COMPARATIVE EFFECTS OF A D2 AND MIXED D1-D2 DOPAMINE RECEPTOR ANTAGONIST ON AMPHETAMINE REINFORCEMENT IN PATHOLOGICAL GAMBLERS AND HEALTHY CONTROLS.

By

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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Comparative Effects of a D2 and Mixed D1-D2 Dopamine Receptor Antagonist on Amphetamine Reinforcement in Pathological Gamblers and Healthy Controls.

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This study used the D2-preferring dopamine antagonist, haloperidol (3mg) and D1-D2 antagonist, fluphenazine (3mg) to investigate the roles of D1 and D2 receptors in d-amphetamine (20-mg) reinforcement in humans with (9 M; 7 F) and without (12 M; 4 F) an addictive disorder, in a placebo-controlled, between-within counterbalanced design. To preclude neurotoxicity, pathological gamblers served to evaluate effects of addiction status. Incentive motivation (e.g., Desire to Gamble), hedonic impact (e.g., Liking) and risky decision-making were assessed. Haloperidol reduced Desire to Gamble in controls, whereas fluphenazine reduced Desire in gamblers. Both antagonists reduced hedonic impact in both groups, with fluphenazine exhibiting stronger effects in gamblers. Both antagonists decreased risky decisions in controls but increased risky decisions in gamblers. Results suggest that D1 mediates amphetamine-induced motivation to gamble; D2 mediates amphetamine’s hedonic effects; D1 function is deficient in gamblers; and D2 blockade may reverse a restorative effect of amphetamine in addicted individuals.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AMPH</td>
<td>Amphetamine</td>
</tr>
<tr>
<td>PG</td>
<td>Pathological Gambler</td>
</tr>
<tr>
<td>HC</td>
<td>Healthy Control</td>
</tr>
<tr>
<td>ARCI</td>
<td>Addiction Research Centre Inventory</td>
</tr>
<tr>
<td>POMS</td>
<td>Profile of Mood States</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analog Scale</td>
</tr>
<tr>
<td>CAMH</td>
<td>Centre for Addiction and Mental Health</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>MAO</td>
<td>Monoamine Oxidase</td>
</tr>
<tr>
<td>SOGS</td>
<td>South Oaks Gambling Screen</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual for Mental Disorders, 4th Edition</td>
</tr>
<tr>
<td>SCID-DSM-IV</td>
<td>Structured Clinical Interview for DSM-IV</td>
</tr>
<tr>
<td>CPP</td>
<td>Conditioned Place Preference</td>
</tr>
<tr>
<td>GO-RT</td>
<td>Go-Reaction Time</td>
</tr>
<tr>
<td>STOP-RT</td>
<td>Stop-Reaction Time</td>
</tr>
<tr>
<td>HAL</td>
<td>Haloperidol</td>
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<tr>
<td>FLU</td>
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1. Introduction

1.1. Background and Rationale

Amphetamine (α-methylphenethyl-amine) is the prototypic psychostimulant that produces a range of pharmacological and behavioral effects on users of the drug (Kuczenski, 2009). Dextro-amphetamine (AMPH), an optical isomer of the original compound is the predominant pharmaceutical form of the drug (Heal, 2009). AMPH was introduced commercially in the 1930s and quickly became popular for its therapeutic role in the treatment of nasal congestion, obesity and depression. Its abuse liability, however, soon became apparent. In fact, several epidemics of AMPH abuse in the United States, Sweden, and Japan have occurred (Kuczenski, 2009; Blum, 1984). Since then, copious research has investigated the biochemical basis of AMPH reinforcement in laboratory animals, and to a lesser extent in human volunteers. To date, relatively few studies have examined the biochemical basis of AMPH reinforcement in addicted individuals, and particularly those who are not primarily addicted to stimulants. Such research would enable a determination of the aspects of the AMPH stimulus that contribute to its generic reinforcing properties as well as features that may make this drug particularly reinforcing to addicted individuals. Finally, by evaluating AMPH effects in addicted individuals who are not stimulant users, restoration of chronic AMPH-induced deficits and conditioned interoceptive properties can be ruled out as explanations for the differential value the drug holds for addicted individuals – that is, what makes AMPH a prototypic drug of abuse.

The overall goal of this thesis is to elucidate the roles of the individual dopamine receptors in AMPH reinforcement in humans who have an addictive disorder, but have never been exposed to stimulants. By reducing receptor availability via pharmacological blockade, one can better understand its functional significance (Brauer and de Wit, 1994,1995; Wachtel et al., 2002; Zack and Poulos, 2007). In their review of evidence linking dopamine in pathological gambling and psychostimulant addiction, Zack and Poulos (2009) outlined a wide body of research that has shown that human pathological gamblers have similar neurochemical dysfunction as individuals addicted to stimulants. Thus, this group provides a good model for testing the roles of the dopamine receptors in putatively ‘addicted’ individuals who have not been exposed to stimulants. A group of healthy control subjects will provide a comparison for these observations.

To provide a context for this investigation, I will first present a summary of findings on the pharmacology and neurochemistry of d-amphetamine, with particular attention to the
monoamines – serotonin, norepinephrine and dopamine. I will then focus on the dopamine system more specifically in light of its central role in reinforcement and outline what is known about the roles of different dopamine receptors in AMPH reinforcement in animals and humans.

Based on the literature, I will propose a hypothesis regarding the respective roles of dopamine D1 and D2 receptors in AMPH reinforcement, and possible differences that may exist between healthy individuals and pathological gamblers in this regard.

1.2. Review of Literature

1.2.1. The Effects of AMPH

1.2.1.1 The Effects of AMPH on Non-dopamine Neurotransmitters

The pharmacological effects of AMPH are a result of its presynaptic action on nerve terminals to increase levels of the biogenic amine transmitters (dopamine, norepinephrine, and serotonin) (Nichols, 1994). While its most significant behavioural effects are regulated by dopamine, AMPH also has been shown to have some affinity for the presynaptic noradrenaline reuptake transporter (NAT) and serotonin reuptake transporter (SERT), and hence an ability to increase synaptic concentrations of both norepinephrine and serotonin (Heal, 2009; Kuczenski, 2009; Nichols, 1994). The affinity for monoamine transporters varies depending on the specific chemical form of AMPH. Briefly, methamphetamine and AMPH/d-amphetamine have similar affinities for the dopamine transporter; however the potency of AMPH at the serotonin transporter is weaker in comparison to methamphetamine (Hilber, 2005). It has been shown that only high doses of AMPH can disrupt the norepinephrine-containing vesicles in such a way as to release transmitter to the cytosol. Since the final stage of norepinephrine synthesis occurs within the vesicle, the cytoplasmic concentration of norepinephrine is normally negligible. Therefore at low doses of AMPH, the increases in extracellular norepinephrine likely occur through simple AMPH blockade of norepinephrine uptake (Hilber, 2005, Kuczenski, 2009).

The effects of AMPH on glutamate transmission appear to be more complex. Xue et al. (1996) reported delayed increases in glutamate release in response to AMPH in the ventral tegmental area (VTA) of the brain. Wolf and Xue (1988) noted that direct administration of AMPH into the VTA resulted in an initial decrease in efflux of glutamate, followed by an increase subsequent to removal of the stimulant. Jones and Kauer (1999) concluded that acute administration of AMPH reduces glutamate release, suggesting that the later glutamate efflux observed by Wolf and Xue
was due to inhibition of glutamate reuptake rather than an increase in glutamate efflux. Additionally, Jones and Kauer (1999) noted that serotonin receptor antagonists blocked this effect and thus the depression in glutamate release was regulated by serotonin. Of the remaining classic neurotransmitters, AMPH has been shown to depress GABAergic transmission via D2 (Centonze et al., 2002), promote release of endogenous opioids, which is believed to contribute to dopamine release in certain parts of the brain (Schad et al., 2002) and increase striatal acetylcholine levels indirectly through the release of dopamine (Ladinsky, 1975; Imperato, 1993).

1.2.1.2. The Effects of AMPH on the Dopaminergic System

The focus of this thesis will be on the dopamine-mediated effects of AMPH, which play a predominant role in its behavioral effects. The effects of AMPH on dopamine occur both inside and outside the nerve terminal. The primary actions of AMPH are a result of AMPH acting as a competitive substrate with dopamine at the dopamine reuptake transporter (DAT) at the presynaptic terminal. When AMPH interacts with DAT, it causes a displacement of dopamine from the storage vesicles to the cytoplasm in the presynaptic neuron (Heal, 2009). Since AMPH is chemically lipophilic, it has the ability to enter the neuron by simple diffusion across the plasma membrane as well as through DAT. Once inside the neuron at an adequate concentration, the lipophilicity of AMPH (a weak base) allows it to enter the acidic vesicle to subsequently become protonated and thereby disrupt the pH that provides the electrochemical gradient that retains the transmitter (Floor, 1996, Sulzer, 2005). Furthermore, AMPH also has been shown to bind the vesicular monoamine transporter-2 (VMT-2), which performs the vesicular uptake of dopamine (Fone, 2005). These two processes contribute to a largely enhanced cytoplasmic pool of dopamine. In an action potential-independent mechanism, the displaced catecholamine is subsequently released into the synaptic cleft by a process called reverse transport via DAT, thus increasing the dopamine concentration in the synapse (Heal, 2009). Moreover, while DAT is occupied by AMPH its ability for reuptake of dopamine into the nerve terminal is reduced, further augmenting synaptic dopamine levels. AMPH has also been shown to inhibit the actions of monoamine oxidase (MAO) in the presynaptic cleft, which acts to metabolize dopamine and other catecholamines. This provides an additional mechanism of preventing dopamine clearance (Miller, 1980). In combining these two mechanisms, AMPH results in a significant and prolonged increase in dopamine concentration available for interaction with post-synaptic as well as pre-synaptic dopamine receptors.
1.2.1.3. Acute Behavioural Effects of AMPH in Animals

AMPH produces a broad spectrum of behavioural effects, including alterations of cognitive, affective, and motor processes. The psychomotor activation and rewarding/hedonic effects of AMPH have been identified in a number of mammals; however rodents have been studied the most thoroughly and systematically (Segal and Kuczenski, 1994; Robinson and Becker, 1986). Acute behavioural responses to AMPH vary based on the administered dose. As per Segal and Schuckit (1983), low doses (0.5-1.5mg/kg) produce a marked enhancement in locomotor activation including varied horizontal and vertical movements. When the dose is increased (1.5-2.5mg/kg) the locomotion enhancement becomes less varied and more perseverative in that rodents continually follow the same paths and patterns. That is, while there is an enhancement in locomotor activation, it is often interrupted by episodes of repetitive acts, referred to as stereotypy. This is often manifested in repetitive sniffing and/or repetitive head and limb movements. At even higher doses (2.5-7.5mg/kg), it has been noted that there is an initial increase in locomotor activity before an intense continuous stereotypy phase where there is an absence of locomotion, which is then followed by another phase of enhanced locomotor activity after the drug has been metabolized. The duration of these phases varies depending on the administered dose (Segal and Schuckit, 1983; Kuczenski and Segal, 1994). Research has shown these effects to depend on the actions of both dopamine and norepinephrine within the medial septal area, lateral hypothalamus, and medial preoptic area sub-cortical regions of the brain (Kuczenski, 2009). Several researchers have noted comparable behavioural characteristics in other mammals such as primates (Ellinwood et al., 1973). A wide range of evidence predominantly developed in rodents has implicated dopamine as the principal neurotransmitter in AMPH-induced motor effects. Specifically, increases in dopamine levels in major projections of the dopamine pathway such as the nucleus accumbens are responsible for mediating locomotor effects (Kuczenski, 2009; Santerre et al., 2012, Delfs et al., 1990), while the caudate (receiving dopaminergic transmission from the substantia nigra) mediates the stereotypic effects. (Cho et al., 1999; Kuczenski, 2009)

1.2.1.4. Chronic AMPH Effects

1.2.1.4.1. Sensitization In Animals

In the case of repeated, intermittent administration of AMPH, the motor behaviours induced by the first treatment show a sensitized or augmented response to subsequent doses. With respect to
AMPH, *sensitization* (also referred to as reverse tolerance) denotes an enhancement of the locomotor and stereotypic effects with repeated exposure to the drug. Interestingly, sensitization to AMPH is quite persistent – once an animal is sensitized, it remains hypersensitive to the psychomotor activation effects of drugs for months or years (Browne and Segal, 1977; Segal and Schuckit, 1983). An example of this can be seen in the work of Segal and Schuckit (1983). In low doses (0.5-1.5 mg/kg), 5-10 days of daily administration results in a progressive increase in locomotion relative to acute administration. At intermediate doses (1.5-2.5mg/kg), the duration of stereotypy is increased after the second administration, and after 3-5 days, a continuous stereotypy phase begins. In addition, post-stereotypy hyperactivity is enhanced as well. At the high dose range (2.5-7.5mg/kg), stereotypy appears more rapidly and is intensified by the second injection, and post-stereotypy locomotion is enhanced progressively depending on the dose also.

It is important to note that repeated AMPH administration does not result in a unitary response modification and that there is enormous individual variation in susceptibility to sensitization depending on genetics, as well as hormonal and experiential factors (Segal and Schuckit, 1983; Segal & Kuczenski, 1994). The effects of sensitization on behaviour appear to arise from changes in certain brain nuclei. Specifically, there appear to be neural restructuring and neurochemical adaptations in the nucleus accumbens (Nordquist, 2008) and prefrontal cortex (Selemon, 2006; Kuczenski, 2009). Much work has shown that augmented dopamine release is a key (although not exclusive) factor in the spectrum of changes that occurs during sensitization. A key study by Robinson et al. (1988) looked at sensitization using escalating doses of AMPH that were not neurotoxic in an animal model designed to mimic the pattern of drug use associated with development of addiction. They found that pretreatment with escalating doses of AMPH produced a persistent hypersensitivity in the locomotor effects of the stimulant. Most importantly, this behavioural sensitization was accompanied by an enhancement in ventral striatal dopamine neurotransmission. Various presynaptic mechanisms have been proposed to mediate the enhanced dopamine response, such as alterations in the terminal auto-receptors (Kalivas and Stewart, 1991) the distribution of intracellular dopamine (enhanced releaseability of dopamine) (Kalivas and Duffy, 1993, Paulson, 1995, Pierce, 1997) as well as alterations in the dopamine reuptake transporter (which would inhibit reuptake of dopamine) (Pierce and Kalivas, 1997; Benmansour et al., 1992). However, most data suggest that the increase in dopamine neurotransmission is due to the former - an increase in the releaseability of dopamine (Kuczenski, 2009, Pierce and Kalivas, 1997). It is important to note that a wide body of research has shown that augmentations of neurotransmitter systems other than dopamine contribute to
sensitization such as, norepinephrine, serotonin, acetylcholine, and glutamate (Segal & Kuczenski, 1994; Tien and Ho, 2011). Nevertheless, enhanced dopamine efflux appears to represent a critical aspect of behavioural sensitization, and studies showing that concomitant treatment with dopamine antagonists prevents sensitization to AMPH, underscore the pivotal role of dopamine. (Kuczenski, 2009; Ujike at al, 1989; Kuribara and Uchihashi, 1993). Since the brain regions altered by sensitization are known to be involved in reward and incentive motivation, sensitization is believed to be a key component in controlling factors related to AMPH abuse such as compulsive drug seeking, and craving. It has been suggested that the neuronal adaptations associated with the process of sensitization contribute to the process of addiction (Robinson and Berridge, 2001; Kuczenski, 2009, Nishikawa et al, 1983). Sensitization to AMPH has been reported in many mammals including primates, and evidence shows that sensitization, in response to repeated doses of AMPH, also occurs in healthy humans (Boileau et al., 2006).

1.2.1.4.2. Tolerance to Amphetamine

Repeated exposure to AMPH does not exclusively lead to sensitization. Many effects of AMPH can undergo tolerance with chronic use. This has been shown in its effects on anorexia (Carlton and Wolgin, 1971), as well as its sympathomimetic (Day and Rand, 1963) and hyper-thermic effects (Sever et al., 1974). The hedonic effects also appear to undergo tolerance. Evidence shows that this tolerance does not have a pharmacokinetic basis, as regular users appear to have similar drug metabolism profiles as naïve subjects (Leith and Barrett, 1976; Kuczenski, 2009). It is likely that tolerance to the hedonic effects of AMPH contributes to the progressive dose escalation that occurs in abusers attempting to maintain initial levels of pleasure from the drug (Kuczenski, 2009).

1.2.1.4.3. Psychosis

Chronic use of AMPH at high doses can lead to the development of schizophrenia-like symptoms such as paranoia, delusions, as well as auditory and tactile hallucinations. The development of these symptoms appears to be related to the amount and duration of drug use, but is more commonly seen in individuals who binge or use the drug throughout the day for several days (Robinson and Becker, 1986). Most data indicate that these symptoms resolve after discontinuation of AMPH administration and thus are likely a result of the neurochemical augmentations induced by the drug (Robinson and Becker, 1986; Kuczenski, 2009), although
psychotic episodes can be reinstated in abstinent methamphetamine abusers when exposed to acute stressors (Yui K et al., 2002).

1.2.1.5. Behavioural Effects of AMPH in Healthy Humans

In healthy humans, acute exposure to AMPH is known for its principal effect of inducing arousal, which refers to the extent to which an organism is responsive to environmental stimuli, and alertness. It has been suggested that AMPH-induced arousal is dependent on both dopamine and norepinephrine in the prefrontal cortex (Berridge CW, 2006). These alerting effects make AMPH an effective clinical treatment for narcolepsy (Parkes and Fenton, 1973). Furthermore, low doses of AMPH are effective at treating attention deficit/hyperactivity disorder due to a range of cognitive effects such as enhanced rate of information processing, as well as an ability to increase attention span and working memory (Heal, 2009). At high doses, human effects on locomotion manifest as movement without an apparent purpose, but are more complex than in rats. For example, human users may repeatedly reposition or sort objects, or compulsively wash their hands (Kuczenski, 2009). Although slower systemic routes, such as oral, can promote elevated mood, rapid-delivery methods such as intravenous or inhalation, which produce marked increases in brain levels of AMPH, result in more intense pleasurable feelings, and are therefore often the preferred route of abusers (Kuczenski, 2009; McGregor and Roberts, 1994).

1.2.1.6. AMPH and Rewarding Effects

The rewarding properties of AMPH have been indexed in animals using a paradigm called conditioned place preference (CPP). A CPP procedure consists of a simple experimental chamber that has been spatially divided into two distinguishable compartments. Animals are periodically exposed to each compartment – one is paired with a reward stimulus (AMPH administration for example) and the other is paired with a neutral stimulus (e.g., saline). After repeated exposures, rats that display a preference in time spent in the compartment previously paired with the reward stimulus (in the absence of reward) are thought to be demonstrating a learned association between the environment and reward (Presley et al, 2010). CPP studies have been used to show the drug reward properties of psychostimulants including AMPH in a variety of mammals (McGregor and Robert, 1994). Notably, pharmacological manipulation and lesion studies have shown a central role for dopamine in mediating AMPH-induced CPP. Spyraki et al., (1982), Mackey and Van der Kooy, (1985) and Mithani et al., (1986) have all noted that AMPH-induced CPP could be negated by pretreatment with dopamine antagonists.
The reinforcing effect of AMPH – that is, the willingness to work to obtain more of the drug - is evident in animal self-administration studies (Mcgregor and Roberts, 1994). Many of these studies provide evidence of the involvement of dopamine in the control of AMPH self-administration. Indeed, treatment with the D2 dopamine antagonist, pimozide has been well established to interfere with the reinforcing properties of AMPH during self-administration. Wilson and Schuster (1972) showed that pretreatment with a dopamine antagonist caused an increase in AMPH intake in monkeys. Risner and Jones (1976) showed that pretreatment with antagonists caused a dose-dependent increase in AMPH intake in dogs. Such effects have been produced in the rat as well (David and Smith, 1974; Yokel and Wise, 1975, 1976). Yokel and Wise suggested that the increase in AMPH intake following pre-treatment with a dopamine antagonist represents a compensatory response to a decrease in drug potency. This is in line with findings by Pickens and Thompson (1968), where animals self-injected at a faster rate on low unit injection doses – animals had the ability of maintaining a relatively constant level of drug intake across a wide range of dose levels by adjusting the infusion interval. Despite these studies, our understanding of the role of dopamine in the reinforcing action of AMPH remains incomplete.

A more sophisticated method of indexing the reinforcing properties of AMPH in animals can be achieved with self-administration progressive ratio schedules. The key feature of the progressive ratio schedule is that the response requirements for the animal (number of lever presses needed to receive an infusion) increase systematically until the performance of the animal falls below the necessary level (the breaking point), and the animal no longer responds. This allows investigators to study the maximum response requirement that will support self-administration behaviour - the maximum effort the animal will expend to receive an infusion of the drug (Richardson and Roberts, 1996). Dopamine has been clearly implicated in supporting self-administration behaviour in the progressive ratio paradigm. Izzo et al. (2001) found that either a partial D2 agonist or D2 antagonist blocks AMPH self-administration in a progressive ratio schedule. That is, the animal will not exert as much effort for a drug infusion of AMPH, as reflected by a lower breakpoint, when dopamine D2 receptors are occluded. Thus, whereas animals that receive a dopamine D2 antagonist will increase AMPH self-administration when doing so causes a proportional increase in drug delivery (fixed ratio), when the ability to restore dopamine transmission by compensating behaviorally is foreclosed (progressive ratio), self-administration (break point) declines.
1.2.2. Dopamine and Reward

1.2.2.1 Dopamine Involvement in AMPH-induced Reward and Reinforcement in Humans

The mesolimbic dopamine pathway is one of the primary pathways that mediate reward (Berridge, 2007, Wise 2009). The dopamine pathway originates in the ventral tegmental area of the midbrain and innervates structures in the limbic system such as the nucleus accumbens, the prefrontal cortex and amygdala (Berridge, 2007). In addition to AMPH, other drugs of abuse have been shown to modulate the dopaminergic system at some level, with the mechanism depending on the particular drug class and interaction with differing molecular targets (Koob, 1992). As discussed earlier, AMPH results in a direct enhancement in dopamine concentration in the synapse by causing reverse transport of dopamine via interaction with DAT (Heal, 2009). On the other hand, opiate narcotics such as heroin for example, indirectly enhance dopamine efflux in the mesolimbic system by decreasing activation of the GABA-ergic system (resulting in disinhibition), which ultimately leads to an enhancement in dopamine release in the nucleus accumbens (Johnson and North, 1992). Another example is the indirect activation of dopamine neurons by nicotine via stimulation of the nicotinic acetylcholine receptors in the ventral tegmental area (Nester, 2005). Moreover, human positron emission topography studies have consistently shown that a variety of drugs of abuse, in addition to stimulants (Volkow et al. 1999, Drevets et al. 2001), lead to an increase in dopamine release in the ventral striatum. Specifically, marijuana (Bossong et al., 2009), nicotine (Montgomery et al., 2007), and alcohol (Boileau et al., 2003) have also been shown to lead to increases in ventral striatal dopamine levels. Studies of healthy volunteers have also shown that the most intense “high” or euphoria is seen in individuals who show the greatest drug-induced enhancement in dopamine levels (Volkow et al., 2009), although the causal role of dopamine in this relationship remains unclear.

