PARALLELBS BETWEEN GAMBLING AND AMPHETAMINE REINFORCEMENT IN PATHOLOGICAL GAMBLERS AND HEALTHY CONTROLS AND THE ROLE OF SENSITIZATION.

By

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A thesis submitted in conformity with the requirements for the degree of Master of Science Graduate Department of Pharmacology and Toxicology University of Toronto

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Parallels between Gambling and Amphetamine Reinforcement in Pathological Gamblers and Healthy Controls and the Role of Sensitization.

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Pathological gambling is a serious disorder with lifetime prevalence between 1.1-3.5%. Evidence suggests commonalities in the neurochemical basis of pathological gambling and psychostimulant addiction. However, parallel effects of gambling and a stimulant drug have not been assessed in the same subjects. This study employed a cross-priming strategy in which 12 male pathological gamblers and 11 male controls were exposed to a 15-minute slot machine game and d-amphetamine (0.4 mg/kg). Subjective, cognitive, electrophysiological, and physiological responses were assessed. Gamblers reported greater desire to gamble after both reinforcers, when baseline motivation was controlled. Conversely, gamblers exhibited diminished cardiovascular response to amphetamine. Gamblers also exhibited decreased pre-pulse inhibition (impaired sensorimotor gating), and deficits on this index predicted greater post-amphetamine desire to gamble and decreased heart rate response to the dose. Results are consistent with possible dopaminergic sensitization in pathological gamblers, but also suggest that central noradrenergic receptor deficits contribute importantly to these effects.
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<td>BEH</td>
<td>Behavioural Session</td>
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<tr>
<td>CAMH</td>
<td>Center for Addiction and Mental Health</td>
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<tr>
<td>CON</td>
<td>Healthy Controls</td>
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<tr>
<td>DA</td>
<td>Dopamine</td>
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<tr>
<td>DDS</td>
<td>Dopamine Dysregulation Syndrome</td>
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<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual for Mental Disorders, 4th Edition</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
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<td>PG</td>
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1. INTRODUCTION

1.1. Background and Rationale

Pathological Gambling is formally classified as an Impulse Control Disorder (American Psychiatric Association 1993). However recent evidence suggests that it shares many similarities to substance addiction (Holden 2001). Currently, there are no approved pharmacological treatments available for pathological gambling (Achab and Yasser 2011). To date, most potential medications for pathological gambling have been derived from the pharmacopeia of substance addiction (Petry 2007). Substance addiction is comprised of a heterogeneous class of disorders. Typically, medications for one substance use disorder do not reliably work for another. For instance, naltrexone is effective in treatment of alcohol dependence, but not nicotine dependence (Wong et al. 1999). This suggests that a more systematic approach to developing medications for pathological gambling might be useful by determining the neurochemical mediators of gambling reinforcement.

Gambling reinforcement is based entirely on neurochemical signals for reward; the signals themselves are arbitrary (e.g., a row of cherries; a pair of face cards), and acquire their meaning by learned associations. It follows that the brain systems which mediate responses to signals associated with reward should play a pivotal role in gambling reinforcement. Dopamine (DA) is the principal mediator of conditioned reinforcement and incentive motivation (Schultz 1998). Dopamine also varies in predictable ways with the uncertainty of reward delivery, which is a defining feature of gambling (Fiorillo et al. 2003). Although all addictive substances activate dopamine, this effect is both necessary and sufficient for the reinforcing effects of only one drug class: psychostimulants (Pierce and Kumaresan 2006). Therefore, if pathological gambling resembles substance addiction, this parallel should be particularly strong.
with respect to the psychostimulant class of addictions. If so, the medications that are helpful in psychostimulant addiction may also benefit pathological gambling. Indirect evidence supports this possibility. Most recently, Mutschler and colleagues (2010) suggested that disulfiram, shown to be effective in treating cocaine addiction, could serve as a potential treatment for pathological gambling. However, direct evidence for parallel patterns of gambling and psychostimulant reinforcement in pathological gambling subjects has yet to be obtained. Thus, the present study was designed to address this issue. In addition, according to the Incentive-Sensitization Theory of Addiction (Robinson and Berridge 1993), repeated exposure to an addictive substance can result in increased drug craving or ‘wanting,’ which is independent from the pleasurable effects of the drug. Robinson and Berridge (1993) proposed that repeated exposure to drugs leads to sensitization of the reward-related dopamine pathways in the brain and compulsive ‘wanting’ which mediates the progression from intermittent drug use to drug addiction. Psychostimulants have been shown to produce the most robust sensitization, therefore if pathological gambling is like psychostimulant addiction then robust sensitization or a hypersensitive dopamine system should also be evident in gamblers. The overall goals of this project were: (1) to assess commonalities between gambling and amphetamine reinforcement in Pathological Gamblers and Healthy Controls; and (2) to investigate cognitive processes (i.e. sensitization) that may contribute to these effects.
1.2 Review of Literature
1.2.1 Pathological Gambling
1.2.1.1 Definition

