvement of 5-HT and serotonergic dysfunction in PG (Grant and Kim 2006), although the benefits of SSRIs in PG have been inconsistent (Bartley and Bloch 2013).

1.2.1.2 Norepinephrine

The NE system is involved in mediating arousal and attention. During a game of Black Jack, heart rate elevation and increases in other NE-related measures have been consistently observed, with elevated levels in PGs compared to controls (Potenza 2008).

Similar to 5-HT, evidence indicates disturbances of NE metabolism in PG subjects. Increased levels of NE and its metabolite, vanillylmandelic acid, have been observed in cerebrospinal fluid and urine samples, and increased central and plasma levels of 3-methoxy-4-hydroxyphenylglycol have been observed in subjects with PG as compared to controls (Pirritano et al. 2014, Roy et al. 1988). Levels of these metabolites also correlate positively with Impulsivity and scores on the Extraversion subscale of the Eysenck Personality Inventory (EPI; Eysenck and Eysenck 1963). Differences in NE transmission may therefore play a role in Impulsive or Sensation Seeking aspects of PG.
1.2.1.3 Dopamine

DA is implicated in reward and reinforcement, and has consistently been associated with maladaptive responses (e.g., cue-reactivity, craving, compulsive seeking) in drug addiction (Nestler 2004, Pirritano et al. 2014). Polymorphisms in D1 and D2 genes have been linked to PG in multiple studies, providing indirect evidence that DA receptor variations contribute to PG (Comings et al. 1996, Comings et al. 1997, Lobo et al. 2010). In addition, numerous functional magnetic resonance imaging (fMRI) studies provide evidence about the involvement of specific brain regions in PG-related behaviours. FMRIIs of the now widely recognized phenomenon of iatrogenic PG after introduction of D2/D3 agonists in Parkinson’s patients (7-10%) show reduced activation of the prefrontal cortex (PFC), ventral striatum, NAc and amygdala (Santangelo et al. 2013, Voon et al., 2007, Pirritano et al. 2014). This suggests a relationship with aberrant reward and response inhibition, and further supports the fundamental role of DA in PG. Whereas inhibition- and reward-related neural activity may be deficient in Parkinson’s patients with DA agonist-induced PG, greater increases in central and plasma levels of DA and its metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, have been demonstrated in non-Parkinsonian PG subjects (Bergh et al. 1997). The DA receptor subtypes, role of DA signaling in reward/reinforcement, and its relevance to PG are reviewed in detail below.

1.2.2 Overview of the DA System

DA is the predominant catecholamine neurotransmitter in the brain. The four major dopaminergic innervations (mesolimbic, mesocortical, mesostriatal and nigrostriatal pathways) initiate in the ventral tegmental area (VTA) or substantia nigra where DA is synthesized, and project to numerous brain areas, where they play a crucial role in voluntary movement, cognition, motivation and reward (Beaulieu and Gainetdinov 2011, Schultz 2001). Most notably, reward processing is signaled by the mesolimbic and mesocortical pathways, which project to the
NAc and PFC, respectively (Anden et al. 1964, Tzschentke and Schmidt 2000). The mesostriatal pathway, projecting to the dorsal striatum, has also been increasingly recognized for its role in reward processing (Tzschentke and Schmidt 2000, Wise 2009). Nigrostriatal DA transmission, from the substantia nigra to the striatum, while traditionally associated with motor control, has also been demonstrated to modulate these processes (Wise 2009). Considerable evidence suggests that natural rewards (i.e., food, sex) and addictive drugs share hedonic properties and elevate DA levels in the mesolimbic system (De Mei et al. 2009, Schultz et al. 1997, Baik 2013).

1.2.2.1 DA Receptor Subtypes

The physiological actions of DA are mediated by five distinct, but closely related G protein-coupled receptors (GPCRs) that are divided into two major groups. D1-like receptors, including D1 and D5, are excitatory GPCRs. Agonist binding to this receptor class activates the $G_{\alpha_{s,olf}}$ family of G proteins to stimulate adenylyl cyclase (AC), increasing cyclic adenosine monophosphate (cAMP) production (Neve et al. 2004). This pathway then induces activation of protein kinase A (PKA), resulting in phosphorylation of downstream substrates, induction of gene expression and modulation of multiple ion channels (Missale et al. 1998, Beaulieu and Gainetdinov 2011).

D1 receptors are localized almost exclusively post-synaptically on DA-receptive cells localized throughout the striatum and PFC, and are primarily involved in reward response and movement control (Beaulieu and Gainetdinov 2011), as well as many aspects of cognition, including working memory, set-shifting, and attention (Fletcher et al. 2005, Castner and Williams 2007). D1 receptors are located mainly in extra-synaptic sites (Caillé et al. 1996) and have lower affinity for endogenous DA relative to the other subtypes. Consequently, they respond primarily to high intensity stimulus-induced bursts of high concentration DA that occur when an unexpected reward is delivered (Schultz et al. 1998, Dreyer et al. 2010). D5 receptor
expression is dispersed throughout several brain regions including the PFC, striatum, substantia nigra, and hippocampus. These receptors are thought to contribute to learning and memory (Beaulieu and Gainetdinov 2011), particularly in the hippocampus, where D1 receptors are less prevalent (Missale et al. 1998).

D2-like receptors (D2, D3, and D4) are inhibitory GPCRs, coupled to the G\(\alpha_{i/o}\) family of G proteins that primarily inhibit AC activity, decreasing cAMP production. This results in decreased PKA activity, activation of potassium channels and modulation of multiple ion channels (Missale et al. 1998, Beaulieu and Gainetdinov 2011). D2 receptors are found in highest concentrations in the striatum, NAc, and olfactory tubercle (Beaulieu and Gainetdinov 2011). D2 receptors have higher affinity for DA relative to D1 receptors, responding primarily to tonic (basal) levels of DA. Consequently, they are sensitive to pauses in DA transmission that occur when an expected reward is omitted (Schultz et al. 1997).

In contrast to D1, D2 and D3 receptors are expressed both post-synaptically on DA target cells and pre-synaptically on dopaminergic neurons (Sokoloff et al. 2006, Rondou et al. 2010). Activation of pre-synaptically localized auto-receptors initiates an important negative feedback signal that decreases neuronal firing, DA synthesis, and pre-synaptic DA release, resulting in decreased locomotor activity (Wolf and Roth 1990, Missale et al. 1998, Sibley 1999). Activation of post-synaptic D2 hetero-receptors (in the striatum), on the other hand, increases downstream DA transmission and stimulates locomotion by inhibiting \(\gamma\)-Aminobutyric Acid (GABA) neurons that tonically suppress mesolimbic DA neurons in the VTA (Beaulieu and Gainetdinov 2011).

D3 receptors are expressed in the greatest density in the NAc (Sokoloff et al. 2006) and contribute to pre-synaptic regulation of DA release. D3 receptor activation appears to inhibit locomotion and can modulate reward-seeking behaviour and cue response in animals (Beaulieu and Gainetdinov 2011, Sokoloff et al. 2006). D4 receptors have comparatively limited expression in the brain, and are involved in specific aspects of cognitive function as well as

1.2.2.2 Tonic and Phasic DA

Receptor-ligand binding assays have provided evidence for differences in DA affinity between D1- and D2-like receptors. D2-like receptors have a 10- to 100-fold greater affinity for DA than the D1-like family, while D1 reportedly has the lowest affinity for DA (Beaulieu and Gainetdinov 2011). These differences in binding affinity support the hypothesized differential role for the two receptors in DA dynamics. DA neurons can have two patterns of DA release – “tonic” or “phasic” based on their firing properties, which engage D2-like and D1-like receptors, respectively (Grace et al. 2007, Atcherley et al. 2015).

Under basal conditions (i.e., without stimulation), DA neurons fire in a slow, asynchronous “tonic” pattern that regulates the steady-state extracellular concentration of DA (Marinelli and McCutcheon 2014). However, following the presence of unexpected salient stimuli or cues predicting reward, the basal signal switches to fast, transient synchronous bursts of “phasic” activity (Grace and Bunney 1984, Grace 2000, Dreyer et al. 2010, Schultz 2007, Atcherley et al. 2015). In fact, mesolimbic DA transforms neutral cues into conditioned cues when they begin to reliably predict reward delivery (Flagel et al. 2011), causing phasic DA release to initiate after delivery of a cue for reward, but before delivery of the reward itself (Redish 2004, Marcott et al. 2014). As such, a substantial body of research posits that phasic DA response to reward delivery does not encode reward, but the reward prediction error – the discrepancy between an organism’s expectation of reward and the actual outcome (i.e., reward or no reward) (Fiorillo et al. 2003). In the context of gambling, Anselme and Robinson (2013) suggest that the motivation to gamble is strongly, though not entirely, determined by the inability to predict reward occurrence. The more reliably a cue predicts a reward, the lower the reward prediction error and hence the lower the post-reward phasic DA release. Conversely, rewards
that are novel or unpredictable cause a substantial reward prediction error and correspondingly larger DA spike. Accordingly, it is theorized that low-affinity D1 receptors are preferentially activated by the transient, high concentrations of phasic DA (Goto and Grace 2005, Grace et al. 2007), and high affinity D2 receptors detect the lower levels of tonic DA release in the absence of reward or reward cues (Goto et al. 2007).

Nonetheless, given that measurements of receptor affinity rely on ligand binding assays from heterologously expressed DA receptors, and do not reflect the receptor’s coupling to downstream signaling cascades, it remains unclear how D1 and D2 receptors participate in different patterns of DA activity in vivo (Baik 2013).

1.2.2.3 DA Signaling and Reward

Berridge and Robinson (1998) argue that reward is not a unitary or subjective process, but instead it is a collection of multiple processes that can be identified by separate brain manipulations. A large portion of the causal evidence supporting a role of the DA system in reward comes from animal studies of DA receptor blockade. DA antagonists reduce reward-directed instrumental behaviour in subtle, yet definite ways. More dramatic evidence is provided by intracranial application of DA-selective neurotoxins, (e.g., 6-hydroxydopamine), that cause extensive DA depletion and destruction of ascending DA neurons, such that animals become unresponsive to food and other rewards (Berridge and Robinson 1998).

D1-mediated phasic and D2-mediated tonic DA signals are both important in reward processing and reinforcement and the response of DA neurons to natural rewards is fairly homogenous. When cells respond to a rewarding stimulus, they almost always increase their firing rate as phasic DA signaling causes spike-dependent/burst DA releases that encode the primary reward signal (Schultz 1998, Marinelli and McCutcheon 2014). Substances of abuse that cause reward-seeking actions often alter this process, which provides another level of proof that
DA signaling is a key component of reward circuitry (Ostlund et al. 2014, Schultz 2007). As rewards become associated with cues over time, DA neurons stop responding to the rewards and instead start responding to the predictive cues or reward prediction error (Schultz et al. 1997), thus, the response to reward becomes heterogeneous.

DA neurons also play a role in coding the uncertainty of reward delivery in the presence of a cue that signals the potential for reward but does not confirm if the reward will actually be delivered. This type of uncertain reward signaling has special relevance to gambling where cues are abundant and rewards are expected, but are never fully predicted. Fiorillo et al. (2003) sought to characterize this process and found that DA cell firing was lowest when the cue predicted reward delivery 100% of the time (no uncertainty), and highest when the cue predicted reward delivery 50% of the time (maximal uncertainty), evoking reward expectancy but essentially providing no information about whether reward would be delivered or not. This finding is noteworthy given its parallel to the frequency of reward delivery (46%) observed over thousands of trials on a standard commercial slot machine (Tremblay et al. 2011).

1.2.3 DA System in PG

A substantial amount of psychopharmacological research on PG has focused on the DA system, due to its established role in signaling reward (Schultz 2006) and in drug addiction (Wise 2004). However, animal and human studies indicate that the role of DA in reward, and in gambling in particular, is more complex than initially believed (Linnet 2013). The role of DA in PG initially surfaced due to reports of emerging uncontrollable gambling behaviours after Parkison’s patients received DA agonists to treat their motor symptoms (Santangelo et al. 2013). The tight temporal relationship between the administration of DA agonists and onset of PG-like behaviour suggested a causal relationship (Dodd et al. 2005). Subsequent research has demonstrated that AMPH (a psychostimulant that increases DA concentration in the synapse)
increases the urge to gamble in PGs (Zack et al. 2004).

Furthermore, Joutsa et al. (2012) showed that DA is released in the ventral striatum during instances of high reward to a larger extent in PGs than non-gambler controls, and that the severity of PG symptoms is associated with a larger DA response (Linnet et al. 2011). Interestingly, Linnet et al. (2010) found that DA release is also greater in PGs losing money than in those same PGs when winning money during a gambling task. This finding is consistent with evidence that “near misses” (gambling losses that closely approximate actual wins – e.g., 3 out of 4 of the same symbol in a row) enhance motivation to gamble and recruit the DA reward circuitry more than “big wins” (Clark et al. 2009). Current evidence therefore suggests that the prospect of winning may engage DA-based motivational pathways more than winning itself.

Two additional areas of differential DA signaling between casual gamblers and PGs that remain unclear are 1) deficient DA activation in response to reward delivery (i.e., tolerance), and 2) increases in DA activation (i.e., sensitization) in response to reward signals in PG. While the majority of the literature consists of data from psychostimulant addiction, evidence of the ever-growing similarities between PG and drug addictions indicate that many of the same processes may apply to PG (Cunningham-Williams 2009, Leeman and Potenza 2012).

1.2.3.1 Deficient DA Activation by Natural Stimuli

In human laboratory models of reinforcement, money has been used as a generic reinforcer, similar to food in animal studies. Since money is also the currency of reward in PG, it is challenging to differentiate whether deficits in reactivity to monetary reward reflect a loss of generic reward function or tolerance to an addictive reinforcing stimulus. Comparing the effects of stimulant reinforcement (in PGs who are stimulant naïve) and monetary reinforcement in PGs provides a means of differentiating systemic deficits in DA function from deficits in response arising from the association between money and gambling. Conversely, responses to monetary
reinforcement can speak to generic deficits in stimulant abusers.

FMRI studies in PGs show significant deficits of DA activation in response to monetary reward compared to controls (Reuter et al. 2005). The deficits were primarily observed in the ventral striatum, a key area targeted by the mesolimbic dopaminergic pathway that is critically involved in reward reinforcement. In addition, there is evidence of a positive correlation between the degree of deficits in DA activation and the severity of PG symptoms (Potenza 2008).

These findings for PGs are noteworthy given that reductions in striatal activation in response to reward have long been considered one of the key characteristics of psychostimulant addiction (Volkow et al. 1997, Volkow et al. 2002). For instance, compared to controls, cocaine abusers show significant reduction in striatal DA release after methylphenidate and report decreases in the pleasurable effects of stimulants. In addition, PET studies have demonstrated a direct correlation between the degree of stimulant-induced striatal DA release and the subjective reinforcing effects (e.g., “high”) of stimulants (Drevets et al. 2001, Volkow et al. 1999). Thus, PG and SUDs may be neurochemically similar syndromes and DA manipulations may have similar effects on responses to gambling-related stimuli as they do for psychostimulant drugs.

1.2.3.2 Sensitization

Sensitization of DA systems is a common neuro-adaptation that occurs in response to repeated exposure to a conditioned stimulus for an addictive drug (Robinson and Berridge 2001, Robinson and Becker 1986). The incentive sensitization theory of addiction hypothesizes that repeated exposure to addictive drugs can persistently change brain cells and circuits that normally regulate the attribution of incentive salience to stimuli, a psychological process involved in motivated behaviour (Robinson and Berridge 2001). In this thesis, sensitization will refer specifically to DA system sensitization.

Several studies have suggested that the increase in tonic DA after chronic exposure to a
reinforcer (e.g., repeated drug administration) opposes phasic DA signaling via the stimulation of D2 auto-receptors, leading to the requirement of a rewarding stimulus with higher intensity (i.e., increased dose or frequency of drug administration) to restore the phasic DA signal (Grace 2000, Wolf et al. 2004).

In both human and animal studies, chronic administration of stimulant drugs, such as AMPH and cocaine, has been demonstrated to reliably produce robust DA sensitization (Robinson and Becker 1986, Boileau et al. 2006). Based on a range of indirect evidence that suggests similar DA disruptions in psychostimulant addiction and PG, it has been suggested that chronic exposure to gambling-related stimuli might induce sensitization in PG (Zack and Poulos 2009, Leeman and Potenza 2012). This was recently demonstrated in a study by Zack et al. (2014), in which chronic exposure to a gambling-like regimen of sucrose reward delivered under 50% (i.e., unpredictable) variable ratio schedule enhanced rat locomotor response to a subsequent low-dose AMPH challenge, compared with chronic exposure to cues that reliably predicted sucrose reward (100% of the time; fully predictable – a regimen unlike gambling). Given the reliable link between striatal DA release and locomotor response to AMPH, the findings were taken to indicate that a gambling-like reward schedule, characterized by uncertainty of reward delivery in the presence of cues for reward, could promote enhanced DA response to AMPH, an outcome that would be expected following chronic exposure to AMPH itself. That is, like drugs of abuse (in animals), chronic exposure to gambling-like stimuli could induce sensitization of DA pathways in originally healthy animals.

In humans, a range of indirect evidence suggests the possibility of DA sensitization in PG (Bergh et al. 1997, Zack and Poulos 2004). The most direct evidence for this comes from a recent PET study, in which stimulant-naïve PG subjects demonstrated increased AMPH-induced DA release in the dorsal striatum compared to healthy subjects (Boileau et al. 2014). In the PG group, the AMPH-induced DA release in the ventral striatum correlated directly with severity of
PG symptoms on the South Oaks Gambling Screen (SOGS; Lesieur and Blume 1987). This result is noteworthy, given that a PET study of PG subjects by Joutsa et al. (2012) found a similar correlation between PG severity on the SOGS and ventral striatal DA release in response to large monetary rewards on a slot machine. In other words, large unpredictable rewards delivered on an actual gambling device exert parallel effects to a moderate dose of AMPH on ventral striatal DA release in PG subjects.

Collectively, the evidence supports the existence of a sensitization-like syndrome in individuals with PG. The rat study on cross-sensitization to AMPH following chronic exposure to cues further suggests that chronic exposure to gambling may have played a causal role in the elevated DA responses found in the PET studies of human PG subjects. More generally, AMPH and gambling appear to recruit a common pattern of severity-related DA responses in PG subjects, consistent with the psychostimulant model of PG (Zack and Poulos 2009).

### 1.2.4 Role of DA receptors in Gambling Reinforcement

Rewards are “objects or events that generate approach and consummatory behaviour, produce learning of such behaviour, represent positive outcomes of economic decisions and engage positive emotions and hedonic feelings” (Schultz 2010). According to Berridge et al. (2009), Liking, Wanting, and learning are three dissociable components of reward.

In principle, Liking and Wanting are separate components of reward, corresponding to the hedonic impact of a reward vs. its attributed incentive salience. Traditional methods of measuring reward (i.e., consumption tests, choice tests, place preference, instrumental performance) directly measure the degree to which a reward is ‘wanted’, and Liking is subsequently inferred, grounded on the assumption that rewards are ‘wanted’ proportionally to the degree they are ‘liked’. However, in order to effectively distinguish the two, Liking and Wanting need to be measured separately.
Wanting, incentive salience to reward-related stimuli, causes the reward, and has distinct psychological and neurobiological features (Berridge 2007). This characterization explains how changes in DA could exert important effects on reward-related behaviour, especially in the context of salient, reward-related cues. Conversely, measures based on affective reactions reflect the hedonic or aversive affect evoked by a stimulus more precisely, and are thus used to provide a more direct measure of whether a stimulus is ‘liked’ (Berridge and Robinson 1998). The finding that rewards may activate the Wanting system and influence behaviour without conscious emotion or desire also helps to elucidate the difference between Liking and Wanting (Schooler and Mauss 2009). This can been observed, for example, when addicts receive doses of drugs too low to produce any conscious experience of pleasure, yet still lead to increased drug-seeking behaviour (Lamb et al. 1991, Fischman and Foltin 1992).

Lastly, learning refers to the acquisition of predictive associations and cognitions that take place when an animal encounters a previously encountered, biologically important (rewarding) stimulus (Bolles 1972, Bindra 1978). However, it is currently unclear if incentive learning maps more onto Liking or Wanting.

1.2.5 Role of DA receptors in AMPH reinforcement

Involvement of the DA system in the neural and behavioural response to addictive drugs has been well documented (Wise 1996, Marinelli and McCutcheon 2014, Robbins and Clark 2015). It is generally accepted that addictive drugs engage the neural circuitry normally involved in pleasure, incentive motivation, and learning (Berridge and Robinson 1998). These brain reward circuits include DA projections from the VTA and substantia nigra to the NAc. Initially, addictive drugs are taken simply to achieve pleasant “highs” (positive reinforcement); however, in addiction, the same drugs are taken to escape withdrawal “lows” (negative reinforcement) (Robinson and Berridge 2003).
AMPH is the prototypical psychostimulant that elicits increased arousal, hyperactivity, and euphoria in humans (Berman et al. 2009), and has been demonstrated to increase implicit gambling-related cognitions and self-reported desire to gamble (DTG; i.e., Wanting) in PG subjects (Zack and Poulos 2004). Traditionally, AMPH has been characterized as a DA releaser that elevates extracellular DA and prolongs DA receptor signaling by three major mechanisms (Calipari and Ferris 2013). First, it competitively inhibits the DA transporter, blocking DA uptake; second, it facilitates release of DA vesicles into the cytoplasm; and third, it inhibits monoamine oxidase activity, reducing cytosolic DA metabolism (Sulzer 2011). Thus, systematic AMPH administration is associated with increased DA efflux and decreased levels of DA metabolites, dihydroxyphenylacetic acid and homovanillic acid, in rat striatum, including the NAc (Miele et al. 2000).