1.2.2.2. Incentive-Sensitization and Addiction in Humans

The pivotal role of dopamine in psychoactive drug effects is well understood. However, the precise roles that dopamine plays in incentive motivation, reward and reinforcement remain controversial (Berridge, 2007). Initially, dopamine release was believed to be responsible for the hedonic effects of a drug (‘liking’), which was believed to be the cause of its addictive potential (Wise and Bozarth, 1987). Additionally, it was thought that increases in dopamine release could be associated with the desire to avoid negative symptoms i.e., relief from aversive withdrawal symptoms (Markou et al., 1993). A great amount of research in this area revealed the functional
limitations of these models. Specifically, the pleasure derived from a drug often declines even as the motivation to seek it increases in addicted individuals (Robinson and Berridge, 1993, 2000).

Robinson and Berridge addressed this inconsistency and suggested that the process of addiction is mediated by sensitization of the reward pathway (Robinson and Berridge, 1993, 2000, 2001; Berridge and Robinson, 1995). As mentioned before, sensitization refers to an enhancement in behavioural and neurochemical response to a stimulus upon repeated exposure to that same stimulus. The Incentive-Sensitization model of addiction (Robinson and Berridge, 2001) postulates that chronic exposure to addictive drugs leads to ‘incentive’ sensitization which refers to the enhanced ability of cues associated with the drug to become more attractive (salient) and highly ‘wanted’. Thus, according to Robinson and Berridge (2001), neurochemical changes caused by sensitization in the dopamine system mediate drug craving and compulsive drug seeking – incentive motivation – in chronic drug users.

Robinson and Berridge (1993, 2001) make the distinction between the term ‘Liking,’ which refers to the euphoric/hedonic effects of a reinforcing stimulus, and ‘Wanting,’ which refers to the incentive motivation and craving for the reinforcer. Thus, the authors suggested that the enhanced dopamine levels associated with sensitization may mediate the ‘Wanting,’ (incentive salience attribution) component but not the ‘Liking’ (pleasurable) component of the reinforcing stimulus. The neural systems mediating ‘Liking’ do not appear to sensitize, and this may explain the observation that as addiction develops, drugs can become pathologically craved and sought after even as a given dose of the drug is enjoyed less and less. The idea that craving of a drug is distinct from its hedonic effects is supported by the observations from a study by Leyton et al. (2002). Using positron emission tomography, these investigators found that dopamine levels in the mesolimbic pathway of healthy volunteers correlated significantly with self-reported drug ‘Wanting,’ as opposed to drug ‘Liking’ following a dose of AMPH.

1.2.2.3. Dopamine and its Receptors

Dopamine is in the catecholamine family of neurotransmitters. Its synthesis initially requires the amino acid l-tyrosine, which then undergoes a series of biochemical reactions until useable dopamine is formed. Once dopamine synthesis is completed within the neuron, it is then packaged into the vesicle (Segal and Kuczenski, 1994). The contents of the vesicle are released either by depolarization or, as discussed earlier, by a stimulant drug such as AMPH. Deactivation occurs by dopamine reuptake via DAT, and can then either be re-stored in the vesicles via
VMAT-2, or broken down enzymatically by MAO. (Heal, 2009) Dopamine acts on two principal types of receptors. Namely, D1-like (D1, D5) and D2-like (D2, D3, D4), which will now be referred to as D1 and D2, respectively. These two receptor subtypes exert opposing effects on the enzymatic activity of adenylate cyclase, which helps convert ATP to cyclic-AMP (cAMP), a molecule responsible for second messenger neurotransmission. Stimulation of D1 receptors activates adenylate cyclase, whereas D2 receptor stimulation results in inhibitory effects on the synthesis of c-AMP. D1 receptors are primarily located outside synapses on the post-synaptic neuron and have a relatively low affinity for dopamine (Shultz, 1998). Due to their lower affinity for dopamine, these receptors normally respond to stimulus-induced, dopamine bursts (phasic transmission). (Caille et al., 1996) Conversely, D2 receptors are primarily located within the synapse on the pre-synaptic as well as the post-synaptic neurons and have a relatively higher affinity for dopamine (Shultz, 1998). Thus, D2 receptors respond to basal dopamine release (tonic transmission) and are saturated during phasic release (Caille et al. 1996).

1.2.2.4. Dopamine Receptors in Chronic Stimulant Users

In the healthy brain, stimulation of D1 and D2 is balanced in that these receptors have both cooperative and countervailing effects (Shi et al., 1997). With chronic hyper-activation of dopamine, as seen in conditions such as schizophrenia or Huntington’s disease, the D1-D2 interactive linkage seems to be disrupted (Seeman et al., 1989). Chronic exposure to stimulant drugs such as cocaine and AMPH may pharmacologically induce similar dysfunction. In animal studies, chronic AMPH (Chen et al., 1999) and cocaine (Volkow et al., 2004) exposure has been shown to reduce D2 receptor availability, a potential manifestation of AMPH sensitization (Chen et al., 1999). Additional animal studies with rats have provided evidence of long-term reduction of both D1 and D2 receptor binding with chronic application of cocaine (Nickolaus et al., 2007). Similarly in humans, post-mortem studies of methamphetamine abusers found a 25-30% reduction in the ability of dopamine to stimulate adenyl cyclase via D1 stimulation (Tong, et. al., 2003), and deficits in D2 receptor binding have been reliably seen in substance abusers using positron emission tomography (Volkow et al., 1999). Thus, structural and functional deficits in D1 and D2 receptors coincide with chronic stimulant use.
1.2.2.5. D1 Receptors and Reward

In healthy humans, D1 receptors regulate many aspects of cognition such as attention, set-shifting (revising one’s response to conform to a change in the criterion) and working memory (ability to simultaneous retain and manipulate information) (Seamans and Yang 2004). Animal studies have shown critical roles for D1 in mediating the rewarding properties of cocaine (as evidenced by CPP) and D2 in the incentive motivational properties for cocaine (as evidenced by progressive ratio responses). Self et al. (1996) found that D2 agonists caused cocaine-seeking behaviour in rat models. In contrast, D1 agonists actually prevented cocaine-seeking behaviour in rats, which they suggest reflects ‘satiation’ of the reward pathways. In human cocaine abusers, data have been gathered supporting a clear role of D1 in cocaine reward. An acute dose of the D1-specific antagonist ecopipam dose-dependently reduced cocaine’s stimulant and euphoric effects, but also led to a dose-dependent decrease in craving in stimulant abusers (Romach et al., 1999). However, chronic administration of ecocipam led to the exact opposite pattern of effects (Haney M, et al. 2001). This same group found that an acute dose of a D1 agonist significantly reduced the subjective pleasurable effects of cocaine (High, Stimulated), but did not alter self-administration of the drug in a laboratory setting (Haney et al., 2001). Thus, preventing dopamine from reaching the D1 receptor or saturating that receptor can each reduce the rewarding properties of a stimulant drug, but tolerance appears to develop quickly to the first effect, while the latter effect does not appear to translate into reduced drug taking.

Animal research provides some insight into the effects of stimulants on D1 function. Chronic cocaine exposure in rats is thought to promote tolerance to the rewarding effects of D1-receptor stimulation (Self, 1998), leading to an increase in cocaine self-administration (Edwards et al. 2007). Direct down-regulation of D1 receptors may partially account for this effect. However, Grace (2000) also proposed that chronic drug use would enhance tonic dopamine levels, resulting in preferential stimulation of high affinity D2 auto-receptors. The resulting increase in negative feedback would attenuate phasic dopamine release, further reducing the net dopamine signal at D1. Although chronic D1 blockade could conceivably up-regulate D1 receptors, acute blockade of D2 auto-receptors would directly remove negative feedback and thereby restore strong phasic D1 activation in chronic stimulant abusers. Such an effect should be functionally similar to acute D1 agonism, i.e., greater satiation of the reward pathways (Self et al., 1996).
1.2.2.6. Inverted U Relationship Between D1 Activation and Cognition/Reward

In their review of the principal features and mechanisms of dopamine modulation and cognition in the prefrontal cortex, Seamans and Yang (2004), noted that there is an inverted-U (bell shaped) function relating cognitive performance and D1 stimulation level. That is, the functional effects of D1 receptor activation are optimal at some point and deviations above as well as below this value lead to sub-optimal cognitive function. Given the evidence linking stimulant reward to D1 stimulation, it is possible that there is also a baseline-dependent inverted U relationship between D1 stimulation and the rewarding effects of a stimulant such as AMPH. If this is the case, subjects with low baseline D1 activation given AMPH might have a shift towards more optimal D1 activation and thus find the drug particularly rewarding (i.e., pleasurable). In contrast, subjects who have optimal or near-optimal baseline levels of D1 activation might find a dose of AMPH less pleasurable or aversive due to supra optimal D1 activation. This logic provides a framework for interpreting existing research on the effects of dopamine antagonists on stimulant reinforcement in healthy human volunteers.

1.2.3. Roles of the Dopamine Receptor Subtypes (D1 and D2) in AMPH Reinforcement

Despite the pervasive use of AMPH as a probe for dopamine release, experimental sensitization agent, and prototypic drug of abuse, there is only limited research into the roles of the individual dopamine receptor subtypes (D1 and D2) on the cognitive and motivational effects of AMPH in human subjects. Furthermore, little research appears to have examined the roles of D1 and D2 receptors in the effects of AMPH in subjects with an addictive disorder.

An early investigation examining how specific dopamine receptor antagonists affect response to AMPH in healthy human subjects was performed by Brauer and de Wit (1995, 1996). The dopamine antagonists used in their study were pimozide and fluphenazine (FLU). The affinity of these drugs for each of the receptors is indexed by their Ki value (inhibition constant), where lower scores indicate a greater affinity for the receptor (Christensen et al, 1984). The Ki values of pimozide and FLU at the D2 receptor are both <3 indicating that they are each potent D2 antagonists. The Ki values of pimozide and FLU at the D1 receptor differ in that FLU has high affinity (Ki<1), whereas pimozide has very low affinity (Ki=250) (See Appendix A, Table 1 and 2). Based on this binding profile, FLU can be considered a mixed D1-D2 antagonist and pimozide can be considered a selective D2 antagonist. Brauer and de Wit (1995, 1996) employed several scales that measured drug-induced euphoria, to assess the effects of AMPH after pre-
treatment with each antagonist. A tool consistently used for measuring drug-induced euphoria in these types of studies is the Morphine-Benzodrine (MBG) scale of the Addiction Research Centre Inventory (ARCI; Haertzen 1965).

1.2.3.1 Effects of Pimozide

When subjects were pretreated with 1-mg of pimozide before AMPH administration (10-mg), MBG ratings were elevated relative to placebo. When subjects were pretreated with 2-mg of pimozide prior to a dose of AMPH, MBG scores did not increase as much as they did with 1-mg of pimozide. In addition, when 20-mg of AMPH was used, the highest scores in MBG were seen when there was no pimozide pretreatment. In fact, the effects of 20-mg AMPH on MBG scores decreased in direct relation to the increase in pimozide doses. The finding of increased reward with low dose pimozide and low dose AMPH but decreased reward with high dose pimozide and high dose AMPH may derive from optimal and supra-optimal D1 signaling and rewarding effects, respectively (i.e., an inverted U relationship), as proposed above.

1.2.3.2. Effects of Fluphenazine

In a companion study, Brauer and de Wit (1995) used varying doses of FLU in combination with 20-mg of AMPH in healthy volunteers. They observed that AMPH alone increased MBG scores relative to placebo. When 3-mg of FLU was given before AMPH, there was a slight upward shift in MBG scores, but when pretreated with 6-mg of FLU before AMPH, there was a downward shift in MBG scores. This pattern contrasts with the pattern for pimozide which dose-dependently decreased MBG scores from 20-mg AMPH. Given the similar Ki values for pimozide and FLU at D2, it seems likely that the differential effect of the antagonists on AMPH-induced euphoria is due to their differing affinity for D1. Thus, partial D1 blockade with 3-mg FLU may have optimized D1 activation in response to 20-mg AMPH in healthy subjects with optimal or near optimal baseline D1 stimulation. The higher dose of FLU (6 mg) would have led to greater effects on AMPH-induced dopamine release due to its stronger effects on D2 autoreceptors (blockade of inhibitory negative feedback), but would also have led to greater antagonism of post-synaptic D1. These two actions could in effect have canceled each other, leading to a situation similar to placebo pretreatment before AMPH administration. Together, the bidirectional effects of low vs. high dose FLU on responses to 20-mg AMPH, coupled with the progressive dampening effects of increasing doses of pimozide on responses to 20-mg AMPH, are consistent with the hypothesized inverted-U relation between D1 activation and subjective
rewarding effects of a stimulant drug.

1.2.3.3. Effects of Haloperidol

In a subsequent study from the same research group, Wachtel et al. (2002) investigated effects of the preferential, high affinity D2 antagonist haloperidol (HAL) on methamphetamine reinforcement in healthy volunteers. The Ki values for HAL at D2 and D1 are <3 and 17 respectively. Thus HAL has similar affinity for D2, but substantially less affinity for D1 than FLU (see Appendix A, Table 1 and 2). This suggests that at the same dose, the enhancement in dopamine release due to D2 auto-receptor blockade under HAL will be similar to that which occurs with FLU; however, there would be greater stimulation of D1 with HAL than with FLU. Relative to placebo pretreatment, 3-mg HAL had a negligible effect on methamphetamine-induced (20-mg) MBG scores. In addition, Wachtel et. al. (2002) had subjects complete the Digital Symbol Substitution Task which measures working memory (linked with D1) at baseline and after methamphetamine. They found that methamphetamine with placebo pretreatment improved performance, but when pretreated with 3-mg HAL, performance was equivalent to that achieved at baseline. These results may also be explained by the inverted-U relationship between D1 activation and stimulant effects. Specifically, the lack of enhancement in MBG scores and in performance on the Digital Symbol Substitution Task may be due to a change in D1 activation from slightly sub-optimal to slightly supra-optimal under HAL, with no net change in reward or cognitive efficiency in subjects with near optimal baseline D1 activation. In this case, it is likely that the differing effects for equal doses of FLU and HAL reflect the differences in their D1 binding affinity. It is important to note however, that the results of HAL pertain to methamphetamine whereas the FLU results pertain to AMPH (d-amphetamine). In fact, there appear to be no previously published studies of the effects of HAL on responses to AMPH in healthy human subjects.

1.2.4. Effects of AMPH in Sensitized Populations

Investigating the effects of AMPH in addicts is difficult due to the possible presence of drug or alcohol-induced neurotoxicity, which could confound the acute effects of experimental drugs. Experimental animals have shown persistent alterations in dopamine neurons that innervate the dorsal striatum after exposure to acute, high doses of stimulants such as AMPH (McCann and Ricaurte, 2004). This is manifested as reduced basal levels of dopamine, its biosynthetic enzymes, as well as both DAT and VMAT-2 (Refer to Gibb et al., 1994; Seiden and Ricaurte,
1987; Lew et al., 1997; McCann and Ricaurte, 2004 for review). Morphological studies strongly indicate that reductions in presynaptic dopamine axonal markers are related to destruction of dopamine axons and axon terminals (McCann and Ricaurte, 2004, Ellison et al., 1978). Accumulating evidence suggests that oxidative stress by drug-induced increases in reactive oxygen/nitrogen radicals and high levels of oxidized cytoplasmic dopamine (caused by drug-induced disruption of vesicular storage) play a primary role in mediating these neurotoxic effects (Lotharius and O'Malley, 2001; Yamamoto and Zhu, 1988). In humans, neuroimaging studies have shown impairments in DAT, a marker of striatal dopamine nerve terminals, in chronic high-dose methamphetamine abusers (McCann et al., 1998). Evidently, chronic stimulant abuse can lead to functional damage or injury to striatal dopamine terminals, and these consequences may contribute to persistent impairments in cognitive/motor functioning seen in previous AMPH addicts (Kuczenski, 2009). Interestingly, research has also shown that even small doses of stimulants like AMPH can contribute to long lasting sensitization in healthy humans (Boileau et al., 2006). In order to examine possible differences in the roles of D2 and D1 receptors in humans who may be ‘sensitized’ to AMPH but have not undergone the neurotoxicity associated with chronic stimulant or alcohol exposure, one can evaluate individuals with a putative behavioural addiction, namely pathological gambling (PG).

1.2.5. Neurobiology of Pathological Gambling

1.2.5.1. Evidence Pathological Gambling is Similar to Chronic AMPH Exposure

Zack and Poulos (2004) compared the effects of AMPH in a group of healthy controls, PG subjects, comorbid gambler-drinkers, and non-gamblers with an alcohol use disorder. They used Visual Analog Scales (VAS) to assess addictive motivation and subjective effects including incentive motivation and euphoria and a computer-based rapid reading task to assess reactivity to words (salience attribution) from motivationally relevant (e.g., wager, whisky) and irrelevant (e.g., window) semantic domains. They found that 30-mg AMPH increased self-reported motivation to gamble in PG subjects and also improved their relative response time to gambling versus neutral words on the reading task (i.e., enhanced salience attribution). The severity of PG was directly proportional to the positive subjective effects of AMPH and its ability to enhance motivation to gamble. Conversely, AMPH did not augment the motivation for alcohol or response time to alcohol-related words on the reading task in gamblers, drinkers, or controls, indicating selective motivational effect of AMPH in PG. When one drug (e.g., AMPH) increases
motivation for another drug (e.g., cocaine), the effect is termed “cross-priming,” and is thought
to indicate a common neurochemical basis for the reinforcing effects of the two drugs (Schenk
and Partridge, 1999). The AMPH data for PG subjects indicate cross-priming of motivation and
semantic memory networks between a stimulant drug and gambling, and thus suggest a
commonality in the neurochemical basis of PG and stimulant addiction.

To the extent that gambling and stimulants recruit common neurochemical processes, chronic
exposure to gambling could conceivably result in repeated activation of dopaminergic neurons as
would occur with chronic AMPH exposure. If so, PG subjects may have a sensitized dopamine
system, akin to animals chronically exposed to low doses of AMPH. However, because no agent
enters the brain during gambling, dopamine levels never exceed physiological levels, so that the
potential for neurotoxicity is greatly reduced compared to stimulant addiction, where high doses
capable of inducing supra-physiological dopamine release are commonly used. The possibility
that gambling exposure can lead to sensitization is supported by recent neuroimaging data
showing that PG subjects exhibit increased striatal dopamine release in response to a slot
machine game in direct relation to the severity of their PG symptoms, a coarse index of ‘chronic
exposure to gambling’ (Joutsa et al., 2012).

Given that the subjective reinforcing effects of AMPH in healthy humans are proportional to
striatal dopamine release (Martinez, 2009), and that increased striatal dopamine release to
AMPH is a marker for sensitization, an increase in reinforcing effects of AMPH in PG subjects
(relative to healthy controls) would indirectly support the existence of sensitization in PG
subjects. Accordingly, Zack and Poulos (2004) found that AMPH resulted in greater reported
‘Good Effects’ and ‘Desire to Take AMPH Again’ in PG subjects vs. healthy controls.

1.2.5.2. The Role of the D2 Receptor in Gambling

Given the evidence suggesting common neurochemical substrates for gambling and stimulant
reinforcement in PG subjects, Zack and Poulos (2007) extended this research to investigate the
role of the D2 dopamine receptor in gambling reinforcement. Previous neuroimaging studies had
found deficits in D2 receptor binding (indicative of lower availability) in individuals addicted to
cocaine, methamphetamine, heroin or alcohol (Volkow et al., 2004). This suggested that
addiction is linked with deficits in D2 availability, and that PG may involve a similar deficit. The
inverse correlation between D2 levels and craving for the preferred drug in Volkow’s studies
further suggested that low D2 might be functionally related to addictive motivation. Zack and
Poulos (2007) explored this possibility by comparing the effects of 3-mg HAL on responses to a 15-min episode of gambling on a commercial slot machine, situated in a mock bar, in PG subjects and healthy controls. Under placebo, the slot machine game significantly increased desire to gamble in both PG subjects and controls. Haloperidol alone had negligible subjective effects in either group; however, in PG subjects, playing the slot machine after pre-treatment with HAL significantly increased scores on all pleasurable effects scales (Enjoyment, Excitement, Involvement) relative to playing the game under placebo. In contrast, in controls, HAL had no apparent effect on responses to the slot machine, relative to placebo. Additionally, in PG subjects, but not controls, HAL significantly increased post-game Desire to Gamble relative to placebo. Lastly, HAL increased facilitation of reading speed to gambling vs. neutral words (i.e., salience) in PG subjects but not controls. In summary, 3-mg HAL had a clear ability to enhance gambling reinforcement, but only in PG subjects.

Pucak and Grace (1994) noted that small acute doses of typical antipsychotics such as HAL bind preferentially to D2 auto-receptors. As a result, HAL would be expected to disrupt the negative feedback mechanism and increase phasic dopamine release into the synapse (Pehek 1999). Shi et al., (1997) noted: “if an antipsychotic drug blocks only D2 receptors, the increased dopamine release would lead to a selective stimulation of D1 receptors” (p. 7993). Thus, increased stimulation of D1 receptors during the slot machine game may account for the effects of HAL in PG subjects seen by Zack and Poulos (2007). The possible mediating role of D1 stimulation in the effects of HAL on gambling reinforcement in PG subjects can be investigated by comparing responses under equivalent doses of HAL vs. FLU.

1.3. Rationale for Employment of Haloperidol and Fluphenazine

Appendix A shows Ki values (inhibition constants) for HAL and FLU at both D1 and D2 (primary relevance in this study) and other receptors (lower scores are indicative of greater binding affinity). While both drugs have similar binding affinity at the D2 receptor (Ki<1 for each), FLU has higher affinity for D1 (Ki<1), while HAL has a considerably lower affinity for D1 (Ki=17). The D1/D2 ratio, which provides a measure of relative affinity (selectivity) of the drugs, with larger scores indicating a stronger affinity for D2 was 2.1 for FLU, and 28 for HAL. Thus, FLU is a mixed D1-D2 antagonist with high affinity for both receptors, whereas HAL is a preferential high affinity D2 antagonist (See Appendix A, Table 1 and 2). Appendix A, Tables 3-8 also shows that the drugs are well matched on affinity for other dopamine receptors. HAL has
low, and FLU modest affinity for serotonin (5-HT) receptors. Also, HAL and FLU have similar low affinity for muscarinic and alpha-2 noradrenergic receptors, and a similar moderate affinity for alpha-1 noradrenergic receptors. The only obvious difference in non-dopamine binding profiles was for histamine (H1) receptors, where HAL has low, and FLU has moderate affinity.

