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

by

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Abstract

Pathological Gambling (PG) is an impulse control disorder with lifetime prevalence of 1-3%. Available treatments are limited by uncertain classification and complexity of implicated neurotransmitter systems. Dopamine (DA), a key neurotransmitter implicated in addictive behavior and reward is elevated in response to gambling and psychostimulants. Based on previous research, it was hypothesized that the D2 blocker, haloperidol (HAL), will enhance slot machine reinforcement in PG but not in Healthy Controls (HC). If this increase reflects preferential stimulation of D1 receptors and group differences in D1 sensitivity, D1-D2 blocker (fluphenazine, FLU) should offset increase in reinforcement seen with HAL in PG subjects. In line with DA’s implicated role in ‘wanting’ vs. ‘liking’ of the addictive reinforcer, the results suggest that DA release mediated partial D1 activation under FLU led to clear differentiation between groups with increased ‘wanting’ seen in controls but not in gamblers. DA’s role in ‘liking’ however remains elusive.
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My contribution to the research study involved: participant recruitment; obtain informed consent; conduct and monitor participants’ activity during study days and follow-ups in compliance with the study protocol, ethics and regulatory requirements; coordinate activities with the research, administrative and clinical staff; collect, analyze data and present the results in a comprehensive format; prepare and manage financial budget for the study; and issue financial compensation to the study participants.
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1. Introduction

Previous research suggested shared neurobiological features between Pathological Gambling (PG) and substance use disorders in terms of the common pivotal role of dopamine (DA) in both these disorders. This thesis will begin by providing an overview of PG and review the complex etiology of this debilitating disorder while focusing on the specific role for DA; discuss the commonality between PG and addictions, particularly stimulant addiction and describe the possible importance of sensitization to this linkage. Subsequently, the roles of DA-D1 and D2 receptors in gambling reinforcement will be discussed with reference to drug challenge studies and their implications for understanding responses to gambling in PG subjects and healthy controls. This overview will end with a rationale and specific hypotheses for the study and brief description of how they were tested.

1.1 Pathological Gambling (PG) - an overview

Gambling is understood as an act of wagering on an activity in which something of value is risked upon an event that has an unpredictable outcome (Korn and Shaffer 1999). Although the majority of people have gambled recreationally at some point in their life, a minority develop a compulsion to engage in this activity, a pattern referred to as problem gambling (ludomania). PG represents the most severe form of gambling disorder (LaPlante et al. 2008a), and it is this syndrome that forms the central topic of this thesis. In the most recent version of the Diagnostic and Statistical Manual of Mental Disorders; Fourth Edition, Text Revision (DSM-IV-TR), PG is represented as a progressive and recurrent maladaptive gambling disorder, characterized by a relentless need and urge to bet money despite harmful negative consequences on personal or professional life (Am. Psychiatr. Assoc. 2000). The DSM-IV-TR classifies PG as an Impulse Control Disorder. However, in the forthcoming 5th version of the DSM, gambling disorder is
proposed to be included under the category of non-substance or behavioral “process” addiction (Am. Psychiatr. Assoc. 2010).

1.1.1 Prevalence and Treatment of PG
PG has been linked with crime (Folino and Abait 2009), broken families and marriages (Shaw et al. 2007), depression, alcohol abuse and mortality (Morasco et al. 2006). PG is reported to have a lifetime prevalence of approximately 1-3% based on epidemiological studies (Petry et al. 2006, Cunningham-Williams et al. 2005). In Canada, PG is found to afflict 1.2% to 1.9% of the adult population (Ladouceur 1996). In the wake of rapid expansion of legalized gambling, the prevalence of this disorder has increased correspondingly (Campbell and Lester 1999; Jacques et al. 2000). This has led to increasing efforts to treat PG.

To date, a number of pharmacological (Kim and Grant 2001; Pallanti et al. 2000; Haller and Hinterhuber 1994) and non-pharmacological trials (Petry et al. 2006; Toneatto and Dragonetti 2008) have proved efficacious in the treatment of PG. Nevertheless, the treatment options have largely been borrowed from the pharmacopoeia of addictions (Petry 2002). There is no medication currently approved for the treatment of PG in Canada or the U.S., and treatment options been limited in part by uncertainty surrounding its classification.

1.1.2 Etiology of PG
Research indicates the influence of a myriad of inter-related biobehavioral factors, such as neurochemical (dysregulation of the neurotransmitters), neuropsychological (dysregulation in certain executive brain functions) and genetic factors (presence of abnormal genes for the neurotransmitter receptors) in the underlying complex etiology of PG (Goudriaan et al. 2004). These factors combined with exposure to gambling activities, contribute to the risk of PG in susceptible individuals.
1.2 Neurobiology of PG

Emerging research on the neurochemical basis of PG has implicated the dysregulation of serotonin (5-HT), norepinephrine (NE) and DA in the etiology of this disorder and genes for these neurotransmitter receptors appear to contribute equally to risk for PG (Comings et al. 2001).

1.2.1 Serotonin (5–HT)

Serotonin, a monoamine neurotransmitter in the brain, is biochemically synthesized from the amino acid L-tryptophan by the action of the enzymes: tryptophan hydroxylase (TPH) and amino acid decarboxylase (DDC). Principally released from the neurons of the raphe nuclei, serotonin exerts its action by binding to the 5-HT receptor family. 5-HT activity is believed to be prominently associated with behavioral inhibition (Soubrie´1986; Coccaro et al. 1989) and aversive processing (Graeff et al. 1996). 5-HT is also implicated in regulating sleep, pain sensitivity, sexual behavior, depression and cognitive functions (Graeff et al. 1996; Harvey and Lints 1971; Ressler and Nemeroff 2000)

Traditionally, impulse control disorders, such as PG have been linked with serotonin dysfunction (Lucki 1998). Accordingly, PG subjects have been found to have reduced levels of 5-HT metabolite, 5-hydroxy indoleacetic acid (5-HIAA) in their cerebrospinal fluid (Nordin and Eklundh 1999). Similar low levels of the 5-HT metabolite in cerebrospinal fluid were reported in subjects with impulsive aggression and mania (Linnoila et al. 1983). Recently, a drug challenge study distinguished PG from control subjects, with greater elevation in prolactin response to postsynaptic serotonergic 5-HT2C receptor stimulation with meta-chlorophenylpiperazine (m-CPP) observed in the gambler group than controls (Pallanti et al. 2006). In response to m-CPP administration, the gamblers also reported a euphoric state which they alluded to as the “high” sensation, comparable to the one reported by alcohol dependent
subjects. Despite their differential response to m-CPP challenge, the few studies that have been undertaken show mixed results for the efficacy of serotonin reuptake inhibitors in treating PG (for review see Brewer et al. 2008). Those findings implied individual differences among PG subjects and call for further research into available treatment options.

1.2.2 Norepinephrine (NE)

Norepinephrine (NE) or Noradrenaline (NA) is a neurotransmitter in the brain, synthesized from DA by the action of the enzyme, dopamine β-hydroxylase (DBH). Released from neurons primarily in the Locus Coeruleus (LC), NE acts via binding to a variety of adrenergic receptors in the Central Nervous System. Because of its link with aspects of excitement and autonomic arousal, NE has long been implicated in PG.

Studies performed in the 1980s reported extraversion as an index of NE function in pathological gamblers (Roy et al. 1989). A comparison between PG subjects and healthy volunteers showed enhanced levels of NE and its metabolites in the blood, cerebrospinal fluid and urine of gamblers (Roy et al. 1988), a result that has since been replicated (Bergh et al. 1997).

NE and heart rate measures were also found to be elevated in PG subjects relative to controls in response to a game of Black jack (Meyer et al. 2004). Apart from having a role in excitement and arousal, NE has been implicated in the functioning of the PFC. Another line of research observed an increase in the growth hormone levels to a challenge dose of the alpha 2-NE agonist, clonidine in male PG subjects relative to controls (Pallanti et al. 2010), suggesting possible dysfunction of the NE system in these individuals co-related with the severity of the disorder.
1.2.3 Dopamine (DA)

DA is a catecholamine neurotransmitter, which through its receptors in the CNS regulates attention, working memory, voluntary movement, cognition, and learning processes. Most importantly, DA (via its action on the D1 and D2 dopamine receptors) has been implicated in the reinforcement and reward circuitry of the brain, the principal neural substrate for addictions, and potentially PG (Nestler 2004; Blum et al. 1995).

1.2.3.1 Biosynthesis and Mechanism of Action

Upon synthesis from L-tyrosine (mainly in the nervous tissue and the adrenal glands) in a two-step process using the enzymes tyrosine hydroxylase and dopa decarboxylase, DA is stored into storage vesicles in the pre-synaptic neuron. Upon release into the synapse, DA is known to act on the dopamine D1-like (D1 and D5) and D2-like (D2, D3 and D4), a set of G-protein coupled receptors herewith referred to as D1 and D2 receptors that are involved in signal transduction. The level and duration of action of DA in the synapse is regulated by the DA transporter (DAT), which transports it back into the pre-synaptic terminal for future use. In animals, chronic exposure to drugs of abuse (such as cocaine, amphetamine) has been shown to enhance DA release and transmission at the synapse (Chiara and Imperato 1988; Azzaro and Rutledge 1973). For instance, chronic exposure to the DA releaser/DAT inhibitor, amphetamine results in a hyper-reactive dopaminergic state or ‘sensitization’. This process has been proposed to directly mediate the cue reactivity and compulsive drug seeking that characterize addiction (Robinson and Berridge 2001). DA-rich brain areas include the ventral tegmental area (VTA), nucleus accumbens (NAc) and prefrontal cortex (PFC), which together comprise the mesocorticolimbic pathway, a key motivational-reward circuit whose disturbance likely contributes to the etiology of PG and Substance Use Disorders (SUDs).
1.3 Dopamine (DA) – Central to Addiction and PG

Although the etiology of PG and SUDs involves multiple neurotransmitter systems, the similarity between the two disorders draws largely from the common key role for DA (Grant et al. 2006). A striking commonality in PG subjects and drug addicts is the way they define the term ‘High or Buzz’ on being exposed to their respective reinforcing stimuli. Initial evidence for this came from an early study in which PG subjects who were asked to describe an imagined episode of gambling endorsed similar items on the Addiction Research Centre Inventory (ARCI, Haertzen 1965) as subjects who had received an acute dose of amphetamine (Hickey et al. 1986). This preliminary finding suggested similarities between gambling reinforcement and the reinforcing effects of drugs, particularly psychostimulants.

1.3.1 Dopamine and its role in Reward

Blum et al. (1996), described addiction as a “Reward Deficiency Syndrome,” such that individuals who have a hypo-dopaminergic state (i.e., deficits in brain DA transmission) are at high risk of engaging in addictive-compulsive behaviors that might temporarily compensate for the scarcity in this neurotransmitter. Alterations in the dopaminergic reward system have frequently been implicated in the genesis of PG and SUDs (Kaasinen et al. 2009; for review see Potenza 2008). Comings et al. (1996), by way of a molecular-genetic study, provided further evidence for the shared genetic component between SUDs and PG, by showing that the Taq A1 variant of the human D2 DA receptor gene is associated with both the disorders, with an increased frequency of the allele seen in the PGs. These findings indicated an important common role for DA in risk for SUDs and PG.

1.3.2 Dopamine Release and links to Sensitization - Basis for Addiction

Drugs of abuse share the ability to produce robust activation of the DA mesolimbic pathway (that connects the VTA to the NAc and striatum) and elevated DA levels in the NAc (Pettit et al. 1991; Bergh et al. 1997). Exposure to reward signals under conditions of uncertainty – key
elements of gambling – activates the same circuitry (Schultz 2007). This elevation in the accumbal DA was earlier believed to correlate with the concept of reward and pleasure directly. Research suggested that alterations in the dopaminergic pathway and drug-taking behavior in the addict may possibly be associated with both positive rewards (pleasure from the addictive reinforcer) (Wise 1982) and negative rewards (relief from the unwanted aversive withdrawal symptoms) (Dackis and Gold 1985). However, considerable evidence in this field put forth the functional limitations of both the positive (Lamb et al. 1991; Haney et al. 1998) and negative (Robinson and Berridge 1993) reinforcement models in that the pleasure-seeking (positive reinforcement) and relief from withdrawal symptoms (negative reinforcement) obtained from exposure to the addictive reinforcer often do not seem to motivate drug-taking and drug-seeking behavior in the addict.

Robinson and Berridge (1993) further addressed this issue and suggested that the ‘process’ of addiction is in turn mediated by ‘sensitization’ of the neural reward pathway which refers to increased responsiveness to drug effect or external stimuli, with repeated administration. The authors implicated that the neural systems which are rendered hypersensitive (‘sensitized’) with repeated drug administration are the same ones that mediate incentive motivation in the addict (in anticipation of reward delivery). In other words, the stimuli then become attractive/ salient and highly ‘wanted’ and in turn confer goal-directed motivation to obtain the target drug (incentive motivation).

In this context, the authors coined the term “liking” to denote to the euphoric or hedonic effects experienced from exposure to the addictive stimulus (i.e., pleasure from obtaining the reward), and the term “wanting” to denote incentive salience of rewards, which indeed are thought to be two dissociable concepts but may interact with each other, along with associate learning of the rewarding stimuli (remembered pleasure) in conferring compulsive-motivational aspect of drug-
taking behavior in the addict. The authors therefore suggested that DA, which is strongly implicated in the reward process, may solely mediate the ‘wanting’ component but not the ‘liking’ component of rewards (for review see Berridge 2007). The notion is supported by evidence from a recent study that enrolled healthy male volunteers and indicated that the mesolimbic dopamine significantly correlates with drug ‘wanting’ (i.e., incentive salience) but not drug ‘liking’ (pleasure) (Leyton et al. 2002). And this pathological incentive sensitization ‘wanting’ may progressively culminate into the phenomena of drug craving.

1.3.2.1 Evidence for Sensitization in PG - from Animal Studies

In a study with primates, Fiorillo et al. (2003) found that phasic release of DA occurred upon exposure to a cue, (an icon; Conditioned stimulus, CS) for the target reinforcer, (juice; Unconditioned Stimulus, US) and that the degree of this mid-brain DA released was affected by variation in the CS-US schedule. The investigators found that the mid-brain DA neurons in the animals were most active in response to the CS in the 50% variable reward schedule, where the CS evokes an expectation of reward but provides no additional information as to whether or not it will be delivered (i.e., maximal uncertainty condition). This closely mirrors the situation in commercial slot machine gambling where initiation of the spin (CS) predicts reward delivery (a monetary payoff; US) on just under 50% of trials (Tremblay et al. 2011). Thus, gambling activates DA release in a manner directly analogous to amphetamine although the pattern of release (series of discrete trials vs. continuous emission) does differ for the two reinforcers.

1.3.2.2 Evidence for Sensitization in PG - from Human Studies

Evidence from neuropsychological studies indicates that PGs exhibit alterations in the brain executive functions consistent with sensitization. PG subjects show impaired set shifting and attention deficits on the Wisconsin Card Sort Task (Rugle and Melamed 1993), much like psychostimulant abusers and patients with schizophrenia, a condition characterized by DA hyper-reactivity (Kalechstein et al. 2009; Barch 2005). PG subjects also exhibit deficits in pre-
pulse inhibition (PPI), a form of rapid habituation that occurs when a startle stimulus (pulse) is preceded by a less intense warning stimulus (pre-pulse) (Stojanov et al. 2003). This pattern is observed in patients with schizophrenia, as well as both animals and humans chronically exposed to amphetamine (Cadenhead et al. 1993; Tenn, Fletcher et al. 2003; Hutchison and Swift 1999), inconsistent results were seen with alcohol administration and no evidence for opiate addicts has been reported (Hutchison et al. 1997; Grillon et al. 1994; Quednow et al. 2008). These findings suggest that decreased PPI is a specific feature of hyper-active DA function, rather than a general indicator of addiction status.