AMPH dependably increases DA neurotransmission in the NAc. Intra-accumbens microinjections of AMPH cause excessive Wanting for reward (including highly palatable food like sugar), but do not increase the hedonic Liking for sugar (Robinson and Berridge 2003). Thus, intra-accumbens increases in DA neurotransmission in the NAc, can magnify Wanting without necessarily changing Liking. These results have important implications for understanding what occurs when DA pathways undergo sensitization. Sensitization enhances the ability of drug-associated cues to trigger intense bursts of Wanting for reward, and in human addicts, who may have many years of drug experience with opportunity for sensitization and learning, this may lead to craving, the compulsive pursuit of drugs, and potentially relapse (Robinson and Berridge 2003). Reports on cocaine and its role in DA neurotransmission can be extrapolated to further understand the role AMPH plays. In rats previously exposed to cocaine, D1 receptors are believed to primarily mediate the rewarding effects, while D2 receptors control craving and motivation (Self et al. 1996). D2 antagonists elicit compensatory self-administration of high doses of cocaine (Caine et al. 2002), suggesting D1 is necessary for cocaine reward and
reinforcement, whereas D2 facilitates acquisition of cocaine reward without mediating its discriminative effects (i.e., subjective effects of the drug). In human cocaine addicts, acute blockade of D1 receptors with ecopipam (10-100 mg) decreased self-reported pleasurable effects and craving ratings in response to intravenous cocaine (30-mg) (Romach et al. 1999), suggesting a role for D1 in the subjective rewarding and incentive motivational aspects of the drug response.

Chronic stimulant use, on the other hand, is associated with decreased binding at both D1 and D2 receptors (Nikolaus et al. 2007). Evidence shows that chronic cocaine exposure leads to increases in tonic DA levels (in animals), which oppose phasic DA release by activating pre-synaptic D2 auto-receptors (Grace 2000). In vitro studies have also demonstrated that AMPH can result in saturation of DA receptors (Richfield et al. 1989), as well as activation of D2 auto-receptors and long-loop feedback pathways that inhibit DA cell firing (Bunney et al. 1973). This DA-mediated feedback inhibition is altered after chronic treatment with AMPH or cocaine, and has been suggested to play an important role in the development of behaviours associated with the abuse of these drugs (White and Wang 1984). Chronic intermittent low-dose AMPH is associated with elevated levels of DA in synaptic clefts, reflecting adaptive changes that promote increased DA release (Robinson and Becker 1986). Sensitization, as reflected by increased AMPH-induced ventral striatal DA release in a PET scan, has been demonstrated in healthy humans after as few as three modest doses of AMPH (0.3-mg/kg), with effects persisting for up to one year (Boileau et al. 2006). The long lasting behavioural and neurochemical changes associated with chronic AMPH use, together with the existence of similar DA disruptions in both SUDs and PG, suggest that sensitization may be relevant to PG.

1.2.6 Trait Factors in Gambling Reinforcement

It is evident that PG results from the culmination of many dynamic variables (e.g., biological, environmental, and/or social). Out of the 60-80% of adults who reported gambling in
the past year, only ~3% developed PG (el-Guebaly et al. 2006, Fong et al. 2005). This suggests that other risk factors must be involved for this syndrome to emerge; it can not solely be the result of chronic exposure.

Of the many possible influences involved, individual differences represent a growing area of interest. Specifically, a subset of research has focused on the examination of a number of different personality traits and their relationship to the predisposition for, and maintenance of gambling behaviour and PG.

1.2.6.1 Reward Delivery and Omission

Analogous to many probabilistic rewarding activities, gambling can have two basic outcomes, reward delivery or reward omission. Neuronal recordings in the striatum of animals have demonstrated that reward delivery (i.e., winning) causes phasically active neurons to increase firing (Brasted and Wise 2004), whereas tonically active neurons may contribute to the storage of stimulus-reward associations or habits (Aosaki et al. 1994, Apicella 2002). The response properties of midbrain DA neurons are thought to encode reward prediction errors, the capacity to carry information about differences between expected vs. actual outcomes, which are crucial for reinforcement learning (Bayer and Glimcher 2005). Because the sensitivity of tonically active neurons to rewarding events shares some features with that described for DA neurons (Apicella 2007), it is conceivable that tonically active neurons contribute to the processing of error prediction signals. In this regard, there are similarities in coding capabilities between tonically active neurons and midbrain DA neurons.

As noted earlier, it is believed that spikes in phasic DA are primarily detected by low affinity D1 receptors. Since DA might play a role in reward prediction error, the riskier a bet is (i.e., low probability, large-sized outcome), the larger the magnitude of DA spike it can evoke (Schultz 2001, Fiorello 2013). Conversely, learning from negative outcomes (i.e., an omission of
reward; loss or non-win) is more controversial. When an expected reward is withheld, there is a temporary pause in tonic DA activity, detected by high affinity D2 receptors (Schultz 1997). However, whether the absence of reward can be encoded in the same graded manner (i.e., variable pauses in tonic DA) as reward delivery is unclear.

Individual differences in learning from positive and negative outcomes are related to striatal D1 and D2 function, respectively (Cox et al. 2015). DA neurons burst fire following the presentation of unexpected rewards, and pause when an expected reward has been withheld, allowing them to encode a reward prediction error signal (Montague et al. 1996, Schultz et al. 1997). The reward prediction error DA signal promotes learning from positive outcomes via stimulation of D1 receptors. While striatal DA signaling is widely thought to play an important role in reward learning (Schultz 2001), its contribution to learning from negative outcomes is more controversial. One proposed model to account for the asymmetrical effects of DA manipulations on reward and punishment or extinction learning suggests that DA modulates both approach and avoidance learning via two separate pathways (Frank 2005, Frank et al. 2006). Striatal medium spiny neurons of the direct pathway, which express D1 receptors, facilitate the selection of rewarding actions encoded in the cortex. Conversely, disinhibition of indirect pathway striatal neurons suppress cortical patterns that encode maladaptive or non-rewarding actions, (Cohen and Frank 2009, Surmeier et al. 2011). Cox et al. (2015) further suggest that the well-documented association between genetic or PET measures of D2 function and drug addiction or PG reflects impaired punishment learning in these disorders.

1.2.6.2 Empathic DA Profile

As noted earlier, the trait dimension of Empathy does not have a universally accepted definition, as the different phenomena it encompasses remain debatable (Batson 2009, de Vignemont and Singer 2006, Preston and de Waal 2002). Nevertheless, it has been broadly
defined as the experiencing of an affective state similar to that expressed by another individual or signal in the environment, where one is aware of the reasons for another’s emotional state and is able to identify with them – that is, the ability to experience these feelings vicariously (Fan et al. 2011, de Waal 2008). While an increasing number of fMRI studies have been conducted on Empathy, there have not been any consistent answers to several fundamental Empathy-related questions (Bernhardt and Singer 2012).

However, conceptual work on Empathy has greatly facilitated the design of empirical studies that assess empathic traits operationally through several relatively easy, reliable, and reproducible self-report questionnaires (Bernhardt and Singer 2012). To measure empathic traits, several self-report questionnaires have been developed, including the Interpersonal Reactivity Index (IRI; Davis 1983) and the Balanced Emotional Empathy Scale (BEES; Mehrabian 1997). Additionally, the Eysenck Impulsiveness Subscale (EIS; Eysenck et al. 1985) has been validated with an internal consistency of 0.84 and has been replicated in different samples with varying age groups (Eysenck et al. 1985).

Furthermore, cognitive-behavioural research has supported the validity of self-report scales of subjective state. Santesso and Segalowitz (2009) showed that self-report scales of Empathy predicted electrophysiological response to negative feedback, mapping onto an objective index of brain sensitivity to negative signals. Amplitude of the Error Report Negativity (ERN) predicted the degree to which individuals learned about the negative consequences (e.g., errors) of their actions; larger ERNs were associated with a bias to learn to avoid negative events more than to seek positive events.

Studies concerning the biochemical foundation of Empathy suggest that many neurotransmitter systems work together in a complex network. Polymorphisms in the oxytocin receptor and the 5-HT transporter promoter region are believed to contribute to social behaviour in a broad range of species from rodents to man (Ebstein et al. 2010). Additionally, the
dopaminergic and noradrenergic systems are crucial for Empathy-related behaviours. Human studies demonstrated that lower DA levels are associated with better performance in a Theory of Mind task measuring the ability to predict the behaviour or thoughts of others in a simple social context (Reuter et al. 2005). This is consistent with the conceptualization of Empathy as the opposite to Psychopathy, a trait profile associated with a general insensitivity to the feelings of others and increased striatal DA response to the prospect of reward and reward delivery.

Similarities between Empathy and Theory of Mind further provide support for the role of DA in Empathy. Theory of Mind, the ability to represent one’s own or another’s mental states, believed to underlie the ability to understand and predict other people’s behaviour is impaired in individuals with autism (Abu-Akel 2003). The broad range of DA associations (e.g., with the cerebellum, amygdala, PFC, parietal lobes, and hippocampus) suggest that the dopaminergic system may play a prominent role in the etiology of autism (Kriete and Noelle 2015). Further electroencephalographic research has shown that the functional development of the dorsal medial PFC is a specific neurodevelopmental correlate of preschoolers’ Theory of Mind development (Sabbagh et al. 2009). Intriguing indirect support for a relation between DA and Theory of Mind comes from research into clinical disorders associated with DA dysfunction. Individuals with late stage Parkinson’s disease, a disorder that also involves impaired DA functioning, show deficits in Theory of Mind (Péron et al. 2009).

Additional studies postulate that DA β-hydroxylase, an enzyme that converts DA to NE, may have broad influences on social functions (Bassett et al. 2007, Harmer et al. 2009). Humans displaying social dysfunctions – and especially a lack of Empathy – such as individuals with autistic spectrum disorder, have lower DA β-hydroxylase enzymatic activity compared to controls, resulting in increased DA and deceased NE levels (Robinson et al. 2001). Another notable study conducted by Gray et al. (1994), which defined personality using the EPI, reported a significant inverse correlation between striatal D2 receptor availability and Psychoticism
(Eysenck’s term for Psychopathy), denoting ‘tough-mindedness,’ or ‘lacking in sensitivity and Empathy’ (Reeves et al. 2007). These findings reveal the importance of polymorphisms as a genetic basis of predicting individual differences in social and affective processing (Gong et al. 2014), and suggest that genetic differences in DA transmission or receptor sensitivity might influence the expression of behavioural and emotional characteristics of Empathy.

Empathy is well characterized by sensitivity to pain. Recent experiments have used pain as an eliciting stimulus to study the neuroanatomical substrates of Empathy in humans (Bernhardt and Singer 2012). For example, when someone empathic sees someone else in pain, they understand what they are going through because they are sensitive to pain themselves. FMRI studies have explained this correspondence by demonstrating that common neural circuits are involved in representing one’s own and others’ affective and motivational aspects of pain (Jackson et al. 2006). When gambling, a negative outcome (e.g., loss) could constitute a painful stimulus to highly empathic individuals who are especially sensitive to negative stimuli. However, a slot machine may be especially reinforcing to highly empathic individuals because it minimizes the salience of loss by providing no sensory signals (lights, bells) when reward is omitted and maximizes the salience of reward delivery by providing these strong audio-visual cues whenever a win occurs.

Sensitivity to social-environmental signals for reward and punishment has been linked with DA transmission. Specifically, Empathy has been associated with hypersensitivity to negative outcomes (or reward omission) and thus may be relevant to gambling in relation to losses or non-wins. Recall that losses cause a temporary dip in tonic DA activity, detected by high affinity D2 receptors, whereas wins cause a spike in phasic DA, detected by low affinity D1 receptors. Socially desirable responding, as indexed by high scores on the Lie scale of the EPI, has been linked with high D1 and low D2 receptors in the striatum, medial PFC and amygdala in healthy individuals (Reeves et al. 2007). The authors suggested that this profile may be
associated with sensitivity to social reward. It is unclear whether this profile is genetically mediated; whether it reflects compensatory responses to variations in tonic or phasic DA transmission, or some combination of these factors. However, in a separate study of healthy volunteers, D1 receptor availability in the striatum was found to directly predict positive incentive learning (reward), while D2 receptor availability in this region directly predicted better learning from negative outcomes (punishment or reward omission), although the latter relationship was non-linear – i.e., optimal avoidance learning with moderate D2 receptor availability (Cox et al. 2015). Given the link between Empathy and hypersensitivity to negative outcomes, receptor binding data suggest that highly empathic individuals may be characterized by relatively lower striatal D1 and higher striatal D2 availability, compared to individuals with low trait Empathy. Whether this profile extends to individuals with PG is unclear.

1.3 Objectives

This study aims to clarify the roles of D1 and D2 receptors in mediating the relationship between Empathy, and the rewarding and reinforcing effects of gambling and a psychostimulant drug in PGs. To do so, we will utilize haloperidol (HAL; 3-mg), a preferential D2 antagonist, and fluphenazine (FLU; 3-mg), a combined D1-D2 antagonist; two drugs which differ mainly in their action at D1. Comparison of the effects of HAL vs. FLU on responses to a 15-minute slot machine game and the prototypical stimulant and DA releaser, AMPH (20-mg) will be carried out during separate sessions.

Empathy will be assessed by self-report from the Eysenck Impulsivity Scale (EIS; Eysenck et al. 1985), a well-validated scale with an internal consistency of 0.84 that has previously been used effectively in multiple studies with PG subjects. Hedonic impact (Liking) of the reinforcers will be assessed by Visual Analog Scales (VAS) Composite Pleasurable Effects and the Addiction Research Center Inventory (ARCI –Amphetamine; AMP) subscale
assessing psychostimulant-like effects. Incentive motivation (Wanting) will be assessed by self-reported DTG (e.g., Zack and Poulos 2007).

1.4 Hypotheses

The literature suggests that Empathy may correlate positively with gambling reinforcement and deficits in baseline D1, or elevations in D2 signaling. However we do not definitively know if variations in D1, D2, or both receptor types impact the relationship between Empathy and reinforcement in PGs.

There are several issues that can make measuring correlations difficult. One issue, restriction of range, refers to the case where one variable is not represented across the entire range of interest. This narrow range makes the observed correlation seem attenuated (weaker) than it is. From a statistical standpoint, differences in maximum scores on a number of dependent variables (e.g., DTG) can restrict range in controls compared to PGs. Therefore, although the effects of Empathy can be meaningfully compared across the two antagonists (HAL, FLU) within the healthy control group, between-group differences (HCs vs. PGs) in the correlation between Empathy and the dependent variables may be biased. Further to this point, from a clinical standpoint, PG is extremely heterogeneous in the development, presentation and maintenance of the disorder, and this may have implications for treatment that are not relevant to controls. Consequently, this paper will focus on analyzing the trends observed amongst PGs, and control data will not be examined.

If D1 mediates reward sensitivity, and D2 mediates sensitivity to reward omission or negative outcomes, then:
In the gambling condition (Phase I), we hypothesize that:

1) By antagonizing D2 auto-receptors and increasing signaling at post-synaptic D1 receptors, HAL should attenuate deficits in sensitivity to reward, and decrease the relationship between Empathy and the reinforcing effects of the slot machine.

2) Conversely, FLU should amplify the relationship between Empathy and the reinforcing effects of the slot machine by antagonizing pre-synaptic D2 auto-receptors, while decreasing signaling at D1.

Conversely, in the psychostimulant condition (Phase II), where AMPH causes unconditioned DA release, such that individual differences in sensitivity to pauses in D2 signaling should be reduced, we hypothesize that:

3) HAL should amplify the relationship between Empathy and AMPH reinforcement by selectively removing AMPH-induced feedback inhibition via D2 auto-receptors and enhancing phasic D1 signaling.

4) Conversely, FLU should attenuate the relationship between Empathy and AMPH reinforcement by removing feedback inhibition via D2 auto-receptors similar to HAL, but also blocking the resulting increase in phasic DA at D1.
2. MATERIALS AND METHODS

2.1 Study Design

This study employed a 2 Group (PG / HC) x 2 Antagonist Group (HAL / FLU) x 2 Pre-Treatment (active antagonist / placebo) x 2 Phase (slot machine / AMPH) repeated measures between-within subjects’ design.

Sixty subjects (30 PGs, 30 HCs) were assigned to receive one of the two antagonists, such that there were 15 PG-HAL, 15 PG-FLU, 15 HC-HAL and 15 HC-FLU. Antagonist treatment was double-blinded and counterbalanced across all subjects. Prior to enrollment, subjects underwent three rounds of screening consisting of a telephone screen, in-person interview and physician’s exam. Eligible subjects attended four test sessions, each separated by a one-week washout period. The first two test sessions (Phase I) assessed the effects of the antagonists on gambling reinforcement with a 15-minute standard session of play on a commercial Electronic Gaming Machine (slot machine). The third and fourth test sessions (Phase II) assessed the effects of the antagonists on AMPH reinforcement with a 20-mg dose of oral d-amphetamine (Dexedrine®). The slot machine session in Phase II was purely exploratory. Thus, the slot machine was played after all AMPH-related tests had been completed and the slot machine data from Phase II do not form part of the analysis of this project.

2.2 Study Medications

2.2.1 Haloperidol

HAL is a high-potency typical antipsychotic agent that is primarily prescribed for management of psychotic disorders (e.g., schizophrenia). It is a DA receptor antagonist with a high affinity for D2 receptors (Ki = 0.6), and a moderate affinity for D1 receptors (Ki = 17). HAL also has high affinity for D3 receptors (Ki = 0.2), moderate affinity for D4 receptors (Ki = 22) and low affinity for D5 receptors (Ki = 169). In addition to DA receptors, HAL also shows
moderate affinity for $\alpha_1$ adrenergic receptors and low affinity for $\alpha_2$, histamine, and muscarinic acetylcholine receptors (Appendix A; Binding Profiles of Haloperidol and Fluphenazine).

An oral dose of HAL has a bioavailability of 60 ± 18%, volume of distribution of 18 ± 7 L/kg, and half-life of 18 ± 5 hours (Froemming et al. 1989). HAL reaches peak plasma concentration after a mean 2.75 hours (Wachtel et al. 2002), wherein a 3-mg oral dose would be expected to occupy approximately 65% of D2 receptors (Nordström et al. 1992). HAL is approximately 92% bound to albumin and is metabolized mainly in the liver.

### 2.2.2 Fluphenazine

FLU is a high-potency, short-acting piperazine phenothiazine antipsychotic. It has high affinity for both D1 ($Ki = 0.85$) and D2 ($Ki = 0.4$) receptors, and low affinity for adrenergic and cholinergic receptors (Appendix A; Binding Profiles of Haloperidol and Fluphenazine). Similar to HAL, FLU has moderate affinity for D3 and D4 receptors ($Ki = 1.4$ and $7.1$, respectively), and low affinity for D5 receptors ($Ki = 54$) (Burstein et al. 2005, D’Aoust and Tiberi 2010).

An oral dose of FLU has a bioavailability of 2.7%, volume of distribution of 11 ± 10 L/kg, and half-life of 14.4 ± 7.8 hours (Jann et al. 1985, Brauer and de Wit 1995). FLU reaches peak plasma concentration after a mean 2 hours and has an elimination half-life of 13-33 hours. The exact proportion of D2 receptors occupied by FLU at peak concentration is unknown, but its similar affinity for D2 as HAL ($Ki = 0.4$ vs. 0.6) suggests comparable D2 occupancy at peak blood levels for an identical dose (i.e., 3-mg).

### 2.2.3 Rationale for Drug Selection

Despite having moderate affinity for D1 receptors, HAL is the most selective D2 antagonist available for human use in Canada. As such, it allows assessment of the effects of D2 blockade, amplifying the signal at D1 receptors. Theoretically, a selective D1 antagonist would
be ideal to identify the role of D1 receptors in these reward responses. However, there currently are no D1 antagonists approved for human use in Canada. Furthermore, using HAL permitted direct comparison of present findings with the results from a previous study that assessed the effects of 3-mg HAL on responses to the slot machine in PGs (Zack and Poulos 2007).

Beside their differences in D1 affinity, HAL and FLU show very similar binding profiles at other DA receptor subtypes (e.g., D2, D3, D4, D5), as well as non-DA receptors including α1, α2 adrenergic receptors and muscarinic acetylcholine receptors. Thus, comparing the effects of the D2 antagonist and combined D1-D2 antagonist will permit inferences about the role of post-synaptic D1 activation in mediating gambling and AMPH reinforcement.

2.2.4 Dextroamphetamine sulfate

Dextroamphetamine sulfate (more commonly known as AMPH) is a sympathomimetic DA releaser and psychostimulant primarily indicated for attention deficit hyperactivity disorder. It is a potent full agonist of trace amine-associated receptor 1, which upon activation, increases cAMP production, inhibits DA transporters and induces monoamine neurotransmitter release. Consequently, it causes increased synaptic DA and produces CNS stimulation. AMPH has an oral bioavailability of over 75%, with peak plasma concentration achieved in 1-2 hours and an elimination half-life of 9-11 hours.

2.2.5 Diphenhydramine

Diphenhydramine (Benadryl®) is a first-generation antihistamine used to treat allergies. It is also frequently applied to the management of extrapyramidal side effects related to first-generation antipsychotics (Dayalu and Chou 2008). Subjects were prescribed 50-mg diphenhydramine and instructed to take it only if they felt delayed side effects (e.g., dyskinesia or akathisia) to the study medications after leaving the laboratory on test days.
2.3 Study Subjects

2.3.1 Inclusion and Exclusion Criteria

This study included subjects between the ages of 19-65. PGs scored ≥5 on both the SOGS and DSM-IV, but were otherwise healthy. PGs who were treatment-seeking or wished to remain abstinent from gambling were excluded due to the procedural requirement to play a slot machine during the study. In addition, any potential subject who was in a treatment program for drugs, alcohol use, or gambling fewer than six months ago was ineligible.