It is important to note that, although a selective D1 antagonist would be a logical choice for comparison to a preferential D2 antagonist, there are no selective D1 antagonists approved for human use in Canada. Hence the proposed research can only address the role of D1 blockade during concomitant D2 blockade. The role of D1 in the absence of D2 blockade is an important question, but is beyond the scope of this project. Additionally, it may be argued that a more selective D2 antagonist such a pimozide, used by Brauer and de Wit (1995), should be used. However this drug is also not available for human use in Canada. HAL is the most selective D2 antagonist approved for human use in Canada, and there exists a wide body of literature characterizing this drug. As well, Zack and Poulos (2007) were able to safely and effectively use HAL to obtain significant effects on gambling reinforcement. This provides an empirical foundation for the proposed study.

1.4. Pharmacokinetics of Haloperidol and Fluphenazine

HAL and FLU belong to the class of typical antipsychotic drugs and are marketed under the names Haldol® and Prolixin® respectively. Both drugs have a similar pharmacokinetic profile (Jorgensen, 1986). Oral bioavailability is reduced by a first-pass effect in the gastrointestinal mucosa or the liver. For FLU, the oral bioavailability is shortened to 40-50% and 60-65% for HAL (Froemming et al. 1989). Bioavailability is increased by intramuscular injection with HAL; however FLU has a slow intramuscular bioavailability due to the ester formation that occurs with the alcohol substituent at the end of the molecule. When FLU is injected, the esters remain as an oil drop in muscle tissue diffusing out slowly due to poor solubility in the tissue. This can be taken advantage of clinically by reducing the inconvenience of having to take a daily pill and ensures patient adherence to the pharmacotherapy (Mamo et al, 2007).

Multiple pathways of biotransformation are important in metabolizing antipsychotic drugs before they are excreted in the urine, such as sulfoxidation, N-dealkylation, ring hydroxylation, and glucoronide conjugation. Studies show that the greatest proportion of the intrinsic hepatic clearance of HAL is by glucuronidation, followed by reduction of HAL to reduced HAL and by cytochrome P450 (CYP)-mediated oxidation (Kudo and Ishizaki, 1999). In vitro research has
shown that CYP 3A4 appears to be the major CYP isoform responsible for HAL and FLU metabolism, and there are no functional polymorphisms in CYP 3A4 identified for the metabolism of these drugs (Froemming, et al., 1989; Dysken et al., 1981). Furthermore, in vivo pharmacogenetic studies have shown that HAL and FLU metabolism may be regulated by genetically determined polymorphic CYP 2D6 activity. There are over 60 functional polymorphisms that have been identified for CYP 2D6 metabolism. In fact, a small percentage of Caucasians (~ 7%) are considered ultra-fast or slow metabolizers of HAL (Brockmöller et al., 2002). Both HAL and its metabolites have been shown to be potent inhibitors of CYP 2D6, and similar selectivity for CYP 2D6 inhibition has been reported with FLU (Shin et al., 1999).

For HAL, peak plasma levels are reached 1.7-3.2 hours after oral administration (Verghese et al. 1991). For this study, the same strategy used by Wachtel et al, (2002) was used, with AMPH administered 2.75 hours after HAL. For FLU, peak plasma levels are reached 2 hours after administration (Midha et al. 1983), which is when Brauer and de Wit (1995) administered AMPH, and which we have adopted for this study. The plasma elimination half-lives for these drugs is in the range of 10-30 hours, however if body fat accumulates a large store of the drug, traces of the drug and/or its metabolites may continue to appear in urine for weeks or months after the final dose (Dahl, 1990). Because these drugs are sequestered in lipid compartments of the body and have very high affinity for selected neurotransmitter receptors in the central nervous system, they generally have a longer clinical duration of action than would be implied from their plasma half-lives (Jann et al, 1985, Mamo et al, 2007). The interval between test sessions in this study will be 1 week, so that carryover effects should be negligible.

1.5. Pharmacokinetics of AMPH

The amphetamine group of drugs has high lipophilicity and is readily absorbed from the gastrointestinal tract so it can be administered orally as well as parenterally. Because they are lipid soluble, they can cross cell membranes quite readily, including the blood-brain barrier. Biotransformation occurs mainly in the liver and involves several processes: hydroxylation of the phenyl ring, deamination, and conjugation reactions (Heal, 2009; Kalant, 2007). The amphetamine metabolites as well as a considerable amount of unchanged drug are eventually excreted in the urine. For the purposes of this study, 20-mg of oral AMPH (d-amphetamine) has been shown to have maximal subjective-behavioral effects at 90 minutes and maximal blood levels after 120 minutes (Brauer and de Wit, 1996). The half-life of the drug varies between 12 and 18 hours (Kalant, 2007).
1.6. Specific Aims of the Current Study

The contribution of the individual dopamine receptor subtypes (D1 and D2) in AMPH reinforcement in PG subjects’ remains undetermined. This is especially important given that PG subjects may represent a sensitized population but with the added virtue of being free of drug-induced neurotoxic effects. In order to delineate the roles of D1 and D2 in AMPH reinforcement, we will adopt the strategy employed by de Wit and colleagues. Accordingly, in the present study, HAL (3-mg) and FLU (3-mg), were used as pharmacological probes to test the roles of D2 and D1, respectively in AMPH reinforcement.

1.7. Hypotheses

**Hypothesis 1:** If stimulation of D2 auto-receptors exclusively mediates the rewarding, reinforcing, and cognitive effects of AMPH, HAL and FLU, which have similar affinity for D2, should reduce these effects of AMPH to a similar degree, compared to placebo.

**Hypothesis 2:** If stimulation of D1 receptors mediates the rewarding, reinforcing, and cognitive effects of AMPH, then HAL should enhance these effects, compared to placebo (by removing inhibitory feedback at D2 auto-receptors), whereas FLU should negate this enhancement (i.e., no difference from placebo) or reduce these effects, relative to placebo.

**Hypothesis 3:** (a) If the relationship between D1 activation and rewarding, reinforcing and cognitive effects of AMPH corresponds to an inverted U, HAL-induced enhancement and FLU-induced reductions in AMPH effects should be more pronounced in PG subjects, reflecting lower baseline D1 function. (b) Furthermore, FLU, but not HAL may enhance rewarding, reinforcing and cognitive effects of AMPH, relative to placebo in control subjects, by offsetting supra-optimal AMPH-induced D1 stimulation in individuals with near optimal baseline D1 function.
2. Materials and Methods

2.1. Study Overview and Design

The study was approved by the Research Ethics Board of the Centre for Addiction and Mental Health (CAMH) (Study Number: RN 52207) and was conducted in accordance with the Declaration of Helsinki (1989). All subjects provided written informed consent before participating (See Appendix C).

The study employed a placebo-controlled, counter-balanced, between-within design (see Figure 1 below). The between-subject variables were Gambler Status (pathological gambler, PG; healthy control, HC), and Drug Group (haloperidol, HAL; fluphenazine; FLU). These groups were crossed to yield four subgroups. The primary within-subject variable effect was the type of Pre-Treatment (antagonist vs. placebo). The order of Pre-treatment was double blind and randomly counterbalanced across subjects. Stimulant reinforcement was assessed based on responses to 20-mg oral Dexedrine ® (dextroamphetamine; AMPH) on each test session.

Subjects attended 6 test sessions in total: a pre-experimental interview to ensure eligibility, a physician’s exam and two procedurally identical test sessions testing amphetamine reinforcement at a one week interval. Subjects underwent two additional test sessions to assess gambling reinforcement under antagonist and placebo, which took place before the two AMPH test sessions. Data from those sessions are not discussed here.

Figure 1: Graphical view of study design
2.2. Subjects

2.2.1. Subject Recruitment Profile

As per figure 2 below, 1130 phone calls inquiring about the study were received in response to recruitment advertisements (HC and PG subjects combined). Of these, 357 said they would be interested in completing the study and thus underwent a telephone screening. Of those screened 116 were considered eligible and invited for a pre-experimental interview at CAMH. A total of 88 subjects underwent the pre-experimental interview and 45 of these were considered eligible and underwent a comprehensive physical examination by a CAMH physician. Of these subjects, 39 were considered eligible after the physical examination and underwent testing. Three HC subjects dropped out of the HAL group (two due to adverse side effects, and one that moved away during testing) and three PG dropped out of the FLU group (all three obtained full time employment during testing) yielding a total of 16 subjects (8 PG, 8 HC) that completed testing in the HAL group and a total of 16 subjects (8PG, 8HC) that completed testing in the FLU group.

**Figure 2:** Graphical flow-chart showing subject recruitment. *N = 16 subjects were assessed by another experimenter; n= 16 subjects were assessed by the author.
2.2.2 Subject Recruitment Media

Subjects were recruited by newspaper advertisements (Jobs Classified) and Internet advertisements (kijiji.ca and craigslist.org). See Appendix B for study advertisement used for recruitment of HC and PG subjects.

2.2.3. Subject Compensation

Subjects were informed that they would receive $920 for their participation. If a subject was ineligible after completion of the psychiatric screening they were given 20 dollars and 2 TTC tokens to cover travel to and from CAMH. If a subject dropped out for any reason, they were compensated on a pro-rated basis. As an incentive to gamble as they might in a real casino, subjects were told they would receive a cash bonus proportional to their winnings on the slot machine game during the test session, payable at the end of the study. We awarded a $80 standard bonus, making total compensation $1000 ($920 + $80) for completing the study. To deter impulsive use of cash in hand to gamble, subjects were paid by cheque 2-3 weeks after study completion.

2.3. Screening

2.3.1. Telephone Screening

Potential subjects initially underwent a telephone screening, and were assessed on the following inclusion criteria (See Table 1 for a summary):

- Between the ages of 19 and 65 years old
- Not seeking treatment for pathological gambling
- Physically and mentally healthy
- Body mass index (BMI) less than 35
- PG subjects scored 5 or above on both the South Oaks Gambling Screen (SOGS; Lesieur and Blume, 1987) and DSM-IV questionnaire for pathological gambling (DSM-IV-PG; Beaudoin and Cox, 1999).
- Healthy controls scored 0 on both SOGS and the DSM-IV questionnaire.
- Grade 7 level English fluency or above (A score of >18 on Wechsler’s Vocabulary scale: WAIS-Vocab; Weschler, 1981)
- Normal or corrected-to-normal vision
Exclusion criteria included:

- Substance abuse or dependence (including alcohol),
- Any prior psychostimulant use
- A score greater than 10 on the Beck Depression Inventory-short form (BDI-sf; Beck and Beck, 1972), to ensure against clinically relevant depression.
- A score greater than 13 on the Alcohol Dependence Scale (ADS; Skinner and Allen, 1982) to ensure against clinically relevant aspects of alcohol misuse.
- Any mental or physical health problems (including diabetes, hypertension, liver cirrhosis, liver failure, epilepsy, asthma, previous heart attack, angina).
- Immediate family history (sibling, parent, child) of schizophrenia and/or bipolar disorder, to minimize potential of psychotic response to AMPH.
- Current use of any psychoactive medications (antidepressants, anxiolytics, etc.).
- Smoking more than 20 cigarettes/day (to minimize nicotine withdrawal during testing where 4-hr abstinence was required).
- Consumption of more than 20 (men) or 15 (women) standard alcoholic beverages/week, the cutoff values for non-problem drinking (cf. Sobell and Sobell 1992).
- Females could not be pregnant or breastfeeding.
Table 1: Summary of Telephone Screening Criteria

<table>
<thead>
<tr>
<th>Scoring Criteria</th>
<th>Pathological Gamblers (PG)</th>
<th>Healthy Controls (HC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>19-65</td>
<td></td>
</tr>
<tr>
<td>Cigarettes/day</td>
<td>&lt;20</td>
<td></td>
</tr>
<tr>
<td>Alcoholic drinks/week</td>
<td>&lt;20 male, &lt;15 female</td>
<td></td>
</tr>
<tr>
<td>Caffeinated beverages/day</td>
<td>&lt;8</td>
<td></td>
</tr>
<tr>
<td>Alcohol Dependency Scale (ADS)</td>
<td>&lt;13</td>
<td></td>
</tr>
<tr>
<td>Beck Depression Inventory (BDI)</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>WAIS-Vocabulary</td>
<td>≥18</td>
<td></td>
</tr>
<tr>
<td>Past and Present drug use</td>
<td>No current medications, no prior use of psychostimulants, &lt;1 marijuana cigarettes/month, &lt;2x ecstasy/hallucinogen use.</td>
<td></td>
</tr>
<tr>
<td>South Oaks Gambling Scale (SOGS)</td>
<td>≥5</td>
<td>0</td>
</tr>
<tr>
<td>DSM-IV Pathological Gambling</td>
<td>≥5</td>
<td>0</td>
</tr>
</tbody>
</table>

2.3.2. Interview Screening

At the pre-experimental interview, The Structured Clinical Psychiatric Interview for DSM-IV (SCID-I; First et al., 1995) was administered by the experimenter in the presence of a psychiatrist. Subjects afflicted with any Axis-I disorder, aside from PG or nicotine dependence, were excluded. The consultant psychiatrist (with expertise in PG) confirmed the presence of a current PG diagnosis according to the SOGS and the DSM-IV criteria. Additionally subjects were administered the drug abuse screening test (DAST; Skinner 1984) to confirm lack of drug use (excluded if >4), and the Fagerstrom Test for Nicotine Dependence (FTND; Heatherton et al., 1991). At the end of the pre-experimental interview, subjects underwent an electrocardiogram (ECG), as well as blood and urine toxicology screens. Prior to inclusion, subjects underwent a comprehensive physician’s exam including approval of the results from the ECG, blood and urine toxicology screens.
2.4. Apparatus and Materials

**J4X-ALERT (Alcohol Countermeasures Inc., Mississauga, Ontario, Canada):** A handheld breathalyzer was used to confirm that the subject had a blood alcohol concentration of 0 at the beginning of each test session.

**HEM-601 wrist-cuff monitor (HEM-601; OMRON, Vernon Hills, IL):** This was used to measure systolic and diastolic blood pressure and heart rate at regular time intervals throughout every test session in order to index physiological response to the medications and experimental procedures.

**Desktop PC equipped with MicroExperimental Laboratories (MEL) software (v. 2.01; Psychology Software Tools Inc., Pittsburgh, PA, USA) connected to an external microphone:** This was used for the Rapid Reading Task (RRT). The software measured vocal response time (ms) to Gambling-related and control words. A serial response box (Psychology Software Tools Inc. Pittsburgh, PA, USA) was used to measure the accuracy of vocal responses by the experimenter.

**Quickvue pregnancy test kit (Quidel Corporation, San Diego, California, USA):** Pregnancy tests were administered at the beginning of every test session to all female participants in order to ensure that no fetus would be exposed to the study medications.

**Haloperidol/Fluphenazine (HAL/FLU):** Depending on Drug Group, subjects were given either 3-mg of haloperidol (Haldol®; HAL) or 3-mg of fluphenazine (Prolixin®; FLU) on drug days. The doses were given in 3 individual 1-mg capsules. On the other test day they received placebo (distributed in 3 capsules visually identical to the active drug). The medication schedule was maintained by the pharmacy at CAMH to preserve double-blind conditions.

**Commercial slot machine (‘Cash Crop’; WMS Gaming Inc., Chicago):** A commercial slot machine, situated in a mock-bar laboratory, was used to provide subjects with an opportunity to gamble. This standardized the treatment during the latter half of the test session with the treatment administered on test sessions 1 and 2 (no AMPH). Provision of an opportunity to gamble not only permitted comparison of pre-gambling data on the AMPH sessions with data from non-AMPH sessions, 1 and 2; it also helped to avoid potential negative affect that can result when addicted subjects are ‘primed’ for their target reinforcer, but denied access to it (Carter and Tiffany, 2001; Davidson et al., 2003; Mackillop and Lisman, 2005). Subjects played
for 15 minutes without supervision. They were allotted 400 cash credits (equivalent to $100; 25 cents per credit) with which to bet at the start of the game. Subjects were told they would receive a monetary bonus proportional to their final credit tally in the game, which would be paid at the end of the study. This provided an incentive for subjects to play the game as they normally would, in terms of trying to maximize winnings.

The object of the slot machine game was to get as many of the same symbols on a single line as possible. Subjects used a touch screen to select any combination of horizontal, diagonal, or vertical lines up to a maximum of 9 lines on any one spin. They could bet anywhere from 1 to 5 credits per line making the maximum bet per trial 45 credits. The more credits they chose per trial, the higher the probability of winning, but also the larger the loss if none of the lines paid off.

A cable feed from the slot machine to an adjacent room electronically recorded the number of credits wagered and won on every trial. Subjects were not aware that their betting pattern was being recorded, to ensure that it would not interfere with the spontaneity of their game play. Final credit count (i.e., winnings) was used as a covariate in statistical analyses to control for the possible impact of this variable on incentive motivational or hedonic effects of the game.

**Dextro-Amphetamine:** On each test session, subjects were given 20-mg oral dextro-amphetamine sulphate (Dexedrine®; AMPH) in 4 separate capsules, when subjects reached expected peak blood levels for the respective antagonists: 2 hours after FLU or 2 hours and 45 minutes after HAL.

### 2.5. Questionnaires

#### 2.5.1 Screening Scales

**The Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I, First et al. 1995):** This was used for making the major DSM-IV Axis I diagnoses that would be considered exclusion criteria for the study. The SCID was administered during the pre-experimental interview and was supervised by the consulting psychiatrist for the study, a clinician-scientist at CAMH trained in SCID administration.

**South Oaks Gambling Screen (SOGS, Lesieur and Blume 1987):** This is a validated 16-item questionnaire used to assess presence of gambling pathology. It is based on the third edition of the Diagnostic and Statistical Manual for Mental Disorders-III-r (APA, 1987) criteria for
pathological gambling and offers a reliable and convenient means to screen clinical populations of alcoholics, drug abusers, and the general population for pathological gambling. Eleven of the 16 items are scored and the maximum score is 20. A score of 5 (or more) denotes “probable pathological gambling.” This questionnaire was administered 2 times: orally on the telephone screening and then in writing – its validated format – as part of a questionnaire package completed at the pre-experimental interview.

Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM-IV) based problem gambling questionnaire (DSM-IV Pathological gambling, Beaudoin and Cox 1999): In order to verify clinically-relevant gambling pathology, the 10-item DSM-IV Pathological Gambling scale was administered both orally on the telephone and during the pre-experimental interview in the written questionnaire package. This scale defines the formal criteria for diagnosing PG and confirms the presence of PG symptoms within the past 30 days.

Beck Depression Inventory Short Form (BDI-sf, Beck and Beck 1972): This 13-item scale is a validated method of detecting depression symptoms in primary care settings. This scale was administered on the telephone screening as an initial method of excluding potentially depressed subjects. Callers who scored ≥10 or ≥1 on a question about suicide were excluded and referred to the Mood and Anxiety Disorders Clinic at CAMH. Lack of depression was confirmed again on the pre-experimental interview by administration of the BDI-sf in the written questionnaire package.

Alcohol Dependence Scale (ADS, Skinner and Allen 1982): This is a 25-item scale that provides an assessment of alcohol use and consequences over the past 12 months. This scale is consistent with the DSM-III for alcohol dependence syndrome. It was administered both on the telephone screen orally and in a written questionnaire package on the pre-experimental interview. A score <9 indicates absence of alcohol use disorder (Ross et al, 1990).

Drug Abuse Screening Test (DAST, Skinner 1982): This 20-item self-report scale was administered on the pre-experimental interview as part of the written questionnaire package to assess presence of drug abuse. Answers were ‘yes or no’ and each question indicative of drug abuse was scored as 1 point. Score ≥4 is indicative of clinically relevant drug abuse.

Wechsler Vocabulary Test (Wechsler 1981): This was used to measure subjects’ proficiency in English. Subjects were asked to define common English words of increasing complexity. This
was necessary to ensure that cognitive task results and self-report scales were not affected by lack of English comprehension. Answers were scored from 0-2 for each word. Half of the items were administered during the telephone screening; the other half during the pre-experimental interview to confirm the results.

2.5.2. Trait Scales
All trait scales were administered in a self-report written questionnaire package during the pre-experimental interview.

**Eysenck Impulsiveness Questionnaire (EIQ, Eysenck et al. 1985):** This scale consisted of 54 “yes” or “no” questions assessing Impulsiveness (scores from 0-16), Venturesomeness (scores from 0-16), and Empathy (scores from 0-19).

**Eysenck Personality Inventory (EPI, Eysenck and Eysenck 1963):** This scale consisted of 57 “yes” or “no” questions that assessed: Extraversion (scores from 0-24), Neuroticism (0-24), and the tendency to Lie (0-9). The Lie scale tested tendency to respond in a socially acceptable manner instead of truthfully, which helped to detect possible dissimulation.

**Fagerström Nicotine Dependence (FTND, Heatherton et al. 1991):** This is a 6-item questionnaire to assess the severity of nicotine dependence. Only subjects who indicated that they smoked were evaluated. A score of 1-2 indicates “very low dependence”; a score of 3 indicates “low to moderate dependence”; a score of 4 indicates “moderate dependence”; and a score of 5 or greater indicates “high dependence”. Each subgroup was matched on nicotine dependence.

**Nicotine Timeline Followback (Lewis-Esquerre et al. 2005, Toll et al. 2006):** Subjects who were regular smokers were asked to complete this assessment. They were asked to indicate on a calendar which days they smoked cigarettes and the quantity smoked within the 7 days preceding the interview. Groups were matched across Drug Group (HAL vs. FLU) and Gambler Status, based on the results.

**Alcohol Timeline Followback (Sobell and Sobell 1992):** Subjects were asked to indicate on a calendar which days they consumed alcohol, and the quantity consumed (number of standard drinks), within the 90 days preceding the pre-experimental interview.
Gambler’s Beliefs Questionnaire (GBQ, Steenbergh et al. 2002): This is a 21-item scale measuring gambler’s erroneous beliefs and cognitive distortions. The questionnaire consisted of two factors: luck/perseverance and illusion of control. For example, the belief that the gambler can influence a positive outcome of a chance-determined event (“My choices or actions affect the game on which I am betting”), and the gambler’s fallacy, which is the notion that future outcomes are dependent on past outcomes (“I should keep the same bet even when it hasn’t come up lately because it is bound to win”). Items were rated on a 7-point scale [1(strongly agree) – 4 (neutral) - 7 (strongly disagree)]. Lower scores on each subscale denote greater distortions.