Evidence from neuroimaging studies in PGs indicates the underlying abnormalities in brain functioning. Although not many studies in this field have been published, fMRI studies conducted in PGs show that the ventral striatum (that comprises of the NAc) and prefrontal cortex, which form part of the brain reward pathway, mediate responses to gambling-like stimuli. Deficits in the temporal and sub-cortical frontal regions have been indicated upon exposure to addictive cues (Potenza et al. 2003; Regard et al. 2003) in PGs but not controls. Greater severity of PG, has also been found to predict greater deficits in the response of the mesolimbic reward pathway to a monetary reward (Reuter et al. 2005), suggesting possible tolerance to a standard ‘dose’ of money similar to the drug tolerance seen in SUD subjects. The above findings are consistent with the notion of the Incentive Sensitization Model of reduced ‘liking’ with severity of dependence (PG), and indicate that stimulant drugs and gambling-like stimuli exert their actions by engaging a common brain reward pathway, in which DA plays a central role.

1.3.3 Dopamine - D1, D2 receptors and links to Sensitization

As noted earlier, D1-like and D2-like DA receptors are key targets for DA. With respect to signal transduction, activation of these receptors exerts contrasting effects on the enzyme,
adenylate cyclase that converts ATP to cyclic-AMP. While D1 receptors stimulate adenylate cyclase, D2 receptors inhibit adenylate cyclase and produce an inhibitory effect on the target neuron. D1 receptors are mostly located post-synaptically and outside the synapse, and thus respond to phasic-stimulus induced DA release, whereas D2 receptors are mostly situated pre-synaptically, and within the synapse, and therefore respond to basal (tonic) DA release (Caille et al. 1996; Schultz 1998). These different sites and modes of action may translate into different subjective-behavioral effects of drugs that bind with D1 vs. D2 receptors.

1.3.3.1 Dopamine - D1, D2 receptor deficits associated with drug addiction and PG
Hyper-dopaminergic tone disrupts the relationship between DA-D1 and DA-D2 receptors within the mammalian brain. Seeman et al. (1989) indicated that the D1-D2 interactive link appears to be lost in hyper-dopaminergic disorders (Schizophrenia and Huntington’s disease) but not in normal controls. Chronic exposure to cocaine and amphetamine can produce similar disruptions. Amphetamine and cocaine challenge studies have detected deficits in the availability and function of D1 and D2 receptors in animal models of stimulant addiction, potentially due to receptor down regulation (Chen et al. 1999; Nikolaus et al. 2007). In addition, evidence from human methamphetamine users found a 25%-30% reduction in the activity of D1- receptor stimulated- adenyl cyclase in the limbic striatum (Tong et al. 2003). If PG is functionally similar to stimulant addiction, PG subjects might also display deficits in the availability and function of D1 and D2 receptors.

1.3.3.2 Dopamine - D1, D2 receptors and Stimulant Reward
Recent studies on cocaine self-administration by animals suggest that the D1-D2 activation is critical process regulating cocaine’s motivational and rewarding properties (Self et al. 1996). These studies indicate a strong relationship between D1 dysregulation and tolerance to the rewarding effects of stimulants drug, as a result of chronic exposure to supra-physiological phasic DA release. Deficits in D2 are thought to arise from elevations in tonic DA. Preferential
activation of high-affinity, pre-synaptic D2 auto-receptors by tonic DA will inhibit phasic release in response to rewards. The consequent deficit in D1 stimulation may contribute to craving. Conversely, stimulation of D1 receptors should promote satiation, and decreased reward seeking (Grace 2000; Self 1998).

In this framework, disrupting D2-autoreceptor-mediated negative feedback (using a specific D2 antagonist) should cause selective stimulation of D1 receptors via enhanced phasic DA release (Shi et al. 1997). By this mechanism, it might be possible to partially restore deficient reward and cortical activation via preferential D1 receptor stimulation in vulnerable (sensitized) individuals.

1.4 Inverted -‘U’ relationship between D1 activation and Cognition/ Reward

In their review of the neuro-modulatory mechanisms of DA in the pre-frontal cortex (PFC), Seamans and Yang (2004), proposed an ‘inverted-U’ dose-response relation between postsynaptic D1 activation and cortical efficiency, in which either too little or too much D1 activation led to sub-optimal processing of salient stimuli. Given the apparent role of D1 in stimulant reward, it is a possible that an inverted-‘U’ relationship also exists between D1 receptor stimulation and the reward derived from a stimulant drug. If this reasoning is valid, then an increase in D1 receptor stimulation would optimize reward in individuals with low baseline-D1 receptor function but would reduce reward in individuals with high baseline-D1 function due to supra-optimal D1 activation.

If gambling exerts similar effects on DA transmission as a stimulant drug, enhancing D1 stimulation should augment the rewarding effects of gambling in subjects with low baseline D1 but reduce gambling reward in subjects with normal or high baseline D1 function. This logic provides the rationale for the present study.
1.5 Drug challenge studies in PGs and implications for understanding Gambling Reward

As discussed, considerable indirect evidence points to a similarity between PG and stimulant addiction. More direct evidence for this similarity comes from research that assessed the effects of the prototypic psychostimulant, d-amphetamine on motivation to gamble in PG subjects (Zack and Poulos 2004). In animals, drugs whose reinforcing properties are mediated by common neurochemical substrates (e.g., amphetamine and cocaine) are capable of substituting for one another in cross-priming paradigms: For example, a dose of amphetamine, elicits cocaine-seeking in animals chronically exposed to cocaine, whereas no such priming is observed in response to opiates, THC or nicotine, whose reinforcing effects are mediated by different neurochemicals than cocaine (Schenk and Partridge 1999). In light of this evidence, it is noteworthy that d-amphetamine selectively increased self reported Desire to Gamble and response time to Gambling words (e.g., wager) on a rapid reading task (an index of salience) in subjects with PG but did not prime desire for alcohol or reading responses to alcohol words (e.g., whisky) in PG subjects, problem drinkers or controls. The findings from this study further supported the similarity between gambling and stimulant reinforcement and indicated potential sensitization (hyper-reactivity to cues for gambling) in PG subjects. However, the study could not isolate the role of DA or the specific receptors involved in primed motivation to gamble as amphetamine enhances 5-HT, NE, as well as DA.

To address this issue, a subsequent study examined the effects of the preferential D2 receptor antagonist, haloperidol (HAL) on responses to an episode of gambling on a commercial slot machine in PG subjects and controls. By blocking inhibitory pre-synaptic auto-receptors, HAL would be expected to increase spontaneous and stimulus-induced DA release, with corresponding increases in D1 activation (Pehek 1999).
The results from the study indicated that relative to placebo treatment, a dose of HAL (3 mg) capable of blocking 65% of D2 receptors significantly enhanced the self-reported post-game Desire to Gamble and subjective effects (e.g., Enjoyment, Excitement) of the game, and led to faster response time to Gambling vs. Neutral words on the rapid reading task, in PG subjects but not in controls. Although the slot machine game primed motivation to gamble in both the groups (under placebo), HAL only augmented this effect in PG subjects. Based on the hypothesized ‘inverted U’ relationship between D1 stimulation and optimal reward, this result suggests that HAL may have restored a deficit in D1 baseline function in PG subjects (who are tolerant to gambling reward), but may have simply shifted D1 function from slightly sub-optimal to slightly supra-optimal, with no net change in subjective reward, in high-baseline controls.

1.5.1 Effects of haloperidol (HAL) on Psychostimulant Reward
In a previous study (Wachtel, Ortengren et al. 2002), HAL (3 mg) caused no alteration in the subjective rewarding effects of methamphetamine (20 mg) in healthy volunteers. These effects directly mirror those of Zack and Poulos (2007) for gambling reward in controls. Another study, using pimozide (4 mg), which is more selective for D2 but somewhat less potent than HAL, had no effect on subjective reward from d-amphetamine (20 mg) in healthy volunteers, confirming the reliability of the results for HAL (Brauer and de Wit 1995). Thus, in subjects with no deficit in D1 function, moderate doses of D2 antagonists have similar null effects on the subjective reward derived from slot machine gambling as well as psychostimulant drugs.

In their program of testing with D2 antagonists and psychostimulant drugs, de Wit and colleagues observed a set of effects that differed from those for HAL and pimozide. Using the drug, fluphenazine (FLU; 3 mg) as the pre-treatment, Brauer and de Wit (1995) observed an increase in the subjective rewarding effects of d-amphetamine (20 mg) along with an improvement in psychomotor tracking (relative to placebo pre-treatment) in control subjects.
By application of the bidirectional inverted ‘U’ relationship between D1 activation and cognition/reward, the above findings could imply that the failure to show amphetamine reinforcement under HAL in controls (with normal-high baseline D1 function) could reflect a shift from slightly sub-optimal to slightly supra-optimal D1 activation, with no net change in reward. By partially reversing supra-optimal D1 stimulation induced by D2 blockade-mediated DA release, FLU would produce optimization of D1 receptor stimulation, resulting in increased amphetamine reward in control subjects.

This interpretation implies that the findings for HAL on responses to the slot machine in PG subjects may reflect optimization of D1 signaling and increased gambling reward in subjects with low baseline D1 function. If this analysis is correct, partial blockade of D1 with FLU should negate the enhancement in gambling reward seen under HAL in PG subjects. This question forms the basis for the present study.

1.6 Specific Aims and Hypotheses

The specific aims and hypotheses of this thesis are:

Aims

1. To replicate the effects of HAL on gambling reward in PG subjects and controls.
2. To investigate the role of D1 activation as the basis for the increase in gambling reward during D2 blockade in PG subjects by comparing the effects of HAL to the effects of FLU.
3. To determine if the enhanced amphetamine reward by FLU seen in the prior study with control subjects also emerges for gambling reward in control subjects.

Hypotheses

- Hypothesis 1: Relative to placebo pre-treatment, HAL (3-mg) will increase gambling reinforcement in PG Subjects but not in controls. This will be demonstrated with a)
subjective, b) cognitive-behavioral, and c) physiological responses to a 15-min slot machine game.

- **Hypothesis 2:** If enhanced D1 activation mediates the effect of HAL, relative to placebo pre-treatment, combined D2 and partial D1 blockade with FLU (3-mg) will lead to a decrease or no change in gambling reinforcement in PG Subjects. This will be demonstrated on the same indices (a-c).

- **Hypothesis 3:** If D1 and D2 receptors play similar roles in psychostimulant and gambling reward, FLU should enhance reinforcement of the slot machine in controls, but not in PG subjects, as demonstrated on indices (a-c).
2. Materials and Method

2.1 Study Overview and Design
The study employed a randomized double-blind, counterbalanced, between-within design: 2 Group (PG, HC) x 2 Antagonist (HAL, FLU) x 2 Treatment (Drug, Placebo) for gambling assessment. On two separate sessions, all subjects received the gambling reinforcer (a 15-min slot machine game) after pre-treatment with the antagonist vs. placebo. Two additional test sessions were also conducted (after the gambling sessions) to evaluate the effect of a stimulant drug (amphetamine, 20 mg) in PG and HC subjects. Those results are not reported in this thesis. Study duration/subject was six weeks on an average. The study involved six visits to CAMH comprising of a screening interview, a physician’s exam, and two test days (held one week apart) that assessed subjects’ gambling behavior. PG and HC subjects were matched on factors that could moderate experimental response (see below) and randomly assigned to an Antagonist Group (HAL, FLU) and a Treatment Sequence (Drug first, Placebo second or vice versa). The study design is summarized in the Figure 1 below.
2.2 Medications

2.2.1 Pharmacokinetics (PK) and Pharmacodynamics (PD) of haloperidol (HAL) and fluphenazine (FLU)

2.2.1.1 Pharmacokinetics (PK)

HAL and FLU belong to the class of typical antipsychotic drugs, sold in Canada under the trade names: Haldol® and Prolixin® (fluphenazine decanoate) respectively. Both the drugs have a very similar PK profile (Jorgensen et al. 1986). Plasma concentrations of the drugs generally reach a low peak 2-3 hr in the range of ng/mL following oral doses and ½- 1 hr following intramuscular (i/m) dosage. Both the drugs exhibit a shortened oral bioavailability of nearly 40-50% (FLU) and 60-65% (HAL) due to extensive hepatic first pass metabolism (Froemming et al. 1989). Whereas FLU has a slow i/m bioavailability, HAL is rapidly absorbed through the i/m route, although depending on the ester formulation used. The drugs’ elimination half lives range between 10-30 hr and a steady-state concentration in plasma is reached with a 2-5 days treatment regime (Dahl 1990).
There are two main Cytochrome-P450 (CYP450) enzymes identified which are involved in the biotransformation of HAL and FLU: CYP3A4 and CYP2D6. For CYP3A4, there are no functional polymorphisms identified that are associated with HAL metabolism. However, over 60 functional polymorphisms have been identified for CYP2D6 metabolism. Glucoronidation regulates a large proportion of HAL’s intrinsic hepatic clearance, followed by oxidation with CYP450 isozymes and reduction to reduced HAL (Gorrod and Fang 1993). Both HAL and its metabolites have been reported to be potent inhibitors of CYP2D6 (Shin et al. 2001). A similar selectivity for CYP2D6 inhibition has been reported with FLU over other CYP isozymes (Shin et al. 1999).

2.2.1.2 Pharmacodynamics (PD)

Drug – Receptor Binding Profile

Appendix A shows Ki values (inhibition constants) for HAL and FLU at DA receptors as well as other major transmitters (lower scores indicate greater affinity). Of primary importance to this study, Table I shows the Ki’s for HAL and FLU at D1 receptor. While FLU had the highest affinity for D1 (Ki < 1), HAL only had intermediate affinity (Ki = 17). The relative affinity (selectivity, larger scores indicate stronger affinity for D2) of HAL for D1: D2 = 28. Whereas the relative affinity of FLU for D1:D2 = 2.1. Thus, HAL is approx. 13 times more selective for D2 than FLU. Thus, FLU could be described as a mixed D1- D2 receptor antagonist with high affinity for both receptors while HAL is a preferential high affinity D2 antagonist with moderate affinity for D1.

Appendix A: Table II, Table III and Table IV show that the drugs are well-matched on affinity for other DA receptors. FLU has modest, and HAL has low affinity for 5-HT receptors. HAL and FLU have similar low affinity for muscarinic and α-2-NE receptors, and similar moderate
affinity for α-1-NE receptors. The only clear difference in binding profiles is for histamine (H1) receptors, where FLU has moderate, while HAL has low affinity.

2.2.2 Rationale for selecting HAL and FLU
Of the DA antagonists available for use in Canada, HAL has the greatest selectivity for the D2 receptor. In addition, use of HAL enabled us to test whether the prior findings for gambling reinforcement could be replicated (Zack and Poulos 2007). As a comparative agent, FLU had very similar affinity to D2 as HAL, but also had very high affinity for D1 (see Appendix A, Table I Binding profiles at Dopamine D1 and D2 receptors).

Use of FLU to assess effects of combined D1-D2 blockade was also based on the existence of an empirical precedent against which to compare our findings (Brauer and de Wit 1995). These investigators observed a clear trend for enhanced AMPH reinforcement following FLU pre-treatment, but this failed to achieve statistical significance due to a small sample (n = 12). Neither HAL nor FLU has ideal selectivity for D1/D2. However, as outlined in Appendix A these antagonists provide the best balance of (i) known effect size, (ii) relative affinity for D1 vs. D2 and (iii) lack of affinity for other neurotransmitter receptors.

2.3 Sample size justification and Blood Genotyping
Sixteen subjects, eight PG subjects and eight healthy controls (HC) completed the study successfully. The entire sample in the course of a projected 3-year study is 80 subjects (80/36 = 2.2 subjects completed per month). The project start-up phase, training, piloting and refinement of protocol lasted 3 months. Testing lasted 9 months = 16/9 = 1.8 subjects per month (Also see Figure 2 Recruitment Flow Chart).

As a possible check for pharmacokinetic variation and the possible role for DA D1 and D2 receptor genes in pathological gambling, blood genotyping was conducted in the sample (as part
of the clinical lab tests, see later in the text). However, future studies would need to increase sample size in order to identify patterns.