In order to minimize confounding effects of co-morbidities, subjects were screened for and excluded on the basis of Axis I diagnoses – current or past manic or depressive episodes (exception of one past depressive episode >1 year prior), current or prior anxiety disorders, alcohol abuse <1 year prior, current or past alcohol dependence, and presence of psychotic symptoms. Subjects with schizotypal or borderline personality disorder were also excluded to avoid possible psychotic response to AMPH.

Subjects with prior exposure to psychostimulants (e.g., cocaine, AMPH), regular recreational drug use, heavy nicotine or alcohol use – as indicated by the Fagerström Test for Nicotine Dependence (FTND) and Alcohol Timeline Follow-Back – were ineligible. Subjects with a first-degree relative with schizophrenia or bipolar disorder were also excluded to avoid any possible psychotic effects of AMPH. Women who were pregnant, or breastfeeding were excluded to avoid potential exposure of fetuses to study medications.
Table 1. Summary of inclusion and exclusion criteria for Pathological Gamblers.

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>PG</th>
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<tbody>
<tr>
<td>Age</td>
<td>19-65</td>
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<tr>
<td>Gambling behaviour</td>
<td>Non-abstinent</td>
</tr>
<tr>
<td>SOGS</td>
<td>≥5</td>
</tr>
<tr>
<td>DSM-IV PG</td>
<td>≥5</td>
</tr>
<tr>
<td>FTND</td>
<td>≤6</td>
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<table>
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<tr>
<th>Exclusion Criteria</th>
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<tbody>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>BDI</td>
</tr>
<tr>
<td>ADS</td>
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<tr>
<td>Alcoholic consumption</td>
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<tr>
<td>Smoking</td>
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<tr>
<td>Caffeine consumption</td>
</tr>
<tr>
<td>DAST</td>
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<tr>
<td>Recreational drug use</td>
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<td>WAIS vocabulary</td>
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PG, Pathological Gambler; SOGS, South Oaks Gambling Screen; DSM-IV PG, Diagnostic and Statistical Manual of Mental Disorders 4e. – pathological gambling; FTND, Fagerström Test for Nicotine Dependence; BMI, body mass index (kg/m²); BDI, Beck Depression Inventory; ADS, Alcohol Dependence Scale; DAST, Drug Abuse Screening Test; WAIS; Wechsler Adult Intelligence Scale.

2.3.2 Screening Instruments

South Oaks Gambling Screen (SOGS) (Lesieur and Blume, 1987)

The SOGS is a validated, widely-used measure of assessing PG status. The scale consists of 16 items, 11 of which are scored for a maximum score of 20. Individuals who scored ≥5 were eligible as PGs. The questionnaire was first administered during the telephone screen and repeated during the interview session. PGs were asked the questions orally by the study psychiatrist to confirm PG status and severity.

DSM-IV PG Questionnaire (Beaudoin and Cox 1999)

The DSM-IV PG questionnaire is based on the DSM-IV diagnostic criteria for PG. It consists of 10 items scored from 0 to 3, based on the time period in which symptoms occurred.
Individuals who scored \( \geq 5 \) were eligible as PGs. Similar to the SOGS, this scale was administered during the telephone screen and again during the interview by the study psychiatrist as a secondary measure of PG status and severity.

**Beck Depression Inventory (BDI)-short form (Beck and Beck 1972)**

The BDI is a widely used instrument to measure severity and depth of depression symptoms. The short form of the BDI consists of 13 items, each scored from 0-3 depending on symptom severity, and is intended for use by primary care (i.e., non-psychiatric) clinicians. Subjects who scored 0 on the suicide item, and <10 overall were eligible for the study. It was administered during the telephone screen, and repeated in the interview screen by self-report as part of the questionnaire package.

**Alcohol Dependence Scale (ADS) (Skinner and Allen 1982)**

The ADS is a reliable tool used in both research and clinical applications. It consists of a 25-item scale used to identify alcohol abuse or dependence within the prior year. Subjects with an ADS score <13 (bottom quartile) were considered for inclusion. This scale was administered during the telephone screen and as part of the questionnaire package during the interview screen.

**Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al. 1991)**

The FTND is a standard instrument for assessing the intensity of addiction to nicotine. It is a 6-item scale, with a maximum score of 10. It was administered during the interview screen and used as an index for matching subjects to the two antagonist subgroups.

**Alcohol Timeline Follow-Back (Sobell and Sobell 1992)**

A 90-day Timeline Follow-Back was administered during the interview screen to assess
patterns of alcohol consumption. Subjects provided retrospective estimates of the number and type of alcoholic drinks consumed each day over the preceding 3-month period. Subjects who consumed >20 (men) or >15 (women) alcoholic drinks per week on average, indicative of possible problem drinking (Sanchez-Craig and Israel 1985), were ineligible for the study.

Nicotine Timeline Follow-Back

A 7-day nicotine Timeline Follow-Back (based on the validated Alcohol Timeline Follow-Back) was administered during the interview screen to confirm smoking status and level of use. Subjects who smoked >20 cigarettes per day on average were excluded to minimize potential withdrawal effects during the 4-hour assessment phase of each test session where smoking was not permitted.

Drug Abuse Screening Test (DAST) (Skinner 1982)

The DAST is a 20-item yes/no screening instrument for the abuse of drugs other than alcohol over the past year. It was administered in the questionnaire package during the interview screen. Subjects who scored ≤4 were eligible.

Structured Clinical Interview for the DSM-IV (SCID) (First et al. 2002)

The SCID is a semi-structured interview used to make diagnoses based on DSM-IV Axis I disorders in psychiatric patients and non-patient research subjects. Open-ended questions are administered by a clinician or trained researcher who is familiar with the DSM-IV diagnostic criteria. A series of pre-set follow up questions are used to clarify the details of all endorsements to achieve a comprehensive assessment of the patient’s psychiatric profile.

The SCID was conducted during the interview screen by the experimenter and study psychiatrist. The version used in this study covered modules assessing mood disorders, panic
disorder, alcohol use, substance abuse and psychotic symptoms. Subjects were excluded if they met the criteria for current or past depressive or manic episodes (except only one past depressive episode >1 year prior), current or past anxiety disorders, current or past alcohol dependence, alcohol abuse (except if >1 year prior), or presence of psychotic symptoms (Appendix B; SCID Inclusion/Exclusion Criteria).

Eysenck Personality Inventory (EPI) (Eysenck and Eysenck, 1963)

The EPI is a self-report questionnaire that consists of 57 yes-or-no questions used to assess Extraversion and Neuroticism. There is also a Lie Scale that measures the likelihood that a subject would modify their responses in order to meet social expectations. Administration of this questionnaire in the interview screen, helped serve as an index of validity for the other self-report questionnaires administered throughout the study.

Eysenck Impulsiveness Scale (EIS; Eysenck et al. 1985)

The EIS is a self-report questionnaire consisting of 54 yes-or-no questions used to assess Impulsivity, Venturesomeness and Empathy. It was administered twice – orally during the telephone screen and via self-report during the interview screen in order to validate the traits that are endorsed. Furthermore, the EIS was selected because it has been used in multiple studies with PG subjects and found to discriminate between subtypes of PG – with and without substance use disorders, to predict performance on laboratory indices of impulsive behaviour and to predict the response to DA enhancing medications in PG subjects (Zack and Poulos 2009).

Gamblers’ Belief Questionnaire (GBQ; Steenbergh et al. 2002)

The GBQ is a 21-item self-report measure of gambler’s cognitive distortions across two domains: Luck/Perseverance (Factor 1) and Illusion of Control (Factor 2), administered as part of
the questionnaire package in the interview session. Each item (e.g., “Gambling is more than just luck”) is rated on a 7-point scale from 1 (Strongly Agree) to 7 (Strongly Disagree). Thus, lower scores indicate greater cognitive distortions.

Wechsler Adult Intelligence Scale (WAIS)-R (Wechsler 1981)

Selected tasks from the WAIS-R were administered during the interview to assess basic cognitive function of all subjects.

WAIS Digit Span The Wechsler Digit Span sub-test assesses attention, short-term rote memory (Forward Subscale), executive function and working memory (Backward Subscale). In the Digit Span Forward, the experimenter read 7 pairs of number sequences (increasing in length from 3 to 9 digits), and subjects repeated each pair in the same order. In the Digit Span Backward, the experimenter read another 7 pairs of number sequences (2 to 8 digits) and subjects repeated each pair in reverse order. Each correct response scored 1 point, with a maximum score of 14 for each subscale, and a combined maximum score of 28.

WAIS Digit Symbol-Coding The Wechsler Digit Symbol-Coding sub-test assesses visual perception/analysis, working memory and motor control. Subjects were provided a legend of 9 digit-symbol pairs and a list of digits. Under each digit, subjects were instructed to write down the corresponding symbol. Subjects were timed to see how many could be completed within 60 seconds. Each correct answer scored 1 point, with a maximum possible score of 92.

WAIS Vocabulary Task The Wechsler Vocabulary sub-test assesses comprehension and English proficiency. It was employed to ensure subjects were able to fully understand the other self-report and cognitive/behavioural tasks administered throughout the study. Subjects were asked to orally define 15 English words of increasing difficulty. Each answer was scored from 0-2, with a maximum score of 30. Subjects who scored <18 were excluded due to probable inability to comprehend other instructions, or stimuli presented throughout the study.
Wisconsin Card Sort Task (WCST) (Heaton 2003)

The WCST assesses the ability to display learning and cognitive flexibility, administered during the interview screen. Subjects were told to match the test card to one of four standard cards. Cards could be matched on one of 3 criteria: colour, number, or shape, and the computer provided feedback as to whether each match was correct or incorrect. The rule for matching changed without notice. Based on the feedback received, subjects had to deduce the rule and follow it until a new rule was implemented. The task did not have a set number of trials; it continued until correct responses were chosen for six new rules. The number of trials required for the subject to learn the correct matching rule after each change was used to assess cognitive flexibility in response to visual cues.

2.3.3 Group Matching

Subjects were assigned to HAL or FLU antagonist groups, matched on gender, age, PG severity (SOGS), impulsivity (EIS), depressive symptoms (BDI), alcohol use (ADS), and nicotine dependence (FTND). A randomization code agreed upon by the Principal Investigator and CAMH Pharmacy blinded the drug sequence received by each enrolled subject. For example, in drug sequence 1, active antagonist pre-treatment was given on session 1 followed by placebo on session 2; in sequence 2, placebo was given on session 1 followed by drug on session 2. Both the experimenter and subjects were blind to drug sequence, and only revealed once the subject had completed testing.

2.3.4 Subject Safety

Potential subjects were systematically screened to ensure physical health and absence of any contraindications for the study medications. The Qualified Investigator reviewed the electrocardiogram (ECG), blood and urine analyses, and each subject underwent a physical exam
by a study physician to further confirm their health status and suitability to receive the study medications. During all test sessions, blood pressure and heart rate were regularly monitored (every 30 minutes in Phase I, and every 15 minutes in Phase II) to gauge physiological reactivity to the stimuli and facilitate rapid intervention in the event of extreme reactions.

A registered nurse examined subjects’ vital signs prior to discharge at the end of each test session. Open-ended questions were continuously posed throughout the test sessions to assess how subjects were feeling and determine if they experienced any adverse effects from the medications. Subjects were prescribed a 50-mg dose of diphenhydramine to take in case of delayed side effects (e.g., dyskinesthia or akathesia) from the study medications, as well as a wallet card noting the appropriate antagonist name, dose, time of administration and contact information in case of emergency. Lastly, subjects were sent home from the lab in a pre-paid taxi, reminded to avoid driving or operating heavy machinery for 24 hours and to abstain from all drugs, medications and alcohol for 72 hours after each test session to avoid possible interactions with the study medications.

2.3.5 Time Commitment and Study Payment

The total time commitment for completion of the study was approximately 42 hours. This encompassed 3 hours for the interview screen, 1 hour for the physician’s exam, 4 test sessions (8 hours each) and a 1-hour commute for each visit. Upon completion of the study, subjects received a cheque for $1000 – $920 for completion, plus a standard $80 bonus for their ‘winnings’ from the slot machine sessions.

2.4 Apparatus

2.4.1 Self-Report Scales

Self-report questionnaire packages were administered at specific time points throughout
each test session: pre-capsule baseline, expected peak antagonist concentration, immediately before and after slot machine play (and expected peak AMPH concentration in Phase II).

*Visual Analog Scales (VAS) (Fischman and Foltin 1991)*

This study employed modified VAS to assess subjective motivation and pleasurable effects in response to gambling and AMPH, a valid tool to assess the subjective effects of drugs (Fischman and Foltin 1991). Each scale ranged from 0 to 10 with half-point gradations. Slot machine VAS questions, derived from an initial study on HAL (Zack and Poulos 2007), assessed DTG, as well as Enjoyment, Excitement, Involvement and perceived High from the game. AMPH VAS questions, derived from an initial study on AMPH (Zack and Poulos 2004), assessed Liking, High, Good Effects and [Desire to] Take Again. Desire for Alcohol was also assessed to determine whether the various manipulations selectively affected gambling compared to other types of addictive reinforcement.

*Profile of Mood States (POMS), short form (Shacham 1983)*

The POMS was used to track changes in mood across six domains (Tension-Anxiety, Depression-Dejection, Anger-Hostility, Fatigue-Inertia, Vigor-Activity, and Confusion-Bewilderment) over the course of each test session. Subjects rated their experience of 37 mood adjectives from 0 (Not at all) to 4 (Extremely) as part of the self-report questionnaire packages.

*Addiction Research Centre Inventory (ARCI) (Haertzen 1965)*

The ARCI is a standardized questionnaire for assessing subjective effects of psychoactive drugs, consisting of 49 true-or-false statements that cover six drug effect domains: Amphetamine (AMP) measures AMPH-specific effects; Morphine/Benzedrine Group (MBG) for euphoria; Lysergic Acid Diethylamine (LSD) for dysphoria; Benzedrine Group (BG) for stimulant effects;
and Pentobarbital-Chlorpromazine-Alcohol Group (PCAG) for sedation. Previous research has demonstrated the validity of ARCI in assessing subjective effects of drugs, including AMPH (Chait et al. 1986).

*Side Effect Checklist (Zawertailo et al. 1994)*

Previously validated for medication studies, a Side Effect Checklist was administered at the end of each session to assess the presence and severity of 24 potential adverse drug effects (e.g., headache, blurred vision) from 0 (Absent) to 5 (Needs intervention). This was used in complement to open-ended questions that asked subjects how they were feeling.

*Capsule Contents Evaluation*

The Capsule Contents Evaluation was administered at the end of the second (Phase I) and fourth (Phase II) test sessions, wherein subjects indicated which capsule(s) they believed were active (compared to placebo) from the previous two test sessions.

### 2.4.2 Devices

**Breathalyzer**

A calibrated J4X Alert Breathalyzer or DriveSafe Breathalyzer (Alcohol Countermeasure Systems Inc., Mississauga, Ontario) was used to confirm absence of alcohol at the beginning of each test session.

**Blood Pressure/Heart Rate Monitor**

A HEM-601 (OMRON; Vernon Hills, IL) wrist cuff or Physiologic automatic wrist blood pressure monitor with smart valve technology (AMG; Montreal, QC) was used to measure blood pressure and heart rate regularly during each test session.
2.4.3 Slot Machine

On each test session (at expected peak antagonist level in Phase I, or after a verbal cue salience task – approximately 20 minutes post-expected peak AMPH in Phase II), subjects played ‘Cash Crop’, a commercial video lottery terminal-style Electronic Gaming Machine (WMS Gaming; Detroit, MI) for 15 minutes or until 400 pre-loaded credits ($100 equivalent) ran out. To enhance external validity and mimic a casino environment, the slot machine session was unsupervised in a mock bar separate from the other testing rooms. To further encourage spontaneous betting behaviour, subjects were informed that a cash bonus proportional to the credits remaining at the end of each session would be added to their agreed upon compensation.

The object of the game was to get as many of the same symbol on a line as possible. For each spin, subjects selected the number of lines to bet on (1-9), and the number of credits to bet on each line (1-5). Thus, subjects were allowed to bet between 1 and 45 credits on any spin. Wins (credits >0 received) were accompanied by visual and auditory cues (flashing lights and bells proportional to the number of credits won), while losses did not generate any sensory feedback. Boxes on screen displayed the payoff from the last spin and total number of credits remaining. To encourage spontaneous patterns of play, subjects were not aware their betting behaviour (bet size, line selection and payoff for each spin) was recorded electronically until debrief at the end of the final session. Subjects were offered the option of having their slot machine data omitted since it was obtained without prior consent.

2.4.4 Computer Tasks

Rapid Reading Task (RRT) (Zack and Poulos 2004)

Subjects completed the RRT directly after the slot machine game in Phase I, and at expected peak AMPH in Phase II. The task was administered on a computer with MicroExperimental Laboratory (MEL, v. 2.01, Psychology Software Tools; Pittsburgh, PA)
software. A warning signal (&&&&&&) appeared before each trial to focus subjects’ attention on the target location in the center of the screen. One at a time, words appeared on the screen with asterisks between each letter (e.g., p*e*n*c*i*l) to enhance the magnitude of priming. Subjects were asked to pronounce each word aloud as quickly and accurately as possible. A microphone attached to the computer measured the vocal response time with millisecond precision. During the task, the experimenter coded response accuracy (correct, incorrect or spoiled response, e.g., if the microphone picked up a cough) after each trial using a serial response box (Psychology Software Tools; Pittsburgh, PA). The events on each trial were identical: warning signal (350ms) → blank (250ms) → target word → subject response → experimenter coded response accuracy → inter-trial interval (550ms).

Subjects performed 20 practice trials prior to 150 test trials, where the words were drawn in random order from 5 categories: Gambling-Related (e.g., blackjack), Alcohol-Related (e.g., martini), Positive Affect (e.g., hopeful), Negative Affect (e.g., sad), and Neutral (e.g., lattice).

Stop-Signal Task (SST) (Logan et al. 1997)

The SST was administered during the interview screen and on each test session (post-RRT in Phase I; immediately after the slot machine in Phase II) to assess subjects’ motor speed and inhibitory control. Visual stimuli (‘a’ / ‘b’ [interview, test session 1, and 3] or ‘c’ / ‘d’ [test session 2 and 4]) appeared on the screen and subjects were instructed to press a corresponding key (‘z’ or ‘/’) in response to each letter as quickly as possible. On a random 25% of trials, an auditory tone (‘stop’ signal) sounded, which indicated for subjects to withhold their planned response and not press any keys. Each time, subjects performed two sets of practice trials in order to account for day-to-day variability.
**Game of Dice Task (GDT) (Brand et al. 2005)**

The GDT was administered during the interview and on every test session to assess reward sensitivity and risk-taking behaviours. The computer rolled a virtual die and subjects bet on the outcome of each roll by choosing from a single number or a combination of two, three, or four possible outcomes. Indicated beside each option, was the amount they were wagering (e.g. how much they would win if the rolled number was among the numbers selected, or how much they would lose if they were incorrect). Since betting on a single number was maximally risky (odds = 1/6), whereas betting on four possible numbers was minimally risky (odds = 4/6), bet size varied from $1000, $500, $200, or $100 for a single number, a combination of two, three and four numbers, respectively. Subjects started with $1000 and were instructed to play out all 18 rolls, even if they had a negative balance with the aim of maximizing their winnings.

**2.5 Procedure**

**2.5.1 Telephone Screening**

Subjects were recruited from across the Greater Toronto Area via classified advertisements posted online (e.g., Kijiji.ca) and in print (e.g., Metro, NOW Magazine) (Appendix C; Recruitment Ad for Pathological Gamblers). Potential candidates who called the CAMH study line were given a brief overview of study objective, requirement and procedure. They were informed about the study medications: Haldol, Prolixin and Dexedrine as well as possible side effects. If interested, a standard 20-minute telephone screen was administered to assess eligibility. It included questions covering demographics, health status, and current/past substance use, as well as SOGS, DSM-IV PG, BDI, ADS, EIS-Impulsivity and WAIS-Vocabulary Task (if English comprehension was borderline). If subjects were eligible based on the above measures, an interview screen was scheduled at CAMH.
2.5.2 Interview Screening

The interview screen began by having all potential subjects read and sign the informed consent form (Appendix D; Informed Consent Form), provided with ample opportunity to ask any outstanding questions they may have had. Then, subjects’ blood alcohol content (0 required), heart rate, blood pressure, height and weight were measured. A mid-stream urine pregnancy test was given to all female subjects. Subsequently, the experimenter administered the SCID to determine current and past psychiatric profile. Potential PG subjects underwent an additional screening by a study psychiatrist to verify PG status and severity (SOGS and DSM-IV PG questionnaire administered verbally), and gather a detailed history of the individual’s gambling habits. The psychiatrist interview was designed in part to identify and exclude individuals who falsely reported PG symptoms to enter the study simply for the sizeable compensation.

Eligible subjects then completed a written screening questionnaire package to confirm telephone screen response validity and gather more information. This included the SOGS, BDI, ADS, FTND, DAST, Alcohol and Nicotine Timeline Follow-Backs, EIS, EPI, and GBQ. The experimenter then administered the DSM-IV PG questionnaire, as well as the WAIS Digit Span, WAIS Digit Symbol-Coding, and WAIS Vocabulary tasks, and subjects proceeded to complete the WCST, SST and GDT computer tasks.