2.5.3. Other Pre-Experimental Interview Measures – Basic Cognitive Functioning

Digit Span Task (Wechsler 1981): This task was used to test short-term rote and working memory. In the first part of the task (digits forward), the experimenter reads out a series of one-digit numbers and the subject must repeat the numbers orally in the same sequence. The length of the sequence (up to 9 digits) progressively increased over trials. The second part of the task (digits backward) required the subject to repeat the sequence of numbers in reverse order. Again the sequence of numbers increased as the trials progressed. For each sub-task, testing was stopped after two consecutive incorrect responses.

Wechsler Digit Symbol (Wechsler 1981): This scale measured psychomotor speed, and associative working memory. The task required subjects to match a series of numbers on a piece of paper to corresponding symbols listed in a legend at the top of the paper. Subjects were to work as quickly and as accurately as possible. The number of correctly matched symbols within the 60-second time allotment was scored.

2.5.4. Experimental Self-Report Scales

Visual Analog Scale (VAS, Fischman and Foltin 1991): These scales quantified the intensity of subjects’ incentive motivation (e.g., Desire to Gamble) as well as perceived good and bad drug effects. These scales were administered at baseline, peak blood concentration of antagonist (to assess antagonist effects), peak subjective-behavioural effects of AMPH (to test reinforcing effects) as well as 120 minutes after AMPH (peak blood concentration), and after the detoxification period. Subjects reported the extent to which they agreed with a number of statements. The scale ranged from 0-10 (with ½ increments; Not At All – Moderate – Extreme), unless otherwise noted.
Gambling: The two statements regarding feelings and attitudes towards gambling were:
(1) Right now, I desire or feel like going gambling. (2) Right now, I am confident I could resist going gambling (if there were a casino across the street).

Drug (AMPH) effects: VAS assessed Liking (-10 to +10), ‘High’, perceived Good and Bad Effects, and Desire To Take The Capsule (AMPH) Again, under antagonist and placebo.

Profile of Mood States-Short Form (POMS-sf, Shacham 1983): This questionnaire was administered concurrently with the VAS to assess transient mood effects of the antagonist and AMPH relative to baseline. This scale consisted of 37 mood-related adjectives (i.e. confused, sad, furious, etc.) and subjects were to indicate whether they believed they felt each adjective by rating 0-4; Not At All-Extremely. Six mood-related factors were calculated: (1) tension-anxiety, (2) depression-dejection, (3) anger-hostility, (4) fatigue-inertia, (5) vigor-activity and (6) confusion-bewilderment.

Addiction Research Center Inventory (ARCI, Haertzen 1965): This is a 49-item “true” or “false” scale that was administered concurrently with VAS and POMS to assess subjective psychoactive effects of antagonist and AMPH, based on standardized items for these effects, relative to baseline. The ARCI consists of five sub-scales: (1) the Amphetamine sub-scale (AMP, measures stimulant effects), (2) Morphine-Benzedrine group (MBG, measures euphoria), (3) Lysergic Acid Diethylamide sub-scale (LSD, measures dysphoria), (4) the Pentobarbital-Chlorpromazine-Alcohol group (PCAG, measures sedation), and the Benzedrine group (BG, an additional measure of stimulant-like effects).

Symptom Side Effect Checklist (Zawertailo et al. 1995): This was a 47-item scale administered at the end of each test day to assess possible side effects from the medications (e.g., headache, nausea) that might affect other outcome measures. The subject rated severity of any side effects from 0-5; (Absent – Needs Intervention).

Capsule Contents Evaluation: This was performed at the end of the study. Subjects were asked to guess which days and times they believed they received active medication vs. placebo.

2.6. Experimental Computer Based Tasks
This task was used to assess the incentive salience of target stimuli (words) shown on a computer screen by measuring latency of the subject’s vocal response. The sequence of events on each
reaction time trial was the same: First, a warning signal (i.e. & & & & ) was shown on the screen for 350 milliseconds to show the subject where the target stimulus would appear. After 250 milliseconds the target stimulus appeared in the same location. Subjects were instructed to read each word aloud as quickly and accurately as possible. The word remained on the screen until the subject made a vocal response, and then a new trial was initiated after a 550-millisecond interval. After 20 practice trials, 150 test trials were administered (5 categories with 30 words per category, randomized over trials).

The stimuli represented the following categories: (1) Gambling-related (i.e. jackpot, casino), (2) Alcohol-related (i.e. vodka, lager), (3) Positive Affect (i.e. cheerful, excited), (4) Negative Affect (i.e. upset, unhappy), (5) Neutral (i.e. ceiling, window). To enhance priming effects, the target items were degraded with asterisks (e.g., j*a*c*k*p*o*t). The task was administered at peak subjective-behavioral effects of AMPH (90 minutes post-capsule) on both test sessions.

2.6.2. Wisconsin Card Sort Task (WCST, Heaton 2003)

This task is a validated neuropsychological task used for assessing cognitive “set-shifting” – the ability to shift response strategy when faced with changes in the designated criterion response. The task presents four stimulus cards that remain at the top of the screen throughout the duration of the task and subjects are required to match a set of cards one at a time to each of these four key cards. The four key cards differed in three dimensions: color, quantity, and shape. The computer determined which dimension was the criterion for a given series of trials, during the task. The accuracy of the subject’s response (correct or incorrect) was indicated by the computer after each trial. Using this feedback the subject had to identify the correct dimension for each particular set of trials. After 10 correct responses, the computer changed the criterion dimension without informing the subject. After the first incorrect response under the new criterion dimension, the subject had to deduce that their response had to be changed to match the new criterion. The task continued until 6 categories were correctly identified. The computer recorded the number of trials that the subject required to learn each new criterion dimension. Cognitive rigidity (perseveration) was indicated by failure to adopt the new criterion dimension in a timely manner (perseverative errors). This task was administered once during the pre-experimental interview to determine if the groups differ in regards to basic neuropsychological function.
2.6.3. Game of Dice Task (GDT, Brand et al. 2005)

This task was given to assess risk-taking behavior. The computer displayed the roll of a virtual die 18 times on each of 18 individual decision trials. Each time the subject was required to guess which number would be thrown (between 1 and 6). The subject could guess a single number or different combinations of numbers. The monetary risk/reward was based on the probability of the chosen outcome. That is, if the subject chose a single number (one possible outcome, corresponding to maximal risk win-probability of 1/6), they would win $1000 if the selected number was thrown, and lose $1000 if any other number than the one selected was thrown. If two numbers were chosen (two possible outcomes with a win-probability of 2/6), they would win or lose $500 depending on the guess and number that was thrown. If three numbers were chosen (three possible outcomes with a win-probability of 3/6) the subject would win or lose $200. Lastly, if four numbers were chosen (four possible outcomes with a minimal risk win-probability of 4/6), the subject would win or lose $100. The number of possible outcomes selected operationally defined ‘risk-taking’ (from maximal risk=1 possible outcome selected to minimal risk = 4 possible outcomes selected). If a subject won a particular trial, the corresponding amount (determined by the alternative selected) was credited to their account, and losses were subtracted in the same manner if a trial was lost. Subjects began with a total of $1000. Prior to commencing the task, subjects were instructed to attempt to win as much money as possible and to avoid losing money. The computer recorded the number thrown, the alternative selected by the subject, the corresponding loss or gain as well as the remaining balance. The GDT was administered on both test sessions towards the end of the test phase to minimize potential carryover 'priming’ effects of this gambling-like task on performance of subsequent tasks.

2.6.4. Stop Signal Task (SST, Logan et al. 1997)

The Stop-Signal Task was used to assess inhibitory control of a pre-potent psychomotor response. The task required the subject to press one of two keys (“z” or “/”) with their left and right index fingers respectively as quickly and accurately as possible, depending on the visual stimulus [“a” or “b”(Test Session 1) and “c” or “d” (Test Session 2)]. Two versions of the task were used on each test session (before and after AMPH) in order to minimize repetition priming. The visual stimuli that appeared on the computer screen acted as a ‘GO’ signal. Each trial commenced with a focal point for fixation in the center of the computer screen (“+”), which appeared for 500 ms, and was quickly followed by the visual stimulus (“a” or “b” and “c” or “d) that was presented for 1000 ms. On a random 25% of the trials, a stop signal (1000-Hz tone) was
presented for 100 ms shortly after the ‘GO’ stimulus, indicating that the subject should withhold their response (not press either key). The stop signals were split evenly between “a” and “b” trials for version 1 and between “c” and “d” trials for version 2. This task was preceded by two sets of practice trials followed by 256 test trials (two blocks of 128 separated by two 40-second breaks).

The interval between the ‘GO’ and ‘STOP’ stimuli (the stop-signal delay) is the critical index of inhibitory efficiency. This interval was initially set at 250 ms and subsequently adjusted, depending on the performance of the subject. Each time a subject was successful in withholding a response (inhibition), the delay was increased by 50 ms on the next stop signal trial, which resulted in greater difficulty for the subject to inhibit their response. Conversely, in the event the subject failed to inhibit their response on a stop trial, the delay was decreased by 50 ms to make it easier for them to inhibit on the next stop signal. Over the course of the task, the adjustments in these delays were designed to result in 50% successful inhibition. The stop signal delay that corresponded with 50% successful inhibition reflected mean inhibitory efficiency. By subtracting the mean stop signal delay from the ‘GO’ response time, it was possible to determine the average time required to inhibit the response, the stop signal reaction time (SSRT). A faster ‘GO’ response time indicated greater psychomotor fluency, and faster SSRT was indicative of greater inhibitory efficiency (decreased impulsivity), controlling for overt response latency.

2.7. Procedure

2.7.1. Pre-Experimental Interview

Subjects who were eligible for either the PG or HC group were invited for a pre-experimental interview in a laboratory located at the 33 Russell Street location of CAMH. The pre-experimental interview lasted approximately 3 hours. As seen in Table 2, upon arrival, subjects were initially briefed on the study requirements. At this point, they provided written consent by signing a consent form containing detailed information about study requirements and possible side effects associated with the medications (see Appendix C). Subjects were given a copy of the signed consent form. A breathalyzer test was administered to ensure a blood alcohol concentration of 0, and blood pressure/heart rate were measured to ensure absence of hypertension. The height and weight of each subject were also measured in the laboratory to ensure a body mass index no greater than 35. Additionally, female subjects were required to perform a urine-based self-test to confirm lack of pregnancy.
Subsequent to these tests, subjects underwent a psychiatrist-supervised structured clinical psychiatric interview for DSM-IV (SCID) to ensure no psychiatric comorbidity. For potential pathological gamblers, the psychiatrist performed a professional assessment of gambling severity to ensure the subjects’ level of gambling pathology met with the study criteria. Subjects who were eligible after the SCID were asked to complete a questionnaire package including the trait scales (EPI, EIQ, FTND, Alcohol and Nicotine Timeline Followback, GBQ as well as written versions of the SOGS, DSM-IV, BDI-Sf, ADS and DST to confirm results from the telephone screening. Subjects who were excluded based on their SCID or questionnaires were dismissed and compensated with $20 and two transit tokens.

Eligible subjects then completed the Wechsler package (Vocabulary, Digit Span, Digit Symbol), followed by a series of exploratory cognitive tasks. Table 2 shows they were completed in the following order: WCST, SST, GDT.

After completion of the computer tasks, the subject was escorted to the Clinical Lab at CAMH for blood and urine tests as well as an ECG. After the results of the assays had been transmitted to and approved by the Qualified Investigator (Study Physician), the subject was scheduled for a complete physician’s exam at the Addiction Medicine Clinic to ensure they were fit to receive the study medications. Subjects whose physical exam results deviated from prescribed inclusion criteria (e.g., blood pressure > 30% above normative values) were excluded, provided with copies of their lab results, instructed to follow-up with their family physician, and compensated for their time. This concluded the screening phase. Eligible subjects were then matched on age, gender and gambling severity and randomly assigned to Drug Group (HAL, FLU) and scheduled for their test sessions. Subjects were instructed to not consume alcohol or caffeinated beverages for 12 hours before a test session, and to fast after midnight on the evenings before a test session.
**Table 2: Pre-experimental Interview Timeline**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Procedure</th>
</tr>
</thead>
</table>
| 0              | Briefing on study  
                | Consent form read and signed  
                | Breathalyzer test to ensure blood alcohol level of 0  
                | Blood Pressure/Heart Rate measurement  
                | BMI calculation |
| 40             | Structured Clinical Interview for DSM-IV Disorders (Supervised by psychiatrist, Dr. Daniela Lobo):  
                | For potential pathological gamblers, the psychiatrist verified gambling pathology. |
| 80             | Screening Questionnaire Package: (SOGS, DSM-IV-PG, BDI, ADS, EPI, EIQ, DAST, Alcohol Timeline Followback, Nicotine Timeline Followback, FTND, GBQ). |
| 120            | Wechsler’s Package (Vocabulary, Digit-Span Forward/Backward, Digit-symbol) |
| 130            | Stop Signal Task |
| 145            | Wisconsin Card Sort Task |
| 160            | Game of Dice Task |
| 170            | Blood Test (Routine Chemistry, Blood Glucose, Liver Enzymes)  
                | Urine Screen (Urinanalysis, Drug Toxicology)  
                | Electrocardiogram (ECG) |
| 200            | Dismissed |

**2.7.2. Experimental Test Sessions**

Aside from Pre-treatment (antagonist/placebo) and debriefing (end of second AMPH session), the procedure for both test sessions was identical. The test day timeline is shown in Table 3 and Figure 3. The order of pre-treatment (HAL/placebo or FLU/placebo) was counterbalanced across subjects within each group (PG, HC). Both the experimenter and subject were blind to the contents of the first capsule. The subject was blind to the contents of the second capsule (AMPH on both test sessions).

As indicated in Table 3, subjects arrived at the CAMH laboratory by 8:30AM on test days. Initially, they were briefed about the study procedure for the day. Next they received the
breathalyzer to ensure a blood alcohol concentration of 0, and also had their baseline heart rate/blood pressure assessed. Females completed a urine-based pregnancy test at this point. Subjects were then given a standardized breakfast and smokers were allowed 1 cigarette and required to abstain until testing was over for the day. Subjects then completed a baseline questionnaire package (Package A – VAS-Desire, POMS-sf, ARCI). Next, they were escorted to a waiting room where they received their first capsule. Subjects were allowed to watch movies or read in the waiting room until the blood concentration of the medication reached expected asymptotic levels (2 hours 45 minutes for HAL, Wachtel et al, 2002; 2 hours for FLU, Brauer & De Witt, 1995). A registered nurse took blood pressure/heart rate readings every 30 minutes after administration to ensure no unusual changes. 15-minutes before peak blood concentration of the antagonist, the second questionnaire package was administered (Package B – VAS-Desire, POMS-sf, ARCI).

Subjects then received their second capsule (20-mg AMPH). After administration, the nurse took a reading of blood pressure/heart rate every 15 minutes to ensure no unusual changes. At expected peak behavioral effects for AMPH (90 min post-capsule; Brauer et al. 1996), subjects were escorted back to the laboratory where they completed the RRT, which lasted approximately 15 minutes. After their blood pressure/heart rate reading, they then completed the critical drug effects scales (Package C – VAS-Desire to Gamble, VAS-Liking of Capsule-2, VAS-High from Capsule-2, VAS-Desire to Take Capsule-2 Again, POMS-sf, ARCI). Desire for Alcohol was also assessed as a control for non-specific hedonic motivation.

Next, subjects were escorted to the mock bar laboratory containing the slot machine. Subjects played the game for 15 minutes or until the credits ran out. After having their heart rate/blood pressure taken by the nurse, they filled out a fourth questionnaire package (Package-D – VAS-Desire to Gamble, POMS-sf, ARCI).

Note - Because the game occurred after the peak effects of AMPH were assessed, and administration was consistent on both AMPH sessions, differences in response to AMPH cannot be attributed to events in the game.

After the game, subjects were escorted back to the laboratory where they completed the SST, followed by the GDT. After a final set of physiological measurements by the nurse (heart rate/blood pressure), a final questionnaire package was given (Package E – VAS-Desire to
Gamble, POMS-sf, ARCI, Symptom Side Effect Checklist) and on the last test session the Capsule Contents Evaluation Sheet. Subjects were then given lunch.

Once the subject’s blood pressure and heart rate returned to baseline, the nurse made sure the subject was not feeling any adverse effects from the study medications. This typically occurred within 1.5 hours after lunch by 3:00 pm. For safety, subjects received a wallet card containing information about the medications they may have received and emergency 24-hour contact information for the Study Physician. Additionally, subjects were given a sealed 50 mg dose of diphenhydramine HCl (Benadryl®) to counteract potential delayed extrapyramidal side effects of the antagonists. They were instructed to take it only if they felt it necessary. They were reminded to not operate a vehicle or any heavy machinery for 24 hours. They were also instructed to avoid alcohol or use of drugs apart from caffeine and cigarettes for 72 hours. On the final test session, subjects were fully debriefed and given an opportunity to ask questions. PG subjects were provided with literature on problem gambling and contact information for the Problem Gambling Service at CAMH. All subjects were informed that they would receive their payment by cheque in 2-3 weeks. They were then dismissed and sent home by pre-paid taxi.
Table 3: Experimental Test Session Timeline. Subjects in the haloperidol group had an additional 45-minute wait time before peak blood concentration was reached.

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>Breathalyzer to ensure blood alcohol concentration is 0</td>
</tr>
<tr>
<td></td>
<td>Baseline Heart Rate/Blood Pressure</td>
</tr>
<tr>
<td></td>
<td>Pregnancy Test</td>
</tr>
<tr>
<td></td>
<td>Questionnaire package A (VAS-Desire to Gamble/Drink Alcohol, ARCI, POMS-sf)</td>
</tr>
<tr>
<td></td>
<td>Standardized Breakfast</td>
</tr>
<tr>
<td>0.5 h</td>
<td>Capsule 1 Administered (HAL/FLU or placebo)</td>
</tr>
<tr>
<td>2 h 15 mins (FLU) + 45 minutes (HAL)</td>
<td>Questionnaire package B (VAS-Desire to Gamble/Drink Alcohol, ARCI, POMS-sf)</td>
</tr>
<tr>
<td>2 h 30 mins (FLU) + 45 minutes (HAL)</td>
<td>Capsule 2 (20 mg d-amphetamine) administered</td>
</tr>
<tr>
<td>4 h (FLU) + 45 minutes (HAL)</td>
<td>Rapid reading task</td>
</tr>
<tr>
<td>4 h 15 mins (FLU) + 45 minutes (HAL)</td>
<td>Questionnaire package C (VAS-Desire to Gamble/Drink Alcohol, VAS-Liking of capsule-2, VAS-High from capsule-2, VAS-desire to take capsule-2 again, POMS-sf, ARCI)</td>
</tr>
<tr>
<td>4 h 30 mins (FLU) + 45 minutes (HAL)</td>
<td>Video slot machine game in mock-bar</td>
</tr>
<tr>
<td>4 h 45 mins (FLU) + 45 minutes (HAL)</td>
<td>Questionnaire package D (VAS-Desire to Gamble/Drink Alcohol, POMS-sf, ARCI)</td>
</tr>
<tr>
<td>5 h (FLU) + 45 minutes (HAL)</td>
<td>Stop Signal Task</td>
</tr>
<tr>
<td>5 h 15 mins (FLU) + 45 minutes (HAL)</td>
<td>Game of Dice Task</td>
</tr>
<tr>
<td>5 h 30 mins (FLU) + 45 minutes (HAL)</td>
<td>Questionnaire package E (VAS-Desire to Gamble/Drink Alcohol, POMS-sf, ARCI, Symptom Side Effect Checklist)</td>
</tr>
<tr>
<td>5 h 45 mins (FLU) + 45 minutes (HAL)</td>
<td>Lunch</td>
</tr>
<tr>
<td>6 h 15 mins (FLU) + 45 minutes (HAL)</td>
<td>Discharged by registered nurse</td>
</tr>
</tbody>
</table>

2.8. Data Analysis

All statistical analyses were conducted using SPSS (v. 15, Chicago, IL).
Analysis of variance (ANOVA) was used to analyze experimental effects. Equal ‘n’ in each subgroup offset concerns around heterogeneity of variance or non-normal distribution of scores with the relatively modest sample size (Howell 1992).

Simple effects analysis compared means for subject "background characteristics" and also allowed detection of significant hypothesized Pre-Treatment effects (drug vs. placebo) for key outcome measures.

Subjective questionnaire scores and physiological indices were analyzed by 2 Group (PG, HC) x 2 Antagonist (HAL, FLU) x 2 Pre-treatment (Drug, Placebo) x 4 Time Point (peak antagonist effect, peak AMPH effect, post-slots game, post-cognitive tasks) repeated measures analyses of covariance (ANCOVAs) using baseline scores and slot machine winnings as covariates.

A 2 Group (PG, HC) x 2 Antagonist (HAL, FLU) x 2 Pre-treatment (Drug, Placebo) x 5 Subscale (Liking, Good Effects, Bad Effects, High, Desire to Take Again) repeated measures analysis of variance (ANOVA) was performed for the VAS assessing the reinforcing effects of AMPH as it was only measured once at expected peak subjective-behavioral effects.

A 2 Group (PG, HC) x 2 Antagonist (HAL, FLU) multivariate analysis of variance (MANOVA) analyzed the subject background characteristics and basic cognitive functioning. A 2 Group (PG, HC) x 2 Antagonist (HAL, FLU) x 2 Pre-treatment (Drug, Placebo) MANOVA assessed slot machine betting behaviour. Word Type was included in the 2 Group (PG, HC) x 2 Antagonist (HAL, FLU) x 2 Pre-treatment (Drug, Placebo) x 5 Word Type ANOVA analyzing response time on the RRT. The data for the GDT were compiled into 3 Blocks (@ 6 consecutive trials) for analysis—in a 2 x 2 x 2 x 3 repeated measures ANCOVA with winnings from the slot machine game as the covariate. Finally, a 2 Group (PG, HC) x 2 Antagonist (HAL, FLU) x 2 Pre-treatment (Drug, Placebo) MANOVA was used to analyze each measure (Go-RT, Stop-RT) on the SST.

Due to the acknowledged limitation in power with the current sample size, effect sizes were reported for analyses that yielded only non-significant or marginal effects. By convention, small, medium, and large effect sizes were denoted by $\eta_p^2$ values of 0.01, 0.06, and 0.14, respectively (Cohen, 1988).

3. Results
3.1. Subject Background Characteristics

3.1.1 Subject Demographics

Table 4 reports the mean (SD) background characteristics scores for all 4 subgroups as determined during the initial telephone screening. A 2 (Group: PG, HC) x 2 (Antagonist: HAL, FLU) MANOVA of these scores yielded a significant group difference between PG and HC for age, F(1, 28) = 5.60, p = 0.025, which was reflective of the PG group being younger on average than the HC group. The lack of any significant effects involving Antagonist indicates that matching across these two conditions was effective, so that differences in the effects of the two Antagonists are not attributable to differences in age or any other background variables.