2.4 Ethical Considerations

This study was approved by the CAMH Research Ethics Board (Study Number: RN 52207) and was conducted in accordance with the Declaration of Helsinki (1975; updated 1989). All subjects provided written informed consent and a Social Insurance Number before participating. Upon completion of the study, subjects were compensated $1000 for participation.

2.5 Subjects

2.5.1 Recruitment

Subjects were recruited by posting advertisements on Craigslist.org, NOW magazine online, and Kijiji (See Appendix B - Study Advertisement for Pathological Gamblers and Appendix C - Study Advertisement for Healthy Controls). The study Advertisements were approved by the CAMH Research Ethics Board (REB).

Prior to inclusion in the study, all subjects underwent a comprehensive telephone screening, an interview screening with EKG, blood and urine toxicology screens, and a physician’s exam. Figure 2 below shows the flow chart for each stage of the recruitment process.
As indicated in Figure 2, 650 individuals responded to our advertisement (gamblers and control ads). 225 subjects underwent the telephone screening and the rest could not be contacted or were not interested in participating. 79 subjects passed the study inclusion criteria as assessed over the phone screening (see below). The pre-screened subjects were then requested to attend the interview screening at CAMH. 52 subjects underwent the interview screening; however only 23 of them could make it to the physical exam. Furthermore, 19 subjects were rendered eligible based on the physician’s exam. Out of the 18 eligible participants that made it to the study test phase, 9 Subjects were randomized to the HAL group while the other half randomized to the FLU group. From the PG-FLU group, 1 subject withdrew from the study due to unforeseen work commitment, and another 1 subject withdrew from the HC-HAL group due to
experienced side effects from the test drug (akathisia; listed on the consent form). As a result, 16 subjects successfully completed the study, 8 in each group (PG/ HC).

### 2.5.2 Inclusion Criteria

In order to participate, all subjects needed to pass the following inclusion criteria:

a) Between the ages of 19 and 65 years of age

b) Non-treatment seeking

c) Physically and mentally healthy - per blood/urine and EKG examinations; Body Mass Index $< 35$ for both males and females as per the physician’s exam.

d) No first order biological relative with schizophrenia or bipolar disorder

e) No prior use of any psychostimulant; scored 0 on the Drug Abuse Screening Test (DAST, Skinner 1982)

f) Non- co-morbid – i.e., no DSM Axis I diagnosis apart from Pathological Gambling or nicotine dependence based on Structured Clinical Interview for DSM Axis I disorders (SCID- I, First et al. 1995)

g) Able to understand English language - grade 7 level English language fluency and a score of $\geq 18$ on the Wechsler’s Vocabulary scale (WAIS-Vocab) was required (to facilitate comprehension of word stimuli on the cognitive computer-based tasks) (Wechsler 1981).

h) Normal or corrected-to-normal vision

i) Scored less than 4 on the Fagerstrom Test for Nicotine Dependence (FTND, Heatherton, Kozlowski et al. 1991)

j) Smoked $< 20$ cigarettes/day for male and $< 15$ cigarettes/ day for females (to minimize nicotine withdrawal during test phase)

k) Scored less than 10 on the Alcohol Dependence Scale (ADS, Skinner and Allen 1982) to rule out any moderate alcohol dependence
1) Scored less than 10 on the Beck Depression Inventory-short form ≤ 10 (BDF-sf, Beck and Beck 1972) to rule out clinically relevant depression

m) Men were required to drink less than 20 alcoholic drinks/week and women less than 15/week, based on the 90-day Timeline Followback (TLFB, Sobell and Sobell 1992)

n) Resting systolic blood pressure ≤ 140mmHg

o) Women may not be pregnant or breastfeeding

p) HC subjects needed to score 0 on South Oaks Gambling Screen (SOGS, Lesieur and Blume 1987) and DSM-IV PG criteria (Beaudoin and Cox, 1999)

q) HC Subjects must have played a slot machine game at least *once* in their lifetime to minimize differences in novelty of gambling reinforcer that could interact with treatment sequence

r) PG subjects needed to score ≥ 5 on both SOGS and DSM-IV criteria

2.5.3 Study Payment

Subjects who completed the study successfully received a payment of $1000- $920 participation + $80 ‘standard bonus’ from playing the slot machine on test days, as a cheque, 2 weeks after completion.

2.6 Study Testing Timeline
Figure 3 Timeline of Events for Test Sessions

Figure Legend: BAS, Baseline; HR/P, Heart Rate/ Blood Pressure; Preg Test, Pregnancy Test; HAL, haloperidol (3 mg); FLU, fluphenazine (3 mg); Pkg- A, Baseline Questionnaire Package A; Pkg- B, Questionnaire Package B (15-min before peak dose-1); Pkg- C, Questionnaire Package C (post slot machine game); Pkg- D, Questionnaire Package D (post RRT); Pkg- E, End Questionnaire Package E; VAS, Visual Analogue Scale; POMS-sf, Profile Of Mood States-short form; ARCI, Addiction Research Centre Inventory; VLT, Subjective Slot Machine Effects questionnaire; SSEC, Symptoms Side-Effects Checklist. (See next page for explanation)
Schedule of Events on Test Sessions (both test day 1 and 2)

Arrival, 8:30 am - Baseline measures:
- Breathalyzer
- BP/HR recorded (Baseline)
- Pregnancy test
- Questionnaire package (A): VAS-Desire to Gamble, ARCI and POMS-sf
- Standard breakfast

Post-breakfast, 9:00 am:
- Capsule 1 (Hal/Flu, Placebo) administered
- Questionnaire package (B) 15-min prior to peak medication levels

FLU group Subjects
- 11:00 am: Capsule 2 administered
- 11:10 am: Subjects play Slot Machine Game (15-min)
- 11:25 am: BP/HR post game, Questionnaire package (C) administered: VAS Desire to Gamble/Enjoyment/Liking, POMS-sf, ARCI, VLT.
- 11:40 am: Subjects play Rapid Reading Task (RRT), Questionnaire package (D) administered: VAS-Desire to Gamble/Enjoyment/Liking, ARCI and POMS-sf
- 12:15 pm: Subjects play SST
- 12:35 pm: Subjects play GDT
- 1:00 pm: Subjects complete Pilot Number Task, Questionnaire package (E) administered: VAS “Feel Drug,” POMS-sf, ARCI, CCE (test day-2) and SSEC.
- 2:00 pm: Subjects receive lunch

HAL group Subjects
- 11:45 am: Capsule 2 administered
- 11:55 am: Subjects play Slot Machine Game (15-min)
- 12:00 pm: BP/HR post game, Questionnaire package (C) administered: VAS Desire to Gamble/Enjoyment/Liking, POMS-sf, ARCI, VLT and DEQ.
- 12:15 pm: Subjects perform RRT, VAS-Desire to Gamble, ARCI and POMS-sf
- 12:50 pm: Subjects perform SST
- 1:10 pm: Subjects complete GDT
- 1:35 pm: Pilot Number Task, Questionnaire package (E) administered: VAS “Feel Drug,” POMS-sf, ARCI, CCE (test day-2) and SSEC.
- 2:00 pm: Subjects receive lunch

Detoxification, 3:00 pm:
- HAL/FLU group Subjects take rest
- Pre-discharge assessment by Registered Nurse

Dismissal, 3:30 pm

2.7 Materials

2.7.1 Apparatus

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

**Heart Rate - Blood Pressure (HR-BP) monitor**

The wrist cut-off (HEM-601; OMRON, Vernon Hills, IL) was employed to measure heart rate and blood pressure at the beginning of the interview/ baseline assessment day, and at regular time intervals throughout each test session in order to assess cardiovascular reactivity to the experimental manipulations.

**Cognitive task software for vocal response task**

The Rapid Reading task (see below) was administered on a PC equipped with Microexperimental Laboratories (MEL) software (v. 2.01; Psychology Software Tools Inc., Pittsburgh, PA, USA) connected to a microphone. This system was used to record subjects’ latencies in voice responses. A serial response box (Psychology Software Tools Inc., Pittsburgh, PA, USA) was used to record the accuracy of vocal responses during this task.

**2.7.2 In-person Screening Interview Tools**

**SCID- Structured Clinical Interview for DSM criteria**

All subjects who passed the telephone screen underwent the Structured Clinical Interview (SCID-I) for DSM-IV (First et al. 1995). The SCID-I is a validated, short structured psychiatric interview, composed of questions designed to make a current diagnosis of psychiatric or substance use disorder and current and lifetime criteria for bipolar and psychotic disorders, in order to assess Axis I DSM-IV disorders. The SCID was supervised by Dr. Daniela Lobo, a psychiatrist trained in its administration and scoring.

**Gambling Severity Assessment Tests**
To verify/confirm subjects’ PG status after the phone screening, SOGS (score ≥ 5) was repeated in person on the day of the interview. A psychiatrist further verified that subjects’ gambling problems were current rather than historic by evaluating subjects against the DSM-IV PG criteria (score ≥ 5, Beaudoin and Cox 1999).

**Pregnancy Test**

To ensure against unintended exposure of a foetus to any of the study medication, all female participants were required to take a urine-based pregnancy self test at the laboratory before the start of the interview screening and each test session.

**Clinical Lab Tests (Urine/Blood and EKG)**

The clinical lab tests were conducted at the end of the interview screen. The blood sample corresponding to an amount equal to 2-3 finger length tubes (30 ml) was drawn from the subjects’ arm, by a registered nurse. The urine sample was used to confirm lack of recent drug use and to confirm that females were not pregnant. The EKG was done to confirm the absence of heartbeat anomalies. The laboratory reports from the Urine, Blood and EKG were reviewed by the study physician later to determine subjects’ eligibility on the study.

### 2.7.3 Initial Screening Scales (Questionnaires)

#### 2.7.3.1 Recruitment and Telephone Screening Assessment Scales

**South Oaks Gambling Screen**

The South Oaks Gambling Screen (SOGS, Lesieur and Blume 1987) identified the problem gambling status of potential gambling volunteers. SOGS is a validated 20-item questionnaire based on DSM-III criteria for pathological gambling, wherein the ad respondents were asked to describe their lifetime gambling habits (i.e., questions such as: “have you ever gambled more than you intended to” or “have you ever borrowed money from someone and not paid them back as a result of your gambling” etc.). Eleven items were scored in total, and a SOGS score ≥ 5 was used to identify “probable pathological gamblers”. Therefore, to be eligible for the PG group, all
subjects needed to score ≥ 5 on the SOGS. However, to be eligible for the social control group, all subjects needed to score 0 to rule out any gambling pathology.

**Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition**

Apart from SOGS, the Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM-IV) based gambling questionnaire (DSM-IV, Beaudoin and Cox 1999) was administered to make a clinical diagnosis of PG. Subjects were enquired about the time in their lifetime when they were gambling the most and for each question four options were provided that targeted different time frames. A score ≥ 5 was required to qualify for the PG group, and a score of 0 was required for the HC group.

**Beck Depression Inventory- short form**

The Beck Depression Inventory- short form (BDI-sf, Beck and Beck 1972) is a 13-item validated tool designed to detect depression in a primary care population. The scale assessed the level of subjects’ depression symptoms at the time of the telephone and interview screening. Individuals with a total score ≤10, indicating at most low-level of depressive symptoms, were eligible for the study.

**Alcohol Dependence Scale**

The Alcohol Dependence Scale (ADS, Skinner and Allen 1982) assessed the problem drinking status of all potential candidates over the past year (12 months time period). The questionnaire was administered on the telephone screen and repeated as a self-report measure on the interview screen. Its 25 items are used to make an overall assessment of alcohol withdrawal symptoms, impaired control over drinking, awareness of a compulsion to drink, increased tolerance to alcohol and salience of drink-seeking behavior. In order to participate in the study, all subjects had to obtain a total score of < 10 on the ADS (Skinner and Allen 1982).

**Eysenck Impulsiveness Questionnaire**
The Eysenck Impulsiveness Questionnaire (EIQ, Eysenck et al. 1985) was administered on the telephone screen and assessed Impulsiveness, Venturesomeness and Empathy. It consists of 54 ‘yes’ or ‘no’ answer questions.

**Wechsler Vocabulary**

Wechsler- vocabulary is a clinical instrument used to measure adult and adolescent intelligence and proficiency of the English language (Wechsler 2001). The test was administered in order to ensure that the subsequent cognitive task results were not affected by lack of comprehension.

**2.7.3.2 Interview Screening Assessment Scales**

**Eysenck Personality Inventory**

The Eysenck Personality Inventory (EPI, Eysenck and Eysenck 1963) was used to assess subjects’ personality. The test was administered both during the telephone and the interview screen. It required subjects to complete 57 “yes” or “no” situational questions by thinking about the way they might act or feel in a given situation. The EPI was used to assess Extraversion (0-24), Neuroticism (0-24), Impulsivity (0-9) and Tendency to Lie (0-9).

**Drug Abuse Screening Test**

The Drug Abuse Screening Test (DAST, Skinner 1982) was designed to provide a brief instrument for clinical screening and treatment evaluation research. The 28 self-report items tap various consequences that are combined in a total DAST score to yield a quantitative index of problems related to drug misuse. To be eligible, subjects had to score < 4 indicating no evidence of drug abuse.

**Fagerstrom Test of Nicotine Dependence**

The Fagerstrom Test of Nicotine Dependence (FTND, Heatherton, Kozlowski et al. 1991) is a 6-item questionnaire used to assess the subjects’ level of nicotine dependence. PG and HC who
smoked were evaluated. A score of 1-2 implies “very low dependence”; a score of 3 implies “low to moderate dependence”; a score of 4 implies “moderate dependence” and a score of 5 or more implies “high dependence”.

**Nicotine Timeline Followback**

For subjects who smoked, the Nicotine Timeline Followback was administered on the interview screening day. Subjects were asked to complete a history of their smoking behavior over the last seven days. By making use of a calendar, they were asked to record the number of cigarettes they smoked in the past week, counting backwards from the day of the interview.

**Alcohol Timeline Followback**

The Alcohol Timeline Followback (TLFB, Sobell and Sobell 1992) was administered on the interview screening day. Subjects were asked to complete a history of their drinking behavior over the last 3 months (90- days). Using a calendar, they were asked to record the number, amount and the type of alcoholic beverage they drank over the past 90 days, counting backwards from the day of the interview.

**Wechsler Digit-Span**

The Wechsler Digit Span test (Wechsler 2001) was used to assess the basic cognitive proficiency of the subject. This verbal test was administered on the interview screen and was divided into two parts- digits forward and digits backward. During the Digits Forward phase, a series of one-digit numbers were called out in a random sequence (e.g. 4, 5, 8) at the rate of one number per second, following which the subject had to repeat the numbers in the same sequence (i.e. 4, 5, 8). During the Digits Backward phase, a series of one-digit numbers in a random sequence (e.g. 4, 5, 8) were called out. However, this time the subject was asked to repeat the numbers back in the reverse sequence (i.e. 8, 5 and 4). In each version of the task, the number of digits increased by one until the subject failed to complete 2 trials of the same span length.
Subjects scored 1 point for each series of digits correctly repeated. The maximum score on the digit-forward and digit-backward tests is 14 points for a total maximum score of 28 points.

**Wechsler Digit-Symbol**

The paper-and-pencil based Wechsler Digit-Symbol task (Wechsler 2001) was administered on the interview screen and measured psychomotor speed and retention of symbolic associations. The task required subjects to match a series of numbers laid out on a test sheet to the corresponding symbols listed on top of the sheet, as quickly and accurately as possible in a one minute (60 seconds) time frame. After finishing 10 practice trials, Subjects were timed to complete the test trials. Total correctly coded trials were the dependent measure.

2.7.3.3 **Self Report - Test Day Assessment Scales**

2.7.3.3.1 **State Scales**

**Visual Analog Scale**

Modified visual analog scale (VAS, Fischman and Foltin 1991) was used to quantify the subjects’ Desire to Gamble and desire for drinking alcohol. Ratings were scored using a numbered scale ranging from 0 (not at all) to 10 (extremely), with 0.5-point gradations.