The American Psychiatric Association (1993) defines pathological gambling as ‘persistent and recurrent maladaptive gambling behaviour’ characterized by the inability to control gambling, leading to significant deleterious psychosocial consequences: personal, familial, financial, professional and legal. In the Diagnostic and Statistical Manual for Mental Disorders (DSM IV-TR) (American Psychiatric Association 2000, p. 671) pathological gambling is formally classified as an ‘Impulse Control Disorder not elsewhere categorised’ along with pyromania, kleptomania and trichotillomania. Impulse control disorders are characterized by repeated engagement in impulsive behaviours and diminished ability to inhibit participation in these behaviours despite adverse consequences (American Psychiatric Association 2000). Unlike other impulse control disorders, pathological gambling criteria share similarities with the criteria for substance use disorder. Hence, pathological gambling is informally recognized as a behavioural or substance-less addiction (Potenza 2008). During the process of the development of the fifth edition of the DSM issues around the inclusion of ‘behavioural addictions’ were debated. In 2010, it was announced that, for the first time, ‘behavioural addictions’ will be included in the upcoming DSM-V. However, only one disorder - Pathological Gambling - has qualified and is set to join the substance use disorders category as a full-fledged addiction (Holden 2010).

Substance-use disorder is defined in the DSM-IV-TR as a ‘maladaptive pattern of substance use, leading to clinically significant impairment or distress’ (American Psychiatric Association 2000, p. 192). Both pathological gambling and substance use disorder share criteria related to tolerance, withdrawal, repeated unsuccessful attempts to cut back or quit; and
interference in major areas of life functioning. In substance use disorders, tolerance describes the use of increasing amounts of a substance in order to achieve an equivalent desired effect of previous consumption or a diminished desire following the use of the same amount of a substance (Grant et al. 2002; Wareham and Potenza 2010). In pathological gambling, tolerance is operationalized as gambling with increasing amounts of money in order to achieve the desired subjective effects (e.g., excitement) or that the same level of gambling leads to diminished subjective response. Withdrawal in substance use disorders relates to onset of physiological or psychological symptoms upon abrupt cessation or diminished usage of substance. This is also evident in pathological gamblers, where symptoms such as anxiety and irritability are reported when quitting or cutting down (Wareham and Potenza 2010; Way et al. 1981). In addition, many of the inclusionary criteria specific to pathological gambling can also be analogous to behaviours evident in substance use disorders. For example, chasing in gambling refers to going back to a gambling venue shortly after losing in an effort to recoup money that was lost. This shares the common feature of ‘chasing a high’ or being on a ‘drug run’ evident in substance use disorders (Wareham and Potenza 2010). Other examples include lying about gambling and partaking in illegal behaviours. The core components of addiction are: (i) continued engagement in behaviour despite adverse consequences; (ii) diminished self-control over engagement in the behaviour; (iii) compulsive engagement in the behaviour; and (iv) appetitive urge or craving state prior to the engagement in the behaviour (Potenza 2006). Pathological gambling fits well within this framework.

In addition, a variety of indirect evidence suggests that pathological gambling may be especially similar to the psychostimulant class of substance use disorders. The Addiction Research Center Inventory scale discriminates between the subjective effects of Amphetamine,
Morphine, LSD, alcohol and other drug classes (Haertzen 1965). When asked to imagine a bout of gambling, pathological gamblers endorsed a strikingly similar profile of items as psychostimulant users did in response to a dose of amphetamine on the Addiction Research Center Inventory (Hickey et al. 1986). This suggests a possible link between the subjective effects of psychostimulants and gambling that is not evident with other drug classes.

1.2.1.2 Prevalence

Epidemiological studies estimate that the prevalence of adult pathological gambling is between 1.1-3.5% in the United States and Canada. This large variation across studies is probably due to sampling and measurement artefacts (Lorains et al. 2011). In certain groups, such as young adults, people with mental health or substance use disorders and prison inmates, rates of gambling problems are several-fold higher (Shaffer et al. 1999). With growing availability of gambling opportunities through legalized and online gambling, the prevalence of pathological gambling is expected to continue to rise (Goudriaan et al. 2004). According to Raylu and Oei (2002), between 70-90% of the North American population have engaged in some form of gambling activity, and approximately 10% meet criteria for ‘problem gambling.’ The term ‘problem gambling’ is often used to describe an intermediate or subclinical form of pathological gambling and is a good indicator of future diagnosis. ‘Probable pathological gambling’ is indicated if individuals score ≥ 5 on the South Oaks Gambling Screen (SOGS; Lesieur and Blume 1987), a common tool used to measure gambling severity. Problem gamblers score 3 or 4 on the SOGS. A formal pathological gambling diagnosis is indicated by endorsement of five or more DSM-IV criteria for the disorder (Beaudoin and Cox 1999).
Both pathological gambling and problem gambling are associated with impaired psychological functioning, reduced quality of life, legal problems and high rates of bankruptcy, divorce and incarceration (Shaffer et al. 2001). Pathological gambling also has a substantial burden on society: e.g., in the United States, the financial burden was estimated at $5 billion in 1999 (National Gambling Study Commission Final Report 1999). Therefore this disorder poses serious public and mental health concerns, with implications for individuals, families and communities (Shaffer et al. 2001).