As expected, there were significant group differences between PG and HC in regards to scores obtained on the SOGS, F(1, 28) = 105.95, p<0.001, and the DSM-IV-PG Scale, F(1, 28) = 117.54, P<0.001. Additionally, there were group differences between PG and HC with respect to the ADS, F(1, 28) = 4.43, p = 0.044, which was indicative of the fact that the PG group on average, reported more negative consequences of drinking than the HC group did. However, it is important to note that the average ADS scores achieved by the PG group were still far from reaching clinical significance, confirming the lack of co-morbid alcohol use disorder in these subjects. With respect to BDI scores, there was a group difference between PG and HC, F(1, 28) = 33.02, P<0.001, although again both groups were below the cutoff for clinical depression. A significant interaction of Group x Antagonist, F(1, 28) = 4.836, p = 0.036 in BDI scores was observed, reflecting a larger discrepancy in mean BDI scores between HC and PG in subjects assigned to FLU vs. those assigned to HAL.

Table 4: Mean (SD) background characteristics in each subgroup: Healthy Controls assigned to the HAL group (n=8) and the FLU group (n=8) and Pathological Gamblers assigned to the HAL group (n=8) and the FLU group (n=8).
SOGS, South Oaks Gambling Screen; DSM-IV PG, Diagnostic and Statistical Manual Pathological Gambling Scale; BDI-sf, Beck Depression Inventory – Short form; ADS, Alcohol Dependence Scale; WAIS-Vocabulary, Wechsler Adult Intelligence Scale. Significant group difference, *p<0.05; Significant Antagonist difference, **p=0.019; ***p=0.036.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Haloperidol Group</th>
<th>Fluphenazine Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>PG</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>5:3</td>
<td>6:2</td>
</tr>
<tr>
<td>Age*</td>
<td>41.63 (12.39)</td>
<td>34 (9.41)</td>
</tr>
<tr>
<td>SOGS*</td>
<td>0 (0)</td>
<td>10.63 (4.41)</td>
</tr>
<tr>
<td>DSM-IV PG*</td>
<td>0 (0)</td>
<td>13.25 (4.71)</td>
</tr>
<tr>
<td>BDI-sf*,<strong>,</strong>*</td>
<td>1.00 (1.41)</td>
<td>4.13 (3.68)</td>
</tr>
<tr>
<td>ADS*</td>
<td>0.50 (1.07)</td>
<td>1.50 (1.69)</td>
</tr>
<tr>
<td>WAIS-Vocabulary</td>
<td>28.00 (3.55)</td>
<td>28.25 (1.91)</td>
</tr>
</tbody>
</table>

Significant group by Antagonist interaction, ***p=0.036.

3.1.2. Personality and Addiction-related Characteristics

Table 5 reports the mean (SD) scores of PG and HC groups on personality and addiction-related characteristics. A series of 2 (Group: PG, HC) x 2 (Antagonist: HAL, FLU) MANOVAs yielded significant group differences between PG and HC with respect to the EIQ-Impulsiveness subscale, F(1, 27) = 9.22, p = 0.05. Additionally group differences were observed on both subscales of the Gamblers’ Belief Questionnaire (lower scores = greater distortion). On the Luck/Perseverance subscale, F(1, 27) = 71.174, p<0.001; and for the Illusion of Control subscale, F(1, 27) = 73.41, p<0.001. No significant group differences were found for the Eysenck Personality Inventory–Neuroticism or Extraversion subscale. Low overall scores and lack of group difference on the Lie scale confirm that neither group exhibited a tendency to misrepresent themselves to create a favorable impression, which strengthens confidence in the accuracy of the other self-report scores. The PG group did report greater alcohol consumption as per the TLFB- drinks/week, F(1, 27) = 4.42, p = 0.045; however their average consumption per week still remained well below clinical significance.
Table 5: Mean (SD) trait characteristics in each subgroup: Healthy Controls assigned to the HAL group (n=8) and the FLU group (n=8) and Pathological Gamblers assigned to the HAL group (n=8) and the FLU group (n=8).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Haloperidol Group</th>
<th>Fluphenazine Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>PG</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>PG</td>
</tr>
<tr>
<td>EIQ – Impulsiveness*</td>
<td>2.63 (2.26)</td>
<td>6.00 (4.41)</td>
</tr>
<tr>
<td>EPI- Neuroticism</td>
<td>4.38 (3.46)</td>
<td>5.36 (3.85)</td>
</tr>
<tr>
<td>EPI- Extroversion</td>
<td>10.50 (3.30)</td>
<td>13.13 (3.27)</td>
</tr>
<tr>
<td>EPI- Lie</td>
<td>3.88 (1.96)</td>
<td>3.13 (1.64)</td>
</tr>
<tr>
<td>GBQ – Luck/perseverance*</td>
<td>85.50 (5.88)</td>
<td>54.75 (16.66)</td>
</tr>
<tr>
<td>GBQ – Illusion of control*</td>
<td>47.00 (8.26)</td>
<td>23.50 (9.21)</td>
</tr>
<tr>
<td>GBQ – Total score*</td>
<td>132.50 (11.17)</td>
<td>78.25 (25.09)</td>
</tr>
<tr>
<td>TLFB- drinks/week*</td>
<td>0.75 (0.47)</td>
<td>2.64 (2.67)</td>
</tr>
<tr>
<td>TLFB – cigarettes/week</td>
<td>0.16 (0.45)</td>
<td>0.18 (0.50)</td>
</tr>
<tr>
<td>FTND</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>DAST</td>
<td>0.63 (0.74)</td>
<td>0.25 (0.71)</td>
</tr>
</tbody>
</table>

EIQ, Eysenck Impulsiveness Questionnaire – Impulsiveness subscale; EPI, Eysenck Personality Inventory-Extroversion, Neuroticism, Lie subscale; GBQ, Gamblers beliefs questionnaire – Luck/perseverance, illusion of control subscale; TLFB, Timeline Followback of average number of drinks per week in the preceding 90 days before testing, and number of cigarettes in the preceding 7 days before testing; FTND, Fagerström Nicotine Dependence; DAST, Drug Abuse Screening Test.
Significant group difference, *p<0.05.

3.1.3. Basic Cognitive Functioning

3.1.3.1. Wechsler Intelligence Scales

Table 6 reports mean Wechsler Intelligence Scale Scores for all four subgroups. A 2 (Group: PG, HC) X 2(Antagonist: HAL, FLU) MANOVA did not reveal any significant effects. This indicates no overall difference in verbal knowledge/comprehension, short-term memory, or working memory among the 4 subgroups.
Table 6: Mean (SD) results in several basic cognitive functioning measures: vocabulary (verbal intelligence), Digit span (short-term memory), and digit symbol substitution (working memory) for each subgroup.

<table>
<thead>
<tr>
<th>Wechsler Test</th>
<th>Haloperidol Group</th>
<th>Fluphenazine Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>PG</td>
</tr>
<tr>
<td>Vocabulary (Out of 30)</td>
<td>29.00 (1.60)</td>
<td>27.50 (2.61)</td>
</tr>
<tr>
<td>Digit Span (Out of 28)</td>
<td>18.88 (4.64)</td>
<td>20.88 (4.05)</td>
</tr>
<tr>
<td>Digit Symbol Substitution (Out of 93)</td>
<td>41.25 (10.54)</td>
<td>50.25 (21.29)</td>
</tr>
</tbody>
</table>

HC, Healthy Controls; PG, Pathological Gamblers. No significant, group, Antagonist, or group by Antagonist interactions, p>0.05

3.1.3.2. Wisconsin Card Sort Task

Table 7 reports mean perseverative errors and non-perseverative errors on the computer-based Wisconsin Card Sort Task (WCST) – a reflection of cognitive “set-shifting” ability. A 2(Group: PG, HC) x 2(Antagonist: HAL, FLU) MANOVA did not yield any significant effects, p’s > 0.16. Thus, there was no overall difference among the 4 subgroups in cognitive “set-shifting”.

Table 7: Mean (SD) scores in perseverative errors and non-perseverative errors in the Wisconsin Card Sort task in each subgroup.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Haloperidol Group</th>
<th>Fluphenazine Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>PG</td>
</tr>
<tr>
<td>Perseverative Errors</td>
<td>9.50 (10.07)</td>
<td>6.88 (2.70)</td>
</tr>
<tr>
<td>Non-perseverative Errors</td>
<td>5.63 (4.41)</td>
<td>6.25 (1.75)</td>
</tr>
</tbody>
</table>

No significant, group, Antagonist, or group by Antagonist interaction, p>0.05

3.2. Slot Machine Betting Behaviour

3.2.1. Trials Played

Figure 3a and 3b show the mean number of trials played under both levels of drug pre-treatment (placebo and antagonist) for both HC and PG under each Antagonist (HAL and FLU). A 2 (Group; HC, PG) x 2 (Antagonist; HAL, FLU) x 2 (Pre-treatment; drug, placebo) ANOVA did not yield any significant effects, p’s > 0.120 (effect sizes, $\eta^2_p < 0.084$). Therefore, the number of trials played, an indication of the speed of play during the 15 minute slot machine game, did not differ with respect to the test groups, antagonists or pre-treatments.
Figure 3a: Mean (SE) total trials played on a 15-minute slot machine game in HC subjects (n=8) and PG subjects (n=8) under HAL (3mg, oral) and placebo.

Figure 3b: Mean (SE) total trials played on a 15-minute slot machine game in HC subjects (n=8) and PG subjects (n=8) under FLU (3mg, oral) and placebo.
3.2.2. Total Bet per Trial

Figure 4a and 4b show the mean total bet per trial for each subgroup under both pre-treatment levels. A 2 (Group; HC, PG) x 2 (Antagonist; HAL, FLU) x 2 (Pre-treatment; drug, placebo) ANOVA did not yield any effects, p’s >0.285, (effect sizes, $\eta^2_p < 0.041$). Therefore, the amount of credits wagered during the 15 minute slot machine game was not dependent on the groups, antagonists, or pre-treatments.

Figure 4a: Mean total bet per trial on a 15-minute slot machine game in HC subjects (n=8) and PG subjects (n=8) under HAL (3mg, oral) and placebo.

Figure 4b: Mean total bet per trial on a 15-minute slot machine game in HC subjects (n=8) and PG subjects (n=8) under FLU (3mg, oral) and placebo.
3.2.3. Lines Selected per Trial

Figure 5a and 5b show the mean number of lines selected per trial for each subgroup under both drug and placebo. A 2 (Group; HC, PG) x 2 (Antagonist; HAL, FLU) x 2 (Pre-treatment; drug, placebo) ANOVA did not yield any significant effects, p’s > 0.423 (effect sizes, $\eta_p^2$<0.023). Therefore, no variability in response selection (distribution of risk) was observed between the groups, antagonists, or pre-treatments during the 15 minute slot machine game.

**Figure 5a:** Mean number of lines selected per trial on a 15-minute slot machine game in HC subjects (n=8) and PG subjects (n=8) under HAL (3mg, oral) and placebo.

**Figure 5b:** Mean number of lines selected per trial on a 15-minute slot machine game in HC subjects (n=8) and PG subjects (n=8) under FLU (3mg, oral) and placebo.
3.2.4. Final Credit Total (Winnings)

Figure 6a and 6b show the mean number of final credits won for both HC and PG in each Antagonist for both levels of drug pre-treatment (placebo and antagonist). A 2 (Group; HC, PG) x 2 (Antagonist; HAL, FLU) x 2 (Pre-treatment; drug, placebo) ANOVA did not yield any significant effects, p’s > 0.116 (effect sizes, $\eta_p^2 < 0.086$) However, inspection of the figures indicates substantial differences in mean winnings as a function of Pre-treatment (drug vs. placebo) and Antagonist. This reflects the high variability that occurs in random events (i.e., gambling outcomes) with relatively small samples. Based on the sizeable error bars, the lack of significant effects likely derives from high variability within each level of Pre-treatment and Antagonist, which obscured differences between them. Although no significant effects were seen for final winnings, this variable could clearly still affect the subjective reinforcing effects of the game (and increase error variance), and was therefore incorporated into the analyses of self-report measures as a covariate.

Figure 6a: Mean final credits won ('winnings') at the end of a 15-minute slot machine game in HC subjects (n=8) and PG subjects (n=8) under HAL (3mg, oral) and placebo.
Figure 6b: Mean final credits won (‘winnings’) at the end of a 15-minute slot machine game in HC subjects (n=8) and PG subjects (n=8) under FLU (3mg, oral) and placebo.
3.3. Subjective Effects - Self-Report Measures

3.3.1. Visual Analog Scale

3.3.1.1. Desire to Gamble

A preliminary ANOVA of baseline Desire to Gamble on each session confirmed higher ‘unprimed’ scores in PG subjects $F (1, 26) = 78.01, p<0.001$. To isolate effects of Antagonist and AMPH, baseline scores were controlled by analysis of covariance along with ‘winnings’. Figures depict covariate-adjusted means. A 2 (Group; HC, PG) x 2 (Antagonist; FLU, HAL) x 2 (Pre-treatment: Drug, Placebo) x 4 (Time Point; peak antagonist effect, peak AMPH effect, post-slots game, post-cognitive tasks) ANCOVA showed significant group differences between PG and HC, $F (1, 26) = 27.7, p<0.001$. Figures 7(a,b) and 8(a-d) clearly show higher Desire to Gamble in PG subjects. Moreover, a significant Group x Antagonist x Pre-treatment interaction was observed $F (1, 26) = 6.20, p=0.020$. Inspection of Group means in Figure 7a and 7b for each level of Antagonist and Pre-treatment revealed that the interaction occurred because Desire to Gamble, collapsed across all time points, was significantly decreased by HAL pre-treatment in HC but not PG subjects. In contrast, Figure 7b shows that pre-treatment with FLU significantly reduced Desire to Gamble relative to placebo in PG but not HC subjects.
Figure 7a: VAS-Desire to Gamble mean scores graphed for each level of Group, HC=8, PG=8, and Pre-treatment (HAL vs. placebo) in the HAL Antagonist group.

Figure 7b: VAS-Desire to Gamble mean scores graphed for each level of Group, HC=8, PG=8, and Pre-treatment (FLU vs. placebo) in the FLU Antagonist group.
A significant Time x Group interaction was also found F(3, 78) = 9.29, p<0.001. PG scores rose more consistently than HC scores after AMPH (and the slot machine). In both groups, scores rose after AMPH administration and increased further after the slot machine game – scores then decreased by the end of the test session [see Figure 8 (a-d)]. Overall, these effects appeared to be more robust in PG. In PG, placebo pre-treatment was associated with a more dramatic increase in scores after AMPH administration than with HAL pre-treatment or FLU pre-treatment. The highest order trend noted was a quadratic Group x Pre-treatment x Time interaction F(1, 26) = 5.63, p = 0.025. This reflected the fact that scores rose and fell more sharply with placebo pre-treatment than drug pre-treatment in PG subjects.

Thus, despite controlling for baseline differences between PG and HC in desire to gamble, PG subjects clearly responded more strongly to AMPH than HC subjects in terms of ‘primed’ motivation to gamble. Moreover, pre-treatment with both HAL and FLU appeared to have directionally opposite effects as a function of PG status, in that HAL but not FLU reduced AMPH-induced gambling motivation in HC subjects, whereas FLU but not HAL decreased AMPH-induced gambling motivation in PG subjects.
Haloperidol-Healthy Controls

Figure 8a: VAS-Desire to Gamble scores for each level of time in healthy controls (n=8) that received HAL and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Haloperidol – Pathological Gamblers

Figure 8b: VAS-Desire to Gamble scores for each level of time in pathological gamblers (n=8) that received HAL and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
Fluphenazine – Healthy Controls

![Graph showing VAS-Desire to Gamble scores for healthy controls](image)

Figure 8c: VAS-Desire to Gamble scores for each level of time in healthy controls (n=8) that received FLU and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Fluphenazine – Pathological Gamblers

![Graph showing VAS-Desire to Gamble scores for pathological gamblers](image)

Figure 8d: VAS-Desire to Gamble scores for each level of time in pathological gamblers (n=8) that received FLU and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
3.3.1.2. Desire to Consume Alcohol

Group differences in baseline Desire for Alcohol scores provided a rationale for including them as a covariate along with ‘winnings’ $F(1, 26) = 47.63, p<0.001$. A 2 (Group; HC, PG) x 2 (Antagonist; FLU, HAL) x 2 (Pre-treatment: Drug, Placebo) x 4 (Time Point; peak antagonist effect, peak AMPH effect, post-slots game, post-cognitive tasks) ANCOVA yielded a main effect of Time, $F(3, 78) = 4.17, p = 0.009$, as well as a Group x Time interaction, $F(3, 78) = 2.84, p = 0.043$, and an Antagonist x Time interaction, $F(3, 78) = 4.95, p = 0.003$. No effect of Pre-treatment was observed (effect size, $\eta^2_p=0.101$). As per figure 9a and 9b, in the HAL condition, it appears that Desire for Alcohol rises systematically after AMPH administration and continues to rise after the slot machine game in both HC and PG, although the effects of AMPH appear to be more pronounced in PG. Drug pre-treatment appears to have a negligible effect on the scores. As per Figure 9c and 9d, it can be seen that in the FLU condition, the change over time is more modest in both groups and both levels of pre-treatment. This difference would appear to account for the Antagonist x Time interaction.

Overall, the magnitude of Desire for Alcohol scores (2-4 out of 10) was similar to that of Desire to Gamble scores in HC subjects, but considerably more modest than Desire to Gamble scores in PG subjects, even though baseline differences in Desire for each reinforcer were controlled in their respective analyses. Thus, AMPH primed motivation to gamble more robustly than motivation for alcohol in PG subjects.
**Haloperidol – Healthy Controls**

Figure 9a: Mean VAS-Desire for Alcohol scores in healthy controls (n=8) that were given HAL and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

**Haloperidol – Pathological Gamblers**

Figure 9b: Mean VAS-Desire for Alcohol scores in pathological gamblers (n=8) that were given HAL and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
Fluphenazine – Healthy Controls

**Figure 9c:** Mean VAS-Desire for Alcohol scores in healthy controls (n=8) that were given FLU and placebo on separate sessions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Fluphenazine – Pathological Gamblers

**Figure 9d:** Mean VAS-Desire for Alcohol scores in pathological gamblers (n=8) that were given FLU and placebo on separate sessions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
3.3.1.3. Subjective Reinforcing Psychoactive Drug Effects

Figure 10 a-d shows the mean subjective reinforcing effects of AMPH for each group and antagonist. Because this VAS was only measured once per test session and ratings were made before the slots game, no covariates were used in the analysis. A 2 (Group; HC, PG) x 2 (Antagonist; HAL, FLU) x 2 (Pre-treatment: Drug, Placebo) x 5 (Subscale; Liking, High, Good Effects, Bad Effects, Desire to Take AMPH Again) ANOVA yielded a 4-way interaction, $F(4, 112) = 3.22, p = 0.015$, which reflected differences in the cubic trend across the subscales, $F(1, 28) = 8.85, p = 0.006$. Comparison of the Drug and Placebo panels for each group and antagonist shows that the relative reduction in scores under drug was greater for HC vs. PG subjects in the HAL condition, whereas the relative reduction in scores under drug was greater for PG than for HC subjects in the FLU condition.

Although not significant, the directional difference in effects of HAL vs. FLU on Bad Effects, which declined under HAL but increased under FLU in PG subjects, but increased slightly under each antagonist in HC subjects, is noteworthy.
Haloperidol – Healthy Controls

Figure 10a: Mean Subjective Effects of AMPH in healthy controls (n=8) that received HAL and placebo on separate occasions. Effects include Liking, Good Effects, Bad Effects, and High from AMPH as well as Desire to take AMPH again. * p<0.05

Haloperidol – Pathological Gamblers

Figure 10b: Mean Subjective Effects of AMPH in pathological gamblers (n=8) that received HAL and placebo on separate occasions. Effects include Liking, Good Effects, Bad Effects, and High from AMPH as well as Desire to take AMPH again.
**Fluphenazine – Healthy Controls**

![Bar chart showing mean subjective effects of AMPH in healthy controls.](image)

Figure 10c: Mean Subjective Effects of AMPH in healthy controls (n=8) that received FLU and placebo on separate occasions. Effects include Liking, High, Good Effects, and Bad Effects of AMPH as well as Desire to take AMPH again. * p<0.05

**Fluphenazine – Pathological Gamblers**

![Bar chart showing mean subjective effects of AMPH in pathological gamblers.](image)

Figure 10d: Mean Subjective Effects of AMPH in pathological gamblers (n=8) that received FLU and placebo on separate occasions. Effects include Liking, Good Effects, Bad Effects and High from AMPH as well as Desire to take AMPH again. * p<0.05
3.3.2 Profile of Mood States

3.3.2.1. Depression-Dejection Subscale

Baseline POMS – Depression score differed significantly between groups F(1, 26) = 94.61, p<0.001, and was therefore used as a covariate in the analysis of experimental effects, in addition to ‘winnings’. A 2 (Group; HC, PG) x 2 (Antagonist; FLU, HAL) x 2 (Pre-treatment; Drug, Placebo) x 4 (Time Point; peak antagonist effect, peak AMPH effect, post-slots game, post-cognitive tasks) ANCOVA did not yield any significant interactions or trends, p’s > 0.145 (effect size, ηp²<0.067). Figure 11 a-d shows overall low, and generally inconsistent mean results for every subgroup.
Haloperidol – Healthy Controls

Figure 11a: Mean POMS-Depression Scores in healthy controls (n=8) that received HAL and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Haloperidol – Pathological Gamblers

Figure 11b: Mean POMS-Depression Scores in pathological gamblers (n=8) that received HAL and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
Fluphenazine – Healthy Controls

Figure 11c: Mean POMS-Depression Scores in healthy controls (n=8) that received FLU and placebo on separate sessions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Fluphenazine – Pathological Gamblers

Figure 11d: Mean POMS-Depression Scores in pathological gamblers (n=8) that received FLU and placebo on separate sessions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
3.3.2.2. Vigor-Activity Subscale

Baseline POMS-vigor-activity score differed significantly between groups F(1, 26) = 14.75, p = 0.001 and was therefore used as a covariate along with ‘winnings’ in the ANCOVA. A 2 (Group; HC, PG) x 2 (Antagonist; FLU, HAL) x 2 (Pre-treatment: Drug, Placebo) x 4 (Time Point; peak antagonist effect, peak AMPH effect, post-slots game, post-cognitive tasks) ANCOVA yielded a main effect of Time, F(3, 78) = 14.77, p<0.001. This effect was associated with a significant cubic trend F(1, 26) = 8.748, p = 0.007. Figure 12 a-d shows that POMS-vigor scores consistently increased after AMPH in all experimental cells and declined at the end of the session, when AMPH effects would be expected to subside. As in the case of subjective reinforcing effects of AMPH, vigor scores tended to be lower under HAL than FLU in HC subjects but tended to be lower under FLU than HAL in PG subjects, although not significantly so.
Haloperidol – Healthy Controls

Figure 12a: Mean POMS-Vigor scores in healthy controls (n=8) that received HAL and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Haloperidol – Pathological Gamblers

Figure 12b: Mean POMS-Vigor scores in pathological gamblers (n=8) that received HAL and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
Fluphenazine – Healthy Controls

Figure 12c: Mean POMS-Vigor scores in healthy controls (n=8) that received FLU and placebo on separate sessions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Fluphenazine – Pathological Gamblers

Figure 12d: Mean POMS-Vigor scores in pathological gamblers (n=8) that received FLU and placebo on separate sessions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
3.3.2.3. Anger-Hostility Subscale

Baseline POMS-anger-hostility score differed significantly between groups $F(1, 26) = 8.94, p = 0.006$ and therefore baseline was used along with ‘winnings’ as a covariate in the analysis. A 2 (Group; HC, PG) x 2 (Antagonist; FLU, HAL) x 2 (Pre-treatment: Drug, Placebo) x 4 (Time Point; peak antagonist effect, peak AMPH effect, post-slots game, post-cognitive tasks) ANCOVA yielded a marginal Group x Pre-treatment interaction $F (1, 26) = 4.05, p = 0.055$. The marginal interaction is illustrated in Table 8. Here, it can be seen that, averaged over Antagonists (HAL and FLU), Drug pre-treatment resulted in slightly higher mean POMS-anger scores relative to Placebo in HC subjects. In contrast, PG subjects tended to have lower mean anger scores with Drug pre-treatment relative to Placebo.