**Profile of Mood States – short form**

The self-reported POMS-sf (Shacham 1983) questionnaire was administered to measure a range of subjective states: Anger-Hostility, Confusion, Depression-Dejection, Vigor, Fatigue, Tension- Anxiety, at specific time points throughout the testing phase. It consisted of 37 mood-related adjectives (e.g., tired, excited, happy). The subjects were required to rate their feeling on a scale from 0-5 corresponding to “not at all” to “extremely”.

**Addiction Research Center Inventory**

The Addiction Research Center Inventory (ARCI, Haertzen 1965) is a standardized 550-item questionnaire used to assess drug-like subjective reactions to the test drugs. The conventional, derived short form of the inventory was used that was composed of 49 true or false items that
subjects filled out at specific times during test days (Martin, Sloan et al. 1971). The short form consisted of five scales: Pentobarbital-Chlorpromazine-Alcohol Group (PCAG, a measure of sedation), Morphine-Benzedrine Group (MBG, a measure of euphoria), Lysergic acid diethylamide (LSD, a measure of dysphoria and psychotomimetic changes), Amphetamine (A, a measure of stimulant effect), and Benzedrine Group (BG, a measure of stimulant effect like amphetamine).

**Slot Machine Subjective Effects**

The Slot machine subjective effects questionnaire was administered on test days right after subjects’ played the slot machine game. It consisted of four visual analogue scale-questions that enquired about subjects’ “Enjoyment”, “Excitement”, “Involvement” and "Buzz/High" from playing the game.

### 2.7.3.3.2 Additional Test Day Assessment scales

**Capsule Content Evaluation**

At the end of the study, the Capsule Content Evaluation (Zack and Poulos 2007) sheet was administered to the subjects. The subjects were asked to make a guess and report on the sheet which day they thought they received the active medication vs. placebo.

**Symptoms Side-Effects checklist**

The Symptoms side-Effects Checklist consisting of 47-items was administered to the subjects at the end of each test day (Zawertailo, Busto et al. 1995). The questionnaire is administered to assess for possible side-effects and control for any adverse effects that the subject might experience from taking the study medication. On the checklist, the subject checks off any symptoms felt and rate the severity of occurrence on a scale of 0-4.

### 2.7.3.4 Cognitive (computer-based) Experimental Tasks

**Wisconsin Card Sort Task (WCST)**
The WCST is a well-established neuropsychological test of "set-shifting", i.e. the ability to display flexibility in the face of changing response rules (Marazziti, Dell’Osso et al. 2008). The computerized version of the task presented four stimulus cards that appeared at the top of the screen. The four key cards were different in three aspects: color, quantity and design. The computer decided which one of these three aspects was the criterion dimension for a given series of trials throughout the task. During the course of the task, the matching aspects changed randomly. On each trial, the subject was required to select one of four response cards presented at the bottom of the computer screen based on its correspondence with the criterion dimension for that trial. After each selection, the subject was told whether his response was right or wrong (the word Correct or Incorrect appeared at the bottom of the screen). However, the computer did not tell the subject how to match the cards correctly.

**Rapid Reading Task**

This task assessed the time and accuracy of the subjects’ vocal response to target stimuli (words) shown on a computer screen (Zack and Poulos 2004, 2007). Before each trial, a warning signal “&&&&” appeared in the centre of the screen to show where the target stimulus would appear. Subjects were required to read the individual words that appeared on the screen as quickly and accurately as possible. Five word categories were employed: Gambling e.g., wager, Alcohol e.g., Vodka, Positive Affect e.g., Cheerful, Negative Affect e.g., upset, Neutral e.g., window. To enhance priming effects the target items were degraded with asterisks (e.g., w*a*g*e*r). Subjects performed 20 practice + 150 (5 categories @ 30 words) test trials, with items and categories randomized throughout. The pace of the task was controlled and response accuracy (correct/misread) was coded after each trial by the experimenter, using a button box.

**Slot machine Game**
A commercial slot machine (‘Cash Crop,’ WMS Gaming; Detroit, MI) was the gambling stimulus/reinforce on test days. The game was played in a simulated bar environment (mock-bar laboratory) to enhance the external validity of the test. Subjects started out with an initial stake of $200 (400 cash credits) on each session and could wager anywhere from 1-45 credits (1-5 credits x 1-9 ‘lines’ per spin). Subjects were advised that the cash credits accumulated at the end of the game were redeemable for real money and would be added on to their participation fee at the end of the study. Thus, although the ratio of credits to cash was not stated, the proportional relationship between final credit tally and cash bonus provided an incentive to play the game as they normally would out at a casino. Trial-by-trial bet size, line selection and credits won were recorded electronically on a hidden computer monitor (subject was not identifiable). To encourage spontaneous betting behavior, subjects were not told that their behavior was being monitored until debriefing.

**Game of Dice Task**

The Game of Dice Task was administered to assess subject’s risk-taking behavior (Brand et al. 2005). At the beginning of the game, subjects were advised that their aim should be to win as much money as possible, and to avoid losing money. The computer rolled a ‘virtual’ die 18 times on the computer screen. Each of these times, the subject was required to “guess” which number (between 1 and 6) will be thrown. The starting balance was a thousand dollars. Before each throw, subject could select a single number or different combinations of numbers from the various options presented on the four displayed rows. The first row was composed of individual numbers (e.g., 1, 2, 6) and corresponded to maximal risk (exact match with the outcome of the die toss required to win) – maximum payoff or loss ($1000) whereas the fourth row was composed of a combination of four numbers (e.g., 1234) and corresponded to minimal risk (outcome of die toss could match any of 4 numbers in the combination and win) – minimum
payoff or loss ($100). Selecting a number from the second and third row (two- and three-number combinations, respectively) corresponded to medium risk ($500, $200). Thus, risk-taking was operationally defined by the number of possible outcomes (row) selected. The number that was thrown on any trial was random. Wins were credited to the current balance that appeared on the top right corner of the computer screen. Losses were recorded in the same way and the corresponding amount was subtracted from the balance. The subjects were informed of a loss or a win by way of a low-pitched tone. If the final balance increased, a green bar would get bigger indicating ‘winning’ and if the balance reduced to a negative value, a red bar would get bigger indicating the amount of money the subject ‘owed’.

**Stop Signal Task**

The Stop-Signal Task was used to assess subjects’ inhibitory control of a pre-potent psychomotor response (Logan, Schachar et al. 1997). The task required subjects to make a quick decision by pressing one of two keys (‘z’ or ‘/’) with the index of finger of the left or right hand respectively, depending on the stimulus (alphabets ‘a’ or ‘b’/ ‘c’ or ‘d’) that appeared on the computer screen. Two key alphabet versions were used in the task: ‘a’ or ‘b’ on test day 1 and version ‘c’ or ‘d’ on test day 2, in order to minimize repetition priming.

The visual stimuli (‘a’ or ‘b’/ ‘c’ or ‘d’) acted as a GO signal in this task. Each trial consisted of a focal point ‘+’ followed by the visual stimuli. On a random 25% of trials, a tone (stop signal) occurred briefly after the onset of the visual stimulus, which indicated that subjects should withhold their response, i.e. not press either key upon the appearance of the visual stimulus on that particular trial. The stop signal was a 100 ms / 1000 Hz tone played through the internal speaker of the computer and was presented at varying intervals after the visual cue appeared. The interval between the onset of the go signal and stop signal (stop signal delay) determined the difficulty of the task. The stop signal delay was initially set at 250 ms and
adjusted automatically depending on subject’s performance. Each time subject was successfully able to withhold a key press (inhibition) following a stop signal the delay was subsequently increased by 50 ms thereby making it more difficult for subject to inhibit a response on the next stop signal trial. However, each time subject failed to inhibit his response to a stop signal, the delay was decreased by 50 ms thereby making it easier to inhibit a response on the next stop signal trial. Subjects completed 2 practice blocks before the test trials began and over a course of 256 test trials administered that were split into 3 blocks with 40 second rest periods in between, the computer generated a range of intervals that progressively approximated the subjects’ average level of inhibitory control (the stop signal delay that coincided with 50% successful inhibition).

The task lasted approximately 15 minutes. The mean time required to pre-empt the key press with 50% success denotes the stop signal reaction time (SSRT). SSRT is calculated by subtracting the mean stop signal delay on stop trials (mean SSdelay) from subject’s mean response time to the GO signal (mean Go RT) on non-stop trials. Shorter SSRT scores denoted greater inhibitory efficiency/ control while shorter Go RT denoted greater psychomotor fluency.

2.8 Study Procedure

2.8.1 Pre- interview (Telephone) Screening
Subjects were pre-screened on major inclusion/exclusion criteria by telephone. Subjects were provided with a brief introduction of the study, description of financial compensation and an overview of the experimental procedure, including self-report questionnaires, computer tasks and slot machine. During the telephone interview, subjects were scored based on their response on the SOGS, DSM-IV, BDI-sf, ADS, EIQ and WAIS- vocabulary. The scoring criteria for the telephone screening are shown below.

Telephone Scoring Sheet
Subjects were assigned to the PG or HC group based on their individual score on the criteria mentioned in the Table 1 below.

<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 (Males and Females)</td>
<td>19-65 (Males and Females)</td>
</tr>
<tr>
<td>English Language Fluency</td>
<td>Equivalent to Grade 7</td>
<td>Equivalent to Grade 7</td>
</tr>
<tr>
<td>Body Mass Index (BMI)</td>
<td>&lt;35</td>
<td>&lt;35</td>
</tr>
<tr>
<td>South Oaks Gambling Screen (SOGS)</td>
<td>≥5</td>
<td>= 0</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>≥5</td>
<td>= 0</td>
</tr>
<tr>
<td>Alcohol Dependence Scale (ADS)</td>
<td>≤10</td>
<td>≤10</td>
</tr>
<tr>
<td>Beck Depression Inventory-short form (BDI-sf)</td>
<td>≤10</td>
<td>≤10</td>
</tr>
<tr>
<td>Alcoholic drinks per week</td>
<td>≤20 drinks/week (Males) &lt;15 drinks/week (Females)</td>
<td>≤20 drinks/week (Males) &lt;15 drinks/week (Females)</td>
</tr>
<tr>
<td>Cigarettes smoked per day</td>
<td>≤20 Cigarettes/day (Males and Females)</td>
<td>≤20 Cigarettes/day (Males and Females)</td>
</tr>
<tr>
<td>WAIS-Vocabulary</td>
<td>≥18</td>
<td>≥18</td>
</tr>
</tbody>
</table>

Table 1 Telephone Screening Sheet

**Subject Matching**

PG and HC subjects were matched on age, gender and smoker status. 16 subjects completed the study, which represents 20% of the full sample for the 3-year project. As far as possible, within each group, pairs of subjects were matched on factors that may influence experimental response and randomly assigned to HAL or FLU antagonist groups. PG severity (SOGS), gender, age, Impulsivity (Eysenck Impulsiveness Questionnaire; EIQ), ethnicity, sub-clinical alcohol use (ADS), sub-clinical depressive symptoms (BDI-sf), and nicotine dependence (FTND). Perfect matching was impossible with 8 variables. Thus, priority was given per the order above.

**2.8.2 Pre-Test Interview Screening**

Subjects that passed the telephone screen were invited to attend a pre-test assessment interview at the CAMH laboratory. At the beginning of the interview, subjects were provided detailed
information about the study, and interested subjects provided written informed consent. An overview of the study procedures and the possible side effects of the test medications were clearly described and listed in the consent form (See Appendix D). To confirm alcohol abstinence, a breath sample was obtained using a breathalyzer. A blood alcohol concentration of 0 was required to proceed further. Baseline blood pressure and heart rate readings were also recorded using the HR-BP monitor. Subjects’ body mass index was calculated as a measure of their height and weight using a standard scale. Female candidates were administered a urine-based pregnancy test. After the baseline measures, subjects underwent the structured psychiatric interview (SCID), supervised by a psychiatrist. For potential gamblers, an assessment of gambling severity was also made by repeating SOGS and DSM-IV in person on the interview.

Subjects who remained eligible based on the SCID completed the trait scales composed of SOGS, DSM-IV, BDI-sf, ADS, DAST, GBQ, FTND, EPI, EIQ, Alcohol Timeline Followback and Nicotine Timeline Followback. Subjects that were considered ineligible on the SCID were dismissed and financially compensated for their time.

Furthermore, eligible subjects completed the Wechsler (vocabulary, digit span and digit symbol) package. After a short rest period, subjects were administered a series of computer-based tasks. The WCST was administered first, followed by the Stop Signal Task. Subjects performed the Game of Dice Task next to last on the interview day, followed by a pilot task (not reported).

After completing the computer tasks, blood and urine samples were collected along with the EKG. Following collection of these samples, subjects were escorted back to the laboratory and reminded to refrain from taking any drugs/medications, or drinking any alcohol for 12 hours prior to the physician’s exam.
2.8.3 Physical Examination
Eligible subjects attended a physical examination where they were briefly examined by a nurse followed by a physician at the CAMH on-site clinic. The physician reviewed all clinical laboratory results in order to determine subjects’ continued eligibility for the study. Following that, subjects were escorted back to the laboratory and were provided instructions to refrain from taking any drugs or medications, and/or drinking any alcohol/caffeinated beverages 12 hours prior to the beginning of the first test day. The subjects were also required to fast after midnight on the evenings before each test session.

The subjects were then randomly assigned to the medication groups- HAL and FLU. A phone call and e-mail were made to the eligible subjects to confirm the date and time of the test days.

2.8.4 Test Sessions
The procedure for each test session was identical: the slot machine game occurred immediately after subjects completed the assigned wait period. This enabled assessment of the cognitive priming effects of the slot machine. The timeline for testing is outlined in Figure 3 with major elements described thereafter. The treatment (antagonist- HAL/placebo or FLU/placebo) was counterbalanced over sessions.

Subjects reported to the laboratory at 8:00am for HAL or 8:30am for FLU group. At the beginning of each test day, subjects were briefed about the study day procedure, which included filling out questionnaires, doing some computer tasks and playing the video slot machine game at specified times. Subjects were told that they would receive a standard breakfast and lunch on each test session. Smokers were allowed 1 cigarette at the start of each test session, and none until testing ended 7-8 hrs later. Heart rate and blood pressure were assessed every 30-min during each session, and right before and after the slot machine.
Upon briefing, subjects received a baseline breathalyzer assessment to confirm alcohol abstinence followed by the blood pressure and heart rate readings. After the baseline measures, subjects filled out the first questionnaire package (Package A). Subjects then received a standard breakfast and a post-breakfast HR/BP reading was recorded. Immediately after breakfast, subjects were relocated to a waiting room where they received their study dose (Antagonist/Placebo). Subjects were allowed to watch movies or read magazines while they waited for the medication blood levels to reach asymptote (2 hr for FLU, Midha, Mckay et al. 1983 and 2.75 hr for HAL, Nordstrom, Farde et al. 1992). 15-min prior to expected peak blood levels for the first dose; the second questionnaire package (Package B) was administered.

Subjects were then relocated to the mock bar laboratory, where the slot machine game was administered. While in the bar, another set of blood pressure and heart rate measures were taken and the third questionnaire package (Package C) was completed. After completing these scales, subjects were escorted back to the testing lab where the Rapid Reading Task was administered. After the task, another set of blood pressure and heart rate measures was recorded and the fourth questionnaire package (Package D) was administered.

Subjects then performed the Game of Dice task followed by the pilot Number task. The number task was intended to evaluate variations in reward expectancy on a slot machine-type game. The task was included to obtain data for possible future investigation. Because of its length (20 min) and the likelihood of considerable practice effects, the pilot Number task was administered as the last task on each session.

After another set of physiological readings, the last questionnaire package (Package E) plus other assessment day scales (Capsule Contents Guess and Side-Effects Checklist) were completed. Subjects then received lunch.
Prior to departure on each test day, subjects were given a reminder sheet that had the date and time for the next scheduled appointment to return in exactly one week’s time. In addition, they received a sealed 50-mg dose of diphenhydramine HCl (Benadryl) to counteract any possible delayed side effects, plus a wallet card stating which drugs they may have received (in case of emergency), and the 24-hour contact number of the study physician.