Pathological gambling is also a highly co-morbid disorder, with the greatest co-morbidity occurring with substance use disorders. It is evident in 4% of treatment seeking alcohol dependant outpatients (Sellman et al. 2002). Steinberg et al.’s (1992) study of cocaine dependant patients found a life-time prevalence of pathological gambling of 14.8%, which was approximately 10 times the rate in healthy population at that time. Another study found a prevalence of gambling disorder of 8% in cocaine dependant inpatients, of whom 72% reported that pathological gambling preceded cocaine dependence (Hall et al. 2000). The exceptionally high rate of gambling disorder in cocaine addicts indirectly supports a particular similarity between pathological gambling and stimulant dependence.
1.2.1.3 Neurobiology of Pathological Gambling

Based on a range of evidence, serotonin has been assigned a prominent role in impulse control disorders due to its link with behavioural inhibition and satiety; noradrenaline has been linked with arousal and excitement, endogenous opioids to pleasure and urges, glutamate to cue reactivity and neuroplasticity; and dopamine to attention, reward and reinforcement.

a) Serotonin

Pathological gamblers have demonstrated low-levels of the serotonin metabolite, 5-hydroxy-indole-acetic acid (5-HIAA), suggesting evidence of a hypoactive serotonin system (Nordin and Eklundh 1999). Studies using serotonergic drugs have demonstrated that gamblers reported a ‘high’ following administration of meta-chlorophenylpiperazine (m-CPP), a partial serotonin 2c receptor agonist (DeCaria et al. 1998, Pallanti et al. 2006). However, other studies have found no serotonin abnormalities in gamblers (Roy et al. 1988). In terms of treatment, several placebo-controlled, clinical trials of serotonin-reuptake inhibitors have obtained both positive and negative evidence for efficacy (Brewer et al. 2008)

b) Noradrenaline

Pathological gamblers have been shown to have lower plasma noradrenaline metabolites but higher cerebrospinal fluid and urinary metabolites compared to controls (Roy et al. 1988; Bergh et al. 1997). Pallanti et al. (2010) found that gamblers had a decreased neuroendocrine response to clonidine challenge, indicating hypo-sensitive post-synaptic alpha-2 adrenergic receptors, which the authors attributed provisionally to chronic noradrenergic ‘overdrive.’ Meyer et al. (2004) found that, during casino blackjack gambling, heart rate and noradrenergic measures were elevated to a greater degree and for a longer duration in men with gambling
problems than in controls. Such evidence supports early claims that the noradrenergic system mediates heightened risk-induced arousal in gambling individuals (Blanco et al. 2000).

c) Opioids

Opioids antagonists have been widely studied in the treatment of pathological gambling and other addictions. For example, placebo-controlled double blind randomized trials have evaluated the efficacies of naltrexone and nalmefene and found them to be superior to placebo in pathological gambling (Grant et al. 2008). Beneficial effects of opiate antagonists are thought to reflect a decline in opiate-stimulated dopamine release and associated craving states (Grant et al. 2008).

d) Glutamate

Glutamate is the most abundant excitatory neurotransmitter and has been implicated in motivational processes and drug addiction (Kalivas and Volkow 2005). N-acetylcysteine, a glutamergic pro-drug, has been found to significantly improve craving in gamblers (Grant et al. 2007). Somewhat paradoxically, the glutamate antagonist, memantine, has also shown some therapeutic effects in pathological gamblers (Grant et al. 2010).

e) Dopamine

Due to dopamine’s involvement in reward and reinforcement processes, pathological gambling has been closely linked with the dysfunction of this system (Nestler 2004, Potenza 2008). There have been three published studies that have evaluated cerebrospinal fluid levels of dopamine and its metabolites in pathological gambling. Roy et al. (1989) found no differences between gamblers and controls. However, more recently, Bergh et al. (1997) and Nordin and Sjödin (2007) each found elevated dopamine metabolites in the cerebrospinal fluid of gamblers compared to controls.
In summary, the literature indicates that a variety of neurochemical anomalies exist in pathological gambling subjects, which may contribute to their symptoms. In view of its critical role in reward seeking and conditioned reinforcement, dopamine has received special attention. The linkage of dopamine, compulsive reward-seeking and monetary risk taking has been demonstrated by neuroimaging studies. Although a full review of this literature is beyond the scope of this thesis, a sample of relevant studies is presented to convey some of the essential findings.