Table 8: Mean (SE) POMS anger-hostility scores both Antagonists (HAL and FLU) by drug pre-treatment (drug or placebo) and group [HC (n=16), PG (n=16)].

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-treatment</th>
<th>Mean (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Controls</td>
<td>Drug</td>
<td>0.38 (0.26)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.05 (0.19)</td>
</tr>
<tr>
<td>Pathological Gamblers</td>
<td>Drug</td>
<td>0.17 (0.26)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.50 (0.19)</td>
</tr>
</tbody>
</table>

The ANCOVA also yielded a marginal Group x Antagonist x Pre-treatment x Time cubic trend $[F(2, 26) = 4.06, p = 0.054]$. Figure 13 a-d below shows the means for each Antagonist condition and time point. The figure shows that AMPH generally led to a reduction in anger scores and that a modest reinstatement of anger occurred at the end of the session when the dose was eliminated in PG subjects in the FLU condition (under both drug and placebo).
Figure 13a: Mean POMS-anger scores in healthy controls (n=8) given HAL and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Figure 13b: Mean POMS-anger scores in pathological gamblers (n=8) given HAL and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
Fluphenazine – Healthy Controls

Figure 13c: Mean POMS-anger scores in healthy controls (n=8) given FLU and placebo on separate sessions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Fluphenazine – Pathological Gamblers

Figure 13d: Mean POMS-anger scores in pathological gamblers (n=8) given FLU and placebo on separate sessions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
3.3.3 Addiction Research Center Inventory

3.3.3.1. Amphetamine (AMP) Subscale

Baseline ARCI-AMP scores differed significantly between groups F(1, 25) = 18.29, p<0.001. Therefore, baseline was used as a covariate along with ‘winnings’ in the analysis. A 2 (Group; HC, PG) x 2 (Antagonist; FLU, HAL) x 2 (Pre-treatment: Drug, Placebo) x 4 (Time Point; peak antagonist effect, peak AMPH effect, post-slots game, post-cognitive tasks) ANCOVA yielded a main effect of Time, F (3, 75) = 12.17, p<0.001, and a Group x Antagonist x Time interaction, F (3, 75) = 3.20, p = 0.028. The interaction pertained to the quadratic trend, F(1, 25) = 7.89, p = 0.009. Inspection of Figure 14 a-d shows that the curvilinear profile of scores over time, (corresponding to the change in blood levels of AMPH), was the same for HC under drug and placebo pre-treatment, and for PG under placebo. In contrast, in PG subjects, HAL coincided with a linear increase over time in AMPH-induced prototypic stimulant effects and FLU coincided with a linear decrease over time in these effects.
Haloperidol – Healthy Controls

Figure 14a: Mean ARCI-AMPH scores in healthy controls (n=8) given HAL and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Haloperidol – Pathological Gamblers

Figure 14b: Mean ARCI-AMPH scores in pathological gamblers (n=8) given HAL and placebo on separate sessions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
Fluphenazine – Healthy Controls

Figure 14c: Mean ARCI-AMPH scores in healthy controls (n=8) given FLU and placebo on separate sessions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Fluphenazine – Pathological Gamblers

Figure 14d: Mean ARCI-AMPH scores in pathological gamblers (n=8) given FLU and placebo on separate sessions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
3.3.3.2. Morphine-Benzodrine (MBG) Subscale

Baseline ARCI-MBG score differed significantly between groups F (1, 25) = 13.25, p = 0.001. Therefore baseline was used as a covariate along with ‘winnings’ in the analysis. A 2 (Group; HC, PG) x 2 (Antagonist; FLU, HAL) x 2 (Pre-treatment; Drug, Placebo) x 4 (Time Point; peak antagonist effect, peak AMPH effect, post-slots game, post-cognitive tasks) ANCOVA yielded a main effect of Time, F(3, 75) = 16.18, p<0.01, and a Group x Antagonist x Time interaction F(3, 75) = 3.60, p = 0.018. A significant quadratic trend was noted for this interaction, F (1, 25) = 7.69, p = 0.010. Figure 15 c-d shows that this interaction reflected similar curvilinear profiles over time for each group and pre-treatment in subjects assigned to FLU. This common profile also emerged in both groups under placebo in subjects assigned to HAL (figure 15 a-d), but differed under the Drug pre-treatment – decreasing in a linear manner in HC but increasing linearly in PG. The pattern in PG under HAL resembles the pattern they displayed for ARCI-AMP, indicating a concordant effect of the antagonist on stimulant and euphoric effects of AMPH in PG subjects. The linear decline in MBG scores in HC subjects under HAL is similar to the pattern displayed by PG subjects for ARCI-AMP scores under FLU. Thus, preferential D2 blockade exerted a progressive inhibition of AMPH-induced euphoria in HC subjects whereas combined D1 and D2 blockade exerted this profile of inhibitory effects effects in PG subjects.
Haloperidol – Healthy Controls

Figure 15a: Mean ARCI-MBG scores in healthy controls (n=8) given HAL and placebo on separate occasions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Haloperidol – Pathological Gamblers

Figure 15b: Mean ARCI-MBG scores in pathological gamblers (n=8) given HAL and placebo on separate occasions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
Fluphenazine – Healthy Controls

Figure 15c: Mean ARCI-MBG scores in healthy controls (n=8) given FLU and placebo on separate occasions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Fluphenazine – Pathological Gamblers

Figure 15d: Mean ARCI-MBG scores in pathological gamblers (n=8) given FLU and placebo on separate occasions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
3.3.3.3. LSD Subscale

Baseline ARCI-LSD score differed significantly between groups $F(1,25) = 18.51, p<0.001$ and was therefore used as a covariate along with ‘winnings’ in the analysis. A 2 (Group; HC, PG) x 2 (Antagonist; FLU, HAL) x 2 (Pre-treatment; Drug, Placebo) x 4 (Time Point; peak antagonist effect, peak AMPH effect, post-slots game, post-cognitive tasks) ANCOVA yielded no significant effects or trends, p’s > .10 (effect size, $\eta^2_p<0.071$), reflecting a similar moderate pattern of dysphoric effects for each group, antagonist and pre-treatment (figure 16 a-d).
Haloperidol – Healthy Controls

Figure 16a: Mean ARCI-LSD scores in healthy controls (n=8) given HAL and placebo on separate occasions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Haloperidol – Pathological Gamblers

Figure 16b: Mean ARCI-LSD scores in pathological gamblers (n=8) given HAL and placebo on separate occasions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
**Fluphenazine – Healthy Controls**

**Figure 16c:** Mean ARCI-LSD scores in healthy controls (n=8) given FLU and placebo on separate occasions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

![Graph showing mean ARCI-LSD scores for healthy controls](image1)

**Fluphenazine – Pathological Gamblers**

**Figure 16d:** Mean ARCI-LSD scores in pathological gamblers (n=8) given FLU and placebo on separate occasions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

![Graph showing mean ARCI-LSD scores for pathological gamblers](image2)
3.4. Experimental Computer-Based Tasks

3.4.1. Rapid Reading Task

A 2 (Group; HC, PG) x 2 (Antagonist; HAL, FLU) x 2 (Pre-treatment; antagonist, placebo) x 5 (Word Type; Gambling, Alcohol, Positive, Negative, Neutral) ANCOVA, with ‘winnings’ as a covariate, yielded a significant effect of Word Type, $F(4, 108) = 9.54, p<0.001$. No other significant effects were observed. Figures 17 a-d shows that this reflected consistently slower reaction time to neutral words than to affective or addiction related words regardless of other factors.

In order to observe the word category involved in the interaction more closely, within-subjects contrasts were performed to compare the response time of each word category to the neutral control words. The within-subjects contrasts showed a significant Word Type x Group x Antagonist interaction for Gambling vs. Neutral words ($p=0.046$), with no other reliable differences in response time for any other test category vs. neutral words. This is observable in Figure 17 a-b. Across both pre-treatments, PG and HC groups assigned to the HAL condition differed significantly from each other in that Gambling words were more salient in PG than in HC.
Haloperidol – Healthy Controls

Figure 17a: Mean reaction time (ms) to different categories of words in healthy controls (n=8) on a rapid reading task when given HAL and placebo. Word categories include: gambling, alcohol, positive, negative and neutral type words.

Haloperidol – Pathological Gamblers

Figure 17b: Mean reaction time (ms) to different categories of words in pathological gamblers (n=8) on a rapid reading task when given HAL and placebo. Word categories include: gambling, alcohol, positive, negative and neutral type words.
Fluphenazine – Healthy Controls

Figure 17c: Mean reaction time (ms) to different categories of words in healthy controls (n=8) on a rapid reading task when given FLU and placebo. Word categories include: gambling, alcohol, positive, negative and neutral type words.

Fluphenazine – Pathological Gamblers

Figure 17d: Mean reaction time (ms) to different categories of words in pathological gamblers (n=8) on a rapid reading task when given FLU and placebo. Word categories include: gambling, alcohol, positive, negative and neutral type words.
3.4.2. Stop Signal Task

Table 9 shows mean Go response time (ms) and Stop response time for each level of Group, Antagonist, and Pre-Treatment. HAL slightly impaired and FLU slightly improved overt psychomotor response (Go-RT) in HC subjects. HAL but not FLU slightly improved psychomotor response in PG. Conversely, HAL slightly improved and FLU slightly impaired inhibitory control (Stop-RT) in HC subjects. Both HAL and FLU were associated with slightly impaired inhibitory control (Stop-RT) in PG subjects. It is important to note that subjects cannot improve their inhibitory control (Stop-RT) simply by slowing their psychomotor response (Go-RT) since the task is designed to correct for speed/accuracy trade-offs.

Table 9: Mean (SE) Go-response time (Go-RT) and Stop signal-response time (Stop-RT) in the Stop Signal Task in HC (n=8) and PG (n=8) subjects under HAL (3mg, oral) and placebo, and in HC (n=8) and PG (n=8) subjects under FLU (3mg, oral) and placebo.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Antagonist</th>
<th>Haloperidol</th>
<th>Fluphenazine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug</td>
<td>Placebo</td>
<td>Drug</td>
</tr>
<tr>
<td>Healthy Controls</td>
<td>Go – RT</td>
<td>528.7 (105.9)</td>
<td>485.4 (77.9)</td>
</tr>
<tr>
<td></td>
<td>Stop -RT</td>
<td>193.21 (105.0)</td>
<td>217.1 (111.3)</td>
</tr>
<tr>
<td>Pathological Gamblers</td>
<td>Go – RT</td>
<td>546.4 (138.8)</td>
<td>569.9 (156.2)</td>
</tr>
<tr>
<td></td>
<td>Stop - RT</td>
<td>181.8 (53.1)</td>
<td>168.5 (38.7)</td>
</tr>
</tbody>
</table>

A 2 (Group; HC, PG) x 2 (Antagonist; HAL, FLU) x 2 (Pre-treatment; antagonist, placebo) x 2 (Measure; Go-RT, Stop-RT) MANOVA that used ‘winnings’ as a covariate, did not yield any significant effects, p’s > 0.110 (effect sizes, ηp^2<0.052). Thus, the trends in mean psychomotor fluency and inhibition reported above were obscured by high within-cell variance.
3.4.3. Game of Dice Task

Table 10 shows mean line selection over three consecutive 6-trial blocks (6 dice tosses/block). Smaller scores are indicative of more risky selections. This table shows that HC made slightly riskier selections overall than PG subjects did, with 3 exceptions.

Table 10. HC, healthy controls; PG, pathological gamblers. Mean (SE) risk-taking scores on the Game of Dice Task in HC (n=8) and PG (n=8) subjects under HAL (3mg, oral) and placebo, and in HC (n=8) and PG (n=8) subjects under FLU (3mg, oral) and placebo. Smaller scores indicate more risky betting behaviour.

| Antagonist | Haloperidol | | | Fluphenazine | | | |
|——|——|——|——|——|——|——|——|——|
| | Drug | Placebo | | Drug | Placebo | | | |
| Block | Block 1 | Block 2 | Block 3 | Block 1 | Block 2 | Block 3 | Block 1 | Block 2 | Block 3 |
| HC | 3.60 (0.73) | 3.64 (0.70) | 3.57 (0.69) | 3.44 (0.91) | 3.44 (0.90) | 3.42 (0.68) | 3.33 (0.54) | 3.50 (0.53) | 3.29 (0.52) |
| PG | 3.81 (0.40) | 3.63 (0.75) | 3.71 (0.58) | 3.77 (0.41) | 3.75 (0.53) | 3.83 (0.32) | 3.19 (1.03) | 3.46 (0.47) | 3.50 (0.64) |

A 2 (Group; HC, PG) x 2 (Antagonist; HAL, FLU) x 2 (Pre-treatment; antagonist, placebo) x 3 (Block; 1, 2, 3) ANCOVA, using ‘winnings’ from the slot machine as a covariate, yielded a marginal Group x Pre-treatment interaction, F (1, 26) = 3.09, p = 0.091. Therefore, risky betting behaviour was dependent on the group (HC and PG) and pre-treatment (drug or placebo) across both antagonists (HAL and FLU) and blocks (1-3). Table 11 illustrates this interaction and shows that HC made slightly riskier selections under Placebo relative to Drug (HAL or FLU) pre-treatment, whereas PG appeared to make slightly riskier selections with Drug relative to Placebo pre-treatment.
Table 11: Mean (SE) line choice per drug pre-treatment for both HC and PG subjects collapsed across Antagonist and block on the game of dice task.

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-treatment</th>
<th>Mean (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Healthy Controls</em></td>
<td>Drug</td>
<td>3.48 (0.15)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>3.31 (0.16)</td>
</tr>
<tr>
<td><em>Pathological Gamblers</em></td>
<td>Drug</td>
<td>3.55 (0.15)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>3.70 (0.15)</td>
</tr>
</tbody>
</table>

3.5. Physiological Measures.

3.5.1. Heart Rate

Baseline differences between groups were observed, F(1, 26) = 30.0, p<0.001, and thus baseline scores were used as a covariate in the ANCOVA of heart rate, along with ‘winnings’. A 2 (Group; HC, PG) x 2 (Antagonist; HAL, FLU) x 2 (Pre-treatment; antagonist, placebo) x 4 (Time Point; peak antagonist effects, peak AMPH effects, post-slots game, post-cognitive tasks) yielded a marginal Pre-treatment x Time interaction, F(3, 78) = 2.43, p = 0.072, (effect size, $\eta^2_p < 0.085$). The means for this interaction are shown in Table 12. These means indicate that, at peak antagonist effects, and immediately after the slot machine game, heart rate was relatively greater under the drug than the placebo.
Table 12: Mean (SE) HR for all time points in both drug and placebo pre-treatments collapsed across group and Antagonist. Time point (1) = peak antagonist HR, (2)=peak AMPH HR, (3)=post slot machine HR, (4)=end of study day HR.

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Time Point</th>
<th>Mean (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>1</td>
<td>66.8 (1.1)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>66.5 (1.4)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>72.6 (2.0)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>70.6 (1.9)</td>
</tr>
<tr>
<td>Placebo</td>
<td>1</td>
<td>64.2 (1.2)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>66.7 (1.6)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>70.7 (2.0)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>70.0 (1.5)</td>
</tr>
</tbody>
</table>

A quadratic trend was noted for the Pre-treatment x Time interaction, F (1, 26) = 4.81, p = 0.037. This is reflective of the rise and decline in heart rate before and after the slot machine game, which is more pronounced under Drug than Placebo (see figures 18a-d).
Haloperidol – Healthy Controls

Figure 18a: Mean heart rate (beats/minute) in healthy controls (n=8) given HAL and placebo on separate occasions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Haloperidol – Pathological Gamblers

Figure 18b: Mean heart rate (beats/minute) in pathological gamblers (n=8) given HAL and placebo on separate occasions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
Figure 18c: Mean hear rate (beats/minute) in healthy controls (n=8) given FLU and placebo on separate occasions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Figure 18d: Mean hear rate (beats/minute) in pathological gamblers (n=8) given FLU and placebo on separate occasions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
3.5.2. Systolic Blood Pressure

Group differences in baseline scores were observed, F(1, 26) = 52.08, p<0.001, and therefore, baseline was used as a covariate along with ‘winnings’ in the analysis. A 2 (Group; HC, PG) x 2 (Antagonist; HAL, FLU) x 2 (Pre-treatment; antagonist, placebo) x 4 (Time Point; peak antagonist effects, peak AMPH effects, post-slots game, post- cognitive tasks) ANCOVA yielded no significant interactions or trends, p’s > 0.136 (effect size, $\eta^2_p<0.068$). In examining figures 19 a-d however, it can be seen that AMPH consistently results in an enhancement in systolic blood pressure after pre-treatment, regardless of Group, Antagonist or Pre-Treatment. Therefore, the lack of significant effects likely reflects the within-group variation at each time point, and suggests that the effects of AMPH will be detectable as sample size increases and within-group variation declines.
Figure 19a: Mean systolic blood pressure (mmHg) in healthy controls (n=8) given HAL and placebo on separate occasions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Haloperidol – Pathological Gamblers

Figure 19b: Mean systolic blood pressure (mmHg) in pathological gamblers (n=8) given HAL and placebo on separate occasions. Time point (1) = peak HAL effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
Figure 19c: Mean systolic blood pressure (mmHg) in healthy controls (n=8) given FLU and placebo on separate occasions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.

Figure 19d: Mean systolic blood pressure (mmHg) in pathological gamblers (n=8) given FLU and placebo on separate occasions. Time point (1) = peak FLU effects, (2) = peak AMPH effects, (3) = post slot machine game, (4) = end of study day.
3.6. Additional Self-Report Measures

3.6.1 Capsule Contents Evaluation

Upon completion of the final test session, subjects were asked to report which of the two test sessions they believed they had received drug versus placebo pre-treatment. A 2(Pre-treatment Sequence; drug on test session 1, drug on test session 2) x 4 (Response Choice: felt drug on day 1, felt drug on day 2, felt drug on both days, don’t know) chi-square test of independence was not significant, p > 0.215 (Refer to Appendix E, Tables 9-12 for relative frequency). Thus, subjects were unable to reliably distinguish between Drug (antagonist) and Placebo. This helps to ensure that differences in self-report under each Pre-treatment are not due to attributions associated with subjects’ belief that they had received the antagonist.

3.6.2. Symptoms Side-Effects Checklist

A 2 (Group; HC, PG) x 2 (Antagonist; HAL, FLU) x 2 (Pre-treatment; antagonist, placebo) ANOVA did not yield any significant interactions, p’s>0.158 (effect size $\eta_p^2 < 0.070$). The lack of significant effects shows that all of the drugs were well tolerated and further suggests that differences in other self-report measures as a function of Group, Antagonist, or Pre-Treatment are not attributable to side effects from the medications. Refer to Appendix E, Table 13, for mean (SE) scores.
4. Discussion

This study investigated the effects of the preferential D2 dopamine receptor antagonist, haloperidol (HAL) and the mixed D1-D2 receptor antagonist, fluphenazine (FLU) on responses to a 20-mg oral dose of d-amphetamine (AMPH) in human subjects. Given that no previous research appeared to have examined the roles of the main dopamine receptor subtypes (D1 and D2), in the reinforcing effects of AMPH in addicted individuals, the current study included a group of pathological gamblers (PG) who are believed to exhibit some similarities to individuals afflicted with stimulant addiction, but lack the stimulant-induced neurotoxicity. As such, PG subjects provided a good comparison to healthy control (HC) subjects for assessing addiction-related alterations in the subjective-behavioral effects of a prototypical stimulant drug.

Based on the binding profiles of the dopamine antagonists used in this study, it was hypothesized that, if stimulation of D2 autoreceptors mediates rewarding, incentive-motivational, and cognitive effects of AMPH, HAL and FLU, which have equal affinity for the D2 receptor, should cause similar reductions in the effects of AMPH, compared to placebo. However, if D1 receptors mediate some or all of these effects, then HAL should enhance AMPH effects, compared to placebo, due to increased D1 stimulation during preferential blockade of inhibitory D2 autoreceptors. Furthermore, FLU should reduce AMPH effects relative to placebo. Lastly, if PG subjects resemble other addicted or at-risk populations, they should experience a greater facilitative effect of HAL (by restoring deficits in reward signaling at D1) and correspondingly greater reduction in reward due to FLU than HC subjects.