2.8.5 Subject Safety
On test days, in order to address potential delayed effects of HAL or FLU, subjects remained under observation post lunch until approx. 3 p.m. They were examined by a registered nurse before departure. Upon discharge, the registered nurse assessed the subjects’ vital signs and enquired about any adverse events that they might be experiencing. Subjects also received a wallet card stating which drugs they may have received (in case of emergency) and the 24-hour emergency contact number of the study physician. They were also dispensed a capsule of 50-mg diphenhydramine HCL (sealed), which is commonly known as Benadryl, as an antidote for any delayed side effects they might experience from the test medication. Whether or not they took the antidote, they were reminded not to drive or operate heavy machinery for 24 hours. Aside from the Benadryl, subjects were asked to abstain from taking all drugs, medications, and alcohol for 72 hours following the test day.

2.8.6 End of Study/Debriefing
At the end of the study, subjects were debriefed prior to dismissal and thanked for their participation. In addition to the standard compensation of $920, they were told that they received a cash bonus of $80, which represented their earnings from playing the slot machine. They were told that their cheque for compensation of $1000 would be mailed to their mailing address. At the completion of this, PG subjects were provided literature on problem gambling and were offered the option of a referral to the Gambling Service at CAMH that provides support and counseling to PG Subjects (however, no PGs showed interest in referral).
2.8.7 Adverse Event Reporting

As part of Good Clinical Practice, subjects were given a follow-up call the day following the test day to enquire about any adverse events that they might have experienced following dismissal from the laboratory. If subjects reported any adverse events, further detailed information regarding the symptoms was collected.

2.9 Data Analysis Approach

Primary outcome measures (indices a-c in hypotheses) were analyzed with the basic design: 2 Group (PG, HC) x 2 Antagonist (HAL, FLU) x 2 Treatment (Drug, Placebo) Analyses of Variance (ANOVA). Where appropriate, repeated measures (e.g., time of test - baseline, post-capsule (pre-game), post-game; word type on Rapid Reading Task) within a session were incorporated into the analysis with Bonferroni correction for multiple comparisons. Furthermore, it should be noted that ANOVA was considered as the best choice for analyzing data in the present study due to the large number of observations per subject and the equal n in each experimental cell which offset concerns around heterogeneity of variance or non-normal distribution of scores. ANOVA is robust to violation of assumption of normality for groups of scores when the cell size across all cells/comparisons is equal. Considering that the present study required between – within comparisons, ANOVA enabled assessment of variation in response at different time points tested during the study testing phase. Power analysis was also conducted for observed effects to identify potential type II errors (failures to detect an hypothesized effect due to too few observations).

Simple effects analysis compared means for subjects’ background characteristics and end of study capsule guess scores. Furthermore, variation in winnings (end credit tally) from playing the slot machine game could hinder detection of treatment related effects, therefore total credits
from the game were included as a co-variate in the ANOVA analysis for the primary outcome variables (b – e) mentioned below to control for this random variance.

2.10  Primary Outcome Variables

Hypotheses testing for the study involved an investigation of the following five primary outcome measures: (a) Betting behavior on the slot machine game; (b) Visual Analogue Scale (VAS) self-reports of Desire to Gamble, Enjoyment/Liking of the slot machine game; (c) Euphoria ratings on the Morphine-Benzedrine (MBG) scale of the Addiction Research Center Inventory (ARCI) before and after the game. ARCI – MBG reflects drug-induced euphoria, a reasonable index of subjective reward. It was also the main index reported in the previous study (Brauer and de Wit 1995) against which we compared the findings from the present study; (d) Cognitive priming effects: Faster reading response time (RT) to Gambling vs. Neutral words on a Rapid Reading Task (incentive salience) (e) Physiological response as indexed by blood pressure.

3.  Results

3.1  Background Characteristics: Subject(s) Eligibility and Demographics

<table>
<thead>
<tr>
<th>Variable Name</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
</tr>
<tr>
<td>Age</td>
<td>40.5 (3.8)*</td>
</tr>
<tr>
<td>Gender</td>
<td>Females:5, Males:3</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>SOGS</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>BDI- sf</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Alcohol Dependence Scale</td>
<td>0.25 (0.7)</td>
</tr>
<tr>
<td>DSM-IV-PG</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>EPI - Extroversion subscale</td>
<td>12.25 (4.1)</td>
</tr>
<tr>
<td>EPI - Neuroticism subscale</td>
<td>4.625 (4.8)</td>
</tr>
<tr>
<td>EPI - Lie subscale</td>
<td>5.12 (1.8)</td>
</tr>
<tr>
<td>EIS- Impulsiveness subscale</td>
<td>4.5 (3.0)</td>
</tr>
<tr>
<td>DAST</td>
<td>0.88 (0.9)</td>
</tr>
<tr>
<td>FTND</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Alcohol- TFB</td>
<td>0.57 (0.5)</td>
</tr>
<tr>
<td>Nicotine- TFB</td>
<td>0.16 (0.4)</td>
</tr>
<tr>
<td>WAIS-Vocabulary</td>
<td>27.65 (2.4)</td>
</tr>
<tr>
<td>WAIS-Digit span</td>
<td>19.25 (8.3)</td>
</tr>
<tr>
<td>WAIS-Digit symbol</td>
<td>35.12 (11.3)</td>
</tr>
</tbody>
</table>

Table 2 reports the mean (SD) background demographic scores for Healthy Controls (HC) and Pathological Gamblers (PG) taken during the initial screening interview. A 2(Group: PG, HC) x 2 (Antagonist: HAL, FLU) Analysis of Variance (ANOVA) of age ratings yielded a significant main effect of Group, F (1, 12) = 6.452, p = 0.026, reflecting that the gambling participants were on an average comparatively younger than the control participants. Significant Group differences were also observed for Alcohol- TFB scale, p = .02 and for the EPI- Lie subscale, p = .04, reflecting a stronger effort to create a favorable impression by controls than gamblers. No other significant group differences were found. Neither group demonstrated clinically significant elevations in depression, nicotine dependence, drug abuse (low DAST scores) nor
alcohol use. Comparable scores on the WAIS sub-scales indicate similar verbal IQ in each group.

### 3.2 Betting behaviour during slot machine game

**Figure 4** Mean (SE) credits bet per total spins on a 15-min slot machine game in HC subjects (n = 4) and PG subjects (n = 4) under HAL (3 mg, oral) and placebo

**Figure 5** Mean (SE) credits bet per total spins on a 15-min slot machine game in HC subjects (n = 4) and PG subjects (n = 4) under FLU (3 mg, oral) and placebo

Figure 4 and Figure 5 show the slot machine betting behavior in HC and PG subjects under HAL and FLU, relative to placebo pre-treatment respectively.

**Analysis of slot machine betting scores**

A 2 (Group: HC, PG) x 2 (Antagonist: HAL, FLU) x 2 (Treatment: Drug, Placebo) ANOVA of mean bet scores yielded a marginal Treatment x Group interaction, F (1, 12) = 4.15, p = .064, with observed statistical power of 46.6%. Comparison of the left-hand (blue) bars in Figure 4
and Figure 5 reveals that this result reflected larger bets under both HAL and FLU than placebo in Group HC, but comparable bet size under drug and placebo in Group PG.

A parallel 2 x 2 x 2 ANOVA of spins per game yielded a significant effect of Group, $F(1, 12) = 6.59, p = .025$ reflecting more spins (faster rate of play) in PG subjects than controls regardless of treatment or antagonist.

![Figure 6](image-url)

**Figure 6** Mean (SE) credits won (winnings) from playing the 15-min slot machine game in HC Subjects ($n = 4$) and PG Subjects ($n = 4$) under HAL (3mg, oral) and placebo, and FLU (3 mg, oral) and placebo.

Differences in winnings are possible with a random payout schedule and a small sample. Such differences could impact on the reinforcing effects of the game. To assess this possibility, two sets of analyses were performed for relevant variables, one examining the scores per se, and one examining the scores when variation in winnings was controlled by including winnings as a covariate. The ANCOVA yielded a significant Treatment x Drug group interaction, $F(1,12) = 6.58, p = 0.025$, reflecting an increase in the mean bet and total spins scores under HAL vs. placebo and a decrease across the scores under FLU vs. placebo, regardless of the group. There were no other significant higher order effects found, $p’s > 0.1$.

### 3.3 Self-reported priming effects of slot machine game

#### 3.3.1 VAS – Desire to Gamble
Figure 7 Mean (SE) self-reported Desire to Gamble at baseline (arrival at the lab), before and after a 15-min slot machine game in HC subjects (n = 4) and PG subjects (n = 4) under HAL (3mg, oral) and placebo. Scores shown are adjusted means, when variance in winnings is controlled by ANCOVA.

Figure 8 Mean (SE) self-reported Desire to Gamble at baseline (arrival at the lab), before and after a 15-min slot machine game in HC subjects (n = 4) and PG subjects (n = 4) under FLU (3mg, oral) and placebo. Scores shown are adjusted means, when variance in winnings is controlled by ANCOVA.

Figure 7 shows scores for HAL Subjects and indicates that, Desire to Gamble scores differed for drug vs. placebo at baseline for both HC and PG subjects. Differences at post-capsule and post-game must be interpreted in the context of these pre-existing (chance) differences. Figure 7 also shows that scores rose consistently from post-capsule to post-game, confirming that the slot machine successfully primed motivation to gamble in HC and PG subjects. HAL slightly increased pre-game (un-primed) Desire to Gamble, and slightly diminished post-game (primed) desire relative to placebo, in both HC and PG subjects.
Figure 8 shows scores for FLU and indicates that relative to baseline and pre-game scores under placebo, FLU had no effect on pre-game Desire to Gamble in either group. In HC subjects, FLU led to a somewhat greater increase in post-game Desire to Gamble relative to placebo. In PG subjects, FLU led to a somewhat smaller increase in post-game desire compared to placebo, reversing the pattern seen at pre-game and essentially restoring the pattern seen at baseline.

**Analysis of VAS - Desire to Gamble**

A 2 x 2 x 2 ANOVA of Desire to Gamble scores yielded a significant main effect of Group, $F(1, 12) = 10.22, p = .008$ and a marginal Time x Group interaction, $F(1, 12) = 4.47, p = .056$. The ANCOVA using winnings as the co–variate yielded effects of Group, $F(1, 11) = 9.87, p = .009$ and Time x Group interaction, $F(1, 11) = 4.43, p = .059$, with observed power of 17.6%. With 2 measures of incentive motivation (Desire/Confidence to Refrain) Bonferroni $\alpha = .025$.

The statistical results confirm that PG subjects reported greater Desire to Gamble than HC subjects across all time points, drug treatment and antagonist condition. In addition, the group difference in Desire tended to be more pronounced at post-game than at pre-game. Baseline differences in Desire scores and high within-group variability appear to have hindered detection of reliable effects of treatment or antagonist.

### 3.3.2 VAS - Desire to Drink Alcohol

<table>
<thead>
<tr>
<th>Drug Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAL</td>
</tr>
</tbody>
</table>
Table 3 Mean (SD) self-reported desire to drink alcohol at baseline, before and after a slot machine game in HC (n = 4) and PG subjects (n = 4) under HAL (3mg, oral) and placebo, and FLU (3mg, oral) and placebo respectively.

Table 3 indicates that desire for alcohol scores were modest at all time points, with an increase in both groups at post-game relative to pre-game under HAL and placebo. In contrast, there was no appreciable change in desire for alcohol scores at pre- vs. post-game under drug or placebo in either group in subjects who received FLU.

**Analysis of VAS - Desire to Drink Alcohol**

A 2 x 2 x 2 x 3 ANOVA of Desire to drink Alcohol scores yielded a significant linear trend for Time, F (2, 24) = 7.42, p = .019 and no other effects, p’s > 0.1. Thus, Desire for Alcohol increased with the passage of time, regardless of other factors in HC and PG subjects.

3.3.3 Subjective rewarding effects of slot machine game

Figure 9 Mean (SEM) self-reported rewarding (pleasurable) effects of a 15-min slot machine game in HC subjects (n = 4) and PG subjects (n = 4) under HAL (3mg, oral) and placebo.
Inspection of Figure 9 above shows that, in HC subjects, HAL led to an appreciable increase in Buzz / High, despite a modest decrease in Enjoyment, relative to placebo. In PG subjects, HAL led to an appreciable increase in High, which unlike HCs coincided with a modest increase in Enjoyment. Figure 10 shows that FLU led to a sizeable increase in Excitement and High relative to placebo in HC subjects, which coincided with a slight decrease in Enjoyment. Figure 10 also shows that in PG subjects, FLU led to a similar increase in High relative to placebo and no other appreciable effects. Thus, HAL and FLU led to a sizeable increase in perceived intoxicating effects of the game in both groups but in HCs, this drug related increase was associated with relatively less Enjoyment.

**Analysis of subjective rewarding effects of slot machine**

A 2 x 2 x 2 x 2 x 4 (Subscale) ANOVA yielded a significant Group x Treatment x Subscale interaction for the quadratic trend $F(1, 12) = 5.95, p = .031$, and no higher order trends or effects. The follow-up ANCOVA yielded the same effect, $F(1, 11) = 5.45, p = .040$, and no other significant trends or effects. With four sub-scales, the analysis did not meet significance at Bonferroni $\alpha = .0125$. 

**Figure 10** Mean (SE) self-reported rewarding (pleasurable) effects of a 15-min slot machine game in HC subjects ($n = 4$) and PG subjects ($n = 4$) under FLU (3mg, oral) and placebo.
Inspection of Figure 9 and Figure 10 indicates that this result reflected a quadratic pattern of scores across subscales under active drug (HAL, FLU) but not placebo in HC subjects, whereas a no clear quadratic trend across subscales under drug or placebo was evident in PG subjects.

For HC subjects, playing the game under the drug led to increased Excitement, High/Buzz but a decrease in Enjoyment, relative to placebo, whereas in PG subjects, both drugs led to comparative Enjoyment and a selective increase in High/Buzz, relative to placebo.

VA S - Confidence to Resist Gambling

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time of Test</th>
<th>Healthy Controls</th>
<th>Pathological Gamblers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAL</td>
<td>Placebo</td>
<td>FLU</td>
</tr>
<tr>
<td>Baseline</td>
<td>9.484 (0.289)</td>
<td>9.003 (0.973)</td>
<td>9.766 (0.289)</td>
</tr>
<tr>
<td>Pre-Game</td>
<td>6.836 (1.725)</td>
<td>9.252 (0.616)</td>
<td>9.914 (1.725)</td>
</tr>
<tr>
<td>Post-Game</td>
<td>9.252 (0.616)</td>
<td>8.248 (1.301)</td>
<td>9.247 (0.973)</td>
</tr>
</tbody>
</table>

Table 4 Mean (SD) self-reported confidence to resist gambling at baseline (arrival at the lab), before and after a 15-min slot machine game in HC subjects (n = 4) and PG subjects (n = 4) under HAL (3mg, oral) and placebo, and FLU (3mg, oral) and placebo respectively.

Analysis of VAS - Confidence to Resist Gambling

A 2 x 2 x 2 x 3 (Time: Baseline, Pre-Game, Post-Game) ANOVA of mean confidence to resist gambling scores yielded significant main effects of Group, F (1, 12) = 9.57, p = .009 and Time F (1, 12) = 6.94, p = .022 and no other higher order effects, p’s > .1. The analysis revealed that the confidence ratings differed over time during the session across the assigned group (PG or HC) regardless of assigned treatment (Drug or Placebo).

The ANCOVA with winnings as covariate, yielded the identical main effect of Group, p = .009, with no higher order effects. Separate ANCOVAs for each group found no significant effects in HC subjects, p > .20, and a marginally significant quadratic trend for Time, F (1,5) = 5.49, p = .066 in PG subjects, reflecting a modest increase in confidence from baseline to post-
capsule/pre-game, followed by a modest decrease in confidence from pre-game to post-game.