1.2.2 Dopamine and Reward

The mesolimbic dopamine pathway is one of the primary pathways involved in reward (for review see Berridge 2007 and Wise 2009). It originates in the ventral tegmental area of the midbrain and innervates structures in the limbic system, including the nucleus accumbens, the prefrontal cortex and amygdala (Berridge 2007). It has been established that all drugs of abuse, either directly or indirectly, increase dopamine neurotransmission within this pathway through their interaction with different molecular targets depending on the drug class (Koob 1992, Hyman et al. 2006, Peirce and Wise 2004). For example, amphetamine, a prototypical psychostimulant, directly increases dopamine transmission by entering dopamine neurons and interacting with the dopamine transporter (DAT) to reverse the transport of dopamine out of the neuron into the synapse, thereby increasing availability in the synapse (Ritz et al. 1990, Seiden et al. 1993, Sulzer et al. 1993). On the other hand, heroin, an opiate narcotic, indirectly increases dopamine in the mesolimbic system by acting on opiate receptors, which decrease inhibitory GABAergic neurotransmission, leading to disinhibition of dopamine neurons. These dopamine neurons are then responsible for the release of dopamine in the nucleus accumbens.
(Johnson and North, 1992, Hakan and Henriksen, 1989, Lee et al. 1999). Nicotine increases dopamine by stimulating the nicotinic acetylcholine receptors leading to the activation of ventral tegmental dopamine neurons (Nestler 2005).

In humans, positron emission tomography (PET) studies have reliably shown that substances from a number of drug classes such as stimulants (Volkow et al. 1999, Drevets et al. 2001), nicotine (Brody et al. 2009), alcohol (Boileau et al. 2003), and marijuana (Bossong et al. 2009) lead to increases in dopamine release in ventral striatum (where nucleus accumbens is located). PET studies utilize radiotracers such as [11C] Raclopride and [11C] PHNO, which bind to unoccupied dopamine D2 receptors (for review, Volkow et al. 2009). Binding profile after drug and placebo is compared to estimate decreases in dopamine D2 receptor availability induced by drug, which is proportional to increases in dopamine (Breier et al. 1997). Most studies have demonstrated that healthy individuals who display the greatest drug-induced dopamine increases also report the most intense “high” or “euphoria” (Volkow et al. 2009). In contrast, cocaine abusers display decreased dopamine release in response to a standard dose of a stimulant drug (methylphenidate) than controls do (Volkow et al. 1997). If pathological gambling is like stimulant addiction, a similar pattern of activation in the mesolimbic dopamine system should be evident in response to gambling-like stimuli in subjects with pathological gambling (see description of Reuter et al. 2005 below).
1.2.2.1 Sensitization

Extensive research has demonstrated that dopamine plays a role in reinforcement, incentive motivation and reward (Wise 2004, Schultz 1998). However, the precise role that dopamine plays is still under debate (Berridge 2007). Originally, dopamine activation was thought to reflect the pleasurable effects of a drug, or drug “liking” which was thought to lead to addictive behaviour, due to the pleasure derived from drug. However, it has been shown that animals still display pleasurable responses to natural reward and drugs of abuse even in the absence of dopamine. For example, lesions of the dopamine neurons in the nucleus accumbens and pharmacological blockade of dopamine transmission do not alter hedonic effects or liking of sucrose in rats (Berridge et al. 1989, Pecina et al. 1997). It was also observed that, although pleasurable effects of drug diminish over time, addicts still found it difficult to reduce or terminate drug use (Robinson and Berridge 2008). Evidence in humans using PET also indicated that dopamine levels in the mesolimbic pathway of healthy volunteers correlate significantly with drug “wanting” as opposed to drug “liking” (Leyton et al. 2002). Therefore, it appears that dopamine affects craving for drugs and this may be dissociable from their pleasurable or hedonic effects.

Increased drug craving has also been observed in clinical populations. A sub-set of patients with Parkinson’s disease develop an addiction-like profile with their dopamine-enhancing medication. These patients compulsively consume greater amounts of the dopamine pre-cursor, L-DOPA, despite the external appearance of being well medicated. Evans et al (2006) found that these Parkinson’s patients with Dopamine Dysregulation Syndrome (DDS) displayed enhanced dopamine release in the mesolimbic pathway such as the ventral striatum.
when challenged with L-DOPA compared to non-DDS Parkinsonian patients. This increase also correlated with increased self-reported drug “wanting” as opposed to drug “liking”. These findings further support the argument that dopamine plays a major role in increased drug “wanting” as opposed to the pleasurable or hedonic effects of drugs.