For the final portion of the interview session, subjects were escorted to the CAMH Clinical Laboratory to complete an ECG, blood and urine analyses. Blood samples (enough to fill 3 finger-length tubes) were drawn by a CAMH nurse. Urine samples were obtained to confirm lack of recent drug use, while ECGs were conducted to confirm the absence of any heart problems. Lab results were sent to the study’s Qualified Investigator for review, and a physician’s exam was scheduled.
2.5.3 Physician’s Exam

Eligible subjects underwent a standard physical examination by a nurse and doctor to gather a medical family history and further confirm suitability to receive the study medications (Appendix E; Physical Exam Inclusion/Exclusion Criteria). Both the physician conducting the physical exam, and the Qualified Investigator signed off on the prescriptions, and eligible subjects were scheduled to begin their test sessions.

2.5.4 Test Day Procedure

There were four test sessions with a minimum one-week washout period between each session. Test days started around 8:30 am. Upon subjects’ arrival, the experimenter measured their blood alcohol content (0 required), baseline blood pressure and heart rate. Subjects were provided with a standard breakfast and the first questionnaire package (VAS-Desire to Gamble/Drink Alcohol, POMS, and ARCI). Urine pregnancy tests were then collected from all female subjects, and a cigarette break was provided if requested. This was restricted to a single cigarette, and smoking was prohibited thereafter until completion of the test session.

The first dose of study medication was administered in the form of three capsules, each of which contained either 1-mg of HAL/FLU, or visually identical placebo. Subjects relaxed in a waiting room with a TV and magazines, until the antagonist reached expected peak concentration (2.75 hours or 2 hours for HAL or FLU, respectively). During the waiting period, blood pressure and heart rate were recorded every 30 minutes. A second questionnaire package was administered 15 minutes before the expected peak blood drug level was reached. The second dose was administered at expected peak antagonist concentration in the form of four visually identical capsules, each of which contained placebo (‘dummy’) in Phase I (to standardize potential expectancies related to capsule administration in Phase I vs. Phased II), or 5-mg of AMPH in Phase II. In Phase I, subjects proceeded directly onto the tasks at this point. However,
in Phase II, subjects waited another 90 minutes for AMPH to reach expected peak concentration. From this point on, a registered nurse was present and measured blood pressure and heart rate every 15 minutes in Phase II.

In Phase I, after consuming their four placebo capsules, subjects were escorted to a mock bar room where they played the slot machine for 15 minutes. Immediately after the gambling session, they completed the third questionnaire package. Subjects then proceeded to the RRT, followed by a fourth questionnaire package.

In Phase II, subjects first completed the RRT (at expected peak AMPH concentration), followed by the third questionnaire package. Afterwards, they played the slot machine for 15 minutes and completed the fourth questionnaire package in the mock-bar room. The RRT was administered after AMPH but before the slot machine in Phase II, in order to capture cognitive priming effects of AMPH.

Regardless of the order of completing the slot machine and RRT, subjects then proceeded to complete the remaining computer tasks: SST and GDT, followed by the final questionnaire package. This package included the Side Effect Checklist to summarize any adverse side effects felt throughout the day. The Capsule Content Evaluation was administered at the end of the second and fourth test sessions to allow subjects to indicate which doses they believed were active and which were placebos. Subsequently, subjects ate lunch and relaxed in the waiting room until a registered nurse confirmed that their blood pressure and heart rate met discharge criteria, and confirmed the absence of adverse effects. Subjects were provided with 50-mg diphenhydramine to take in case of delayed side effects, a wallet card stating the name of the study medication and emergency contact information and sent home by pre-paid taxi.
Table 2. Outline of test day procedure in Phase I and Phase II.

<table>
<thead>
<tr>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline physiological measurements (HR/BP, BAC)</td>
<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td></td>
</tr>
<tr>
<td>Package A (DTG/Drink Alcohol; POMS; ARCI)</td>
<td></td>
</tr>
<tr>
<td>Pregnancy test</td>
<td></td>
</tr>
<tr>
<td>Cigarette break</td>
<td></td>
</tr>
<tr>
<td>Dose 1 administered (HAL/FLU vs. placebo)</td>
<td></td>
</tr>
<tr>
<td>Waiting period: 2.75 hr (HAL); 2 hr (FLU), HR/BP measured every 30 min</td>
<td></td>
</tr>
<tr>
<td>Package B (DTG/Drink Alcohol; POMS; ARCI) – 15 min before expected peak</td>
<td></td>
</tr>
<tr>
<td>Dose 2 administered at expected peak concentration (dummy placebo in Phase I; AMPH in phase II)</td>
<td></td>
</tr>
<tr>
<td>15-min slot machine game</td>
<td>Waiting period: 90 min, HR/BP measured every 15 minutes</td>
</tr>
<tr>
<td>Package C (DTG/Drink Alcohol, VAS-Enjoyment/Excitement/Involvement/High of slot machine game; POMS; ARCI)</td>
<td>RRT Package C (DTG/Drink Alcohol, VAS-Liking/High/Good Effects/Take Again; POMS; ARCI)</td>
</tr>
<tr>
<td>RRT</td>
<td>15-min Slot machine game</td>
</tr>
<tr>
<td>Package D (DTG/Drink Alcohol; POMS; ARCI)</td>
<td>Package D (DTG/Drink Alcohol; POMS; ARCI)</td>
</tr>
<tr>
<td>SST</td>
<td></td>
</tr>
<tr>
<td>GDT</td>
<td></td>
</tr>
<tr>
<td>Package E (DTG/Drink Alcohol; POMS; ARCI; Side Effect Checklist)</td>
<td></td>
</tr>
<tr>
<td>Capsule Content Evaluation (on session 2 and 4)</td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td>Detoxification period</td>
<td></td>
</tr>
<tr>
<td>Discharge by registered nurse</td>
<td></td>
</tr>
</tbody>
</table>

HR, heart rate; BP, blood pressure; BAC, Blood Alcohol Content; DTG, Desire to Gamble; POMS, Profile of Mood States; ARCI, Addiction Research Center Inventory; HAL, Haloperidol; FLU, Fluphenazine; AMPH, Amphetamine; VAS, Visual Analog Scale; RRT, Rapid Reading Task; SST, Stop Signal Task; GDT, Game of Dice Task.

2.6 Data Analysis

All data analyses from this repeated measures between-within subjects’ design were performed using SPSS (v. 15, Chicago IL). As previously noted, only data from the PG subjects will be reported here.
2.6.1 Subject Characteristics

Background characteristics including age, gender, SOGS, and smoker status were analyzed with t-tests or chi-square as appropriate to identify potential differences in the profile of background characteristics between PG-HAL and PG-FLU groups. Other background characteristics, trait scores, and scores for measures of basic cognitive function (e.g., short term memory) were analyzed with multivariate analysis of variance (MANOVA).

2.6.2 Statistical Checks

SPSS EXPLORE analyses were conducted to confirm that EIS-Empathy did not deviate from normal and that there were no outliers or extreme scores (any score >1.5 box lengths away from the upper or lower edge). Composite VAS scores were computed for each phase by gathering the responses to multiple self-reported VAS measures of subjective Pleasurable Effects from the slot machine (sum of Enjoyment, Excitement, Involvement and High) and AMPH (sum of Liking, High, Good Effects and [Desire to] Take Again). Data were analyzed separately for Phase I and Phase II in order to identify the patterns of the response to slot machine game and AMPH, respectively.

Bivariate exploratory correlations assessed the relationship between EIS-Empathy and background characteristics to detect correlates of Empathy that could potentially account for correlations between Empathy and the dependent measures (i.e., mediators). Point bi-serial correlations were conducted for gender and smoker status.

2.6.3 Primary Analyses

Bivariate correlations were conducted between EIS-Empathy and each of the dependent variables to examine overall patterns of shared variance. The primary analyses employed partial correlations to control for extraneous factors that could influence responses to the reinforcers. In
Phase I, separate partial correlations for each antagonist group under each treatment (antagonist, placebo) controlled for factors that could influence affective-motivational responses to the slot machine game, including credits won and, where appropriate, pre-capsule baseline measures of the dependent variable for the respective test session. In Phase II, partial correlations controlled for baseline measures of the dependent variable where appropriate. Regression residuals were generated to depict the scatterplots of the partial correlations for the principal results.

2.6.4 Statistical Comparisons of Correlations

Comparison of $r$ values was performed using t tests for within-subjects’ comparisons (antagonist vs. placebo) in each antagonist group (Williams 1959); $z$ tests were performed for between-subjects’ comparisons (PG-HAL vs. PG-FLU) under each treatment (Fisher, 1921).
3. RESULTS

3.1 Subject Background Characteristics

In total, 60 subjects, 30 PGs (and 30 HCs) completed the study (Appendix F, Flow Chart of Subject Recruitment, Eligibility and Group Assignment). The results reported here come exclusively from PGs, who were matched on potential mediating and moderating factors and assigned to receive HAL (n = 15; 10 males) or FLU (n = 15; 11 males).

Table 3 displays a comparison of mean (range) background characteristics for both PG-HAL and PG-FLU antagonist groups, and confirms that the groups did not differ significantly on any of the key background variables (e.g., age, gender ratio, SOGS, etc.; p’s > 0.18). This ensured that the overall composition of the two antagonist groups was well-matched and consistent with respect to dispositional factors that could influence response to HAL, FLU or placebo. Similarity in range across antagonist groups and treatments helps to rule out bias in r scores due to range restriction.

Table 3. Mean (range) background characteristics for PG-HAL and PG-FLU antagonist groups.

<table>
<thead>
<tr>
<th></th>
<th>PG HAL</th>
<th>PG FLU</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Age</td>
<td>35.8 (22-53)</td>
<td>33.5 (19-49)</td>
</tr>
<tr>
<td>Gender (Male : Female)</td>
<td>10 : 5</td>
<td>11 : 4</td>
</tr>
<tr>
<td>Smokers : non-smokers</td>
<td>1 : 14</td>
<td>2 : 13</td>
</tr>
<tr>
<td>SOGS</td>
<td>11.1 (6-18)</td>
<td>11.7 (5-20)</td>
</tr>
<tr>
<td>DSM-IV PG</td>
<td>14.6 (5-26)</td>
<td>15.8 (6-26)</td>
</tr>
<tr>
<td>BDI</td>
<td>4.3 (0-9)</td>
<td>5.6 (0-13)</td>
</tr>
<tr>
<td>ADS</td>
<td>1.1 (0-4)</td>
<td>1.6 (0-11)</td>
</tr>
<tr>
<td>Drinks per week</td>
<td>2.3 (0-8)</td>
<td>1.5 (0-3)</td>
</tr>
<tr>
<td>DAST</td>
<td>0.2 (0-2)</td>
<td>0.4 (0-2)</td>
</tr>
</tbody>
</table>

PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine; SOGS, South Oaks Gambling Screen; DSM-IV PG, Diagnostic and Statistical Manual of Mental Disorders 4e. – pathological gambling; BDI, Beck Depression Inventory; ADS, Alcohol Dependence Scale; DAST, Drug Abuse Screening Test; p > 0.18.
Table 4 shows mean (range) personality traits for both PG-HAL and PG-FLU antagonist groups, and confirms that the groups also did not differ significantly on any of the measured personality traits assessed by the EPI and EIS ($p > 0.55$). This ensured that the two groups were similar in personality profiles that could influence response to HAL, FLU or placebo.

Table 4. Mean (range) scores on personality traits for PG-HAL and PG-FLU antagonist groups.

<table>
<thead>
<tr>
<th></th>
<th>PG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAL</td>
</tr>
<tr>
<td><strong>EPI - Extraversion</strong></td>
<td>13.5 (5-19)</td>
</tr>
<tr>
<td><strong>EPI - Neuroticism</strong></td>
<td>5.8 (0-13)</td>
</tr>
<tr>
<td><strong>EPI - Lie</strong></td>
<td>3.2 (1-6)</td>
</tr>
<tr>
<td><strong>EIS - Impulsiveness</strong></td>
<td>9.2 (2-18)</td>
</tr>
<tr>
<td><strong>EIS - Venturesomeness</strong></td>
<td>9.7 (3-15)</td>
</tr>
<tr>
<td><strong>EIS - Empathy</strong></td>
<td>12.1 (7-17)</td>
</tr>
</tbody>
</table>

*PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine; EPI, Eysenck Personality Inventory; EIS, Eysenck Impulsiveness Scale; $p > 0.55$.*

SPSS EXPLORE analyses were run for EIS-Empathy and each of the dependent variables to ensure there were no outliers or extreme scores, and that the range of scores was similar between antagonist groups, in order to ensure that range restriction did not influence the opportunity to detect correlations.

The distributions for EIS-Empathy in the PG-HAL and PG-FLU antagonist groups did not contain outliers or extreme scores, and had a very similar range (HAL – mean = 12.07, range 7 – 17 vs. FLU – mean = 12.53, range 6 - 16, respectively; Figure 1).
Figure 1. Boxplots illustrating the range of scores on the EIS-Empathy subscale for PG-HAL (top panel) and PG-FLU (bottom panel).

PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine.
Table 5 shows the inter-correlations between EIS-Empathy and background characteristics for PG-HAL and PG-FLU antagonist groups. In general, the correlations were modest and non-significant ($p > 0.11$). Application of Bonferroni correction to control for multiple exploratory tests, yielded a minimum $p > 0.99$. Therefore, Empathy appeared to be a distinct dimension. The lack of significant correlation with gender confirms that there was no relationship between gender and Empathy in either group. This ensured that gender did not contribute to individual differences in Empathy despite a prevailing assumption that women are more empathic than men.

Although not statistically significant, the moderate correlations between EIS-Empathy and smoker status in the PG-FLU group, and EIS-Empathy and EPI-Extroversion and EIS-Impulsiveness in the PG-HAL group were sufficiently large to warrant follow-up analyses with the key dependent variables to rule out these factors as potential mediators of the observed relationship with Empathy. None of the correlations between EIS-Empathy and smoker status, EPI-Extroversion, EIS-Impulsiveness or any of the other dependent variables were significant, $p > 0.19$. Collectively, the lack of significant inter-correlations or correlations with other trait factors ensures that the critical correlations - between Empathy and the dependent variables - are not attributable to factors aside from Empathy. That is, in this sample, the dimension tapped by EIS-Empathy is distinct from smoker status, Impulsiveness, Extroversion, and SOGS, the index used to classify PG severity, as well as the tendency to misrepresent self-report information (Lie scale), giving us confidence that the relationships reported reflect Empathy, and not another trait.
Table 5. Inter-correlation ($r$) of EIS-Empathy and background characteristics and personality traits for PG-HAL and PG-FLU antagonist groups.

<table>
<thead>
<tr>
<th></th>
<th>PG</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAL</td>
<td>FLU</td>
</tr>
<tr>
<td>Gender*</td>
<td>-0.06</td>
<td>0.19</td>
</tr>
<tr>
<td>Smoker Status*</td>
<td>0.10</td>
<td>0.38+</td>
</tr>
<tr>
<td>SOGS</td>
<td>0.24</td>
<td>0.00</td>
</tr>
<tr>
<td>DSM-IV PG</td>
<td>0.20</td>
<td>-0.25</td>
</tr>
<tr>
<td>BDI</td>
<td>0.05</td>
<td>-0.10</td>
</tr>
<tr>
<td>ADS</td>
<td>-0.20</td>
<td>-0.29</td>
</tr>
<tr>
<td>DAST</td>
<td>-0.01</td>
<td>-0.25</td>
</tr>
<tr>
<td>EPI - Extraversion</td>
<td>0.43+</td>
<td>-0.23</td>
</tr>
<tr>
<td>EPI - Neuroticism</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>EPI - Lie</td>
<td>-0.13</td>
<td>-0.28</td>
</tr>
<tr>
<td>EIS - Impulsiveness</td>
<td>0.36+</td>
<td>-0.04</td>
</tr>
<tr>
<td>EIS - Venturesomeness</td>
<td>0.15</td>
<td>-0.19</td>
</tr>
</tbody>
</table>

PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine; SOGS, South Oaks Gambling Screen; DSM-IV PG, Diagnostic and Statistical Manual of Mental Disorders 4e. – pathological gambling; BDI, Beck Depression Inventory; ADS, Alcohol Dependence Scale; DAST, Drug Abuse Screening Test; EPI, Eysenck Personality Inventory; EIS, Eysenck Impulsiveness Scale; p > 0.11.

* Values for gender and smoker status reflect the results of a point bi-serial correlation ($r_{pb}$); $p$’s > 0.15.

+ $r$ values for smoker status, EPI-Extraversion and EIS-Impulsiveness were further investigated; $p > 0.19.$

3.2 Dependent Variables and Transformation

VAS Composite Pleasurable Effects and ARCI-AMP assessed the subjective pleasurable and stimulant properties (i.e., Liking) of the slot machine and AMPH. DTG indexed the incentive motivation (i.e., Wanting) of the slot machine and AMPH.

3.2.1 Phase I

Table 6 shows the mean (range) for each of the dependent variables, along with the random outcome variable, Winnings (credits remaining at the end of the slot machine game under each treatment in Phase I). Winnings were included in the table as they were used in all Phase I partial correlations. The overall magnitude of scores on these dependent variables was very similar in the two antagonist groups. Furthermore, inspection of the range for each score
under placebo and antagonist in each group confirms the absence of large differences within groups under the two treatments, or between groups under either treatment. This helps to rule out range restriction as an influence on the magnitude of the \( r \) values with Empathy.

Table 6. Mean (range) dependent variables of gambling for PG-HAL and PG-FLU in Phase I.

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Placebo</th>
<th>Antagonist</th>
<th>Placebo</th>
<th>Antagonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS Composite</td>
<td>28.0 (0-40)</td>
<td>25.7 (2-37)</td>
<td>27.7 (14-40)</td>
<td>27.2 (9.5-40)</td>
</tr>
<tr>
<td>Enjoyment</td>
<td>7.0 (0-10)</td>
<td>6.4 (0-10)</td>
<td>7.5 (3-10)</td>
<td>7.1 (3-10)</td>
</tr>
<tr>
<td>Excitement</td>
<td>7.3 (0-10)</td>
<td>6.7 (2-10)</td>
<td>7.1 (3-10)</td>
<td>7.1 (3-10)</td>
</tr>
<tr>
<td>Involvement</td>
<td>7.6 (0-10)</td>
<td>6.7 (0-10)</td>
<td>7.3 (4-10)</td>
<td>7.0 (3-10)</td>
</tr>
<tr>
<td>High</td>
<td>6.1 (0-10)</td>
<td>6.0 (0-8)</td>
<td>5.8 (0.5-10)</td>
<td>6.0 (0.5-10)</td>
</tr>
<tr>
<td>ARCI-AMP 1</td>
<td>2.1 (0-6)</td>
<td>2.1 (0-5)</td>
<td>3.7 (0-8)</td>
<td>4.2 (0-9)</td>
</tr>
<tr>
<td>ARCI-AMP 2</td>
<td>1.6 (0-5)</td>
<td>1.9 (0-4)</td>
<td>3.4 (0-9)</td>
<td>4.5 (0-11)</td>
</tr>
<tr>
<td>ARCI-AMP 3</td>
<td>3.3 (0-9)</td>
<td>2.3 (0-8)</td>
<td>3.6 (0-8)</td>
<td>5.0 (0-10)</td>
</tr>
<tr>
<td>DTG 1</td>
<td>4.3 (0-8)</td>
<td>4.0 (0-10)</td>
<td>4.2 (0-10)</td>
<td>4.9 (0-10)</td>
</tr>
<tr>
<td>DTG 2</td>
<td>3.9 (0-8)</td>
<td>4.2 (0-7)</td>
<td>4.1 (0-9)</td>
<td>3.8 (0-8)</td>
</tr>
<tr>
<td>DTG 3</td>
<td>6.6 (2-10)</td>
<td>6.1 (2-8)</td>
<td>6.6 (0-10)</td>
<td>6.1 (0-10)</td>
</tr>
<tr>
<td>Winnings*</td>
<td>562.4 (0-2380)</td>
<td>239.1 (0-1159)</td>
<td>140.9 (0-1266)</td>
<td>277.8 (0-810)</td>
</tr>
</tbody>
</table>

PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine; Enjoyment, Excitement, Involvement and High are all subscales of Visual Analog Scale (VAS) Pleasurable Effects of the slot machine that were averaged to create a VAS Composite Pleasurable Effects score; ARCI-AMP, stimulant (amphetamine-like) effects from the Addiction Research Center Inventory; DTG, Desire to Gamble. Time points 1, 2, and 3 represent baseline, expected peak blood levels of the antagonist (2.75 hours for HAL/2 hours for FLU), and post-slot machine game, respectively.

* Winnings = random outcome variable that was controlled for in Phase I partial correlations.

The inter-correlations among the individual VAS subscales (Enjoyment, Excitement, Involvement, and perceived ‘High’) assessing Pleasurable Effects of the slot machine was \( r_{\text{mean}} = 0.84 \) (0.68 \( \leq r \leq 0.95 \)) in PG-HAL and \( r_{\text{mean}} = 0.91 \) (0.86 \( \leq r \leq 0.97 \)) in PG-FLU, with \( p < 0.01 \) (Table 7). Therefore, in both antagonist groups, these individual scales tapped a common underlying construct. The sum of these individual scales (0-10) was combined to create a
The composite VAS score (0-40). The composite helps to minimize the occurrence of Type I errors that can increase when correlations are conducted on multiple scales that are largely overlapping. The composite VAS score is also beneficial as it retains variance from each of its components and provides a reliable, parsimonious index of the target construct (i.e., Pleasurable Effects of gambling) (Murphy and Davidshofer 1994).

Table 7. Inter-correlation (r) of individual VAS subscales of Pleasurable Effects of the slot machine in Phase I for PG-HAL (top panel) and PG-FLU (bottom panel).