The observed power for the Group x Time interaction was 19.8%, and Bonferroni $\alpha = .0125$. 
3.3.4 Self-reported Subjective Effects of Capsule

3.3.4.1 Addiction Research Center Inventory

Figure 11 Mean (SE) self-reported subjective effects of capsule (3mg HAL; Placebo) at baseline, before the slot machine game or peak blood levels (2.75h post-administration of the capsule, pre-game) and after the game, post-game on three ARCI subscales, in HC subjects (n = 4) under HAL (3mg, oral) and placebo
Figure 12 Mean (SE) self-reported subjective effects of capsule (3mg HAL; Placebo) at baseline, before the slot machine game or peak blood levels (2h post-administration of the capsule, pre-game) and after the game, post-game on three ARCI subscales, in PG subjects (n = 4) under HAL (3mg, oral) and placebo.

Figure 11 and Figure 12 above show the 5 ARCI sub-scale scores for HC and PG subjects under HAL and placebo at each time point. The figures reveal that, for HC subjects who received HAL, MBG scores (scale 2) were greater under drug than placebo at pre-game, but greater under placebo than drug at post-game. As seen in HC Subjects, in PGs who received HAL, MBG scores were greater under drug than placebo at pre-game, but this pattern was reversed at post-game.
Figure 13 Mean (SEM) self-reported subjective effects of capsule (3mg FLU: Placebo) at baseline, before the slot machine game or peak blood levels (2.75h post-administration of the capsule, pre-game) and after the game, post-game on three ARCI subscales, in HC subjects (n = 4) under FLU (3mg, oral) and placebo.
Figure 14 Mean (SE) self-reported subjective effects of capsule (3mg FLU; Placebo) at baseline, before the slot machine game or peak blood levels (2h post-administration of the capsule, pre-game) and after the game, post-game on three ARCI subscales, in PG subjects (n = 4) under FLU (3mg, oral) and placebo
Figure 13 and Figure 14 above show the corresponding scores for HC and PG subjects in the FLU antagonist group. In HC subjects who received FLU, the opposite pattern emerged for MBG, with the difference for drug vs. placebo smaller at pre-game than at post-game, such that the game restored the pattern seen at baseline. In PG subjects who received FLU, the drug alone increased AMPH scale scores somewhat compared to baseline, and this effect was not altered by playing the game. In contrast, the drug alone did not appreciably change MBG scores relative to baseline, but playing the game led to a dramatic and selective increase in MBG scores under drug vs. placebo.

Taken together, the results indicate a similar pattern of scores in HC and PG subjects for both HAL and FLU. In each group, HAL alone slightly enhanced MBG ‘euphoria’ but decreased euphoria following the game. Conversely, FLU alone did not alter MBG euphoria but enhanced euphoria after the game, with this effect being especially pronounced in PG subjects.

**Analysis of Addiction Research Center Inventory**

A 2 x 2 x 2 x 3 x 5 (Subscale) ANOVA of ARCI subscale ratings yielded a marginally significant Group x Treatment x Time x Subscale interaction, $F(8, 96) = 2.02, p = .052$ that did not interact with Antagonist condition. The ANCOVA controlling for ‘winnings yielded the same pattern of effects although the 4-way interaction was somewhat attenuated, $p = .062$. The observed power associated with this effect was 46%.
3.3.4.2 Subjective Mood Effects - Profile of Mood States (POMS-sf)

Figure 15 Mean (SE) self-reported subjective mood effects reported at baseline, before the slot machine game at peak blood levels (2.75h post-administration of the capsule) and after the game on the six POMS subscales, in HC subjects (n = 4) and PG subjects (n = 4) under HAL (3mg, oral) and placebo.
Figure 16 Mean (SE) self-reported subjective mood effects reported at baseline, before the slot machine game at peak blood levels (2h post- administration of the capsule) and after the game on the six POMS subscales, in HC subjects (n = 4) and PG subjects (n = 4) under FLU (3mg, oral) and placebo.
Figure 15 shows that, in HC subjects who received HAL the drug somewhat diminished Vigor before and after the game, and somewhat diminished Depression at post-game. In contrast, HAL had no effect on the pattern of scores relative to placebo in PG subjects.

Figure 16 shows that FLU had no differential effect on the pattern of scores relative to placebo in HC subjects. In PG subjects, FLU was associated with a selective increase in Vigor relative to placebo at post-game, and no other differential effects relative to placebo.

**Analysis of POMS**

A 2 x 2 x 2 x 3 x 6 (Subscale) ANOVA of POMS ratings yielded a Time x Subscale x Group interaction, F (10, 110) = 2.3, p = .01. The ANCOVA controlling for winnings yielded a main effect of Group, F (1, 110) = 5.18, p = .04. A higher order Treatment x Time x Subscale x Antagonist interaction, F (10, 100) = 2.0, p = .03 was also found. This result would appear to have emerged because in, both HC and PG groups, playing the game did not alter the effects of HAL relative to placebo on Vigor scores. In contrast, playing the game appeared to reduce the effect of FLU on all sub-scales except Vigor.

**3.4 Cognitive Effects (Computer-Based Tasks)**

**3.4.1 Mean (SE) Response Time on the Rapid Reading Task**

![Mean (SE) reading response time (milliseconds; ms) on Rapid Reading Task in HC subjects (n = 4) and PG subjects (n = 4) under HAL (3mg, oral) and placebo](image-url)
Figure 18 Mean (SE) reading response time (ms) on Rapid Reading Task in HC subjects (n = 4) and PG subjects (n = 4) under FLU (3mg, oral) and placebo.

Figure 17 indicates that in HC Subjects, response time (RT) to Gambling words, and to a lesser extent Alcohol words, was slower than RT to Neutral words (i.e., less salient) under HAL (difference = + 36 ms) relative to placebo treatment (difference = −10 ms). In contrast, RT to Gambling words remained salient under HAL in PG subjects, although RT to Neutral words also improved under HAL, so the degree of salience was somewhat less under drug (difference = −72 ms) than placebo (difference = −121 ms).

Figure 18 indicates that under FLU, Gambling words (and the other word categories) were more salient under both FLU (difference = −135 ms) than placebo (difference = −88 ms) in HC subjects. In PG subjects, Gambling words had little salience under FLU (difference = −7 ms) relative to placebo (difference = −30 ms). Thus, HAL negated while FLU enhanced the salience of gambling cues in control subjects, whereas both HAL and FLU diminished the salience of gambling cues relative to placebo in gamblers.

**Analysis of Rapid Reading Task**

A 2 x 2 x 2 x 6 (Word type: Gambling, Alcohol, Positive, Negative, Neutral) ANOVA of RT scores yielded a significant Group x Antagonist x Word Type interaction, $F(1, 11) = 12.27, p =$
The ANCOVA using ‘winnings’ as the covariate yielded the same interaction, $F(4, 40) = 3.779, p = .01$.

Post-hoc contrasts indicated that RT was faster to Gambling vs. Neutral words under FLU but slower to Gambling vs. Neutral words under HAL in controls, although the effect was marginal, $p = .061$. In gamblers, RT was faster to Gambling vs. Neutral words under both drugs, but the RT difference (salience) was greater under HAL than FLU, $p = .042$.

### 3.4.2 Stop Signal Task

<table>
<thead>
<tr>
<th>Measure</th>
<th>Healthy Controls</th>
<th>Pathological Gamblers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAL</td>
<td>Placebo</td>
</tr>
<tr>
<td>Go – RT</td>
<td>587.6 (72.4)</td>
<td>531.7 (65.2)</td>
</tr>
<tr>
<td>Stop – RT</td>
<td>207.3 (60.1)</td>
<td>242.8 (56.4)</td>
</tr>
</tbody>
</table>

Table 5 Mean (SE) GO- response time (GO-RT) and STOP signal- response time (STOP-RT) on a game of Stop Signal Task in HC subjects ($n = 4$) and PG subjects ($n = 4$) under HAL (3mg, oral) and placebo, and FLU (3mg, oral) and placebo.

Table 5 shows that HAL but not FLU slowed overt psychomotor responses (Go-RT) in HC subjects. Neither drug affected Go RT in PG subjects. In contrast, HAL but not FLU improved Stop RT in HC subjects, whereas both drugs impaired Stop RT in PG Subjects. The task corrects for speed-accuracy trade-offs so that slowing of Go RT cannot account for improvements in inhibitory/Stop RT in HCs under HAL.

**Analysis of Stop Signal Task**

A $2 \times 2 \times 2 \times 2$ (Measures: GO- RT, STOP- RT) multivariate ANOVA yielded no significant effects or trends, $p > .39$. Furthermore, multivariate ANCOVA performed by using ‘winnings’ as the co-variate again yielded no significant effects, $p’s > .18$. Thus, the pattern of mean scores was not statistically reliable with the present sample size.
3.4.3 Game of Dice Task (GDT)

**Table 6** Mean (SE) risk-taking scores on a Game of Dice task in HC subjects (n = 4) and PG subjects (n = 4) under HAL (3mg, oral) and placebo, and FLU (3mg, oral) and placebo

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAL</td>
</tr>
<tr>
<td>Block</td>
<td></td>
</tr>
<tr>
<td>Block-1</td>
<td>3.268 (0.389)</td>
</tr>
<tr>
<td>Block-2</td>
<td>3.399 (0.385)</td>
</tr>
<tr>
<td>Block-3</td>
<td>3.218 (0.327)</td>
</tr>
<tr>
<td>Block-1</td>
<td>3.149 (0.389)</td>
</tr>
<tr>
<td>Block-2</td>
<td>3.048 (0.523)</td>
</tr>
<tr>
<td>Block-3</td>
<td>3.104 (0.339)</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.256 (0.55)</td>
</tr>
<tr>
<td>Pre-Game</td>
<td>2.869 (0.523)</td>
</tr>
<tr>
<td>Post-Game</td>
<td>2.813 (0.339)</td>
</tr>
</tbody>
</table>

Table 6 shows mean line selection over three consecutive 6-trial (6 tosses of the die) blocks.

Smaller scores indicate more risky wagers.

**Analysis of Game of Dice Task**

A 2 x 2 x 2 x 3 (Block) ANOVA of high vs. low risk response selections yielded a marginally significant Treatment x Block interaction, $F(1, 12) = 4.30, p = .06$, suggesting that the change in risk-taking over the course of trials differed under drug vs. placebo. The ANCOVA controlling for ‘winnings revealed a significant Treatment x Block interaction, $F(1, 11) = 5.70, p = .03$. The interaction reflected a difference in the quadratic trend across blocks which increased and decreased under drug (inverted U-shape), but decreased and increased (U-shape) under placebo.

3.4.4 Wisconsin Card Sort Task (WCST)

A 2 x 2 x 2 (Response Type: Perseverative Errors, Non-Perseverative Errors) ANOVA of error scores yielded no significant effects or trends, $p > .18$. Somewhat unexpectedly, gamblers exhibited less evidence of perseveration than controls. Mean (SD) error scores: 8.5 (3.1) vs. 13.1 (3.1). However, as expected, perseverative errors appeared to be more frequent than non-perseverative errors in both the groups: 7.0 (1.5) for gamblers and 7.6 (1.5) for controls. (Scores for Perseverative Errors and Non-Perseverative Errors not shown here, see Appendix E, Table V for mean (SE))
3.5 Physiological Measures- Blood Pressure

Analysis of the physiological effects

A 2 x 2 x 2 x 3 multivariate ANOVA of Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) scores yielded a significant Treatment x Time x Group x Antagonist interaction for DBP, F (1, 12) = 5.76, p = .033, but not SBP, p > 0.2 (Scores for SBP not shown here, see Appendix E, Table VI for mean (SE))

The ANCOVA with ‘winnings’ as the co-variate, revealed a significant quadratic trend in DBP, F (1, 11) = 13.84, p = .003, which varied as a function of Treatment, Group and Antagonist.

To isolate the source of the interaction, separate ANCOVAs were performed in each group. The ANCOVA in HC subjects yielded a significant Treatment x Time interaction, F (1, 5) = 7.226, p = 0.043, which was not moderated by Antagonist. The ANCOVA in PG subjects yielded a significant Treatment x Time x Antagonist interaction, F (1, 5) = 22.04, p = .005. Figure 19 and Figure 20 below illustrate the significant effects for HC and PG subjects.

**Figure 19** Mean (SE) diastolic blood pressure (mm Hg) at pre-capsule baseline, peak capsule dose or pre-game and after a 15-min slot machine game in HC subjects (n = 4) and PG subjects (n = 4) under HAL (3 mg, oral) and placebo.
Figure 20 Mean (SE) diastolic blood pressure (mm Hg) at pre-capsule baseline, peak capsule dose or pre-game and after a 15-min slot machine game in HC subjects (n = 4) and PG subjects (n = 4) under FLU (3 mg, oral) and placebo.

The left-hand panels in the Figure 19 and Figure 20 above show scores for HC subjects and indicate that, under placebo, DBP declined from baseline to pre-game and increased from pre-game to post-game in subjects assigned to both HAL as well as FLU antagonist groups.

In contrast, under drug, DBP remained stable or increased slightly but did not decline at any point in the session. The consistent profile for placebo and consistent but alternative profile under HAL and FLU accounts for the Treatment x Time interaction and lack of moderating effects of Antagonist.

The right-hand panels in the figures show scores for PG subjects and indicate a different profile of scores for placebo, HAL and FLU. Like HC subjects, PG subjects in the HAL group, displayed a decline in DBP from baseline to pre-game then recovered from pre-game to post-game under placebo. Also like HC subjects, PG subjects in the HAL group, displayed very stable DBP over the course of the session under HAL itself. In contrast, PG subjects in the FLU group, displayed a modest but steady decline in DBP over the session under placebo, but exhibited a pattern akin to that of the other sub-groups under placebo when they received FLU itself. In sum, HAL stabilized physiological arousal in HC and PG subjects relative to placebo.
FLU also stabilized physiological arousal relative to placebo in HC subjects but not in PG subjects. In the latter group, FLU dampened arousal during the pre-game phase and this dampening effect was reversed after the subjects played the game, thus exhibited a pattern akin to that of the other sub-groups under placebo itself.

3.6 Procedural Checks

3.6.1 Capsule Content Evaluation- Effects of Capsule

At the end of test day 2, subjects were asked to recall the perceived effect from the capsule they received during each session and report which of the two days they believed they received the active drug. A 2 (Treatment Sequence: Drug on Session 1 vs. Drug on Session 2) x 3 (Response Option: Felt Drug on Day 1, Felt Drug on Day 2, Don’t Know) chi-square test of independence was not significant, p > 0.25 (Refer Appendix E, Table VII for mean (SE) scores) Thus, subjects were unable to reliably distinguish active drug from placebo.

3.6.2 Symptoms Side-effects Checklist

A 2 x 2 x 2 x 2 ANOVA of symptom side effect scores yielded a significant effect of Treatment, F (1, 12) = 4.64, p = 0.05 and no other significant effects. Out of a possible 6, mean (SD) scores under drug, 2.3 (0.5) were somewhat greater than placebo, 1.0 (0.4) (Refer Appendix E, Table VIII for mean (SE) scores). However, the lack of significant Group or Antagonist-related effects suggests that differences in other dependent measures as a function of Group or Antagonist are not attributable to side effects.
4. Discussion

This study compared the effects of a preferential D2 receptor antagonist (haloperidol, HAL) and a mixed D1- D2 receptor antagonist (fluphenazine, FLU) on responses to an episode of slot machine gambling: in pathological gamblers (PG) and healthy controls (HC). Based on previous research that found an increase in the subjective reinforcing effects of gambling in PG subjects under HAL (Zack and Poulos 2007), it was predicted that this result would occur again in the current sample. Based on evidence that HAL increases DA release and the fact that it has low affinity for D1, increased gambling reinforcement under HAL was thought to reflect a preferential increase in DA signaling at D1 receptors. If so, FLU should not increase and may possibly decrease gambling reinforcement in PG subjects. If PG and HC subjects differ in their D1 receptor sensitivity, FLU may enhance gambling reinforcement in controls by augmenting DA release (like HAL) while preventing over-stimulation of D1 in subjects with normal baseline D1 function.