Increased dopamine response or increased “wanting” in response to a standard dose of a drug is termed sensitization. More generally, sensitization refers to an increase in behavioural and neurochemical response to a stimulus with repeated exposure to that stimulus. Robinson and Berridge’s Incentive Sensitization Model of Addiction (1993) postulates that chronic exposure to addictive drugs leads to ‘incentive’ sensitization, which is the increase in the ability of drug cues to recruit attention (i.e.: salience) and the appetitive response of craving/compulsive drug seeking. Therefore, it can be argued that sensitization and associated neuroplastic changes in the dopamine system mediate drug craving or “wanting” in chronic drug users.

The Incentive-Sensitization model considers chronic, drug-induced elevations in phasic (burst) dopamine to be the primary (though not exclusive) process underlying sensitization in substance use disorders (Robinson and Berridge 1993). In animals, sensitization is manifested as an increase in locomotor response to a drug after repeated dosing relative to the first exposure (Robinson and Becker 1986). The greater the locomotor response to the drug, the greater is the presumed dopamine sensitization (Piazza et al. 1990). Chronic use of most drugs of abuse can produce sensitization; however there is considerable variability among different classes. For example, alcohol does not produce as reliable sensitization effects as psychostimulants do (Vezina et al. 1999, Zapata et al. 2006). If pathological gambling closely
mimics psychostimulant addiction, then robust sensitization should also be evident in pathological gamblers.

Sensitization is also remarkably persistent: increased locomotor response to dopamine challenge with d-amphetamine has been observed in animals even months to years after prior exposure to the drug (Robinson and Becker 1986, Paulson et al. 1991). Recently, evidence of sensitization has also been observed in humans. Boileau and colleagues (2006) exposed healthy stimulant-naive subjects to 3 moderate doses of amphetamine (0.3 mg/kg). Using PET, they showed that this minimal drug regimen was sufficient to induce long-lasting increases in dopamine release in the ventral striatum in response to an amphetamine challenge 1 year after the initial exposure.

If drugs of abuse lead to increased ‘wanting’ or a sensitized state, then human addicts should display increased dopamine release in response to a dopamine-releasing agent. However, current literature consists of conflicting reports on brain dopamine changes in addicts. For example, detoxified cocaine addicts have demonstrated blunted dopamine release rather than a sensitized or increased response (Volkow et al. 1997, Martinez et al. 2007). Alcohol–dependant detoxified subjects also showed blunted dopamine release from a stimulant challenge compared to controls (Volkow et al. 2007). These findings are complicated and need to be interpreted with caution as numerous limitations exist. Firstly, prolonged use of drugs of abuse can lead to neurotoxicity; therefore damage to the dopamine neurons may mask or alter the expression of stimulant induced dopamine release in PET (Abekawa et al. 1997). Secondly, many variables interact to determine whether sensitization is present at any particular time or place. Therefore, context is crucial in gating the expression of sensitization (Leyton 2007). Animal studies have consistently shown that expression of sensitization is powerfully
modulated by context in which the drug was administered (Robinson et al. 1998). For example, sensitization and enhanced dopamine release typically do not manifest in animals that have been tested in a novel context where drug was never administered before (Fontana et al. 1993, Anagnostaras and Robinson 1996). Hence, on the basis of animal literature, human drug addicts may not display the typical sensitized dopamine release response if the environment is different from the context where drug was taken (e.g., PET scanner). The best demonstration of sensitization in humans is evident when the investigator kept the context similar (Boileau et al. 2006). Thirdly, current human PET studies have been conducted when addicted individuals were in the withdrawal or detoxified state. Therefore, both tolerance and withdrawal may have masked the expression of sensitization, as it is said to best expressed after a period of incubation (Robinson and Becker 1986). To date, no PET studies appear to have been performed after a period of incubation to test this assumption. Lastly, these drugs of abuse, due to their supra-physiological action on the synapse and receptor sites, lead to rapid depletion of the dopamine stores over time. Therefore, most addicts have depleted vesicular dopamine stores which may contribute to the blunted dopamine response. In contrast, pathological gamblers may evince sensitization as gambling does not involve an agent directly altering synaptic or neuronal dopamine levels, but instead involves physiologically-mediated dopamine release in response to signals for reward. This differential profile of acute effects may mitigate the potential neurotoxic effects of gambling relative to drugs of abuse and thereby permit expression of the sensitized dopamine response in pathological gamblers which has been elusive in substance abusers.
1.2.2.2 Prepulse Inhibition: Marker for Sensitization