<table>
<thead>
<tr>
<th></th>
<th>Enjoyment</th>
<th>Excitement</th>
<th>Involvement</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enjoyment</td>
<td>0.95**</td>
<td>0.79**</td>
<td>0.68**</td>
<td></td>
</tr>
<tr>
<td>Excitement</td>
<td>0.95**</td>
<td>0.89**</td>
<td>0.78**</td>
<td></td>
</tr>
<tr>
<td>Involvement</td>
<td>0.79**</td>
<td>0.89**</td>
<td>0.93**</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>0.68**</td>
<td>0.78**</td>
<td>0.93**</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Enjoyment</th>
<th>Excitement</th>
<th>Involvement</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enjoyment</td>
<td>0.97**</td>
<td>0.95**</td>
<td>0.86**</td>
<td></td>
</tr>
<tr>
<td>Excitement</td>
<td>0.97**</td>
<td>0.97**</td>
<td>0.86**</td>
<td></td>
</tr>
<tr>
<td>Involvement</td>
<td>0.95**</td>
<td>0.97**</td>
<td>0.88**</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>0.86**</td>
<td>0.86**</td>
<td>0.88**</td>
<td></td>
</tr>
</tbody>
</table>

** PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine. **\(p < 0.01\).

ARCI-AMP measured the current experience of stimulant-like effects at key time points: pre-capsule baseline, expected peak blood levels of the antagonist (2.75 hours for HAL/2 hours for FLU) immediately before the slot machine game, and immediately post-slot game.

DTG was reported at pre-capsule baseline, expected peak antagonist effects (immediately before the slot machine game in Phase I), and immediately after the slot machine.

Winnings (final credit tally or number of credits won on each session) were used in partial correlations to control for random variation in reward magnitude across subjects and
treatment conditions in Phase I.

3.2.2 Phase II

Table 8 shows the mean (range) for each of the dependent variables in Phase II. As was the case in Phase I, Table 8 shows that the overall magnitude of scores on these dependent variables was very similar in the two antagonist groups and the similar range for each score under placebo vs. antagonist in each group helps to rule out range restriction as an influence on the $r$ values with Empathy. Winnings was not included in the table since the slot machine game was played after expected peak AMPH effects and was purely exploratory in Phase II.
Table 8. Mean (range) dependent variables of AMPH for PG-HAL and PG-FLU in Phase II.

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>HAL Placebo</th>
<th>HAL Antagonist</th>
<th>FLU Placebo</th>
<th>FLU Antagonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS Composite</td>
<td>13.3 (-3.5-40)</td>
<td>13.6 (-6-40)</td>
<td>22.7 (-10-40)</td>
<td>19.2 (0-40)</td>
</tr>
<tr>
<td>Liking</td>
<td>1.5 (-7-10)</td>
<td>2.0 (-9-10)</td>
<td>4.4 (-10-10)</td>
<td>4.1 (-2-10)</td>
</tr>
<tr>
<td>High</td>
<td>4.4 (0-10)</td>
<td>4.1 (0-10)</td>
<td>6.2 (0-10)</td>
<td>5.2 (0-10)</td>
</tr>
<tr>
<td>Good Effects</td>
<td>4.2 (0-10)</td>
<td>4.0 (0-10)</td>
<td>6.6 (0-10)</td>
<td>5.3 (0-10)</td>
</tr>
<tr>
<td>Take Again</td>
<td>3.1 (0-10)</td>
<td>3.6 (0-10)</td>
<td>5.5 (0-10)</td>
<td>4.6 (0-10)</td>
</tr>
<tr>
<td>ARCI-AMP 1</td>
<td>1.7 (0-5)</td>
<td>1.3 (0-5)</td>
<td>3.3 (0-9)</td>
<td>2.8 (0-9)</td>
</tr>
<tr>
<td>ARCI-AMP 2</td>
<td>1.2 (0-5)</td>
<td>1.1 (0-3)</td>
<td>3.2 (0-10)</td>
<td>2.9 (0-9)</td>
</tr>
<tr>
<td>ARCI-AMP 3</td>
<td>2.4 (0-8)</td>
<td>2.2 (0-8)</td>
<td>5.6 (1-10)</td>
<td>4.9 (0-10)</td>
</tr>
<tr>
<td>DTG 1</td>
<td>3.6 (0-9)</td>
<td>3.9 (0-8)</td>
<td>3.7 (0-8)</td>
<td>3.6 (0-8)</td>
</tr>
<tr>
<td>DTG 2</td>
<td>3.8 (0-8)</td>
<td>3.9 (0-8)</td>
<td>3.9 (0-8)</td>
<td>3.5 (0-7)</td>
</tr>
<tr>
<td>DTG 3</td>
<td>5.5 (0-10)</td>
<td>5.4 (1-10)</td>
<td>5.5 (0-10)</td>
<td>5.1 (0-10)</td>
</tr>
</tbody>
</table>

PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine; Liking, High, Good Effects and Take Again are all subscales of Visual Analog Scale (VAS) Pleasurable Effects of AMPH that were averaged to create a VAS Composite Pleasurable Effects score; ARCI-AMP = stimulant (amphetamine-like) effects from the Addiction Research Center Inventory; DTG = Desire to Gamble. Time points 1, 2, and 3 represent baseline, expected peak blood levels of the antagonist (2.75 hours for HAL/2 hours for FLU), and expected peak AMPH, respectively.

The inter-correlations among the individual VAS subscales (Liking, High, Good Effects, and Take Again) assessing Pleasurable Effects of AMPH were $r_{\text{mean}} = 0.89$ ($0.76 \leq r \leq 0.96$) in PG-HAL and $r_{\text{mean}} = 0.80$ ($0.69 \leq r \leq 0.92$) in PG-FLU (Table 9). Therefore, these individual scales tapped a common underlying construct in both antagonist groups, similar to Phase I. The sum of these scales was computed to create a composite VAS score as a reliable index of the Pleasurable Effects of AMPH.
Table 9. Inter-correlation ($r$) of individual VAS subscales of Pleasurable Effects of AMPH in Phase II for PG-HAL (top panel) and PG-FLU (bottom panel).

<table>
<thead>
<tr>
<th></th>
<th>Liking</th>
<th>High</th>
<th>Good Effects</th>
<th>Take Again</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liking</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High</strong></td>
<td>0.76**</td>
<td></td>
<td>0.89**</td>
<td>0.87**</td>
</tr>
<tr>
<td><strong>Good Effects</strong></td>
<td>0.89**</td>
<td>0.96**</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Take Again</strong></td>
<td>0.70**</td>
<td>0.91**</td>
<td>0.96**</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Liking</th>
<th>High</th>
<th>Good Effects</th>
<th>Take Again</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liking</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High</strong></td>
<td>0.69**</td>
<td></td>
<td>0.83**</td>
<td>0.92**</td>
</tr>
<tr>
<td><strong>Good Effects</strong></td>
<td>0.83**</td>
<td>0.65**</td>
<td></td>
<td>0.86**</td>
</tr>
<tr>
<td><strong>Take Again</strong></td>
<td>0.92**</td>
<td>0.86**</td>
<td>0.83**</td>
<td></td>
</tr>
</tbody>
</table>

**PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine.**

** $p < 0.01$.

In Phase II, the ARCI-AMP again measured stimulant-like effects, reported at pre-capsule baseline, as well as at expected peak blood levels of the antagonist alone, and then again at expected peak blood levels of AMPH (90 minutes after expected peak antagonist effects).

DTG was similarly reported at pre-capsule baseline, expected peak antagonist effects, and again at expected peak AMPH effects on each session.

3.3. Sequential Correlational Analysis of Empathy-related Effects

3.3.1 Bivariate Correlations

3.3.1.1 Phase I

Table 10 reports the zero-order bivariate correlations for EIS-Empathy and the dependent variables in Phase I, to convey the overall pattern of associations before controlling for potential mediators and moderators.
Table 10. Bivariate correlations ($r$) for EIS-Empathy and the dependent variables for PG-HAL (top panel) and PG-FLU (bottom panel) in Phase I.

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Treatment</th>
<th>VAS Composite</th>
<th>ARCI-AMP</th>
<th>DTG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.45</td>
<td>0.44</td>
<td>0.60*</td>
<td></td>
</tr>
<tr>
<td>Expected Peak Antagonist</td>
<td>N/A</td>
<td>0.19</td>
<td>0.56*</td>
<td></td>
</tr>
<tr>
<td>Post-Slot Machine</td>
<td>N/A</td>
<td>0.49</td>
<td>0.57*</td>
<td></td>
</tr>
<tr>
<td><strong>Antagonist</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.04</td>
<td>0.37</td>
<td>0.58*</td>
<td></td>
</tr>
<tr>
<td>Expected Peak Antagonist</td>
<td>N/A</td>
<td>0.05</td>
<td>0.58*</td>
<td></td>
</tr>
<tr>
<td>Post-Slot Machine</td>
<td>N/A</td>
<td>0.04</td>
<td>0.42</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Treatment</th>
<th>VAS Composite</th>
<th>ARCI-AMP</th>
<th>DTG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>N/A</td>
<td>-0.26</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Expected Peak Antagonist</td>
<td>N/A</td>
<td>0.06</td>
<td>0.71**</td>
<td></td>
</tr>
<tr>
<td>Post-Slot Machine</td>
<td>0.39</td>
<td>-0.10</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td><strong>Antagonist</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>N/A</td>
<td>0.01</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Expected Peak Antagonist</td>
<td>N/A</td>
<td>0.07</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Post-Slot Machine</td>
<td>0.64*</td>
<td>0.21</td>
<td>0.42</td>
<td></td>
</tr>
</tbody>
</table>

EIS, Eysenck Impulsiveness Scale; PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine; VAS Composite, Visual Analog Scale Composite Pleasurable Effects of the slot machine; ARCI-AMP, stimulant (amphetamine-like) effects from the Addiction Research Center Inventory; DTG, Desire to Gamble.

* $p < 0.05$.
** $p < 0.01$.

### 3.3.1.1 HAL

**VAS Composite Pleasurable Effects**

The bivariate correlation between EIS-Empathy and VAS Composite Pleasurable Effects was moderate under placebo $r = 0.45$, and negligible under HAL, $r = 0.04$.

**ARCI-AMP**

The bivariate correlation between EIS-Empathy and ARCI-AMP was
weak to moderate under placebo \((0.19 \leq r \leq 0.49)\) and HAL \((0.04 \leq r \leq 0.37)\).

**DTG**  The bivariate correlation between EIS-Empathy and DTG was highly consistent and significant at pre-capsule baseline, expected peak effects of the antagonist (2-2.75 hours later) pre-slot machine, and again immediately after the slot machine \((0.56 \leq r \leq 0.60, p^{'s} < 0.05)\) under placebo (encased within the box; Table 10, top panel). Therefore, in the absence of any drug, greater Empathy reliably coincided with greater DTG before, as well as after, playing the slot machine. The bivariate correlation between EIS-Empathy and DTG was also moderate under HAL \((0.42 \leq r \leq 0.58)\).

### 3.3.1.1.2 FLU

**VAS Composite Pleasurable Effects**  The bivariate correlation between EIS-Empathy and VAS Composite Pleasurable Effects was moderate under placebo \(r = 0.39\) and strong under FLU, \(r = 0.64\).

**ARCI-AMP**  No consistent correlation was observed between EIS-Empathy and ARCI-AMP under placebo \((-0.26 \leq r \leq 0.06)\) or FLU \((0.01 \leq r \leq 0.21)\).

**DTG**  Similar to the HAL group, there was a consistent correlation between EIS-Empathy and DTG at pre-capsule baseline, expected peak effects of the antagonist, and again after the slot machine \((0.43 \leq r \leq 0.71)\); however the correlation was only significant at peak FLU \((p < 0.01; \text{encased within the box}; \text{Table 10, bottom panel})\). Emergence of this pattern *in both HAL and FLU groups* suggests a reliable pre-existing relationship between Empathy and motivation to gamble that was not merely induced by the study manipulations. The bivariate correlation between EIS-Empathy and DTG was also moderate under FLU \((0.35 \leq r \leq 0.46)\).

### 3.3.1.2 Phase II

Table 11 reports the zero-order bivariate correlations for EIS-Empathy and the dependent
variables in Phase II, to convey the overall pattern of associations before controlling for potential mediators and moderators.

Table 11. Bivariate correlations ($r$) for EIS-Empathy and the dependent variables for PG-HAL (top panel) and PG-FLU (bottom panel) in Phase II.

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Treatment</th>
<th>VAS Composite</th>
<th>ARCI-AMP</th>
<th>DTG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td>Baseline</td>
<td>N/A</td>
<td>0.01</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Expected Peak Antagonist</td>
<td>N/A</td>
<td>0.31</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Expected Peak AMPH</td>
<td>-0.13</td>
<td>0.08</td>
<td>0.56*</td>
</tr>
<tr>
<td><strong>Antagonist</strong></td>
<td>Baseline</td>
<td>N/A</td>
<td>0.01</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Expected Peak Antagonist</td>
<td>N/A</td>
<td>-0.22</td>
<td>0.52*</td>
</tr>
<tr>
<td></td>
<td>Expected Peak AMPH</td>
<td>0.22</td>
<td>0.33</td>
<td>0.63*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Treatment</th>
<th>VAS Composite</th>
<th>ARCI-AMP</th>
<th>DTG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td>Baseline</td>
<td>N/A</td>
<td>0.17</td>
<td>0.57*</td>
</tr>
<tr>
<td></td>
<td>Expected Peak Antagonist</td>
<td>N/A</td>
<td>0.26</td>
<td>0.65**</td>
</tr>
<tr>
<td></td>
<td>Expected Peak AMPH</td>
<td>0.39</td>
<td>0.26</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Antagonist</strong></td>
<td>Baseline</td>
<td>N/A</td>
<td>0.15</td>
<td>0.55*</td>
</tr>
<tr>
<td></td>
<td>Expected Peak Antagonist</td>
<td>N/A</td>
<td>0.09</td>
<td>0.62*</td>
</tr>
<tr>
<td></td>
<td>Expected Peak AMPH</td>
<td>-0.07</td>
<td>0.03</td>
<td>0.57*</td>
</tr>
</tbody>
</table>

EIS, Eysenck Impulsiveness Scale; PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine; VAS Composite, Visual Analog Scale Composite Pleasurable Effects of AMPH; ARCI-AMP, stimulant (amphetamine-like) effects from the Addiction Research Center Inventory; DTG, Desire to Gamble.

* $p < 0.05$.
** $p < 0.01$.

3.3.1.2.1 HAL

**VAS Composite Pleasurable Effects**

The bivariate correlation between EIS-
Empathy and VAS Composite Pleasurable Effects was very weak under placebo ($r = -0.13$) and modest under HAL ($r = 0.22$).

**ARCI-AMP**  No consistent correlation was observed between EIS-Empathy and ARCI-AMP under placebo ($0.01 \leq r \leq 0.31$) or HAL ($-0.22 \leq r \leq 0.33$).

**DTG**  The bivariate correlation between EIS-Empathy and DTG (encased within the box; Table 11, top panel) was moderate and consistent under placebo (i.e., AMPH, without any antagonist pre-treatment) ($0.41 \leq r \leq 0.56$), as well as under HAL ($0.32 \leq r \leq 0.63$).

### 3.3.1.2.2 FLU

**VAS Composite Pleasurable Effects**  The bivariate correlation between EIS-Empathy and VAS Composite Pleasurable Effects was modest under placebo ($r = 0.39$) and weak under FLU ($r = -0.07$).

**ARCI-AMP**  The bivariate correlation between EIS-Empathy and ARCI-AMP was modest under placebo, ($0.17 \leq r \leq 0.26$) and minimal under FLU ($0.03 \leq r \leq 0.15$).

**DTG**  The bivariate correlation between EIS-Empathy and DTG (encased within the box; Table 11, bottom panel) was moderate under placebo ($0.57 \leq r \leq 0.65$), as well as under FLU ($0.55 \leq r \leq 0.62$).

### 3.3.2 Partial Correlations

When variance due to baseline ratings and winnings on the slot machine was removed by partial correlation, inspection of scores for the placebo session revealed that Empathy continued to correlate with VAS Composite Pleasurable Effects of the slot machine in Phase I and with DTG at expected peak AMPH effects in Phase II. The partial $r$ scores for the variables under each treatment in the two antagonist groups are described below. Correlations for all other variables under placebo were non-significant, $p > 0.28$. 
3.3.2.1 Phase I

3.3.2.1.1 HAL

**VAS Composite Pleasurable Effects**  
Figure 2 (top panel) shows the scatterplot of the partial correlation between EIS-Empathy and VAS Composite Pleasurable Effects of the slot machine under placebo (in terms of regression residuals) and reveals a clear upward trend in ratings with increasing Empathy under placebo ($r = 0.50$). Figure 2 (bottom panel) shows the corresponding scatterplot for the same subjects under HAL. The antagonist completely negated the relationship between Empathy and the pleasurable effects of the game ($r = 0.02$).
Figure 2. Scatterplot of the correlation ($r$) between EIS-Empathy and VAS Composite Pleasurable Effects following a 15-minute slot machine session in the PG-HAL group under placebo (top panel) and antagonist (bottom panel) treatments. Scores shown are regression residuals, which exclude variance due to winnings on the slot machine under each of the respective treatments.

EIS, Eysenck Impulsiveness Scale; PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine; VAS Composite, Visual Analog Scale Composite Pleasurable Effects of the slot machine.
3.3.2.1.2 FLU

VAS Composite Pleasurable Effects  

Figure 3 (top panel) shows the scatterplot of the partial correlation between EIS-Empathy and VAS Composite Pleasurable Effects under placebo and reveals a similar, although somewhat more modest upward trend in ratings with increasing Empathy under placebo in the PG-FLU group ($r = 0.38$). Figure 3 (bottom panel) shows the corresponding scatterplot for these subjects under the antagonist, and shows that in contrast to HAL, FLU enhanced the relationship between Empathy and the pleasurable effects of the game ($r = 0.48$).
Figure 3. Scatterplot of the correlation ($r$) between EIS-Empathy and VAS Composite Pleasurable Effects following a 15-minute slot machine session in the PG-FLU group under placebo (top panel) and antagonist (bottom panel) treatments. Scores shown are regression residuals, which exclude variance due to winnings on the slot machine under each of the respective treatments.

EIS, Eysenck Impulsiveness Scale; PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine; VAS Composite, Visual Analog Scale Composite Pleasurable Effects of the slot machine.
3.3.2.2 Phase II

3.3.2.2.1 HAL

DTG Figure 4 (top panel) shows the scatterplot of the partial correlation between EIS-Empathy and DTG at expected peak AMPH effects under placebo and reveals a clear upward trend in ratings with increasing Empathy under placebo ($r = 0.48$). Figure 4 (bottom panel) shows the corresponding scatterplot for these subjects under the antagonist and shows that HAL enhanced the relationship between Empathy and DTG ($r = 0.59$).
Figure 4. Scatterplot of the correlation ($r$) between EIS-Empathy and Desire to Gamble following a low dose of d-Amphetamine (AMPH, 20-mg) in the PG-HAL group under placebo (top panel) and antagonist (bottom panel) treatments. Scores shown are regression residuals, which exclude variance due to baseline DTG scores under each of the respective treatments.

EIS, Eysenck Impulsiveness Scale; PG, Pathological Gambler; HAL; Haloperidol; FLU, Fluphenazine; DTG, Desire to Gamble.
3.3.2.2.2 FLU

**DTG** Figure 5 (top panel) shows the scatterplot of the partial correlation between EIS-Empathy and DTG at expected peak AMPH effects under placebo in PG-FLU subjects and reveals a similar, but somewhat more modest increasing pattern of ratings with increasing Empathy as was seen in the PG-HAL group ($r = 0.34$). Figure 5 (bottom panel) shows the corresponding scatterplot for these subjects under the antagonist and shows that FLU considerably attenuated the relationship between Empathy and expected peak AMPH DTG ratings ($r = 0.20$).
Figure 5. Scatterplot of the correlation ($r$) between EIS-Empathy and Desire to Gamble following a low dose of d-Amphetamine (AMPH, 20-mg) in the PG-FLU group under placebo (top panel) and antagonist (bottom panel) treatments. Scores shown are regression residuals, which exclude variance due to baseline DTG scores under each of the respective treatments.

EIS, Eysenck Impulsiveness Scale; PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine; DTG, Desire to Gamble.
3.3.3 Within-Subject and Between-Group Comparisons

Further statistical tests were conducted in order to determine whether the observed changes in $r$ values were statistically significant across treatment conditions and between groups.

Table 12 shows the correlation coefficients ($r$ values) and the effect size or percent change in variance ($\%\Delta s^2$) explained by the relationship between EIS-Empathy and the dependent variable under the two treatments (placebo/antagonist) in each antagonist group, as assessed by $t$ tests. Table 13 reports the between-group differences in strength of the correlation with EIS-Empathy under each treatment, as assessed by $z$ tests.

Considering the effects of treatment, the strength of the relationship between Empathy and VAS Composite Pleasurable Effects of the slot machine in Phase I decreased significantly under HAL vs. placebo ($\%\Delta s^2 = 1.00; p < 0.001$). The increase in the relationship under FLU vs. placebo ($\%\Delta s^2 = 0.24$) was not statistically significant. In Phase II, the increase in the strength of the relationship between Empathy and DTG at expected peak AMPH effects under HAL vs. placebo ($\%\Delta s^2 = 0.21$) was not significant; nor was the decrease in the strength of this relationship under FLU vs. placebo ($\%\Delta s^2 = 0.49$). According to Cohen (1988), effect size for HAL in Phase I would be considered very large, while the effect size for FLU in Phase II would be considered medium.