These hypotheses were tested on a range of outcome measures – broadly classified as self-report, cognitive-behavioral and physiological. With respect to subjective effects - incentive motivation was assessed using: Visual Analog Scale (VAS) ratings of Desire to Gamble and Desire for Alcohol (non-gambling addictive motivation). Hedonic impact of AMPH was assessed with VAS ratings of Liking, High, Good Effects, and Bad Effects of AMPH. Desire to Take AMPH Again captures incentive motivation, but also the perceived rewarding properties of the drug. To maximize parsimony, Desire to Take AMPH Again was analyzed together with the other hedonic ratings measured once only at the time of expected peak subjective effects. The Addiction Research Center Inventory (ARCI) subscales provided a standardized index of psychoactive drug effects. The amphetamine subscale (AMP) assessed stimulant-like effects, the Morphine-Benzedrine subscale (MBG) assessed euphoric effects; and the Lysergic Acid Diethylamide
subscale (LSD) assessed dysphoric effects. Lastly, the Profile of Mood States-short form (POMS-sf) Depression, Vigor and Anger subscales were used to assess mood effects of AMPH not captured by the drug effects scales.

The data described in this thesis (n = 16 PG; n = 16 HC) represent a subset of the total sample (N = 80; 40 PG and 40 HC) required to reliably test the hypotheses (with power 0.80 and probability of a Type I error, $\alpha = .05$). As the current sample only includes 40% of the proposed total sample, all interpretations are provisional. In light of this relatively small sample size, marginal effects ($p < 0.10$) will also be interpreted.

4.1. Betting Behaviour on the Slot Machine Game

No significant effects involving group, antagonist, or pre-treatment were observed on any of the indices of betting behaviour on the slot machine game: number of trials played, total bet per trial, number of lines selected per trial, and overall winnings. This pattern of effects suggests that neither D1 nor (pre-synaptic) D2 receptors mediate the mean effects of AMPH on slot machine gambling behaviour in PG or HC subjects. As noted in the Results, the figures indicated noticeable differences in mean winnings despite the lack of statistical significance. This is likely due to the high levels of variability in winnings (a random variable) within each level of pre-treatment and antagonist, obscuring differences between them. Since overall winnings could clearly influence perceived reinforcing effects of the slot machine, this variable (final credit tally) was incorporated into the analyses of self-report measures as a covariate.

4.2. Hypothesis Testing

4.2.1. Subjective Effects: Incentive Motivation

4.2.1.1. Visual Analog Scale - Desire to Gamble

The ANCOVA of VAS-Desire to Gamble revealed a significant Group x Antagonist x Treatment interaction. Simple effects showed that relative to placebo pre-treatment, HAL significantly reduced overall Desire to Gamble under AMPH in HC subjects, but did not reliably alter Desire to Gamble in PG subjects. In contrast, FLU significantly reduced overall Desire to Gamble under AMPH in PG subjects, but did not reliably alter Desire to Gamble in HC subjects. Thus, when feedback inhibition was removed by D2 auto-receptor blockade, preferential stimulation of D1
under HAL reduced AMPH-primed motivation to gamble in HC, whereas decreased stimulation of D1 under FLU reduced AMPH-primed motivation to gamble in PG subjects.

### 4.2.1.2. Visual Analog Scale – Desire to Consume Alcohol

The ANCOVA of VAS-Desire to Consume Alcohol scores revealed Time x Group and Time x Antagonist interactions, but no significant effects of pre-treatment (drug vs. placebo). The lack of pre-treatment-related effects may reflect a lack of statistical power as the effect size was moderate ($\eta_p^2 = 0.101$). Desire for Alcohol increased more under AMPH in PG than HC regardless of other factors. The overall increase (2-3 VAS points) was smaller than for Desire to Gamble (5-7 points) in both antagonist groups among PG subjects; and similarly the increase (~1 VAS point) was smaller than for Desire to Gamble (~2 points) in both antagonist groups among HC subjects. This pattern indicates that AMPH is associated with a greater increase in motivation to gamble and drink alcohol in PG vs. controls subjects, but also a relatively greater motivation to gamble than to drink alcohol regardless of PG status.

The Time x Antagonist interaction reflected greater overall Desire for Alcohol in subjects assigned to HAL versus FLU, regardless of PG status or Pre-treatment. Because HAL and FLU subjects were matched on PG severity and other potential moderators of motivation, this effect appeared to reflect random trait differences not controlled by matching with the small sample.

Overall, the results show that AMPH was associated with greater motivation for alcohol in PG than HC subjects. Although the time-dependent increase in VAS scores is consistent with a priming effect of AMPH, because AMPH was administered on both sessions (i.e., no placebo AMPH challenge), the role of expectancies cannot be established. In contrast, the differential effects of the antagonists on Desire to Gamble under AMPH in the two groups cannot be attributed to expectancies alone because antagonists were administered double-blind and subjects were unable to discriminate the antagonist from the placebo pre-treatment.

### 4.2.2. Subjective Effects: Indices of Hedonic Impact

#### 4.2.2.1. Visual Analog Scale – Subjective Reinforcing Effects of AMPH.

The ANCOVA of hedonic effects ratings yielded a four-way interaction. Simple effects showed that there was no significant difference in subjective AMPH-induced Liking, High or Desire to Take Again in HC subjects as a function of Pre-treatment with either antagonist. However, HAL,
but not FLU did significantly reduce perceived Good Effects in HC. In contrast, FLU but not HAL consistently reduced subjective Liking, Good Effects, High, and Desire to Take Again in PG. Simple effects of the VAS ‘Bad Effects’ subscale did not show any effect of Pre-treatment in any of the subgroups. Overall the pattern of subscale effects shows that increased dopamine release and preferential stimulation of D1 receptors reduced the positive subjective effects of AMPH to some degree in HC subjects, whereas decreased availability of D1 led to a consistent reduction in a range of hedonic effects of AMPH in PG, despite the expected increase in dopamine release due to auto-receptor blockade (Pehek, 1999). The relatively modest overall ratings and minimal impact of the antagonists on ‘Bad Effects’ suggest that D2 blockade did not appreciably increase the aversive properties of AMPH, but instead reduced the ability to detect its pleasurable effects.

4.2.2.2 Profile of Mood States

4.2.2.2.1. Depression- Dejection Subscale

POMS Depression-Dejection scores were generally low and inconsistent and no significant effects involving pre-treatment (drug/placebo) were observed.

4.2.2.2.2. Anger-Hostility Subscale

There was a marginally significant interaction of Treatment x Group for the POMS Anger-Hostility subscale. HAL tended to increase Anger scores relative to placebo in HC, but not PG. On the other hand, FLU did not reliably alter Anger scores in either group. Thus, preferential stimulation of D1 under AMPH appears to promote irritability in HC subjects.

4.2.2.2.3. Vigor-Activity Subscale

The ANCOVA of POMS – Vigor-Activity scores yielded a main effect of Time, and a marginally significant Group x Antagonist x Pre-treatment interaction (effect size, $\eta_p^2<0.074$). The increase in Vigor under AMPH was reduced by HAL in HC but not PG. Conversely, the increase in Vigor scores under AMPH was reduced by FLU in PG but not HC. This pattern of effects, although marginal, is similar to the pattern observed for VAS subjective reinforcing effects, and suggests that the behavioral activating effects of AMPH were linked to its subjective reinforcing effects, in terms of the mediating role of D1.
4.2.2.3. Addiction Research Centre Inventory

4.2.2.3.1. Amphetamine (AMP) Subscale

Under both placebo and HAL, AMPH increased ARCI-AMP scores more sharply in HC at the time of peak effects, versus a steadier, more gradual increase observed in PG over the course of the test session. However, this group difference was reversed in subjects assigned to FLU, with a sharper increase in scores seen under drug and placebo in PG and a gradual increase across the test session seen in HC subjects were associated with a sharper increase in PG than in HC. The lack of Pre-treatment-related effects indicates that these group differences were related to trait factors in the subjects assigned to HAL vs. FLU, which can emerge despite matching and randomization, with a relatively modest sample.

4.2.2.3.2. Morphine-Benzedrine (MBG) Subscale

Under placebo as well as HAL pre-treatment, AMPH consistently increased ARCI-MBG scores at the time of peak AMPH effects in HC and PG. On the other hand, with both placebo and FLU pre-treatment, the AMPH-induced increase in ARCI-MBG scores was larger in PG than in HC. As with the ARCI-AMP subscale, these data appear to reflect trait differences in the specific subjects assigned to HAL vs. FLU rather than differential effects of the antagonists themselves.

4.2.2.3.3. LSD Subscale

The ANCOVA of the ARCI LSD subscale did not yield any significant effects. That is, AMPH did not elicit any significant dysphoric effects in either HC or PG under either antagonist or placebo. This is consistent with the modest VAS Bad Effects ratings.

4.2.3. Cognitive Effects

4.2.3.1. Rapid Reading Task

The ANCOVA of the rapid reading task yielded a significant effect of Word Type, and no other significant effects. Within-subjects contrasts showed a significant difference in response time to Gambling versus Neutral words with no other reliable differences in response time for motivationally relevant vs. Neutral words. Thus, regardless of Group or Pre-Treatment, Gambling words were more salient than Neutral words under AMPH, although the role of expectancies or baseline/trait salience of Gambling words cannot be established. Additionally, PG responded fastest overall to Gambling words, while HC responded fastest to Negative Affect
words. Overall, this pattern of effects provides evidence of salience for Gambling-related stimuli in PG. The lack of Pre-treatment effects provides no evidence for the role of D1 and/or D2 stimulation in cue salience under AMPH. However, given the relatively modest effect sizes for reading tasks (Neely, 1991), it is possible that antagonist-related effects will only become discernible as the sample N approaches its target size.

4.2.3.2. Stop Signal Task

Neither antagonist reliably altered GO- or Stop-Response Time in HC or PG subjects, suggesting that blockade of D1 and D2 receptors (at least at the doses tested) did not reliably affect psychomotor fluency or inhibitory control under AMPH, regardless of PG status.

4.2.3.3. Game of Dice Task

An ANCOVA yielded a marginal Group x Pre-Treatment interaction p=0.091 (moderate effect size, $\eta^2 = 0.106$). Both HAL and FLU pretreatment appeared to slightly reduce risky bets in HC relative to placebo. On the other hand, both HAL and FLU pretreatment appeared to slightly increase risky bets in PG relative to placebo. Thus, interruption of D2-mediated feedback under AMPH may alter risky decision-making and the effects of this manipulation are directionally opposite depending on the presence of gambling pathology.

4.2.4. Physiological Measures

4.2.4.1. Heart Rate

The ANCOVA of heart rate scores yielded a marginal Pre-Treatment x Time interaction p=0.072 (moderate effect size, $\eta^2 = 0.085$). This interaction reflected slightly higher heart rate after the slot machine game under both antagonists relative to placebo in both groups, (See Table 12). The timing and pattern of these effects suggests that removal of D2 auto-receptor feedback may disinhibit the sympathetic activating effects of gambling under AMPH (Misu Y, et al., 1985), without altering the sympathetic effects of AMPH per se.
4.2.4.2. Systolic Blood Pressure

The ANCOVA of systolic blood pressure readings did not yield any significant effects. Detectible effects of AMPH on systolic blood pressure may emerge as the sample size increases and within-group variation declines.

4.3. General Discussion

This study yielded two main findings with respect to self-report (VAS-Desire to Gamble and VAS-subjective rewarding effects of AMPH) and one main behavioral result (on the Game of Dice Task). Desire to Gamble and subjective reward correspond to Robinson and Berridge’s (2003) concepts of ‘wanting’ (incentive motivation) and ‘liking’ (hedonic impact), respectively.

The Desire to Gamble results will be considered first. Simple effects analyses showed that HAL led to a significant reduction in motivation to gamble relative to placebo in HC but not PG subjects, whereas FLU led to a significant reduction in gambling motivation relative to placebo in PG but not HC subjects. Preferential blockade of D2 auto-receptors by HAL would be expected to increase basal DA release as well as AMPH-induced DA release (Pehek 1999). This in turn would have led to preferential stimulation of D1 receptors, which respond primarily to high intensity signals (i.e., phasic DA) under normal conditions. The decline in Desire to Gamble in HC subjects under these conditions is consistent with supra-optimal D1 stimulation in individuals with normal baseline D1 receptor availability and/or sensitivity. Preferential stimulation of D1 receptors is thought to “satisfy” incentive motivation for stimulant rewards (e.g., cocaine)(Self DW, 1998).

In the case of HC in the FLU condition, no significant difference was observed in motivation to gamble depending on pre-treatment. While FLU disrupts D2-mediated auto-receptor feedback, and increases basal and AMPH-induced DA release, like HAL, it also reduces post-synaptic D1 availability. Thus, post-synaptic D1 blockade under FLU would counteract supra-optimal D1 stimulation, leading to no appreciable net change in D1 signal in individuals with normal baseline D1 function – i.e., HC subjects.

The pattern of effects for the two antagonists in PG subjects was opposite to that seen in HC subjects. The reduction of Desire to Gamble in PG subjects under FLU but not HAL suggests that sub-optimal rather than supra-optimal D1 stimulation accounted for this effect. This interpretation fits with our hypothesis that the relationship between D1 signaling and stimulant...
reinforcement conforms to an inverted-U, much like the proposed relationship between D1 signaling and cognitive acuity/flexibility (Seamans and Yang, 2004). According to this formulation, under placebo pre-treatment, AMPH would have increased D1 signaling from deficient to near-optimal in PG subjects (i.e., just below the apex of the inverted-U). HAL pre-treatment would have augmented AMPH-induced D1 signaling, and the lack of change in Desire to Gamble in PG subjects under HAL suggests this may involve a shift to just beyond the apex of the inverted U, with no net change in subjective effects (i.e., stable motivation). In contrast, FLU would have negated the restorative effect of AMPH on D1 signaling in PG subjects, reducing the priming effects of the drug. The inverted-U account also explains the pattern of effects in HC subjects, who shifted from near-optimal signaling under AMPH (plus placebo pre-treatment) to well beyond the apex of the inverted U under AMPH plus HAL, but experienced no net change in D1 signal (no decline from the apex of the inverted U) when increased dopamine release was offset by blockade of D1 receptors by FLU.

In short, the inverted-U hypothesis for the relationship between D1 signaling and motivation to gamble appears to provide a parsimonious explanation for the incentive motivational effects of HAL and FLU in the two groups, and further indicates a possible deficit in baseline D1 function in PG subjects. More generally, the increased Desire to Gamble under AMPH plus placebo Pretreatment in both groups (relative to pre-capsule baseline), suggests that increased dopamine release enhances the incentive value of gambling regardless of gambling pathology.

The pivotal role of D1 in motivation for a target reinforcer has been observed in previous studies. Khroyan et al. (2003) found that monkeys with extensive histories of cocaine self-administration displayed a rightward and downward shift in the dose-response relationship for reinstatement of cocaine-seeking when pretreated with either a D1 agonist or D1 antagonist. That is, when cocaine’s ability to stimulate D1 was made partially redundant by a pre-existing D1 signal (agonist) or prevented by direct blockade (antagonist) a larger dose of cocaine was needed to reinstate (i.e., prime) motivation for drug seeking. This finding agrees with the results of the current study in that preferential stimulation of D1 by HAL rendered the priming effects of AMPH redundant in PG subjects (like cocaine treated rats); and direct blockade by FLU prevented AMPH from transmitting its signal to the D1 receptor with concomitant decline in priming in PG subjects (akin to decreased reinstatement by a previous effective dose).
The study by Khroyan et al. (2003) suggests that a higher dose of AMPH would have directionally opposite effects on priming in HC vs. PG subjects in the present design. A higher dose of AMPH would further over-stimulate D1 in HC subjects, which would be expected to further reduce their desire to gamble under HAL relative to placebo. In contrast, a higher dose of AMPH would be expected to partially restore the deficit in D1 signaling in PG subjects and thereby restore their desire to gamble under FLU relative to placebo.

Anderson et al., (2003) found that administration of a D1 antagonist directly into the medial nucleus accumbens dose-dependently attenuated drug seeking induced by a cocaine prime in rats that were trained to lever press for cocaine. This is also in line with our finding that FLU significantly reduced AMPH-induced priming of gambling motivation in PG subjects. The similar profile of effects of D1 antagonism in the present PG subjects and cocaine-treated animals, suggests that chronic heavy gambling may be functionally similar to chronic exposure to a stimulant drug. The decline in priming by a central antagonist infusion in animals raises the possibility that D1 receptors in the terminal region of the mesolimbic dopamine pathway may have partly mediated the decline in AMPH priming of desire to gamble seen in PG subjects in this study.

Romach et al. (1999) obtained similar effects in human cocaine abusers. In this case, pre-treatment with the selective D1 antagonist, ecopipam led to a dose-dependent reduction in craving in response to a priming dose of cocaine. Together, the animal and human data in subjects with a history of cocaine administration correspond well with our finding that FLU resulted in a reduction in AMPH-primed motivation to gamble in PG subjects. The lack of such effects in HC subjects is also consistent with the idea that dopamine plays a similar role in PG and stimulant addiction (i.e., a brain state associated with repeated stimulant activation).

Turning to the VAS results for subjective rewarding effects of AMPH (hedonic impact or ‘Liking’): Simple effects showed that in HC, perceived Good Effects from AMPH were significantly reduced with HAL pretreatment; and the other subscales (Liking, High, Desire to Take Again), apart from Bad Effects, also showed consistent but more modest reductions relative to placebo. On these indices, HC subjects also showed consistent decreases under FLU pre-treatment, relative to placebo, although less sizeable than for HAL pre-treatment.
In PG subjects, consistent decreases were seen on all subscales, apart from Bad Effects with both HAL and FLU pretreatment relative to placebo. In this case, the extent of the reduction was greater under FLU rather than HAL.

Given that FLU and HAL have similar affinity for D2, the reduction in subscale scores under both drugs in both groups suggests that decreased D2 availability was the primary mechanism mediating the hedonic effects of AMPH. At the same time, group differences in the magnitude of this effect under HAL vs. FLU, suggested a role for D1 in hedonic effects of AMPH that mirrored the pattern for the two groups seen on incentive motivation. However, for hedonic effects, the role of D1 appeared to be secondary to or ‘moderating’ (i.e., influencing the degree of effect) rather than mediating (i.e., playing a causal role), relative to D2.

The observation that D2 plays a primary role in the hedonic effects of AMPH appears to agree with several lines of previous research. Furmidge et al. (1991) found that systemic injection of raclopride (a selective D2 antagonist) in rats significantly inhibited their ability to discriminate AMPH from saline, suggesting that D2 receptors play a primary role in the internal stimulus or subjective effect of d-amphetamine. Beninger et al. (1989) noted that D2 antagonists block place preference learning based on AMPH. Conditioned place preference is a standard paradigm for drug reward in animals and indicates that the experience associated with a particular context was favorable – i.e., subjectively positive.

Overall, the decline in AMPH hedonic effects was more consistent across groups and antagonists than the decline in incentive motivation. This implies that availability or sensitivity of D2 autoreceptors may not be the principal feature distinguishing between PG and HC subjects, at least based on the effects of the drug doses employed here.

PET studies have consistently found deficits in D2 receptor availability/sensitivity in the striatum, the brain region that encompasses the nucleus accumbens, in cocaine abusers (Volkow et al., 1999; Nader and Czoty, 2005). However, such deficits are also seen in alcohol dependent subjects (Martinez et al., 2005), and indeed in a range of other disorders of motivation and reward (e.g., obesity) (Wang et al., 2001). Thus, D2 deficits appear to be a rather general marker of deficits in brain reward function rather than a distinctive feature of stimulant addiction.

Following a single conditioning trial, Bardo et al (1999) found that “CPP [conditioned place preference]…effects of amphetamine were completely blocked by pretreating rats with the D1
DA antagonist SCH-23390 (0.025 and 0.25 mg/kg) or the D2 DA antagonist eticlopride (0.2 and 2 mg/kg) on the conditioning trial” (p. 39). Therefore, both D1 and D2 stimulation are necessary for the expression of AMPH-induced reward, as removal of either signal negates this effect, and this is evident in the absence of chronic drug exposure. This may explain the decline in hedonic effects of AMPH by both antagonists in both PG and HC subjects.

Increased self-administration of AMPH by a priming dose of the drug in animals previously treated with the drug is an index of sensitization. It also corresponds closely with the concept of incentive motivation or drug ‘wanting.’ Pierre and Vezina (1998) found that “D1 dopamine receptor blockade prevents the facilitation of amphetamine self-administration induced by prior exposure to the drug” (p. 159). Chronic AMPH treatment also facilitates or cross-sensitizes incentive motivation for cocaine under a progressive ratio schedule, which measures the willingness to work for a drug after receiving a priming dose (Suto et al., 2002). Notably, this effect was blocked by local infusion of a D1 antagonist in the ventral tegmental area, site of the cell bodies for mesolimbic dopamine neurons. Taken together, these findings suggest that **D1 plays a relatively more important role in the incentive motivational effects of stimulants in animals that have been sensitized through prior chronic stimulant exposure.**

PET studies have elucidated the role of D1 receptors in humans chronically exposed to stimulants. Martinez et al (2009) found no difference between cocaine dependent (CD) subjects and controls in overall availability of D1 receptors in the striatum. “However, within the CD subjects [but not controls], low D(1) receptor availability in the ventral striatum was associated with the choice to self-administer cocaine, suggesting that low D(1) receptor availability may be associated with an increased risk of relapse in cocaine dependence” (p. 1774) Thus, cocaine users may self-administer cocaine in part to restore a deficit in D1 signaling.

Post-mortem studies of methamphetamine users have also found significant reductions in the ability of dopamine to stimulate adenylyl cyclase via D1 (Tong et al, 2003). Although some co-abuse of cocaine in these subjects cannot be ruled out, these data suggest that a functional deficit in D1 signaling may be a general consequence of chronic stimulant exposure. To the extent that PG corresponds to a stimulant-like addiction syndrome, deficits in D1 signaling may contribute to PG subjects’ relative sensitivity to the decreased priming effects of AMPH during D1 blockade with FLU as opposed to HAL, which had greater impact on controls.
The current results appear to contrast with the results of Brauer and de Wit (1995) discussed earlier, who observed a non-significant enhancement in the hedonic properties of AMPH (ARCI MBG scores) in a small group of HC subjects (n = 12) pre-treated with FLU (3 mg). The current results also appear to contrast with the results of Wachtel et al. (2002) who observed virtually identical ARCI scores in response to 20-mg met-amphetamine under HAL (3 mg) in HC subjects, although the difference in choice of psychostimulant prime may account for this disparity. However, the decrease in hedonic effects of AMPH under HAL in our HC subjects does correspond to a previous study by Brauer and de Wit (1995), which found a near-significant reduction (p < .06) in subjective Elation from 20-mg AMPH by 4-mg pimozide, a D2 antagonist with somewhat lower affinity, but somewhat greater selectivity, for D2 than HAL. The differential effects of FLU and HAL across groups in the present study, and between both medications and previous samples of HC subjects, indirectly suggest that baseline differences in D2 (and/or D1) availability/sensitivity may be an important determinant of response to low doses of dopaminergic drugs (Cools et al., 2009).