In addition to PET, electrophysiological indices can provide an effective, though indirect, way of measuring dopamine function and thus sensitization. Prepulse inhibition of acoustic startle reflects the brain’s ability to gate redundant sensory information, and is widely used to as an experimental tool (for review, Braff et al 2001). The startle response refers to contraction of the skeletal muscles in response to a sudden intense stimulus (Koch and Schnitzler 1997). This startle response is observed translationally in multiple species from rats (indicated by full body flinches) to humans (indicated by eye blinks) (Abduljawad et al. 1999, Davis et al. 1982). The startle amplitude is modulated by stimuli presented prior to the startle stimulus (Graham 1975). Prepulse inhibition is a robust phenomenon that occurs when a weak sensory event (the pre-pulse, 68-80 decibels) is presented a few milliseconds before a strong sensory event (the startle pulse; 105-120 decibels) causing the brain to inhibit the magnitude of response to the latter stimulus (Braff et al 2001). Prepulse inhibition indicates that part of the information from the pulse is redundant, having already been conveyed by the prepulse (Braff and Geyer 1990). It is a cross-species phenomenon that uses similar testing parameters in both humans and animals (Swerdlow et al. 1994). It does not show habituation after multiple sessions and is observed after the first exposure (Blumenthal et al. 1996, Braff et al. 2001).

Normal prepulse inhibition reflects intact sensorimotor gating, while deficient prepulse inhibition (lack of decrease in startle amplitude) reflects deficient sensorimotor gating. This deficient prepulse inhibition is evident in patients with disorders such as schizophrenia, which is considered to be a heightened dopaminergic syndrome (Braff et al. 1990, Braff et al. 1992). Psychotomimetic drugs such as cocaine and amphetamine that produce schizophrenia-like symptoms in humans also disrupt prepulse inhibition in normal individuals and animals. For
example, rats given systemic injections of the dopamine releaser d-amphetamine demonstrate disrupted prepulse inhibition (Mansbach et al. 1988). Numerous studies have also shown that pharmacological increases in dopamine transmission lead to disruption in prepulse inhibition. For example, apart from d-amphetamine, other dopamine agonists such as apomorphine (Mansbach et al. 1988, Davis et al. 1988), quinpirole (Caine et al. 1995) and bromocriptine (Swerdlow et al. 1998) disrupt prepulse inhibition. Furthermore, the disruption of prepulse inhibition caused by dopaminergic drugs is blocked by drugs that antagonise dopamine receptors, such as haloperidol (Mansbach et al. 1988) indicating that dopamine plays a crucial role in the modulation of prepulse inhibition.

Recently, Stojanav et al. (2003) measured prepulse inhibition in pathological gamblers and determined that they exhibit disrupted sensorimotor gating on all measures of prepulse inhibition, with the most profound deficits seen in eye blink response at 120 milliseconds prepulse lead interval. Thus, pathological gamblers exhibit a prepulse inhibition profile consistent with dopamine sensitization. Deficits in prepulse inhibition are evident in stimulant users (Hutchison et al. 1999), but appear to be restricted to the acute withdrawal phase in alcohol dependent patients (Keedwell et al. 2001), and no evidence of decreased prepulse inhibition appears in the literature for opiate-dependant individuals. Nicotine has been shown to increase prepulse inhibition in both animal and human studies (Kumari et al 1996; DellaCasa et al. 1998). Thus, decreased prepulse inhibition is primarily associated with psychostimulant exposure and is not a generic feature of addiction.

However, it is important to note that disruption in sensory motor gating is not exclusive to elevated dopamine transmission; manipulations in other neurotransmitter systems also affect prepulse inhibition. For example, NMDA antagonists such as phencyclidine and dizocilpine
also disrupt prepulse inhibition and these are not reversed by the dopamine antagonist, haloperidol (Bakshi and Geyer 1997, Bakshi et al. 1994). Increases in serotonin through serotonin agonists, such as 3, 4 methyl-dioxy-methamphetamine, also disrupt prepulse inhibition (Mansbach 1989, Kehne et al. 1996). Noradrenaline agonists can also lead to disruption in prepulse inhibition, however this was only found when agents were administered centrally not peripherally (Alsene et al. 2006).

In sum, elevated dopamine transmission most reliably (although not exclusively) leads to disruption in sensory motor gating in both humans and animals, and decreased prepulse inhibition can provide a putative marker for sensitization, although the specific role of dopamine cannot be confirmed without evidence of restoration of prepulse inhibition by a dopamine antagonist.

1.2.2.3 Functional Relationship between Dopamine and Gambling Related Stimuli

A functional magnetic resonance imaging (fMRI) study by Reuter et al. (2005) found that, relative to controls, pathological gamblers showed a blunted response to monetary payoffs from a card-guessing task in the ventral striatum (location of nucleus accumbens); and the degree of deficit correlated with the severity of gambling symptoms. This suggests that, like cocaine abusers in a response to a modest dose of a stimulant drug, pathological gamblers display a ‘tolerance’-like response to stimuli that mimic gambling. In healthy subjects, ventral striatal activity is associated with decisions to chase monetary losses in a probabilistic risk-taking task (Campbell-Meiklejohn et al. 2008). The Incentive Sensitization model of addiction (Robinson and Berridge 1993) argues that cues for the drug reinforcer, rather than the drug itself elicits a sensitized response. Accordingly, images of gambling elicit increased activation
and concomitant craving in pathological gamblers versus controls (Crockford et al. 2005). This resembles the increased cue-induced response to images of alcohol or cocaine in abusers of these substances (George et al. 2001, Garavan et al. 2000).