Inspection of Table 13 shows that, under placebo, the $r$ scores did not differ appreciably between PG-HAL and PG-FLU in Phase I or Phase II, $p$’s > 0.25. The $r$ scores for the two groups differed considerably under the active antagonist in Phase I ($\%\Delta s^2 = 1.00$) and Phase II ($\%\Delta s^2 = 0.80$), corresponding to very large and large effect sizes, respectively (Cohen 1988). However, with the reduced power of a between-groups test (relative to a within-groups test), these differences did not reach significance ($p \approx 0.10$).
Table 12. Within-subjects’ statistical comparisons (t tests) of correlations (r) with EIS-Empathy for Phase I VAS Composite Pleasurable Effects (top panel) and Phase II DTG (bottom panel) separated by antagonist group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Phase I - Slots</th>
<th></th>
<th>Phase II - Amphetamine</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Haloperidol Group</strong></td>
<td></td>
<td><strong>Haloperidol Group</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Variable:</strong></td>
<td><strong>VAS Composite</strong></td>
<td></td>
<td><strong>Variable:</strong></td>
<td><strong>Desire to Gamble</strong>*</td>
</tr>
<tr>
<td><strong>Treatment:</strong></td>
<td><strong>Placebo</strong></td>
<td></td>
<td><strong>Treatment:</strong></td>
<td><strong>Placebo</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Drug</strong></td>
<td></td>
<td></td>
<td><strong>Drug</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Placebo</strong></td>
<td></td>
<td></td>
<td><strong>Placebo</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Drug</strong></td>
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<td><strong>Drug</strong></td>
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<td><strong>0.24</strong></td>
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<td></td>
<td><strong>0.49</strong></td>
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* Partial correlation of VAS composite controls for Winnings on the slot machine under each treatment.

** %Δ s^2 = (r_{placebo} - r_{antagonist}) / (r_{placebo} + r_{antagonist}) = percent change in variance explained under placebo vs. antagonist.

*** Partial correlation of DTG controls for session baseline DTG ratings under each treatment.

EIS, Eysenck Impulsiveness Scale; VAS Composite, Visual Analog Scale Composite Pleasurable Effects; DTG, Desire to Gamble.
Table 13. Between-groups’ comparisons (z tests) of correlations ($r$) with EIS-Empathy for Phase I VAS Composite Pleasurable Effects (top panel) and Phase II DTG (bottom panel) separated by treatment.

<table>
<thead>
<tr>
<th>PHASE I - Slots</th>
<th>Treatment: PLACEBO</th>
<th>Variable: VAS Composite *</th>
<th>Drug Group:</th>
<th>$z$</th>
<th>$p$</th>
<th>%Δ $s^2$ **</th>
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<td>FLU</td>
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<th>Variable: VAS Composite</th>
<th>Drug Group:</th>
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<th>%Δ $s^2$</th>
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<tr>
<th>PHASE II – Amphetamine</th>
<th>Treatment: PLACEBO</th>
<th>Variable: Desire to Gamble ***</th>
<th>Drug Group:</th>
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<th>$p$</th>
<th>%Δ $s^2$</th>
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EIS, Eysenck Impulsiveness Scale; PG, Pathological Gambler; HAL, Haloperidol; FLU, Fluphenazine; VAS Composite, Visual Analog Scale Composite Pleasurable Effects; DTG, Desire to Gamble.

* Partial correlation of VAS composite controls for Winnings on the slot machine under each treatment.

** %Δ $s^2 = (r_{placebo} - r_{antagonist}) / (r_{placebo} + r_{antagonist})$ = percent difference in variance explained under HAL vs. FLU.

*** Partial correlation of DTG controls for session baseline DTG ratings under each treatment.
3.4 Summary of Effects

After controlling for potential mediating factors, moderately strong partial correlations emerged between Empathy and VAS Composite Pleasurable Effects of the slot machine in Phase I and between Empathy and DTG in Phase II in both groups.

Relative to placebo, HAL and FLU had opposite effects on the strength of these correlations in each phase, consistent with the differential binding profile of the antagonists. In addition, within each antagonist group, the directional effect of the antagonist (increase, decrease) on the correlation between Empathy and the reported effects of the reinforcer was opposite for the pleasurable effects of the slot machine and the incentive motivational effects of AMPH. The magnitude of the treatment effect (for antagonist vs. placebo) in terms of the change in variance accounted for was greater for HAL than FLU in Phase I, but greater for FLU in Phase II. Effect sizes for HAL in Phase I and FLU in Phase II would be characterized as very large and medium respectively (Cohen 1988).
4. DISCUSSION

This study set out to assess the relationship between Empathy and the reinforcing properties of gambling and a psychostimulant drug in PGs, and to clarify the roles of D1 and D2 in mediating the observed relationships. Relative to other personality dimensions, Empathy has received less attention as a risk or protective factor for addiction. In the case of a behavioural addiction like PG, we reasoned that sensitivity to and interpretation of motivationally relevant environmental signals may be especially important. This implied a potential role for Empathy. A review of the literature revealed that Empathy was linked with significantly larger Error Related Negativity (ERN) signals – an electrophysiological index of error processing, where larger values denote greater sensitivity to errors – on a visual attention task in healthy male volunteers (Santesso and Segalowitz 2009). In contrast, diminished ERN amplitudes have been reported in individuals high in Psychopathy (i.e. low Empathy) (Munro et al. 2007). The positive correlation between ERN and Empathy therefore indicates that highly empathic individuals are sensitive to feedback signaling negative outcomes.

4.1 Error Processing, DA Transmission and DA Receptor Function

Lower tonic DA transmission has been found to predict greater learning from negative vs. positive feedback in healthy volunteers (Slagter et al. 2015). Negative feedback signals (which encourage discontinuation of behaviour) appear to be primarily processed by D2 receptors, whereas positive feedback signals (which encourage continuation of behaviour) are primarily processed by D1 receptors (Frank and O’Reilly 2006), suggesting that highly empathic individuals’ heightened sensitivity to errors may be partly mediated by sensitive D2 receptors.

As noted earlier, D2 receptors have high affinity for DA, are located within the synapse, and preferentially respond to tonic DA signals, whereas low affinity, extra-synaptic D1 receptors are more sensitive to high intensity phasic (burst) DA signals (Schultz et al. 1998, Dreyer et al.
In terms of the downstream effects of these receptors, stimulation of D2 receptors primarily inhibits adenylyl cyclase activity, whereas D1 receptors primarily enhance it. In addition, D2 receptors exist as both auto-receptors and hetero-receptors. Activation of auto-receptors reduces pre-synaptic DA release, whereas activation of post-synaptic D2 hetero-receptors (in the striatum) can increase downstream DA transmission by inhibiting GABA neurons that tonically suppress mesolimbic DA neurons in the VTA.

Given their differential impact on DA transmission, increased sensitivity of post-synaptic D2 receptors will likely have different subjective-behavioural effects than increased sensitivity of D2 auto-receptors. Therefore, whether Empathy is primarily associated with increased sensitivity of post-synaptic vs. pre-synaptic D2 receptors is an important question. Of the two receptor populations, post-synaptic D2 appears to be more critical for learning from emotionally salient events, such that genetic deletion of post-synaptic D2 receptors greatly reduces electric shock-induced place aversion (but also morphine induced place preference) in rodents (Smith et al. 2002). In a heroin-dependent sample, lower growth hormone response and lack of prolactin suppression following pharmacological challenge with the direct D2 agonist, bromocriptine, has been found in patients with antisocial personality disorder (i.e., low Empathy), but not in those without antisocial personality disorder (Gerra et al. 2003). These neuroendocrine responses are mediated by (low) post-synaptic D2 receptors. Extrapolating that individuals high in antisocial tendencies have low trait Empathy, we could infer that individuals at the other end of this personality dimension (i.e., those with high Empathy) might show an opposite pattern of receptor sensitivity; i.e., they may express sensitive or abundant post-synaptic D2 receptors.

The literature raised the possibility that Empathy is associated with highly sensitive D2 receptors. Whether this relationship extended to individuals with PG was unknown. In addition, whether this sensitivity is primarily expressed at pre-synaptic D2 auto-receptors or at post-synaptic D2 hetero-receptors is not clear. In addition, the possibility that the relative affinity of
D2 vs. D1 receptors was the underlying basis for Empathy-related responses could not be ruled out (Cox et al. 2015).

We addressed these questions by systematically comparing the pattern of correlations between Empathy and the pleasurable (Liking) and subjective motivational (Wanting) effects of a slot machine and a dose of AMPH.

In vivo, pharmacodynamic studies have shown that “acute administration of HAL actually augments dopaminergic activity due to relatively strong pre-synaptic actions and relatively weak post-synaptic blocking effects” (Lidsky and Banerjee 1993). Preferential binding to pre-synaptic D2 receptors is especially likely to occur at relatively low doses (Richfield et al. 1989, Schoemaker et al. 1997).

Thus, acute administration of a modest dose (3-mg) of HAL (D2 antagonist) should provide a means of assessing individual differences in post-synaptic D2 (and D1) receptor response to reward, while comparison with a modest dose (3-mg) of FLU (D1-D2 antagonist) should reveal the relative contribution of post-synaptic D1 vs. D2 receptors in mediating these effects. Observation of parallel effects for both antagonists would indicate a role for pre-synaptic D2 receptors, which should be blocked to a similar degree by HAL and FLU.

### 4.2 Critical Findings

Three major findings emerged from this study. Firstly, in Phase I, the zero-order bivariate correlations under placebo treatment, revealed that EIS-Empathy correlated positively and significantly with Desire to Gamble (DTG) at pre-capsule baseline, expected peak antagonist effect, and again after the slot machine in both the HAL and FLU antagonist groups. This highly reliable pattern, which emerged in the absence of any experimental manipulations (or statistical controls), indicated that self-reported Empathy taps a fundamental aspect of motivation to gamble in individuals with PG. A similar pattern emerged on the placebo session, at baseline and
expected peak AMPH effects, in Phase II, further supporting the reliability of this relationship. Based on the pattern of largely null inter-correlations with the other trait variables, this linkage appears to be distinct from any other dimension. In addition, Impulsivity and Extroversion scores, which have been previously associated with D2 anomalies (Buckholtz et al. 2010, Reeves et al. 2007) and were moderately inter-correlated with Empathy in the HAL group, did not correlate to any degree with the dependent variables. Furthermore, the lack of correlation between Empathy and SOGS in either group indicates that the link between Empathy and the reinforcing effects of gambling and AMPH was not a byproduct of greater PG severity among high Empathy subjects.

From an analytic standpoint, the fact that we observed a consistent correlation between Empathy and addictive motivation in both groups of subjects in the absence of any manipulation is reassuring, as it suggests that the relationship between Empathy and the reinforcing effects of gambling and AMPH was not ‘induced’ by the experimental manipulations (i.e., not an artifact).

To help isolate the effects of the target reinforcers, the critical correlations controlled statistically for extraneous factors: final credit tally (Winnings) on the slot machine in Phase I, and baseline ratings of Desire To Gamble prior to receiving AMPH in Phase II. In Phase I, the key correlations emerged for reported pleasurable effects of the slot machine (i.e., explicit rating of the addictive stimulus) for which no meaningful baseline measure exists. In Phase II, the key correlations emerged for ratings of Desire To Gamble at expected peak AMPH effects, controlling for baseline Desire To Gamble ratings. Therefore, in both Phase I and Phase II, the observed correlations with Empathy can be reasonably attributed to individual differences in response to the reinforcers, rather than pre-existing variation or extraneous factors.

The second major finding of this study pertained to the subjective Pleasurable Effects of the slot machine game in Phase I. Under placebo, EIS-Empathy correlated modestly but consistently with VAS Composite Pleasurable Effects (Liking) of the slot machine, with
statistically equivalent $r$ values in each antagonist group ($0.38 \leq r \leq 0.50$). HAL virtually negated the correlation between Empathy and VAS Composite Pleasurable Effects, whereas FLU increased the strength of this relationship. As a result, the variance explained by the correlation between Empathy and VAS Composite Pleasurable Effects differed considerably for PG-HAL vs. PG-FLU under the active antagonist treatment ($\%\Delta s^2 = 1.00$), although the z-test comparing the two groups’ $r$ scores did not achieve significance ($p = 0.11$).

The third major result of this study pertained to Desire To Gamble ratings following AMPH in Phase II. Under placebo pre-treatment, greater Empathy was moderately, but consistently associated with greater Desire To Gamble at expected peak AMPH, with no difference in the $r$ scores for the two antagonist groups ($0.34 \leq r \leq 0.48$). Therefore, with no antagonist, increased Empathy was moderately associated with increased motivation to gamble in both groups in Phase II as it had in Phase I, except in this case, the correlation was seen at expected peak AMPH levels, and controlled for baseline desire to gamble, indicating that Empathy was specifically linked with the priming effects of AMPH on desire, over and above trait-based variation in desire to gamble.

HAL increased the strength of the correlation between Empathy and desire to gamble at expected peak AMPH levels, whereas FLU decreased the correlation. As a result, the between-group difference in variance explained under the antagonists was considerable ($\%\Delta s^2 = 0.80$), although the z-test was again only marginal, $p = 0.12$.

4.3 Interpretation of Findings

4.3.1 Phase 1: Slot Machine Game

In Phase I, VAS Composite Pleasurable Effects primarily captured the pleasurable effects of gambling. In line with the hypothesis, preferential blockade of pre-synaptic D2 receptors by HAL (Pucak and Grace 1994, Richfield et al. 1989, Schoemaker et al. 1997) negated the
relationship between Empathy and the reinforcing effects of the slot machine, whereas combined blockade of pre-synaptic D2 and D1 receptors by FLU enhanced this relationship. FLU’s affinity for D1 is half as great as it is for D2 (Ki = 0.85 vs. 0.4; Appendix A; Binding Profiles of Haloperidol and Fluphenazine). Therefore, the effects of FLU may reflect an enhanced ability to detect individual differences in D1 sensitivity when overall stimulation of these receptors was reduced. Alternatively, the effects of FLU may reflect the increased focusing of the DA signal at post-synaptic D2 receptors, which, based on the literature, would be expected to remain available (relative to D1 and pre-synaptic D2) under the antagonist.

During the slot machine game in Phase I, reward was signaled by phasic activation of D1 and reward omission was detected by a pause in tonic DA at D2 receptors. Therefore, with no stimulus-independent activation of tonic DA (e.g., by AMPH), both reward delivery and omission were salient under placebo. However, slot machines artificially enhance the salience of rewards with sensory cues such as bells and lights, and diminish the salience of losses by providing no sensory feedback when they occur, and in some cases providing the illusion of reward by delivering credits whose value does not equal the amount of credits wagered (“a loss disguised as a win”) (Dixon et al. 2010). Both HAL and FLU groups exhibited a moderate \( r \) between Empathy and VAS Composite Pleasurable Effects under placebo, consistent with a reinforcer that promotes detection of reward vs. reward omission.

HAL eliminated the correlation, and FLU strengthened the correlation between Empathy and VAS Composite Pleasurable Effects. This can be explained by HAL removing feedback inhibition at D2 auto-receptors, enhancing reward-related phasic DA transmission at D1 as well as post-synaptic D2 receptors. FLU on the other hand, also removed feedback inhibition by blocking D2 auto-receptors, but led to a weak signal at D1 and strong post-synaptic D2 signal due to its additional blockade of D1 receptors. The increased correlation between Empathy and VAS Composite Pleasurable Effects under FLU would be expected if high Empathy individuals...
have low baseline DA transmission (under drug-free conditions) and highly sensitive post-synaptic D2 receptors. Thus, preferential stimulation of sensitive post-synaptic D2 receptors may account for the increased Liking of gambling under FLU in high Empathy subjects.

4.3.2 Phase II: AMPH

In Phase II, desire to gamble captured the incentive motivation to gamble in response to AMPH. HAL and FLU had directionally opposite effects on responses to AMPH in Phase II as they did on responses to the slot machine in Phase I. In line with the hypothesis, preferential blockade of pre-synaptic D2 receptors by HAL enhanced the relationship between Empathy and the reinforcing effects of AMPH, whereas combined blockade of pre-synaptic D2 and D1 receptors by FLU negated this relationship. Consideration of the pattern of DA transmission under the two reinforcers may help to explain this. While the slot machine causes time-limited bursts and pauses in DA release during reward delivery and omission, AMPH causes unconditional, stimulant-independent DA release, stimulating all DA receptors. Under these conditions, individual differences in post-synaptic D2 sensitivity may be obscured.

Recent PET scan evidence has shown that approach motivation in response to signals for reward is primarily mediated by D1 receptors in healthy volunteers. AMPH increases motivation to gamble in PG subjects, and HAL amplifies the increase in motivation to gamble induced by the slot machine in PG subjects. It is conceivable that HAL intensifies the inherent bias of slot machines to render rewards more salient than losses, for the reasons noted above (i.e., bells, lights). Such an effect would likely involve preferential activation of D1 receptors. In contrast, AMPH will exert robust effects at D1, pre-synaptic D2 auto-receptors and post-synaptic D2 hetero-receptors. Given that stimulation of D2 auto-receptors facilitates accurate avoidance of punishment in healthy volunteers (Frank and O’Reilly 2006), negation of this effect by HAL would effectively ‘disinhibit’ responding. Thus, HAL may have negated a bias toward avoidance
responding in punishment-sensitive subjects by removing AMPH-induced DA signaling at D2 auto-receptors. This may partly explain the increase in AMPH-induced Desire To Gamble in high Empathy PG subjects under HAL.

In contrast to HAL, FLU removes the inhibitory effect of D2 auto-receptors but selectively increases the post-synaptic D2 signal rather than the D1 signal that mediates approach (Dagher and Robbins 2009). However, unlike Phase I, in Phase II baseline deficits in signaling at D2 would already be reversed by AMPH (i.e., high tonic DA transmission). Therefore, augmentation of post-synaptic D2 signaling under FLU further obscures pre-existing differences in post-synaptic D2-mediated avoidance bias. In sum, reversal of avoidance under HAL manifests as increased Wanting in high Empathy subjects, although whether this difference reflected an unconditioned increase in D1 signaling due to the pharmacological effect of AMPH, or whether it reflected a conditioned increase in D1 signaling in response to an interoceptive stimulus (i.e., activation of catecholamines and hypothalamic pituitary axis response) that mimics gambling (i.e., cross-priming) is unclear.

Relative to baseline, PG subjects in both antagonist groups were more inclined to gamble when they received AMPH in Phase II, compared to the dummy capsule received in Phase I. Knutson et al. (2004) examined the effects of oral AMPH (0.25 mg/kg) administration on neural and affective responses to an event-related fMRI probe of monetary incentive delay in healthy volunteers. Under placebo, anticipation of gains elicited increased positive arousal and ventral striatum activity in the monetary delay task (Bjork et al. 2004, Knutson et al. 2001a), and delivery of gain outcomes elicited medial PFC activity (Knutson et al. 2001b, 2003). Relative to placebo, AMPH treatment “equalized” levels of ventral striatum activity and positive arousal during anticipation of both gains and losses. In the case of potential loss, AMPH treatment appeared to potentiate right NAc activity, and augment positive arousal. Knutson et al. (2004) suggested this may reflect reframing of negative/fear-based arousal due to anticipation of
potential losses as positive arousal in response to an expectation of potential gains of safety (i.e., averting a loss), consistent with the “safety-seeking” hypothesis, which predicts that negative events should only elicit NAc activity to the extent that an organism believes that it can escape (i.e., anticipate a positive outcome) (Ikemoto and Panksepp 1999). In the present study, AMPH alone may have promoted this reframing effect and HAL may have augmented this shift to a greater extent in high Empathy subjects who were especially sensitive to negative outcomes.

4.4 DA Receptors, Approach/Avoidance Learning and Personality

Consideration of related and opposing personality traits can be advantageous in providing additional insight into the underlying mechanism of Empathy. PET studies have indexed Psychopathy, ‘coarse insensitivity’ to the feelings of others, with low midbrain D2 auto-receptors in healthy volunteers (Buckholtz et al. 2010). However, a similar link between low D2 availability or functionality and Antisocial Personality has been observed using post-synaptic D2 challenge with bromocriptine (Gerra et al., 2003). Since Psychopathy is essentially the antithesis of Empathy, one could infer that Empathy may be associated with high D2 receptor availability. However, given that the literature suggests a role for both pre- and post-synaptic D2 receptors in Psychopathy/Antisocial Personality, low scores on this dimension (i.e., high Empathy) may also involve (high) pre- and post-synaptic D2 receptors. The parallel effects of HAL and FLU at pre-synaptic D2 auto-receptors suggest that differences in Empathy-based responses under the two antagonists are unlikely to be attributable to the drugs’ pre-synaptic D2 effects.

As the data reveal that HAL and FLU had opposite directional effects, and HAL and FLU differ primarily in their action at D1 receptors, it seems fair to assume that at least part of the observed effects were attributable to D1 signaling. Empathy is a very difficult trait to measure because it does not have a universal definition. It has been referenced as Theory of Mind in autism, sense of self in psychology research and vicarious reward/pain sensitivity in terms of
learning and motivation. Wondra and Ellsworth (2015) recently proposed an appraisal theory of vicarious emotional experiences, including Empathy, based on appraisal theories of emotion. According to this theory, emotions for others are based on how we evaluate their situations, just as firsthand emotions are based on how we evaluate our own situations. With respect to the present data, the theory suggests that high Empathy individuals should perceive the risks and benefits of a given situation more accurately than low Empathy individuals. Slot machines preferentially enhance the salience of wins vs. losses and AMPH preferentially enhances the expectation of a positive (reward or escape from harm) vs. negative outcome in conditions of uncertainty. These biasing effects of gambling and AMPH could help to shift the otherwise accurate risk/benefit appraisals of high Empathy individuals towards perceived ‘benefit.’ This in turn could translate into high incentive value of these reinforcers for people with this personality profile. The present data suggest that enhanced stimulation of post-synaptic D2 receptors may contribute to the pleasurable effects – or ‘Liking’ – of slot machine gambling in high Empathy individuals due to the machines’ ability to enhance the salience of reward vs. loss. With regard to incentive motivation, enhanced stimulation of post-synaptic D1 receptors may contribute to reversal of an avoidance bias and increased desire – or ‘Wanting’ – to gamble in high Empathy PGs under AMPH.