One factor that may contribute to these differences is age, which was relatively greater (~17 years) in the present sample than in the studies by Brauer and de Wit (1995). Both D1 and D2 receptor levels decline with age, which may account for differences in the ability of the antagonists to promote or block effects of AMPH in the different samples (Kuwabara et al., 2012; Suhara et al., 1991) In the present sample, age differences between PG and HC groups may contribute to overall differences in the magnitude of drug effects, but cannot explain within-group differences in response to HAL vs. FLU, as subjects were age-matched between Antagonist conditions within each group.

In summary, the current pattern of self-report effects suggests that D1 stimulation plays a primary role in the incentive motivational effects of AMPH in both HC and PG subjects. Moreover, the results appear to support an inverted-U relationship between D1 activation and the change in desire to gamble in the presence of an AMPH prime. There exists an optimal level of D1 stimulation for gambling to be seen as desirable and this level appears to be higher in PG subjects than controls. On the other hand, D2 blockade appears to reduce the hedonic properties of AMPH in both groups; with a secondary role for D1 in this process. It is possible that interruption of D2-mediated auto-receptor feedback with both HAL and FLU (Pucak and Grace, 1994), and consequent increase in basal dopamine release (Pehek, 1999) indirectly masked the hedonic effects associated with AMPH-induced dopamine release (Beninger, 1989).
Turning now to the behavioral effects of the antagonists: only one task - the Game of Dice - showed a clear effect of treatment. Specifically, a marginal Pre-treatment x Group interaction ($p = 0.091$) indicated that both antagonists (HAL and FLU) tended to reduce risky betting in HC, but tended to increase risky betting in PG. The commonality of these effects across both antagonists suggests that this effect is D2-related. The directionally opposite effects of the antagonists in HC vs. PG subjects suggest that removal of inhibitory auto-receptor feedback may have different consequences depending on differences in baseline D2 function linked with gambling pathology.

Previous research has implicated the D2 receptor in mediating inhibitory control (a sub-component of risk-taking during gambling) in both addicted and healthy animal models. Groman et al (2012) showed that chronic escalating doses of methamphetamine in monkeys reduced D2 availability, and proposed that alterations in positive feedback associated with these chronic deficits in D2 availability may be the mechanism by which stimulant users develop dysfunction in inhibitory control. Zeeb et al. (2009) showed that in stimulant-naive rats, D2 blockade resulted in improved performance on a ‘rat gambling task.’ That is, acute blockade of D2 may improve inhibitory control in healthy animals with relatively high baseline D2 availability. Other studies show that AMPH selectively improves inhibitory control in human volunteers with relatively poor baseline inhibitory control (de Wit H et al., 2000), and stimulant-induced restoration of dopamine signaling at D2 receptors appears to predict this effect (Rosa-Neto et al., 2005). Given the group differences in trait impulsivity between HC and PG subjects in the present study, the opposite effects of D2 blockade on risk-taking in the GDT may derive from baseline-dependent effects of AMPH. That is, D2 blockade deterred risky decision-making in HC subjects as it did in healthy rats (Zeeb et al., 2009), whereas D2 blockade may have promoted risky decision-making in PG subjects by negating the beneficial effects of AMPH on inhibitory control in individuals with higher baseline impulsivity.

4.4. Limitations

A number of limitations exist with respect to the current study. First and foremost the interpretation of the study findings is limited by the relatively small sample size, which impeded our ability to detect statistically significant effects on all of the measured indices. Furthermore, inter-individual variability in response to HAL, FLU and AMPH in terms of pharmacogenetic and inter-ethnic differences in pharmacokinetic parameters, including metabolism, may have
obscured the mean effects, as a standard dose of each drug was used in all subjects. Additionally, inter-individual pharmacogenetic variability may exist in terms of pharmacodynamic factors; namely the subjective response to AMPH and its relation to baseline availability of D2 and D1 receptors, by which HAL and FLU exert their effects. Subsequent studies should include a genetic analysis to identify the role of different genotypes in moderating response to the study medications via deactivating enzymes, or via D2 and D1 receptor function. The failure to replicate the effects of FLU on AMPH-induced ARCI MBG scores (euphoria) in healthy controls (Brauer and de Wit, 1995) is another limitation. This may reflect age differences in the samples as noted earlier along with the fact the original study used a single session design whereas the current study employed a repeated measures counterbalanced design. Inspection of the pattern of MBG scores revealed that the inhibitory effect of FLU on various self-report measures including ARCI MBG was more pronounced in subjects that received the drug on the second day as opposed to the first day suggesting a synergistic effect of prior exposure to AMPH and pharmacological effects of D1-D2 blockade.

A more general limitation of the present study is that FLU does not permit us to define the role of the D1 receptor per se in AMPH effects, in the absence of concomitant D2 blockade. Similarly, group differences in outcome measures for which no baseline measures were taken (e.g., GDT) cannot be conclusively attributed to AMPH (as opposed to expectancy of AMPH), and future studies should include an AMPH placebo condition to address this issue.

The present sample of PG subjects was carefully selected to exclude individuals with co-morbid Axis I psychiatric disorders. Although this enables us to attribute group difference to PG status rather than other features, co-morbid alcohol use disorders are found in about 3/4 of all cases of PG, and major depression is found in about 1/2 of all cases of PG (Petry et al, 2005). The extent to which the present findings generalize to PG subjects with these more complicated psychiatric profiles cannot be established from this study.

As noted earlier, although there were no age differences between Antagonist conditions within each group, the mean age did differ between PG and HC subjects. Given that dopamine function is affected by age (Bäckman et al., 2000), efforts to recruit older PG subjects and younger HC subjects would help to mitigate the possible impact of age on group-related effects in the final sample.

Finally, although the final study sample calls for an equal number of men and women in each
group and antagonist condition, neither the present nor the final sample is adequately powered to reliably assess gender differences in response to the experimental manipulations. This is important given differences in the etiology and clinical presentation of PG in men and women (Ibáñez, 2003; Blanco et al., 2006), and more generally, in the effects of dopaminergic drugs on healthy men and women (Walker et al., 2005; Kaasinen et al., 2001; Franconi, 2007)

4.5. Future Directions

It will be necessary to enroll more subjects in each Group and Antagonist condition to enhance the power to detect statistically significant effects. Additionally, the diversity of the sample may need to be balanced in terms of ethnicity and gender. In addition to reducing inter-ethnic and gender differences this may provide an opportunity to explore the effects of genotype and individual pharmacokinetic and pharmacodynamics differences in moderating the experimental responses to the study medications. To provide further evidence that acute AMPH exposure is comparable to an episode of gambling, it will be useful to directly compare the current subjects’ responses to the slot machine with their responses to AMPH under the same antagonist regimen to see if D1 and D2 appear to play similar roles with the game and the drug. Similarly, if pathological gambling and psychostimulant addiction are similar, it would be logical to repeat this protocol including an additional group of psychostimulant dependent subjects to see whether the antagonists reveal similar roles for D1 and D2 in the reinforcing effects of AMPH and gambling in these subjects as they do in PG subjects. Evidence of differences in response to the antagonists would increase understanding of the roles of D1 and D2 in the incentive motivational and rewarding properties of drug and behavioral reinforcers in substance-addicted individuals. Collectively, these studies can inform development of effective medications for both PG and psychostimulant addiction, which have thus far remained elusive.

4.6. Conclusion

The findings from the current study provide evidence for differing roles of D1 and D2 receptors in AMPH reinforcement. Specifically, the findings indicate that D1 primarily mediates stimulant-induced ‘wanting’, whereas D2 primarily mediates stimulant ‘liking’ as well as risk-taking in the Game of Dice Task. Group differences in Desire to Gamble under HAL vs. FLU suggest that PG subjects may have lower baseline D1 function. Group differences in the magnitude of HAL vs. FLU reductions in the hedonic effects of AMPH suggested a moderating role for D1 that mirrored the pattern seen for Desire to Gamble. This in turn suggests that the
mechanisms underlying the incentive motivational and hedonic effects of AMPH are not entirely independent in PG subjects. Finally, group differences in the effect of D2-blockade on risk-taking in the Game of Dice task suggest that baseline differences in impulsivity between HC (lower impulsivity) and PG (higher impulsivity) may determine whether D2 stimulation or blockade is more likely to confer a beneficial effect on gambling behaviour outside the laboratory. Together, the findings provide preliminary evidence that D1 receptor blockade reduces incentive motivation to gamble and that D2 auto-receptor blockade reduces stimulant-related reward in PG subjects. Medications that stabilize the dopamine signal at these receptors may have beneficial effects in PG and stimulant addiction.
REFERENCES


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Mackey, W. B., and D. van der Kooy. "Neuroleptics Block the Positive Reinforcing Effects of Amphetamine but Not of Morphine as Measured by Place Conditioning." Pharmacology, Biochemistry, and Behavior 22 (1985): 101--105.


APPENDIX A: Drug Binding Profiles
Table I: D2 Binding affinity for various ligands including Pimozide, Haloperidol, and Fluphenazine.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Response %</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>K&lt;sub&gt;i&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromperidol</td>
<td>-54 ± 6</td>
<td>2.1 ± 0.6</td>
<td>1.0 ± 0.6</td>
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<tr>
<td>Spiperone</td>
<td>-49 ± 1</td>
<td>0.3 ± 0.1</td>
<td>0.03 ± 0.01</td>
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<tr>
<td>Fluspirolene</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 ± 1.8</td>
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<tr>
<td>Pirodilone</td>
<td>-43 ± 16</td>
<td>0.2 ± 0.0</td>
<td>1.0 ± 0.4</td>
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<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>
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<tr>
<td>Chlorpromazine</td>
<td>-40 ± 14</td>
<td>11 ± 5</td>
<td>11 ± 5</td>
</tr>
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<td>Ractopamine</td>
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<td>0.4 ± 0.2</td>
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<td>Sertraline</td>
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<td>2.0 ± 0.3</td>
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<td>Ocloperidone</td>
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<td>0.1 ± 0.0</td>
<td>0.4 ± 0.3</td>
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<td>Risperidone</td>
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<td>0.6 ± 0.3</td>
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<td>Remoxipride</td>
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<td>16 ± 6</td>
<td>105 ± 38</td>
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<td>Tiapride</td>
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<td>31 ± 13</td>
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<td>Mozerone</td>
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<td>Tefudazine</td>
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<td>Transflupenthixol</td>
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<td>Clozapine</td>
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<td>Sulforidazine</td>
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<td>129 ± 21</td>
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<td>89 ± 26</td>
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Table II: D1 binding affinity for various compounds including Pimozide, Haloperidol, and Fluphenazine.

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<td>Butyrophenones + analogues</td>
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<tr>
<td>Clozapine</td>
<td>670</td>
<td>320</td>
</tr>
<tr>
<td>Benzamides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clebopride</td>
<td>16,000</td>
<td>7,600</td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>28,000</td>
<td>13,000</td>
</tr>
<tr>
<td>(-) Sulpiride</td>
<td>48,000</td>
<td>23,000</td>
</tr>
<tr>
<td>(+) Sulpiride</td>
<td>41,000</td>
<td>19,000</td>
</tr>
<tr>
<td>(-) Sulpiride</td>
<td>46,000</td>
<td>22,000</td>
</tr>
<tr>
<td>Sulpipride</td>
<td>&gt;100,000</td>
<td>&gt;47,000</td>
</tr>
<tr>
<td>YM 08050</td>
<td>13,000</td>
<td>6,200</td>
</tr>
<tr>
<td>Tiapride</td>
<td>&gt;100,000</td>
<td>&gt;47,000</td>
</tr>
<tr>
<td>YM 09151-2</td>
<td>2,800</td>
<td>1,300</td>
</tr>
</tbody>
</table>

Table III: D2, D3, D4 binding affinity of various ligands including haloperidol and fluphenazine.
Table IV: Binding affinity for various drugs including haloperidol and fluphenazine for the serotonergic receptor subtypes.

Table V: Equilibrium dissociation constants ($K_D$’s) of various drugs including haloperidol and fluphenazine for the muscarinic acetylcholine receptor.
Table VI: Equilibrium dissociation constants ($K_D$’s) of various drugs including haloperidol and fluphenazine for H1 histamine receptor

<table>
<thead>
<tr>
<th>Neuroleptics: equilibrium dissociation constants ($K_D$’s) for the histamine H1 receptor of human brain frontal cortex. *</th>
<th>Hill coefficient *</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_D$ (nM) *</td>
<td></td>
</tr>
<tr>
<td>Neuroleptics</td>
<td>Hill coefficient *</td>
</tr>
<tr>
<td>Mesoridazine</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Promazine</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>Clozapine</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>Loxapine</td>
<td>4.9 ± 0.8</td>
</tr>
<tr>
<td>cis-Thiothixene</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>Thiouridazine</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>19.0 ± 0.2</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>62 ± 7</td>
</tr>
<tr>
<td>d-Butaclamol</td>
<td>390 ± 70</td>
</tr>
<tr>
<td>Spiperone</td>
<td>480 ± 70</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>1900 ± 300</td>
</tr>
<tr>
<td>Molindone</td>
<td>124000 ± 12000</td>
</tr>
<tr>
<td>Antihistamines: d-Chlorpheniramine</td>
<td>15 ± 2</td>
</tr>
</tbody>
</table>

*Richelson and Nelson, 1984.*
**Table VII:** Equilibrium dissociation constants (K_D’s) of various drugs including haloperidol and fluphenazine for the α-1 adrenergic receptor.

<table>
<thead>
<tr>
<th>Neuroleptics: equilibrium dissociation constants (K_D’s) for α_1-adrenergic receptor of human brain frontal cortex. * ± S.E.M.</th>
<th>K_D (nM) *</th>
<th>Hill coefficient *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroleptics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spiperone</td>
<td>1.2 ± 0.2</td>
<td>0.82 ± 0.03</td>
</tr>
<tr>
<td>Mesoridazine</td>
<td>2.0 ± 0.5</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>2.6 ± 0.3</td>
<td>0.97 ± 0.08</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>5 ± 1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Fromazine</td>
<td>6 ± 2</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>6.1 ± 0.8</td>
<td>0.81 ± 0.07</td>
</tr>
<tr>
<td>Clozapine</td>
<td>9 ± 3</td>
<td>0.90 ± 0.05</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>9 ± 2</td>
<td>1.02 ± 0.06</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>10 ± 2</td>
<td>1.10 ± 0.04</td>
</tr>
<tr>
<td>cis-Thiopromazine</td>
<td>11 ± 1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Prechloperazine</td>
<td>24 ± 7</td>
<td>1.10 ± 0.05</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>24 ± 3</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Loxapine</td>
<td>28 ± 6</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>d-Butaclamol</td>
<td>56 ± 8</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Molindone</td>
<td>2.500 ± 600</td>
<td>0.71 ± 0.07</td>
</tr>
<tr>
<td>Antibypertensives</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prazosin</td>
<td>0.09 ± 0.01</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>15 ± 4</td>
<td>0.82 ± 0.03</td>
</tr>
</tbody>
</table>

*Richelson and Nelson, 1984.*
Table VIII: Equilibrium dissociation constants (K_D’s) of various drugs including haloperidol and fluphenazine for the α-2 adrenergic receptor.


<table>
<thead>
<tr>
<th>Neuroleptics: equilibrium dissociation constants (K_D’s) for the α_2-adrenergic receptor of human brain frontal cortex. *</th>
<th>Hill coefficient *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroleptics</td>
<td></td>
</tr>
<tr>
<td>Clozapine</td>
<td>160 ± 20</td>
</tr>
<tr>
<td>cis-Thiothixene</td>
<td>200 ± 20</td>
</tr>
<tr>
<td>d-Butaclamol</td>
<td>310 ± 40</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>510 ± 20</td>
</tr>
<tr>
<td>Molindone</td>
<td>640 ± 100</td>
</tr>
<tr>
<td>Spiperone</td>
<td>660 ± 20</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>750 ± 50</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>800 ± 100</td>
</tr>
<tr>
<td>Promazine</td>
<td>900 ± 100</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>1550 ± 20</td>
</tr>
<tr>
<td>Mesoridazine</td>
<td>1600 ± 100</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>1700 ± 100</td>
</tr>
<tr>
<td>Loxapine</td>
<td>2400 ± 600</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>2600 ± 200</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>3800 ± 400</td>
</tr>
<tr>
<td>Antiadrenergic</td>
<td></td>
</tr>
<tr>
<td>Yohimbine</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Rauwolscine</td>
<td>2.7 ± 0.2</td>
</tr>
</tbody>
</table>
APPENDIX B: Study Advertisements
Do you gamble?

You may be eligible for a medication research study.

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. 6574

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.net or call (416) 535-8501, or 1-800-463-6273
Healthy Volunteers

You may be eligible for a medication research study.

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. 6574

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.net or call (416) 535-8501, or 1-800-463-6273
APPENDIX C: Consent Form
Study Information Sheet

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

Principal Investigator: Martin Zack, PhD
Co-Investigators: James Kennedy, MD, PhD
Daniela Lobo, MD, PhD
Daniel DiGiacomo, MD

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

Confidentiality and Continuing Review

As part of continuing review of the research, your study records may be assessed on behalf of the 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.

Purpose:

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.

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 involves 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 registered nurse 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 ¾ - 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.

**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). Neither the experimenter nor you will know which pills you will receive. 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.

e) 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.

f) 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.

g) 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.
h) 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).

i) You will then perform two additional tasks on the computer, this time focusing on decision-making (20-min).

j) Between 1:30 and 2 you will receive lunch after which you can relax and read or watch videos until 5 p.m.

k) 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.

l) 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 operate a motor vehicle or heavy equipment for 8 hours after completion of EACH test session.

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

5. You must follow the experimenter’s instructions during the interview and test sessions. This will include adhering to schedules and arriving at the laboratory on time.

6. Just as you are free to drop out of the study for any reason at any time (for partial payment), the experimenter is 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.

7. The schedule of payment is as follows:

<table>
<thead>
<tr>
<th>Description</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>

8. You will receive a copy of this Study Information Sheet and Agreement to Participate (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 transient 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 subjects 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. Similarly, any reports of the study findings will be made so that you and all study participants remain anonymous.

As part of the Research Services Quality Assurance role, studies may be audited by the Manager of Quality Assurance. Your research records and CAMH records may be reviewed during which confidentiality will be maintained as per CAMH policies and to the extent permitted by law.

Questions
We have used some technical terms in this form. Please 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 you make a decision.

Contact

If you have any further questions, please feel free to contact Dr. Martin Zack at 416-535-8501-ext. 6052 regarding the procedures involved in the study.
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. 6876.

**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.

Print Participant’s Name ___________________________ Date ____________
Participant’s Signature ___________________________
Signature of Individual Obtaining Consent ___________ Date ____________
Signature of Investigator __________________________ Date ____________
(If investigator did not obtained the consent)

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 CAM
HAL-FLU-DEX Study: SIN and T4 advisory

Principal Investigator: Martin Zack, Ph.D. Phone: (416) 535-8501, ext. 6052

As a paid participant in this study, my Social Insurance Number is required by law. Payment I receive for my participation will be reported to Revenue Canada as taxable income, and I will receive a T4-A slip for this income.

I will receive a signed copy of this Agreement.

I agree to the conditions outlined above.

Participant Signature ____________________________ Date __/__/____

Print Name ____________________________ Date __/__/____

DD/MM/YY

Participant’s Address ____________________________

Street

__/__/____________
city prov postal code

Participant’s DOB __________/________/________

dd mm yy

Participant’s S.I.N # ____________________________
(required in order to issue cheque for payment)

Witness Signature ____________________________

Print Name Daniel Tatone Date __/__/____

DD/MM/YY
Appendix E: Additional Result Data
Table IX: Capsule Contents Evaluation for test session 1 showing subjects the number of subjects who guessed a certain way. Capsule 2 was always active AMPH however the contents of capsule 1 varied between drug and placebo depending on the test session.

<table>
<thead>
<tr>
<th>Subjects’ guess of which capsule(s) contained active drug</th>
<th>Subject received active antagonist</th>
<th>Subject received placebo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neither capsule</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Capsule 1 (Antag. or Placebo)</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Capsule 2 (AMPH)</td>
<td>8</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>Capsule 1 and 2</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>16</strong></td>
<td><strong>16</strong></td>
<td><strong>32</strong></td>
</tr>
</tbody>
</table>

Table X: Chi square test of independence for the Capsule Contents Evaluation on test session 1 shows no significant effects (p>0.05).

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Df</th>
<th>Asymp. Sig. (2-sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>2.426</td>
<td>3</td>
<td>.489</td>
</tr>
<tr>
<td>Likelihood Ratio</td>
<td>2.484</td>
<td>3</td>
<td>.478</td>
</tr>
<tr>
<td>Linear-by-Linear Association</td>
<td>1.848</td>
<td>1</td>
<td>.171</td>
</tr>
<tr>
<td>N of Valid Cases</td>
<td>32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table XI: Capsule Contents Evaluation for test session 2 showing the number of subjects who guessed a certain way. Capsule 2 was always active AMPH however the contents of capsule 1 varied between drug and placebo depending on the test session.

<table>
<thead>
<tr>
<th>Subject’s guess of which capsule(s) contained active drug</th>
<th>Subject received active antagonist</th>
<th>Subject received placebo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neither capsule</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Capsule 1 (Antag. or Placebo)</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Capsule 2 (AMPH)</td>
<td>6</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Capsule 1 and 2</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>16</strong></td>
<td><strong>16</strong></td>
<td><strong>32</strong></td>
</tr>
</tbody>
</table>
Table XII: Chi square test of independence for the Capsule Contents Evaluation on test session 2 shows no significant effects (p>0.05).

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Df</th>
<th>Asymp. Sig. (2-sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>4.471</td>
<td>3</td>
<td>.215</td>
</tr>
<tr>
<td>Likelihood Ratio</td>
<td>4.743</td>
<td>3</td>
<td>.192</td>
</tr>
<tr>
<td>Linear-by-Linear</td>
<td>0.336</td>
<td>1</td>
<td>.562</td>
</tr>
<tr>
<td>Association</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N of Valid Cases</td>
<td>32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table XIII: Means (SE) for the total score on the Symptom Side-Effects Checklist in PG and HC subjects in the HAL and FLU antagonist groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Antagonist Group</th>
<th>Treatment</th>
<th>Mean</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Controls</td>
<td>HAL</td>
<td>Drug</td>
<td>1.88</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>1.00</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>FLU</td>
<td>Drug</td>
<td>1.75</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>2.00</td>
<td>2.62</td>
</tr>
<tr>
<td>Pathological Gamblers</td>
<td>HAL</td>
<td>Drug</td>
<td>2.25</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>2.75</td>
<td>2.96</td>
</tr>
<tr>
<td></td>
<td>FLU</td>
<td>Drug</td>
<td>1.88</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>3.13</td>
<td>3.94</td>
</tr>
</tbody>
</table>