Increased recruitment in the ventral striatal region in healthy subjects is also seen with near-miss events, which occur when an unsuccessful outcome is proximal to the designated win (Clark et al. 2009). In pathological gambling subjects, greater striatal activation in response to near-misses (but not wins) directly predicts greater gambling severity on the South Oaks Gambling Screen (Chase and Clark 2010). This suggests that the expectancy of reward may interact with reward-related cues to promote pathological reward seeking and associated consequences in pathological gambling. The authors noted that these effects indicate a selective disturbance in operation of ‘the dopaminergic midbrain’ in gamblers (p. 6180).

Preferential dopamine activation in response to near-misses is consistent with the possibility of sensitization in gamblers. However, the results of this study cannot establish the causal relationship between dopamine response and pathological gambling symptoms. Evidence to support a causal role of dopamine in gambling comes from the influence of dopamine-specific drugs on the development of pathological gambling symptoms in patients with no history of gambling disorder. A growing body of studies has found that drugs that selectively activate dopamine (D2 and D3) receptors can induce problem gambling in patients with Parkinson’s disease, Restless Legs Syndrome, and endocrine disorders who receive these drugs to treat their symptoms (Crockford et al. 2008). Typically, gambling symptoms emerge shortly after the introduction of these drugs and remit rapidly when the drugs are withdrawn (Voon et al. 2006). Low doses of these dopamine-selective drugs (e.g., pramipexole) lead to increased risk-taking in gambling tasks in healthy volunteers (Riba et al. 2008), indicating that
selective dopamine activation is sufficient to cause maladaptive reward-seeking even in the absence of pre-existing neuropathology or psychopathology.

Evidence from animals suggests that gambling-related stimuli selectively recruit dopamine specific neural substrates. Fiorillo et al. (2003) implanted electrodes in the mid-brain dopamine neurons of monkeys and recorded activity during exposure to cues for primary reward (liquid) and the reward itself. The critical independent variable was the reward schedule: 0, 25, 50, 75 or 100%. The investigators found that dopamine activity following reward delivery decreased linearly with its predictability, with maximal responses in the 0% condition (completely unexpected) and minimal responses in the 100% condition (completely expected). Importantly, the investigators also observed dopamine release following exposure to the cue (rather than the reward), which was maximal in the 50% condition (i.e. cue incurs expectancy of reward but provides no information as to whether the reward will or will not be delivered). Lower dopamine release was evident following the cue in the 25% and 75% conditions, each of which provides more information about reward delivery or omission than the 50% condition does. The investigators concluded that cues associated with maximally uncertain reward (50%) elicit maximal dopamine release and proposed that: “This uncertainty-induced increase in dopamine could contribute to the rewarding properties of gambling, which are not readily explained by overall monetary gain or dopamine’s corresponding role in prediction error (as losses tend to outnumber gains)” (p. 1901). If acute exposure to cues for 50% reward elicits maximal dopamine release, chronic or repeated exposure – as in the case of gambling – should lead to repeated bouts of maximal dopamine release. Research on the long-term average frequency of reward delivery (vs. omission) on a commercial slot machine game shows that the percentage of rewarded trials (47%) closely approximates the rate of reward that
would elicit maximal dopamine release to the reward cue (i.e., the lever or button that activates the spin) (Tremblay et al. 2010). To the extent that chronic exposure to dopamine-releasing stimuli mediates incentive sensitization, individuals chronically exposed to gambling should be susceptible to this effect much like individuals chronically exposed to drugs of abuse.

Evidence to suggest a common role for dopamine in psychostimulant addiction and pathological gambling comes from research on pharmacological priming. Priming refers to the instigation of drug seeking by administration of a modest dose of the target drug. Dopamine D1 and D2 receptors are each involved in the priming effects of cocaine in cocaine experienced animals (Khroyan et al. 2000, Graham et al. 2007, Schmidt and Pierce 2006). Cross-priming of cocaine seeking is seen in response to a psychostimulant drug (amphetamine) but not to alcohol, opiates, cannabinoids or even nicotine, a non-stimulant drug with strong pro-dopaminergic effects (Schenk and Partridge 1997). Thus, cross-priming indicates recruitment of common neurobiological substrates and, possibly discriminative (i.e. subjective) features, by the prime and target substance. If pathological gambling and psychostimulant addiction are mediated by common neurobiological substrates, motivation to gamble should be primed by a dose of psychostimulant in pathological gamblers, and this effect should not be found in subjects without this disorder.