While we focused on clarifying the roles of D1 and D2 receptors in this study, we cannot rule out the potential role of D3 in PG. HAL and FLU have different affinities for D3 (Ki = 0.2 vs. 1.4; Appendix A; Binding Profiles of Haloperidol and Fluphenazine), which raises the question of the role of D3 in mediating the observed effects. Furthermore, Dodd et al. (2005) have suggested D3 as a key factor in the PG-inducing effects of DA agonists in Parkinson’s patients. However, a study by Boileau et al. (2014) supports our proposal that D2 is more likely involved, compared to D3. In this study, PET scans examined binding of a preferential D2 ligand (raclopride) and of a preferential D3 ligand (PHNO) in healthy control and PG males. Post-hoc
analysis revealed that Empathy scores (from the EIS) correlated consistently with raclopride binding in the ventral striatum of both controls and PG subjects, whereas PHNO binding was completely unrelated to Empathy. The magnitude of the effect for PG was consistent with what was found in the current study (i.e., including zero-order/baseline correlation) between Empathy and Desire to Gamble under placebo, \( r = 0.41 \) \((r^2 = 0.166)\). The correlation between Empathy and raclopride binding in the ventral striatum in healthy controls was even stronger, \( r = 0.90 \) \((r^2 = 0.817)\). Thus, the direction and strength of the correlation between Empathy and baseline Desire to Gamble in the present study correspond very closely to the direction and strength of the correlation between Empathy and ventral striatal D2 (rather than D3) receptor levels in PG subjects. The emergence of this effect in controls suggests that the relationship is reliable and is a trait factor. The greater strength of the relationship in controls vs. PG subjects is consistent with the idea that PGs are heterogeneous and that Empathy is likely only one dimension that mediates gambling reinforcement in these individuals.

4.5 Limitations

The present study had a number of limitations. Firstly, the sample size of 15 subjects per group is fairly modest. However, given that only 5% of individuals with PG are drug- and medication-free and have no co-morbid disorders, this represents a substantial accomplishment, within the time constraints and resources available to the project.

Second, PGs in this study had moderate symptom severity without any other comorbid disorders. While this enhances internal validity by permitting attribution of the results to PG rather than some alternative dimension, it decreases the generalizability of the findings to the broader population of individuals with PG characterized by diverse comorbidities.

A third issue concerns the attribution of effects to D1. By virtue of the lack of a selective D1 antagonist available for human use in Canada, HAL and FLU were used in a deductive model
to infer the role of D1. However, this design is not able to provide definite evidence on the role of D1 alone (in the absence of D2 auto-receptor blockade).

Fourth, the protocol relied mainly on self-report scales to assess Empathy and changes in Liking and Wanting of gambling. There is some inherent vulnerability to variation between subjects, however, normative scores on the Lie scale of the EPI suggest that the scores reported are likely an accurate reflection of subjective state, providing confidence that the reported scores are valid measures of Empathy, VAS ratings of Pleasurable Effects and Desire to Gamble.

In addition to these limitations, the protocol implemented a standard test session timeline according to the average expected interval for the antagonists and AMPH to reach peak plasma concentration. However, individual differences in metabolism could lead to different plasma levels, and thus contribute to variation in the observed correlations. Using a standard time for expected peak drug levels was pragmatic given that genetic assays for slow/fast metabolizers would not have sufficient power to detect meaningful differences given the small sample size of this study. Similarly, direct assessment of plasma levels would entail invasive procedures (i.e., blood draw), which would likely disrupt subjective motivation and reinforcement.

Lastly, the protocol is based on the assumption that both HAL and FLU primarily antagonize pre-synaptic D2 auto-receptors (compared to post-synaptic D2 hetero-receptors) at the low dose (3-mg) prescribed, although there are limited human studies to support this conclusion. However, as noted earlier, multiple animal studies indicate that D2 antagonists primarily block auto-receptors at modest doses (Richfield et al. 1989, Schoemaker et al. 1997), and that this effect translates into increased DA cell firing and DA release (Grace 1995, Pucak and Grace 1994, Schmitz et al. 2003, Starke et al. 1989).
4.6 Future Directions

Although we are unable to conclusively confirm our interpretation based on the present data alone, there are many steps that could be taken to try and refine the present study. Enhancing the sample size could help to enhance the reliability of our findings. Direct measurement of DA transmission and receptor binding using PET studies, for example, may allow selective study of D1 receptor levels and help to confirm the association between changes in DA release and the subjective-behavioural effects of Empathy in complement to, or instead of, the current deductive analysis using HAL and FLU as two probes to learn about D1 and D2 signaling. Future studies may also help to further clarify if Empathy predicts acute or chronic response to DA-modulating medications for PG, with the end goal of patient-treatment matching for highly empathic individuals with PG.

4.7 Conclusion

As hypothesized, HAL and FLU had opposite patterns of effect on the relationship between Empathy and reinforcement in each phase. This was expected due to their differential signaling at the D1 receptor. HAL negated, whereas FLU enhanced, the correlation between Empathy and pleasurable effects of the slot machine. Furthermore, in the AMPH phase, where AMPH would have caused stimulus-independent unconditional DA firing and obscure pre-existing receptor sensitivities, HAL and FLU had opposite effects compared to Phase I. HAL enhanced, whereas FLU attenuated, the correlation between Empathy and incentive motivation to gamble in response to AMPH. Phase I results suggest that post-synaptic D2 receptors may mediate Empathy-related differences in Liking of gambling, while Phase II results suggest that D1 receptors may mediate Empathy-related differences in Wanting to gamble under AMPH in PGs. Collectively the findings help to advance our understanding of the role of DA in gambling and AMPH reinforcement, which may be beneficial to the development of individually-targeted
interventions for highly empathic PGs.
REFERENCES


Zack, M., Featherstone, R.E., Mathewson, S. and Fletcher, P.J. (2014). Chronic exposure to a gambling–like schedule of reward predictive stimuli can promote sensitization to amphetamine in rats. Front Behav Neurosci 8(36), eCollection.


APPENDIX A:

Binding Profiles of Haloperidol and Fluphenazine
Table A-i. Receptor binding of Haloperidol and Fluphenazine at D2 receptors.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>% Response</th>
<th>IC\textsubscript{50}</th>
<th>K\textsubscript{i}</th>
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</thead>
<tbody>
<tr>
<td>Bromperidol</td>
<td>-54 ± 6</td>
<td>2.1 ± 0.6</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>Spiperone</td>
<td>-49 ± 1</td>
<td>0.3 ± 0.1</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Fluspirilene</td>
<td>-48 ± 6</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Pimozide</td>
<td>-45 ± 14</td>
<td>0.5 ± 0.1</td>
<td>2.4 ± 1.3</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>-43 ± 16</td>
<td>0.2 ± 0.0</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>-43 ± 15</td>
<td>0.8 ± 0.2</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Trifluoperidol</td>
<td>-42 ± 12</td>
<td>0.2 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Cisflupenthixol</td>
<td>-41 ± 7</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Sulpiride</td>
<td>-41 ± 4</td>
<td>11 ± 9</td>
<td>8.6 ± 1.9</td>
</tr>
<tr>
<td>Chlorproethazine</td>
<td>-40 ± 14</td>
<td>10 ± 5</td>
<td>11 ± 5</td>
</tr>
<tr>
<td>Butaclamol</td>
<td>-40 ± 2</td>
<td>0.3 ± 0.3</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>Amoxapine</td>
<td>-40 ± 3</td>
<td>38 ± 6</td>
<td>3.6 ± 1.5</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>-39 ± 6</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Sertindole</td>
<td>-39 ± 4</td>
<td>2.7 ± 1.1</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Oceperidone</td>
<td>-38 ± 11</td>
<td>0.1 ± 0.0</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>Risperidone</td>
<td>-37 ± 3</td>
<td>0.3 ± 0.3</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Remoxipride</td>
<td>-37 ± 3</td>
<td>16 ± 6</td>
<td>105 ± 38</td>
</tr>
<tr>
<td>Tiapride</td>
<td>-35 ± 10</td>
<td>31 ± 13</td>
<td>226 ± 223</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>-34 ± 12</td>
<td>3.0 ± 1.1</td>
<td>5.7 ± 1.5</td>
</tr>
<tr>
<td>Raclopride</td>
<td>-34 ± 9</td>
<td>0.5 ± 0.3</td>
<td>2.4 ± 0.8</td>
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<tr>
<td>Moperone</td>
<td>-34 ± 7</td>
<td>1.0 ± 0.6</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Tefludazine</td>
<td>-33 ± 2</td>
<td>0.3 ± 0.2</td>
<td>0.7 ± 0.2</td>
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<tr>
<td>Transflupenthixol</td>
<td>-32 ± 6</td>
<td>17 ± 6</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>Clozapine</td>
<td>-31 ± 10</td>
<td>71 ± 21</td>
<td>72 ± 20</td>
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<td>Molindone</td>
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<td>20 ± 7</td>
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<td>Chlorpromazine</td>
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<td>1.7 ± 1.1</td>
<td>8.1 ± 2.1</td>
</tr>
<tr>
<td>Sulforidazine</td>
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<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>9-OH-risperidone</td>
<td>-29 ± 13</td>
<td>1.0 ± 0.2</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>Thiothixene</td>
<td>-27 ± 15</td>
<td>0.2 ± 0.0</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Sultopride</td>
<td>-24 ± 6</td>
<td>4.5 ± 0.7</td>
<td>1.6 ± 1.0</td>
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<td>Melperone</td>
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<td>N.D.</td>
<td>3.6 ± 0.5</td>
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<td>Octoclohepin</td>
<td>-24 ± 6</td>
<td>0.2 ± 0.1</td>
<td>0.7 ± 0.3</td>
</tr>
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<td>Promazine</td>
<td>-18 ± 13</td>
<td>N.D.</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>-14 ± 10</td>
<td>N.D.</td>
<td>16 ± 12</td>
</tr>
<tr>
<td>Mesoridazine</td>
<td>-14 ± 9</td>
<td>N.D.</td>
<td>4.3 ± 1.4</td>
</tr>
<tr>
<td>N-Desmethylolanzapine</td>
<td>-14 ± 7</td>
<td>N.D.</td>
<td>32 ± 29</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>-13 ± 12</td>
<td>N.D.</td>
<td>106 ± 43</td>
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<td>Thoridazine</td>
<td>-12 ± 15</td>
<td>N.D.</td>
<td>21 ± 16</td>
</tr>
<tr>
<td>L-745,870</td>
<td>N.D.</td>
<td>N.D.</td>
<td>343 ± 297</td>
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<tr>
<td>Aripiprazole</td>
<td>194 ± 42</td>
<td>N.D.</td>
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<tr>
<td>N-Desmethylolanzapine</td>
<td>129 ± 21</td>
<td>N.D.</td>
<td>89 ± 26</td>
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Table A-ii. Receptor binding of Haloperidol and Fluphenazine at D1 receptors.

Table 6. *Inhibition of* $^3$H-SCH 23390 *binding to rat striatal membranes in vitro*

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC 50 nM</th>
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<td>Thioxanthenes</td>
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<tr>
<td>Cis(Z)-chlorprothixene</td>
<td>3.7</td>
<td>1.8</td>
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<td>Trans(E)-chlorprothixene</td>
<td>270</td>
<td>130</td>
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<td>Zuclopenthixol</td>
<td>1.4</td>
<td>0.66</td>
</tr>
<tr>
<td>Trans(E)-clopenthixol</td>
<td>110</td>
<td>52</td>
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<tr>
<td>Cis(Z)-flupentixol</td>
<td>2.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Trans(E)-flupentixol</td>
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<td>0.19</td>
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<td>2.7</td>
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<td>6.2</td>
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<td>14</td>
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<td>0.85</td>
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<td>10</td>
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<td>Thioridazine</td>
<td>8.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Trifluoperazine</td>
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<td>3.7</td>
</tr>
<tr>
<td>Butyrophenones + analogues</td>
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<td>Bromperidol</td>
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<td>Halopemide</td>
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<td>Diphenylbutylpiperidines</td>
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<td>Clopimozide</td>
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<td>320</td>
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Table A-iii. Receptor binding of Haloperidol and Fluphenazine at D2, D3, and D4 receptors.

**Table A-iv.** Receptor binding of Haloperidol and Fluphenazine at serotonin receptors.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Clinically effective dose (mg)</th>
<th>S-HT₁A</th>
<th>S-HT₁B</th>
<th>S-HT₁D</th>
<th>S-HT₁E</th>
<th>S-HT₂F</th>
<th>S-HT₂A</th>
<th>S-HT₃</th>
<th>S-HT₅A</th>
<th>S-HT₇</th>
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<tr>
<td>Antipiprazole</td>
<td>5-30</td>
<td>5.6</td>
<td>833</td>
<td>63</td>
<td>8000</td>
<td>17.5</td>
<td>0.36</td>
<td>22.4</td>
<td>628</td>
<td>1241</td>
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<tr>
<td>Chlorpromazine</td>
<td>300-900</td>
<td>3115</td>
<td>1489</td>
<td>452</td>
<td>344</td>
<td>3.22</td>
<td>15.55</td>
<td>977</td>
<td>118</td>
<td>12</td>
</tr>
<tr>
<td>Chlorprothixene</td>
<td>50-400</td>
<td>0.43</td>
<td>4</td>
<td>&lt;0.001</td>
<td>0.01</td>
<td>0.66</td>
<td>0.25</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Clozapine</td>
<td>300-900</td>
<td>105</td>
<td>398</td>
<td>2132</td>
<td>966</td>
<td>130</td>
<td>9.15</td>
<td>7.38</td>
<td>14.9</td>
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<tr>
<td>Fluphenazine</td>
<td>2-15</td>
<td>145</td>
<td>334</td>
<td>334</td>
<td>540</td>
<td>21</td>
<td>983</td>
<td>&gt;10000</td>
<td>445</td>
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<td>Haloperidol</td>
<td>2-15</td>
<td>1202</td>
<td>165</td>
<td>7606</td>
<td>&lt;10000</td>
<td>&lt;5000</td>
<td>1186</td>
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<td>25-100</td>
<td>2456</td>
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<td>3468</td>
<td>1399</td>
<td>4.38</td>
<td>133</td>
<td>190</td>
<td>776</td>
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<tr>
<td>Mesoridazine</td>
<td>100-400</td>
<td>150</td>
<td>137</td>
<td>157</td>
<td>300</td>
<td>150</td>
<td>137</td>
<td>157</td>
<td>300</td>
<td>150</td>
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<tr>
<td>Molindone</td>
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<td>3797</td>
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<td>4653</td>
<td>10000</td>
<td>10000</td>
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<td>17</td>
<td>23</td>
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<td>23</td>
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<td>Pimozide</td>
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<td>526</td>
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<td>2240</td>
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<td>1843</td>
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<td>Risperidone</td>
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<td>&gt;10000</td>
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<td>Sertindole</td>
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<td>0.09</td>
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<td>28</td>
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<td>Thioridazine</td>
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<td>109</td>
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<td>&gt;10000</td>
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<td>57</td>
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<td>Thiothixene</td>
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<td>151</td>
<td>659</td>
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<td>50</td>
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<td>1863</td>
<td>361</td>
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<td>Trifluoperazine</td>
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<td>291</td>
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<td>378</td>
<td>144</td>
<td>291</td>
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<tr>
<td>Ziprasidone</td>
<td>80-160</td>
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<td>4</td>
<td>9</td>
<td>1279</td>
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<td>&gt;10000</td>
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<td>61</td>
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</table>

APPENDIX B:
SCID Inclusion/Exclusion Criteria
Module A: Mood
CURRENT MAJOR DEPRESSIVE EPISODE (in last month) → ineligible
PAST MAJOR DEPRESSIVE EPISODE
  • If only one episode, more than 1 yr ago → eligible
  • If more than one episode, or less than one year ago → ineligible
CURRENT MANIC EPISODE (at least 1 week) → ineligible
PAST MANIC EPISODE
  • If only one episode, more than 1 yr ago → eligible
  • If more than one episode, or less than one year ago → ineligible
CURRENT HYPOMANIC EPISODE (at least 4 days) → ineligible
PAST HYPOMANIC EPISODE
  • If only one episode, more than 1 yr ago → eligible
  • If more than one episode, or less than one year ago → ineligible
DYSTHYMIC DISORDER (at least 2 yrs) → ineligible
SUBSTANCE-INDUCED or GENERAL MEDICAL CONDITION → ineligible
BEREAVEMENT; if simple bereavement (sudden loss of loved one, symptoms < 2 mths, no significant loss of function) → eligible

Module F: Anxiety
PANIC DISORDER WITH AGORAPHOBIA (PDA) → ineligible
PANIC DISORDER (PD) WITHOUT AGORAPHOBIA → ineligible
AGORAPHOBIA WITHOUT PD (AWOPD) → ineligible
SOCIAL PHOBIA (if only public speaking AND age <18yrs, at least for 6 mths) → eligible
SPECIFIC PHOBIA (if age <18yrs, at least for 6 mths) → ineligible
OBSESSIVE-COMPULSIVE DISORDER → ineligible
POST-TRAUMATIC STRESS DISORDER (PTSD) (1 mth) → Ineligible
GENERALIZED ANXIETY DISORDER (GAD) (at least 6 mths) → ineligible
ANXIETY DISORDER NOS → ineligible
Anxiety due to substance use → ineligible
Anxiety due to medical condition → ineligible

Module E: Substance Abuse
ALCOHOL ABUSE (if less than 1 yr ago) → ineligible
ALCOHOL DEPENDANCE → ineligible
NON-ALCOHOL SUBSTANCE USE DISORDERS
  • Prior use of any stimulants → ineligible
  • Prior use of ecstasy, hallucinogens, GHB, ketamine, or PCP >2, occasions → ineligible
  • Prior use of marijuana >1 cigarette a month → ineligible
  • If any drug >10 times in one month: drug abuse/dependence → ineligible (e.g., Holistic and herbal-Kava, Valerian, St. John’s wort, Ginseng, gingko biloba, salvinorum A)
  • If reports becoming dependant, or use more than prescribed → ineligible (e.g., Tylenol codeine)

Module B/C: Psychotic
DELUSIONS → ineligible
AUDITORY HALLUCINATIONS → ineligible
VISUAL HALLUCINATIONS → ineligible
OTHER HALLUCINATIONS → ineligible
SCHIZOPRENNIA OR any family history → Ineligible
SCHIZOPHRENIFORM DISORDER (duration less than 6 mths) → ineligible
SCHIZOAFFECTIVE DISORDER → ineligible
DELUSIONAL DISORDER → ineligible
PSYCHOTIC DISORDER DUE TO GMC/SUBSTANCE-INDUCED → ineligible
APPENDIX C:
Recruitment Ad for Pathological Gamblers
If you are:
• 19-65 years of age
• Drug- and Medication-Free
• Available for Weekly Day-long Sessions (M – F)

Call Study Line: (416) 535-8501, ext. 36743

NOTE: This is not a treatment study
FINANCIAL COMPENSATION IS PROVIDED
All Information Provided Will Remain Confidential To The Extent Allowed By Law

CAMH provides treatment options for mental illness and addictions.

For more information about programs and services at CAMH, visit www.camh.ca or call (416) 535-8501, or 1-800-463-6273
APPENDIX D:
Informed Consent Form
Consent to Participate in Research Study

Mental and behavioral effects of central nervous system medications in frequent and occasional gamblers

Qualified Investigator/Study Physician: Daniela Lobo, MD, PhD
Co-Investigators: Martin Zack, PhD
James Kennedy, MD, PhD

Study Site: Centre for Addiction & Mental Health (CAMH), 33 Russell Street & 250 College Street, Toronto Ontario

Introductory Statement

Before agreeing to take part in this research study, it is important that you read the information in this research consent form. It includes details we think you need to know in order to decide if you wish to take part in the study. If you have any questions, ask a study doctor or study staff. You should not sign this form until you are sure you understand the information. All research is voluntary. You may also wish to discuss the study with your family doctor, a family member or close friend. If you decide to take part in the study, it is important that you are completely truthful about your health history and any medications you are taking. This will help prevent unnecessary harm to you.

Conflict of Interest

None of the investigators and none of the experimenters have any conflict of interest in performing their duties on this study. They have no interest, financial or otherwise, in the study outcomes.

Study Sponsor

This study is sponsored by Dr. Daniela Lobo and the Centre for Addiction and Mental Health (CAMH).

Funds to conduct the study are provided by a grant from the Canadian Institutes of Health Research.

Ethics, Confidentiality and Continuing Review:

The study protocol and consent form have been reviewed by a committee called the Research Ethics Board at CAMH. The Research Ethics Board is a group of scientists, medical staff, individuals from other backgrounds (including law and ethics) as well as members from the community. The committee is established by the hospital to review studies for their scientific and ethical merit. The Board pays special attention to the potential harms and benefits involved in participation to the research participant, as well as the potential benefit to society. As part of continuing review of the research, your study records may be assessed on behalf of the CAMH Research Ethics Board and by the Health Canada Therapeutic Products Programme. A person from the research ethics team may contact you (if your contact information is available) to ask you questions about the research study and your consent to participate. The person assessing your file or contacting you must maintain your confidentiality to the extent permitted by law.