To test these hypotheses, a sample of 40 PG and 40 HC subjects was proposed for testing over the course of 3 years. This would ensure adequate power to detect reliable patterns in group mean scores in the presence of inter-individual differences. The present study represents an initial examination of the data from 20% of the total sample. As such, all interpretations of the data are strictly provisional.

As mentioned earlier in the thesis, hypotheses testing involved an investigation of the following five primary outcome measures: (a) Betting behavior on the slot machine game; (b) Visual Analogue Scale (VAS) self-reports of Desire to Gamble, Enjoyment/Liking of the slot machine game; (c) euphoria ratings on the Morphine-Benzedrine (MBG) scale of the Addiction Research Center Inventory (ARCI) before and after the game; (d) Cognitive priming effects: Faster
reading response time (RT) to Gambling vs. Neutral words on a Rapid Reading Task (incentive salience) (e) Physiological response as indexed by Blood Pressure.

**Betting Behavior on the Slot Machine Game**

Comparison of group mean betting scores on the slot machine revealed comparable mean bet size per trial played under both HAL and FLU versus placebo in PG group. On the contrary, the HC group displayed increased betting behavior (larger bets) under both HAL and FLU relative to placebo treatment. These findings are congruent with previous research on reward-related decision-making in healthy subjects, in which Frank and O’Reilly (2006) observed that “haloperidol [group] exhibits greater Go versus No-Go learning due to enhanced dopamine (DA) bursting” (p. 506). In the present case, the act of betting (comparable to a reward-seeking Go response) appeared to be more strongly reinforced under the drug in HC subjects. The lack of such effects in PG subjects may reflect a habitual pattern of play that is relatively insensitive to situational changes in reinforcement, or a disturbance in DA/D2 function, or both.

In addition to the differences in drug-induced betting behavior, a group difference emerged for trials (number of total spins on the game) played, with PG subjects playing more trials (i.e. faster rate of play) than HC subjects under both drug and placebo, a pattern seen similar to the one seen in the previous study under HAL (Zack and Poulos 2007). Due to random variation and a small N, both the groups showed a decrease in final winnings received under HAL vs. placebo, but an increase in final winnings under FLU, relative to placebo. Clearly, winnings could affect the motivational-reinforcing effects of the game. Accordingly, winnings were controlled by covariance in the analysis of other outcome measures.
4.1 Hypotheses testing

4.1.1 Comparison between HAL and FLU on subjective- self reported effects before and after playing the slot machine game in PG and HC Subjects

Visual Analogue Scales

Under placebo, the slot machine game increased Desire to Gamble in both the groups, confirming its effectiveness as a motivational prime. HAL alone slightly increased post-capsule (pre-game) Desire to Gamble in both HC and PG subjects, relative to placebo treatment. However, contrary to the hypotheses, HAL slightly diminished post-game (primed) Desire to Gamble relative to placebo, in PG subjects and HC subjects, although the degree of the reduction was greater in HC than PG. On the contrary, FLU had no effect on pre-game Desire to Gamble in either group, relative to placebo. Whereas FLU led to a somewhat greater increase in post-game Desire to Gamble relative to placebo in HC subjects, FLU led to a somewhat smaller increase in post-game desire compared to placebo in PG subjects, reversing the pattern seen at pre-game and essentially restoring the pattern seen at baseline.

The ability to interpret the post-capsule scores in the HAL antagonist group was impeded by the emergence of higher pre-capsule baseline Desire to Gamble scores on the drug vs. placebo session in both PG and HC groups. Higher baseline scores may have limited the extent of increase in desire that could be brought about by the drug.

The results for VAS ratings of confidence to resist gambling under HAL in PG subjects appear to be generally in line with the desire ratings: a modest increase in confidence from baseline to post-capsule/pre-game, followed by a modest decrease in confidence from pre-game to post-game. However, these effects were not affected by drug treatment. No significant effects were seen in HC subjects.
Desires for alcohol scores were modest at all time points and playing the game appeared to slightly primed motivation to drink alcohol post-game, irrespective of the treatment or antagonist received. The pattern observed might be pointing to the general notion that people often engage in casino gambling in order to obtain alcohol (Giacopassi, Stitt et al. 1998) and specifically that problem gamblers frequently report drinking alcohol while engaging in a gambling episode (Baron and Dickerson 1999). The observed pattern might suggest that playing the slot machine game primed a conditioned association for alcohol in these subjects.

With respect to the rewarding-pleasurable effects of the slot machine game, considering first the findings in PG subjects, both HAL and FLU led to an appreciable increase in Buzz / High and a modest increase in Excitement from playing the game, relative to placebo. Whereas HAL led to a modest increase in Enjoyment, no appreciable difference was observed under FLU, relative to placebo. A similar pattern of response on the Excitement and High/ Buzz scales emerged in HC subjects under both HAL and FLU, relative to placebo. However, unlike the PG subjects, the increase across Excitement and High scales corresponded with a modest decrease in Enjoyment subscale in HC subjects. Thus, both HAL and FLU appeared to increase the perceived ‘intoxicating’ effects of slot machine gambling regardless of PG status, but in HCs, this drug related intoxication was found to be associated with relatively less Enjoyment.

In PG, HAL led to a moderate (17%) increase in Enjoyment of the game, relative to placebo. The direction and size of this effect closely replicate the effects for HAL in PG subjects in the original study (+19%; Zack and Poulos, 2007). The effects for the other sub-scales were more modest and variable in PG subjects. Also, in agreement with the findings for HAL in HC subjects from the previous study (Zack and Poulos 2007), playing the game modestly increased Excitement and reduced Enjoyment experienced from the game in the present study.
Group differences also emerged in the subjective-rewarding effects of the game under FLU. Whereas HC subjects reported a considerable increase in Excitement and High but a slight decline in Enjoyment, PG subjects reported congruent pattern across subscales involving moderate increases in Excitement and Enjoyment and a sizeable increase in High. Based on previous research examining effects of FLU (3-mg) on subjective Liking of d-amphetamine (20 mg) in healthy volunteers (Brauer and de Wit 1995), we expected a similar increase in Enjoyment of the slot machine game under FLU in the present HC subjects. The lack of such an effect, if it persists in the full sample, might suggest that in subjects who gamble infrequently, the biochemical processes that mediate the pleasurable effects of a slot machine may differ from those that mediate the pleasurable effects of a psychostimulant.

**Addiction Research Center Inventory (ARCI)**

The ARCI measured subjective response to the antagonists and the slot machine in terms of standard drug effects. HAL alone showed similar effects in both HC and PG subjects, such that subjective Euphoria (ARCI-MBG) tended to increase modestly under drug vs. placebo at pre-game. However, HAL decreased post-game euphoria scores relative to placebo in HC subjects, but had only a slight attenuating effect on post-game euphoria in PG subjects.

Compared to placebo, FLU alone slightly increased euphoria scores in both HC and PG subjects at pre-game. Interestingly, in PG subjects, FLU alone increased pre-game AMPH (stimulant-like) scores ~75% relative to placebo. In both groups, FLU was associated with a sizeable increase in euphoria scores at post-game, along with a further increase in stimulant-like scores in PG subjects.

These findings, if reliable, are highly novel. First, in the absence of other manipulations or rewarding stimuli, the findings might be indicating that a blockade of D2 and combined
blockade of D1 and D2 lead to a similar modest increase in subjective euphoria regardless of PG status. Second, combined blockade of D1 and D2, in the absence of other manipulations might induce mild subjective stimulant-like effects in PG subjects. Third, selective blockade of D2 may differentially affect response to a slot machine game in PG vs. HC Subjects. In PG subjects, playing the game tended to modestly increase euphoria relative to pre-game under both HAL and placebo, but the relative increase from the game was more modest under HAL. In HC subjects, playing the game appeared to increase euphoria under placebo only but decreased euphoria relative to pre-game under HAL. Together the data suggest that HAL may reduce the relative euphoric effect of the game in PG subjects perhaps due to high pre-game euphoria (i.e., a more modest contrast effect). In HC subjects HAL reduces the game and proved aversive as absolute euphoria scores decline at post-game vs. pre-game. This could conceivably reflect supra-optimal stimulation of D1 receptors in these subjects. Fourth, combined blockade of D1 and D2 under FLU, in contrast to the pattern seen under HAL led to sizeable increase in subjective euphoria after playing the slot machine regardless of PG status. Overall, the profile of effects maybe suggesting that increased gambling-induced DA release during D2 blockade is more pleasant when D1 is partially blocked regardless of PG status, but this effect may be even more pronounced in HC subjects for whom selective D1stimulation proved aversive.

The findings for HAL do not align closely with the literature for HC or PG subjects. HAL (3-mg) has negligible effect on methamphetamine reward in healthy volunteers (Wachtel, Ortengren et al. 2002), but reduced gambling word salience in the present HC subjects. HAL tended to modestly enhance the rewarding (Enjoyment) effects of the slot machine in PG subjects, but did not enhance stimulant-like or euphoria scores on the ARCI in the present PG subjects. The findings for FLU align more closely with the literature, in that FLU (3-mg) increased euphoria scores under d-amphetamine (20 mg) in healthy volunteers (Brauer and de
Wit 1995) and increased post-game euphoria scores in the present HC subjects. However, the corresponding increase in post-game euphoria (and stimulant-like) scores in the present PG subjects does not seem to align with the hypothesis that preferential stimulation of D1 receptors optimizes the subjective rewarding effects of gambling in these subjects.

Profile of Mood States (POMS-sf)

Relative to placebo, HAL by itself and upon playing the game somewhat diminished Vigor in PG subjects and more so in HC subjects. In PG subjects, FLU alone led to a decrease in Vigor ratings at pre-game but a selective increase relative to placebo at post-game. In HC subjects FLU increased Vigor compared to placebo both at pre and post-game. Thus, D2 blockade alone tends to reduce feelings of energy, whereas D2 blockade coupled with partial D1 blockade tends to energize HC subjects, and augments the energizing effects of gambling in PG and HC subjects.

4.1.2 Comparison between HAL and FLU on cognitive-behavioral (computer-based tasks) effects from playing the slot machine game in PG and HC Subjects

Rapid Reading Task

Relative to placebo, HAL reduced while FLU enhanced the salience of Gambling stimuli in HC subjects, as evidenced by relative reading speed of Gambling (faster = more salient) vs. Neutral words under drug on the Rapid Reading Task. In contrast, both HAL and FLU diminished the salience of Gambling cues relative to placebo in PG subjects. This latter effect involved an improvement in reading speed to Neutral words under each drug, coupled with a more modest improvement in reading speed to Gambling words under placebo. Due to the limit on how fast one can respond, it is possible that the reduction in priming of Gambling words partly derived from a floor effect. Whatever the explanation, the improvement in response time under both D2 antagonists in PG subjects is noteworthy.
Game of Dice Task

The Game of Dice Task did not show any appreciable effects of HAL or FLU in either group. The stability of scores on this task suggests it is not sensitive to state manipulations or that it may not be amenable to repeated measures testing.

Stop Signal Task

Neither drug reliably affected GO-Response Time (GO- RT) in HC or PG subjects. Both HAL and FLU slightly improved inhibitory control (SSRT) in HC subjects but slightly impaired inhibitory control in PG subjects. These patterns did not approach significance however.

4.1.3 Comparison between HAL and FLU on the physiological effects before and after playing the slot machine game in PG and HC Subjects

Relative to placebo, HAL appeared to stabilize physiological arousal as measured by diastolic blood pressure in HC and PG subjects. FLU also appeared to stabilize arousal relative to placebo in HC subjects but not in PG subjects. In the latter group, FLU dampened arousal during the pre-game phase and this dampening effect was reversed after PG subjects played the game. Thus, physiological effects of the game seemed to be accentuated by combined D1 and D2 blockade but not selective D2 blockade in PG subjects. In the prior study (Zack and Poulos 2007), HAL reversed a pre-game deficit in systolic blood pressure in PG subjects, whereas in the present study, FLU appeared to exert this effect.

4.2 General Discussion

Considering first the findings for PG subjects, both antagonists increased the rewarding-pleasurable effects (Excitement, Enjoyment, High/Buzz) of the game, relative to placebo. In contrast, both drugs tended to reduce post-game motivational priming (Desire to Gamble) and to diminish post-game semantic priming of gambling cues (incentive salience) on the Rapid Reading Task. However, dissociable effects were seen for the two antagonists on mood state and
physiological measures. Whereas HAL decreased euphoria and vigor ratings before and after the game, FLU enhanced both of these effects in PG subjects.

Considering the findings for HC subjects, both antagonists increased perceived intoxication (High) and Excitement from the game, relative to placebo. However, neither drug led to a corresponding increase in Enjoyment of the game in HC subjects, in contrast to PG subjects. HAL and FLU had a similar modestly favorable profile of ARCI drug effects at pre-game, but had directionally opposite effects on euphoria ratings at post-game. The overall pattern of effects for the two drugs and post-game increase in euphoria under FLU looked similar in HC and PG subjects. The post-game difference in PG subjects under HAL vs. FLU suggests that partial D1 blockade optimized stimulus-induced DA transmission during D2 blockade.

In terms of priming, HAL appeared to mitigate post-game Desire to Gamble in HC subjects as it did in PG subjects. In contrast, FLU appeared to exert directionally opposite effects on post-game Desire to Gamble in the two groups, increasing this effect in HC subjects, but decreasing it in PG subjects. In this context, it is worth noting that a similar pre-game and post-game pattern under both HAL and FLU emerged in the PG group, relative to placebo treatment. In a functional sense, this could suggest that irrespective of antagonist or PG status, DA release due to D2 blockade increases Desire to Gamble primarily in the absence of robust stimulus-induced phasic DA release. In addition, playing the game under both HAL and FLU reduced Desire to Gamble post-game relative to placebo treatment, suggesting a possible satiating effect from increased stimulus-induced DA release during D2 blockade. The findings for HAL and FLU in enhancing the rewarding-pleasurable effects of the game (Enjoyment, Excitement, and Buzz) and impaired (reduced) lexical salience of gambling cues on the Reading task in PG subjects relative to placebo, further maybe supportive of this reasoning.
Physiological effects appeared to correspond to the pattern found for subjective reward as opposed to priming, with HAL attenuating blood pressure reactivity in both groups and FLU enhancing post-game reactivity in both groups, albeit modestly.

In general, the findings for measures of incentive motivation and priming for HC and PG subjects under FLU seemed to be generally in line with the hypotheses, with modest reductions under FLU in the PG group and modest enhancements under FLU in the HC group. The findings for subjective reward and intoxicating effects of the game were not in line with the hypothesis and indicated a parallel increase in these effects in both groups under FLU, although this effect did not translate into more enjoyment of the game in HC subjects. The dissociation of excitement/intoxication and enjoyment in HC subjects contrasts from the congruent pattern seen for these indices in PG subjects, and suggests that arousal and alteration of consciousness may be subjectively favorable for PG subjects but not for controls.