Study Purpose:

v. 4 04/22/2015  I have read this page of the document  Participant’s Initials _______  Page 1 of 8
This study is intended to test the effects of the central nervous system (CNS) medications, Haloperidol, Fluphenazine and Dexedrine on mental processes and feelings in individuals who gamble frequently and in a comparison group of people who gamble rarely or occasionally. The study is not intended to treat any aspect of your gambling. If you are eligible, based on the conditions outlined below (see Study Requirements), you will be one of 80 participants in the study. The study medications are approved in Canada. However, in this study, they are not used to treat any condition, but because they temporarily decrease (haloperidol, fluphenazine) or increase (Dexedrine) activity of a chemical called dopamine, in the brain. For this reason, they are considered investigative probes.

Participant Enrolment:

You have been invited to participate because you indicated during the initial interview that you are a (a) frequent gambler and report some problems related to your gambling; or (b) an occasional gambler and report no problems related to gambling.

Pregnancy and Breastfeeding:

Pregnancy and this study are not compatible. Due to the risk or potential risk to the fetus, women who are pregnant or planning to become pregnant are therefore excluded from this study. Women of childbearing potential are advised to discuss appropriate family planning with their doctor if they are interested in enrolling in this study. Unless you have had a hysterectomy, a tubal ligation, are post-menopausal, or not at risk of pregnancy, you are advised to practice an appropriate method of family planning. Women will be screened for pregnancy before each test session. Because medications consumed by a woman can be passed on to a child through the breast milk, women who are currently breastfeeding a baby are also ineligible.

Study Procedure:

1. Participation involves coming to the 33 Russell Street of the Centre for Addiction and Mental Health (CAMH) 6 separate times: A pre-experimental interview, a physician’s examination, and 4 test sessions, scheduled at 1-week intervals. You will receive transit tokens to cover round-trip fare to CAMH for the interview and physician’s exam, as well as the cost of travel to CAMH on all test sessions. You will be sent home by pre-paid taxi at the end of all test sessions.

2. Pre-experimental Interview. This session will involve answering some questions and filling out some questionnaires about your gambling experiences, alcohol and drug use, and personality characteristics. You will meet with a doctor who will ask you questions about any mental or emotional concerns you may have. In addition, you will be asked to provide a urine sample and a person certified to draw blood will take a blood sample from your arm (3-4 finger-sized vials). The blood sample may cause minor discomfort and temporary bruising on your arm. The urine and blood samples will be used to make sure you have not recently used any mood-altering drugs and will also ensure that you have no health condition that would make it risky to receive the study medications. During this session you will also undergo an electrocardiogram (EKG), administered by a trained technician. The EKG is a harmless test that examines your heart’s activity over the course of several minutes. This session will take 2 - 2.5 hours.

3. Physician’s Exam. If the information from your interview shows that you meet the initial requirements for participation, you will be asked to undergo a physical examination by a doctor at CAMH. The purpose of the exam is to make sure you have no physical condition that would make it risky for you to receive any of the study medications. The exam will take 1/4 - 1 hour.

4. Test Sessions. If your physician's exam shows that you are fit to receive the study medications, you will be asked to attend 4 test sessions scheduled at 1-week intervals. Each test session will be identical in terms of the things you will be asked to do. You will have an opportunity to ask questions throughout the
study. You are free to not answer any question or to not perform any task or withdraw from the study without penalty. Payment for partial participation is pro-rated as outlined below.

5. **Right to opt out.** You have the option to not answer a question or questions, to not participate in an element of the test procedure, or to withdraw from the study. Your decision to do so will not affect your current or future care at CAMH (should you wish to use it).

**Details of Test Sessions:**

a) You will abstain from alcohol and all mood-altering drugs for 12 hours prior to the start of each test session and for 72 hours after the completion of each test session. This is extremely important to prevent potentially dangerous interactions between the study medications and other drugs.

b) You will also abstain from caffeinated beverages and eat no food on the morning of each test session. You will receive a standard breakfast (with coffee if you wish) at the laboratory when you arrive.

c) You will report to the laboratory at 8:15 am on each test session. At that time, you will take a breathalyzer test to ensure there is no alcohol in your bloodstream. You will then receive your breakfast. You will take your first pill after you finish breakfast. You will take a second pill between 2-3 hrs after you receive your first.

d) On your test sessions, you may receive 3-mg Haloperidol, 3-mg Fluphenazine, 20-mg Dexedrine, or a placebo (an inactive pill). Half of the participants in the study will be assigned to receive haloperidol, and the other half will be assigned to receive fluphenazine. Assignment is done randomly, and every participant has an equal chance of receiving either medication.

e) Placebo pills look the same as the pills containing active medication. There will be no harm to you from receiving a placebo. Placebos are used to standardize all aspects of the study procedure except the medication. That way, the investigators can compare your response to the different tasks and questionnaires under normal (i.e., drug-free) conditions with your responses after you have received the study medications.

f) Neither the study personnel nor you will know which pills you will receive on a given session. The pharmacist who provides the pills and the principal investigator on the study will determine which pills you receive. This will be done before the study begins and will be based on a participant number so that all participants will have an equal chance of receiving the different pills on their test sessions.

g) After receiving your pills you will fill out some questionnaires; these questionnaires will be re-administered several times during the session. You will then read magazines or the newspaper for about 2 hours while the first pill is being absorbed before receiving your second pill.

h) At specified intervals throughout the session, the experimenter will assess your heart rate and blood pressure using a small device that slips over your wrist. The device will produce a feeling of mild pressure while it takes the reading but is not painful. Each reading takes about a minute.

i) Next you will play a VLT-style slot machine game, of the kind currently in use in Ontario. You will be provided with cash credits (tokens) for the machine and allowed to play for a standard period of time (10-20 minutes; to be confirmed on test day) or until your tokens run out, whichever comes first. To make the game more interesting, a monetary bonus will be provided based on the amount of your winnings in the game. The bonus will be paid upon completion of the study when you receive your standard payment for participation.

j) Following the VLT-game you will do a short (5-minute) reaction time task on a computer and fill out some more questionnaires dealing with your impressions of the game and how you feel generally (thoughts and feelings).

k) You will then perform two additional tasks on the computer, this time focusing on decision-making (20-min).
l) Between 1:30 and 2 you will receive lunch after which you can relax and read or watch videos until 5 p.m.

m) On the remaining test sessions, you will do the exact same things as you did on the first. In addition, at the end of the final test session, you will be given information about how you did in the various aspects of the study as well as more information about what the study was about.

n) You will be paid by cheque (participation fee plus any bonus payment you may have earned) which you can pick up 2 to 4 weeks after the study is over or have mailed to you.

**Study Requirements:**

1. To be eligible for this study you must have no mental or physical illness apart from problems related to gambling.

2. You must be free of all mood-altering drugs or medication.

3. You must not be pregnant, planning to become pregnant, or breastfeeding a baby.

4. You must not operate a motor vehicle or heavy equipment for 8 hours after completion of EACH test session.

5. You must not take any drugs or alcohol for 12 hours before, and 72 hours after each test session.

6. You must follow the instructions from study personnel during the interview and test sessions. This will include adhering to schedules and arriving at the laboratory on time.

7. Just as you are free to drop out of the study for any reason at any time (for partial payment), study personnel are free to stop your participation before the study is over if you do not follow any of the study requirements. In this case, you would receive the payment earned for your participation up to that point.

8. The schedule of payment is as follows:

<table>
<thead>
<tr>
<th>Activity</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Test Interview</td>
<td>$40</td>
</tr>
<tr>
<td>Physician’s Exam</td>
<td>$30</td>
</tr>
<tr>
<td>Test Session 1</td>
<td>$200</td>
</tr>
<tr>
<td>Test Session 2</td>
<td>$200</td>
</tr>
<tr>
<td>Test Session 3</td>
<td>$200</td>
</tr>
<tr>
<td>Test Session 4</td>
<td>$250</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$920</strong></td>
</tr>
</tbody>
</table>

9. You will receive a copy of this Consent to Participate in Research Study including signature page (below).
Risks:

Haloperidol

Haloperidol has been in use for many years. Many experimental participants in other laboratories have taken the dose being tested in this study (3 mg) without negative effects. Some people may experience temporary muscle stiffness, slowing of movement, difficulty with balance or co-ordination. Both sedation and agitation (feeling 'uptight') have been reported. Although extremely rare, it is possible that this medication could cause difficulty swallowing. Measures have been taken to deal with this possibility should it occur (see below).

Fluphenazine

Fluphenazine is a medication in the same drug class as Haloperidol. As such, its side effect profile and the precautions surrounding its use are the same as those described for Haloperidol above.

Dexedrine

Dexedrine® is a stimulant medication currently in clinical use in Canada for the management of attention deficit hyperactivity disorder (ADHD) and sleep-disorder (narcolepsy). Side-effects of this drug include palpitations, mildly elevated blood pressure, restlessness, headache and dizziness. In some cases, anxiety, euphoria or agitation may occur. All of these effects are temporary and wear off after about three hours. Some may find it hard to fall asleep in the evening following Dexedrine®. Because Dexedrine® can stimulate the heart and the blood vessels there is the rare possibility that the drug could over-stimulate your heart and cause a stroke or even death. All study participants undergo a comprehensive medical exam before testing, which will evaluate risk for such a rare effect. In addition, it has not been confirmed that the low dose of Dexedrine® that you will receive could cause these serious side effects.

The unintended effects of the drugs to be used in this study are uncommon at the doses being tested. Also, the likelihood that they will occur goes down with time as the drug wears off. In addition, we have taken several steps to minimize negative effects:

a) First, we require that you stay under observation at the laboratory until 5 p.m. on test days.

b) Second, prior to leaving the lab at this time, you will be examined by a health care practitioner at our Clinic. She or he will make sure you are feeling all right before you go home that day. If you are experiencing any side effects at that time, the doctor can treat them and you can stay at the Clinic until you are feeling well enough to leave.

c) Third, you will be sent home from the laboratory by pre-paid taxi after all test sessions. Do not drive to the laboratory on test days; use the tokens we provide for you and take public transit.

d) Fourth, you will receive a wallet card stating that you may have received the various study medications as part of a research study and providing the phone number of the study physician who will be on-call after you leave the lab. You should keep the card with you at all times and contact the physician immediately if you experience any side effects.

e) Finally, when you leave the lab after test sessions you will receive a sealed capsule containing 50-mg Benadryl. This safe, non-prescription allergy medication is effective in counteracting the side effects of the study medications and will provide rapid relief if such symptoms do occur. The Benadryl is strictly a back-up measure. Take it ONLY if you are experiencing side effects. If you do take the Benadryl, you should not drive or operate heavy machinery for 8 hours, because it will likely make you sleepy. Regardless of whether or not you take the Benadryl, you should NOT DRIVE or OPERATE HEAVY MACHINERY on test days. If, after
taking the Benadryl, you continue to experience any side effects, apart from drowsiness, contact the study physician at the phone number on your wallet card. He will tell you what to do from there.

f) **Difficulty Swallowing:** If you experience this rare side effect you should immediately take the anti-side effect medication (Benadryl). If this symptom persists or worsens after several minutes, proceed to the nearest hospital emergency room and present your wallet card to the medical staff to inform them that you may have received Haloperidol or Fluphenazine. You are also advised to contact the study physician at the number provided on the wallet card AFTER you go to the emergency room.

**Benefits:**

You should expect no benefit to your gambling from participating in this study. However, you will receive information about your performance on the various tasks at the end of the study that may be interesting to you. Although the research will not directly help your gambling now, the results may help in developing new and better treatments for gambling problems that may assist you or others with gambling difficulties, in the future.

**Payment, Conditions, and Confidentiality:**

If you complete the study you will receive $920. In addition, you will be required to play a slot machine for a short time (10-20 minutes) during each test session (see below). We will provide you with cash credits to play the VLT and you will receive a cash bonus proportional to your winnings from each test session at the end of the study. The bonus, if you win, will be in addition to your standard $920 payment for participation.

You can drop out of the study at any time and receive payment for the parts of the study you have completed (as outlined above). All information you provide will remain confidential to the extent allowed by law. Your name will not appear on any of the test materials (e.g., questionnaires, rating scales) or in any of the data, from the computer task. You will be assigned a participant number which will be used to code all of your data. Names and identifying information will be stored in locked cabinets. Electronic records that include your data will be password-protected and only accessible to study personnel. Any reports of the study findings (e.g., journal articles, conference presentations) will be made so that you and all study participants remain anonymous.

No assurance of confidentiality—whether for research or for care and treatment—can be absolute. In rare circumstances, there are exceptions, such as when disclosures are required by law--to report suspected child abuse or communicable diseases, for example.

As part of the Research Services Quality Assurance Program, this study may be monitored and/or audited by a member of the Quality Assurance Team. Your research records and CAMH records may be reviewed during which confidentiality will be maintained as per CAMH policies and extent permitted by law.

**Safety**

In the unlikely event you suffer a physical injury from (the study medication(s) or participation) in this study; medical care will be provided to you in the same manner as you would ordinarily obtain any other medical treatment. In no way does signing this form waive your legal rights nor release the study doctor(s)/investigators, or CAMH from their legal and professional responsibilities.

**New Findings**

We may learn new things during the study that you may need to know. We can also learn about things that might make you want to stop participating in the study. If so, you will be notified about any new information in a timely manner. You may also be asked to sign a new consent form discussing these new findings if you decide to continue in the research study.
Questions

We have used some technical terms in this form. Feel free to ask about anything you don't understand and to consider this research and the consent form carefully -as long as you feel is necessary- before making a decision.

Contact

If you have any further questions, please feel free to contact Dr. Daniela Lobo at 416-535-8501-ext. 36568 regarding the procedures involved in the study or any other study-related questions.

If you have any questions about your rights as a participant in this study, you may contact Dr. Padraig Darby, Chair, Research Ethics Board, Centre for Addiction and Mental Health, at 416 535 8501 ext. 36876.

Genetics Screen

As part of the blood sample you provide on the Interview session (first visit to CAMH) we will be collecting information about genes that may be related to how people respond to the medications tested in this study. Dr. Kennedy’s laboratory at CAMH looks for genetic variants that are related to preference for gambling and other personality variables. This could help to identify people at risk for gambling problems before they develop.

The genetic sample will be stored in a locked refrigerated cabinet and identified only by a code number. Your name will be stored in a separate area in a password protected computer file, but not on any computer network. Your clinical information will be stored in a locked file cabinet. Stated another way, there will be no direct connection between your blood sample and your name. The stored DNA material will be kept until our research is finished, which may take many years, although the samples will not be kept more than 25 years. The DNA can be used to test any gene that may be relevant to gambling or problem gambling. Other laboratories may be involved in analyzing the genetic material, and if so this will be confidential, and your name will not be given out. Results from this study may be presented at meetings and may be published. Your identity will not be disclosed at these presentations or in any publications.

Your decision to allow your blood to be assessed for genes is COMPLETELY UNRELATED to your decision to participate in the rest of the study. However, because it is so important to have a complete data set we try very hard to obtain genetic information from all research participants.

Please indicate your willingness to allow your blood to be assessed for genes related to gambling (as outlined above):  I do [ ] OR do NOT [ ] wish to have my blood used for genetic analysis.
Mental and behavioral effects of central nervous system medications in frequent and occasional gamblers

- The investigator or a member of the investigator’s staff has discussed with me the risks of participation in this study.
- I have read all of the information in the Study Information Sheet, and I have had time to think about the information, and all of my questions have been answered to my satisfaction.
- I voluntarily agree to be part of this research study, to follow the study procedures, and to provide necessary information to the investigator or other staff members as requested.
- I am under no pressure to participate in the study, and I understand that I may withdraw from the study at any time. I also understand that my participation in the study may be terminated by the study investigator if necessary.
- By signing this consent form, I am not giving up my legal rights or releasing the investigators or sponsors from their legal and professional obligations.
- I have received a copy of the Information Sheet and will receive a copy of this signed consent form.

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Print Participant’s Name ___________________________ Participant’s Signature ___________________________ Date (mm/dd/yyyy) ___________

Print Name of Individual Obtaining Consent ___________________________ Signature of Individual Obtaining Consent ___________________________ Date (mm/dd/yyyy) ___________

Research at CAMH is ongoing and it is often helpful to investigators to contact individuals who have participated in previous studies, who have expressed interest in participating in future research.

Please indicate your interest in being contacted for future studies:

I do [ ] OR do NOT [ ] wish to be contacted for future studies at CAMH.

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v. 4 04/22/2015 I have read this page of the document Participant’s Initials ________ Page 8 of 8
APPENDIX E:
Physical Exam Inclusion/Exclusion Criteria
Inclusion/exclusion criteria:

Subjects will be 30 non-treatment-seeking pathological gamblers and 30 social gambler controls, randomized to receive Haloperidol (HAL; 3-mg)/Placebo or Fluphenazine (FLU; 3-mg)/Placebo and all subjects will receive d-amphetamine (AMPH; 20-mg).

Pre-test physician’s exam (and Blood/urine assays, ECG) is required to confirm that ALL subjects be physically and mentally healthy (apart from PG/nicotine dependence) and be taking no drugs or medications that could interact with any of the 3 study medications.

- **No prior use of psychostimulants.**
- **No current use of psychoactive medication or medication that could interact with AMPH or PHNO** (Ss will explicitly consent NOT to use any psychoactive herbal, holistic or over-the-counter medications (e.g., cold medicines, Tylenol with codeine) that could interact (modify the kinetics or dynamics of) HAL, FLU or DEX for the entire duration of the study (4 weeks: test session 1-4). These include: Kava, valerian, St. John’s Wort, Ginseng, Gingko Biloba, Salvinorin A.)
- Women will not be pregnant or nursing and will complete a urine pregnancy test at the start of each test session.
- At least grade 7 English comprehension.
- No personal/family history of schizophrenia or bipolar disorder.
- No hypersensitivity to sympathomimetic amines or aspirin.
- No low hemoglobin (Hb < 14 gm/dL).
- No hypertension (resting SBP/DBP > 140/90).
- Volunteers with obesity (> 25% mean bodyweight for their gender, height and age will be excluded.
- No metal or paramagnetic prostheses or implants.
- No radiation exposure in workplace or prior nuclear medicine protocols.

Specific Medication-related Contraindications:

**HALOPERIDOL**
- Severe CNS depression, lesions of the basal ganglia, history of spastic disorders or Parkinson’s disease, patients with hypersensitivity to haloperidol.
- Volunteers with cardiovascular disease, hepatic or renal impairment, Diabetes, Orthostatic hypotension, Hypokalemia, Thyrotoxicosis, Agranulocytos, mild and transient, Leukopenia will be excluded.
- Volunteers taking medications that can prolong the QTc, have anticholinergic properties and medications such as phenytoin or rifampin, erythromycin, fluoxetine, fluvoxamine, itraconazole, ketoconazole, paroxetine, quinidine, lithium, levodopa will be excluded.

**FLUPHENAZINE**
- Volunteers must have no known sensitivity to fluphenazine nor can they be taking another phenothiazine.
- **Volunteers at risk for hypotension (the elderly >65 years; or those who misuse alcohol), with abnormal ECG will be excluded.**
- Volunteers who may be exposed to extreme heat or cold will be alerted and must explicitly consent to NOT expose themselves to extreme heat or cold for 24 hours after each test session.
- Volunteers who would be screened positive for exposure to organophosphate insecticide must explicitly consent to NOT expose themselves to these insecticides for 24 hours after each test session.
- Volunteers taking any of the following medications will be excluded: medications that modify CYP2D6: CYP2D6 Inducers and Inhibitors, Levodopa, Lithium, Metoclopramide, Antipsychotics, Anticonvulsants, Anticholinergics, Antidepressants, Tricyclic, Antihypertensives.
- Volunteers who have severe CNS depression due to CNS medications, with a history of head trauma with associated loss of consciousness, those with bone marrow depression and those who are comatose will be excluded.
- Volunteers with brain tumor, any abnormalities in hepatic function, with respiratory difficulties agranulocytosis, hypocalcemia, obstruction or Reye’s syndrome, at risk for retinopathy, with either glaucoma or prostatic hypertrophy or suspicion of any of these syndromes will be excluded.
DEXEDRINE®

- Advanced arteriosclerosis symptomatic cardiovascular disease, cardiomyopathy, moderate to severe hypertension, hyperthyroidism, myocardial infarction, ventricular arrhythmia, hypersensitivity or idiosyncrasy to sympathomimetic amines, history of drug abuse, glaucoma, diabetes, concomitant treatment with MAO inhibitors.
- Patients with known hypersensitivity to dextroamphetamine or to any ingredient in the formulation or component of the container.
- Volunteers with ***history of allergy to ASA*** will be excluded.
- Known interactions with amphetamines are as follows:
  - Synergistic Interactions: tricyclic antidepressants, MAO inhibitors, meperidine, norepinephrine, phenobarbital, phenytoin, propoxyphene, acetazolamide, thiazides, gastrointestinal and urinary alkalinizing agents.
  - Antagonistic Interactions: adrenergic blockers, antihistamines, antihypertensives, chlorpromazine, ethosuximide, guanethidine, haloperidol, lithium carbonate, methenamine, Veratrum alkaloids, gastrointestinal and urinary acidifying agents.
APPENDIX F:
Flow Chart of Subject Recruitment, Eligibility and Group Assignment
131 phone calls received

23 eligible after telephone screen
  3 no shows for interview

20 attended interview
  7 ineligible at interview

13 attended physical exam
  3 excluded post-physical

50 subjects previously enrolled
  10 subjects enrolled

60 subjects total

30 PG
  15 HAL
  15 FLU

30 HC
  15 HAL
  15 FLU