The findings for both the groups under HAL indicated a general countervailing effect of the drug on incentive motivation as well as mood and rewarding effects of the game. These results also differed from the hypotheses, particularly for PG subjects. The pattern of effects on the Rapid Reading Task most closely approximated the previous results for HAL in PG subjects, with RT to Gambling (but also Neutral) words improving to near asymptotic levels under the drug. Outlier scores (RT > 3 SD above mean) are common on reaction time tasks due to momentary lapses of attention, and scores are typically trimmed or log-transformed for this reason. With the very small n of this sample the SD was likely overestimated, so there was no reliable basis for transformation. It is possible that increased sample size will permit detection of differential facilitation in RT to Gambling vs. Neutral words when invalid scores are removed.
## Summary of Effects with Respect to Hypotheses

<table>
<thead>
<tr>
<th>List of Hypotheses</th>
<th>Variables Tested</th>
<th>Result - Consistent with Hypothesis (Yes/No)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HC</td>
</tr>
<tr>
<td><strong>Hypothesis 1:</strong> Relative to placebo, HAL (3 mg) will enhance slot machine reinforcement in PG but not in HC subjects.</td>
<td>a) Slot machine Betting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>b) VAS – Desire to Gamble</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>c) Rewarding Effects (Enj/High)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>d) Euphoria (MBG)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>e) DBP</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>h) VAS – Enjoyment (Enj)/ Excitement/ High, Addiction Research Centre Inventory – Morphine Benzedrine Scale (MBG, Euphoria); Arousal; Diastolic Blood Pressure (DBP)</td>
<td></td>
<td>Partly Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
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<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 7 Summary of Key Result Findings – Determining Consistency with the Study Hypotheses

Figure Legend: Incentive Motivation; Slot machine betting, VAS - Desire to Gamble; Rewarding – Pleasurable effects of the game; VAS – Enjoyment (Enj)/ Excitement/ High, Addiction Research Centre Inventory – Morphine Benzedrine Scale (MBG, Euphoria); Arousal; Diastolic Blood Pressure (DBP)
4.3 Conclusion

Overall, the findings for the role of D1 and D2 receptors in gambling reinforcement in problem gamblers and control subjects reveal some interesting trends. There were marked differences in the subjective effects of HAL vs. FLU. Thus, despite their similar affinity for D2, differences in the drugs’ affinity for D1 might have led to effects that suggest a role for D1 in various aspects of gambling reinforcement. In recent years, a distinction has been drawn between ‘liking’ (subjective pleasure, hedonic effects) and ‘wanting’ (craving, seeking) of psychoactive drugs and natural reinforcers (Robinson and Berridge 2001; Berridge 2007; Berridge and Robinson 2009). The weight of the evidence indicates that dopamine might be playing a clear facilitative role in ‘wanting’, but has a less consistent and potentially inhibitory role in ‘liking’ (e.g., Laviolette and van der Kooy 2003). This framework provides a context for interpreting the results of this study. Specifically, it suggests that activation of D1 might moderate the wanting and incentive salience of gambling in a manner that differentiates PG from HC subjects. Partial blockade of D1 by FLU appeared to increase gambling-induced ‘wanting’ in HC subjects, but appeared to decrease ‘wanting’ in PG subjects. Seamans and Yang (2004) postulated an inverted-U relation between D1 activation and optimal arousal (with respect to cognitive performance): a given increase in DA transmission would optimize D1 signaling and arousal in subjects with low baseline D1 function, but would over-stimulate D1 signaling and excessively arouse subjects with high baseline D1 function. The present findings seem to be consistent with that formulation and suggest that increased post-game ‘wanting’ to gamble in HC subjects involved a restoration of optimal D1 signaling when D2-blockade enhanced DA release was attenuated by FLU. The decline in post-game ‘wanting’ to gamble in PG subjects under FLU vs. placebo suggests that baseline D1 signaling may be deficient in these subjects so that gambling-induced DA release primes wanting to gamble, in part, by restoring this deficit.
The increase in subjective rewarding effects or game-induced arousal under FLU was evident in both groups, while the consistent decrease in reward or arousal emerged under HAL. Thus, as reported by Laviolette and van der Kooy (2003), increased DA release (during selective D2 blockade) during exposure to an addictive reinforcer can sometimes be aversive. More than simply neutralizing this effect, FLU appeared to actually reverse it, such that combined blockade of D2 and D1 augmented ‘liking’ of the game, albeit to varying degrees, in HC and PG subjects. The self-reported increase in post-game Excitement, High, POMS Vigor, and ARCI Euphoria scores might suggest that FLU increased the subjective arousal or exhilaration of the game. D1 receptors are mostly located post-synaptically, suggesting that the downstream effects of FLU on other neurotransmitter systems apart from its predicted action on D1 receptors may have contributed to its effects on ‘liking’ of the game. It is also possible that pre-game effects of HAL and FLU alone may in part reflect enhanced tonic stimulation of post-synaptic D2 receptors and that the drugs’ comparable antagonist effect at D2 auto-receptors accounted for this similarity. The slight decline in post-game diastolic blood pressure observed with HAL may also reflect its action on neurotransmitter systems other than DA, especially the noradrenergic system. In a recent study, HAL (3 mg) attenuated the increase in systolic blood pressure and heart rate seen with alcohol in healthy volunteers (Engasser and de Wit 2001), and alcohol appears to exert an effect on the NA system (Linnoila et al. 1987). Clearly, the above mentioned possibilities form an important matter for future investigation.

4.4 Limitations

As noted earlier, the findings from this study cannot be generalized to the population of PGs, considering the small sample size, and the correspondingly modest statistical power. The pre-treatment interval employed for the antagonists (2 hr for FLU, Midha, McKay et al. 1983; 2.75 hr for HAL, Nordstrom, Farde et al. 1992) may also have led to the high within-subject
variability in response. Previous studies reported differences in the pharmacokinetic profile for FLU (Midha, Hawes et al. 1988) and HAL (Potkin et al. 1984), and thus pharmacogenetic and inter-ethnic individual differences in metabolism might have also affected the study results. Future studies need to be conducted in order to elucidate the trends observed in the present study and the potential role of genotype in modifying response.

It is also important to note that the present study is incapable of defining the role of D1 receptor per se in the absence of D2 blockade in gambling reinforcement. Additionally, external validity could be a limitation for the present study in light of the findings from previous study that used casino gaming as the gambling prime (Meyer et al. 2004). The authors noted that casino gambling as a “real life” situation induces activation of the HPA-axis and the sympatho-adrenal system, with significantly more pronounced changes than were observed here. In the present study, the exposure to slot machine gambling occurred in a simulated mock-bar setting with artificial credits (subjects didn’t use their own money to gamble). Also, the subject stayed alone in the lab for the entire duration of the 15-min slot machine session. However, in the real world, the casino environment may be much more stimulating and interactive.

Furthermore, heterogeneity in nature or reinforcing effects of the gambling stimulus could be another drawback of the present study. Findings from numerous studies emphasize that different gambling primes can have diverse motivational-rewarding effects in PG vs. HC subjects (e.g., video poker, horse racing etc.) as well as among PG subjects themselves (Coulombe, Ladouceur et al. 1992; Coventry and Norman 1997). In such cases, the preferred gambling prime tends to regulate the desired level of ‘arousal’ in gamblers. For instance, Cocco, Sharpe et al. (1995) in their study assessing preferred level of arousal and motivation to gamble between 12 problem machine gamblers and 13 problem horse race gamblers found contrasting results between the two groups. Therefore, employing a slot machine game as the standard prime for all subjects in
the present study could have introduced variability in the size and possibly the direction of its motivational effects in different individuals.

4.5 Future Prospects

In order to further elucidate findings from the present study, it will be necessary to enroll more subjects in each antagonist group and potentially increase the diversity of the sample. This would provide an opportunity to explore the effects of genotype and individual differences in pharmacokinetics in modulating experimental response to the study medications. Evidence for such variation would suggest that PG subjects might respond differently to medications with different receptor binding profiles and provide an opportunity to explore interventions targeted to particular sub-types of PG (see Blaszczynski and Nower 2002).

The current findings also raise the possibility that dopamine D2 and D1 receptors may play a different role in gambling vs. psychostimulant reinforcement, particularly in controls. Parallel analysis of gambling and amphetamine reinforcement under FLU and HAL would provide an opportunity to directly examine the role of D2 and D1 receptors in the incentive-motivational and rewarding properties of these two ‘addictive’ reinforcers. This, in turn, could inform development of reliable, effective medications for PG and psychostimulant addiction, which have so far proven elusive.
References


Appendix A – Binding profiles of Dopamine Antagonists (HAL, FLU) at neurotransmitter receptors

Table I Binding profiles at Dopamine D1 and D2 receptors

<table>
<thead>
<tr>
<th>Ligands</th>
<th>D1</th>
<th></th>
<th>D2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀ (nM)</td>
<td>Ki (nM)</td>
<td>Response %</td>
<td>IC₅₀ (nM)</td>
</tr>
<tr>
<td>haloperidol</td>
<td>36</td>
<td>17</td>
<td>-43 ± 15</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>1.8</td>
<td>0.85</td>
<td>-39 ± 6</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>


Table II Binding profiles at Dopamine D2, D3 and D4 receptors

<table>
<thead>
<tr>
<th>Ligands</th>
<th>D2</th>
<th></th>
<th>D3</th>
<th></th>
<th>D4</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Response %</td>
<td>IC₅₀ (nM)</td>
<td>Ki (nM)</td>
<td>Response %</td>
<td>IC₅₀ (nM)</td>
<td>Ki (nM)</td>
</tr>
<tr>
<td>haloperidol</td>
<td>-43 ± 15</td>
<td>0.8 ± 0.2</td>
<td>0.6 ± 0.3</td>
<td>-53 ± 12</td>
<td>0.6 ± 0.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>-39 ± 6</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>-45 ± 12</td>
<td>2.1 ± 0.3</td>
<td>1.4 ± 0.8</td>
</tr>
</tbody>
</table>


Table III Neuroleptics equilibrium dissociations constants (KD’s) for muscarinic acetylcholine receptor (of human caudate nucleus), histamine H₁ receptor and α₁, α₂- adrenergic receptor (of human brain frontal cortex)

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Muscarinic Acetylcholine Receptor</th>
<th>Histamine H₁ Receptor</th>
<th>α₁ - adrenergic Receptor</th>
<th>α₂ - adrenergic Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kᵥ (nM)</td>
<td>Hill Coefficient</td>
<td>Kᵥ (nM)</td>
<td>Hill Coefficient</td>
</tr>
<tr>
<td>haloperidol</td>
<td>24000 ± 9000</td>
<td>0.7 ± 0.1</td>
<td>1900 ± 300</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>1900 ± 500</td>
<td>1.32 ± 0.08</td>
<td>21 ± 4</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

Table IV Binding profiles of antagonist at Serotonin (5-HT) receptors

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Clinically effective dose (mg)</th>
<th>5-HT&lt;sub&gt;1A&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;1B&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;1D&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;1E&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;1F&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2A&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2B&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2C&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;3&lt;/sub&gt;</th>
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<th>5-HT&lt;sub&gt;7&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluphenazine</td>
<td>2-15</td>
<td>145</td>
<td>334</td>
<td>334</td>
<td>540</td>
<td>21</td>
<td>983</td>
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<td>145</td>
<td>28</td>
<td>8</td>
<td></td>
<td></td>
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<tr>
<td>Haloperidol</td>
<td>2-15</td>
<td>1202</td>
<td>165</td>
<td>7606</td>
<td>&gt;10000</td>
<td>&gt;5000</td>
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<td>1204</td>
<td>5580</td>
<td>&gt;10000</td>
<td>2247</td>
<td>3666</td>
<td>378</td>
</tr>
</tbody>
</table>

Do you gamble?

If so, you may be eligible for a research study.

If you are:

19-65 years of age

Drug- and Medication-Free

Available for Weekday Sessions

Call Aditi Kalia: (416) 535-8501, ext. 6533

NOTE: This is not a treatment study.

- Financial Compensation Is Provided.
- All Information Will Remain Confidential To The Extent Allowed By Law.

CAMH provides other 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 4 Weekly Day-long Sessions (M – F)

Call Aditi Kalia: (416) 535-8501, ext. 6533

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 D - 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 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.

The schedule of payment is as follows:

<table>
<thead>
<tr>
<th>Task</th>
<th>Amount</th>
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<tr>
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<td>Physician’s Exam</td>
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<td>Test Session 1</td>
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<td>Test Session 2</td>
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<td>Test Session 3</td>
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<td><strong>$920</strong></td>
</tr>
</tbody>
</table>

7. 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.

**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 CAMH.
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 __/__/_____  DD/MM/YY

Print Name ____________________________

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)

WitnessSignature ____________________________

Print Name ____________________________  Date __/__/_____  DD/MM/YY
## Appendix E – Result Data

**Table V** Mean (SE) scores for the Perseverative and Non-Perseverative errors on the Wisconsin Card Sorting Task in PG and HC Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Error Type</th>
<th>Mean</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Control</td>
<td>Perseverative Errors</td>
<td>13.125</td>
<td>3.178</td>
</tr>
<tr>
<td></td>
<td>Non-Perseverative Errors</td>
<td>7.625</td>
<td>1.586</td>
</tr>
<tr>
<td>Pathological Gamblers</td>
<td>Perseverative Errors</td>
<td>8.500</td>
<td>3.178</td>
</tr>
<tr>
<td></td>
<td>Non-Perseverative Errors</td>
<td>7.000</td>
<td>1.586</td>
</tr>
</tbody>
</table>

**Table VI** Mean (SE) of the Systolic Blood Pressure (SBP) scores in PG and HC Subjects assigned to the HAL and FLU antagonist groups

<table>
<thead>
<tr>
<th>Measure</th>
<th>Antagonist Group</th>
<th>Drug/Placebo</th>
<th>Time</th>
<th>Mean</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>HAL</td>
<td>Drug</td>
<td>Baseline</td>
<td>113.791</td>
<td>6.695</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-game</td>
<td>116.311</td>
<td>6.255</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post-game</td>
<td>115.372</td>
<td>7.229</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>Baseline</td>
<td>117.732</td>
<td>6.631</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-game</td>
<td>114.409</td>
<td>5.943</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post-game</td>
<td>122.429</td>
<td>7.676</td>
</tr>
<tr>
<td>FLU</td>
<td>Drug</td>
<td>Placebo</td>
<td>Baseline</td>
<td>123.018</td>
<td>6.631</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-game</td>
<td>121.091</td>
<td>5.943</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post-game</td>
<td>120.821</td>
<td>7.676</td>
</tr>
</tbody>
</table>

**Table VII** ANOVA of the Capsule Guess scores on Test session 1 and 2

<table>
<thead>
<tr>
<th>Capsule contents evaluation- Test session 1</th>
<th>Drug Sequence</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active Drug</td>
<td>Placebo</td>
</tr>
<tr>
<td>Capsule contents evaluation- Test session 1</td>
<td>No guess</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>capsule 1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Capsule 2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>
### Chi-Square Tests

<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>$2.667^a$</td>
<td>2</td>
<td>.264</td>
</tr>
<tr>
<td>Likelihood Ratio</td>
<td>3.452</td>
<td>2</td>
<td>.178</td>
</tr>
<tr>
<td>Linear-by-Linear Association</td>
<td>2.143</td>
<td>1</td>
<td>.143</td>
</tr>
<tr>
<td>N of Valid Cases</td>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. 6 cells (100.0%) have expected count less than 5. The minimum expected count is 1.00.

### Capsule contents evaluation- Test session 2

<table>
<thead>
<tr>
<th>Capsule contents evaluation- Test session 2</th>
<th>No Guess</th>
<th>Active Drug</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>capsule 1</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>capsule 2</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>capsule 1 and capsule 2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

| Total                                      | 8        | 8           | 16      |

### Chi-Square Tests

<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>1.667$^a$</td>
<td>3</td>
<td>.644</td>
</tr>
<tr>
<td>Likelihood Ratio</td>
<td>1.726</td>
<td>3</td>
<td>.631</td>
</tr>
<tr>
<td>Linear-by-Linear Association</td>
<td>.238</td>
<td>1</td>
<td>.626</td>
</tr>
<tr>
<td>N of Valid Cases</td>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. 8 cells (100.0%) have expected count less than 5. The minimum expected count is 1.00.

**Table VIII** Mean (SE) for the symptoms side-effects scores in PG and HC Subjects in the HAL and FLU antagonist groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Group</th>
<th>Drug_plac</th>
<th>Mean</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Control</td>
<td>HAL</td>
<td>Drug</td>
<td>2.000</td>
<td>1.188</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>1.750</td>
<td>.854</td>
</tr>
<tr>
<td></td>
<td>FLU</td>
<td>Drug</td>
<td>.750</td>
<td>1.188</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>.250</td>
<td>.854</td>
</tr>
<tr>
<td>Pathological Gamblers</td>
<td>HAL</td>
<td>Drug</td>
<td>3.500</td>
<td>1.188</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>1.250</td>
<td>.854</td>
</tr>
<tr>
<td></td>
<td>FLU</td>
<td>Drug</td>
<td>3.000</td>
<td>1.188</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>.750</td>
<td>.854</td>
</tr>
</tbody>
</table>