Zack and Poulos (2004) adopted a cross-priming strategy to test this hypothesis in pathological gamblers, problem drinkers, co-morbid gambler-drinkers and healthy controls. They found that a moderate dose of d-amphetamine (30-mg oral) led to robust positive subjective effects in all groups (drug liking), with the most pronounced effects in pathological gambling subjects. The drug led to significant bad effects in problem drinkers only. In contrast, amphetamine significantly and selectively increased self-reported Desire to Gamble in
gamblers. Thus, amphetamine only elicited a robust incentive motivational effect in subjects with a gambling disorder. Finally, amphetamine selectively increased fluency of vocal response time to gambling words (e.g., wager) relative to neutral words (e.g., window) in pathological gambling and co-morbid gambler-drinker subjects on a rapid reading task. No such effects were evident for alcohol words. Rapid response to gambling words is indicative of incentive salience; and other research suggests that decreased response latency to verbal drug stimuli is mediated by mesolimbic dopamine (Goldstein et al. 2009). Collectively, these findings were consistent with the possibility of a sensitized incentive motivational response to amphetamine (i.e., primed “wanting” to gamble).

Subsequent research sought to isolate the role of dopamine (D2 receptors) in primed motivation to gamble in response to a brief slot machine game (i.e., a modest dose of gambling) in pathological gamblers and healthy controls (Zack and Poulos 2007). Relative to pre-game, post-game ratings of desire to gamble increased significantly and similarly in both groups. Pre-treatment with the D2 antagonist haloperidol (3-mg) significantly increased the priming effects of the game in pathological gamblers as evidenced by heightened post-game desire to gamble and increased facilitation of responses to gambling words on the reading task. In healthy control subjects, haloperidol had no effect on either of these indices. Although counterintuitive, this effect may nonetheless derive from activation of dopaminergic substrates. Haloperidol, particularly at low to moderate doses, preferentially blocks inhibitory autoreceptors (Pucak and Grace 1994). By removing feedback inhibition, this can result in increased dopamine release under basal conditions as well as in response to amphetamine (Pehek 1999). These results suggest that the incentive motivational effects of the slot machine
game were partly mediated by dopamine, and that the group difference in this effect may reflect a sensitized motivational response to dopamine activation in pathological gamblers.

1.3 Restatement of Purpose:

Taken together, the results of the amphetamine and haloperidol-slot machine studies suggest that amphetamine and gambling may exert parallel effects in pathological gambling subjects, and that sensitization may contribute to group differences in this response relative to controls. However, this inference is based on indirect evidence. The present study sought to directly test the correspondence between gambling and psychostimulant reinforcement by assessing responses to the slot machine and d-amphetamine on separate sessions in the same subjects.

As noted, even low-level exposure to amphetamine can lead to lasting sensitization (Boileau et al. 2006); therefore, the sequence of testing was standardized: Slot machine on session 1, amphetamine on session 2. On test session 1, subjects were also exposed to a battery of cognitive tasks to assess incentive salience, risk-taking behaviour, perseveration, reward sensitivity, and impulse control. Prepulse inhibition was tested as a putative marker for sensitization and to assess its role as a possible mediator of gambling and amphetamine reinforcement. On test session 2, subjects received oral d-amphetamine (0.4 mg/kg = 30 mg for a 75-kg male) and underwent a PET scan (results not reported here). Placebo effects are not typically assessed in PET. Thus, as in the case of the slot machine, the effects of the reinforcing stimulus (game/drug) must be inferred from within-session comparisons before and after its administration.
1.4 Hypotheses

**Hypothesis 1:** If pathological gambling and psychostimulant addiction are mediated by common processes, an episode of slot machine gambling and a dose of d-amphetamine should result in parallel subjective pleasurable, incentive motivational, cognitive, and physiological effects in pathological gamblers.

**Hypothesis 2:** If the pattern of effects seen in pathological gamblers is due in part to disturbances in neurobiological function (i.e., sensitization), healthy controls should exhibit less robust responses to both reinforcers than pathological gamblers do.

**Hypothesis 3:** If sensitization mediates gambling and amphetamine reinforcement, prepulse inhibition should also predict individual differences in incentive motivation (i.e., primed ‘wanting:’ Desire to Gamble) in response to the slot machine and drug, such that greater startle (greater deficit in prepulse inhibition) should be associated with greater reinforcing effects of each stimulus within each group.
2. MATERIALS AND METHODS

2.1 Study Design

This study employed a 2(GROUP: Pathological Gambler [PG], Healthy Control [CON]) x 2 (SESSION: Behavioural Session [BEH], Amphetamine Session [PHARM]) between-within repeated measures design. The study was designed to assess similarities between gambling and psychostimulant addiction. Therefore, each subject was exposed to both of these ‘addictive’ reinforcers: a gambling episode and a dose of a prototypical stimulant. On the BEH test session, the challenge was a 15-minute episode of slot machine gambling; on the PHARM session, the challenge was a 0.4 mg/kg oral d-amphetamine. This was the first phase of a proposed two-phase study, employing 12 pathological gamblers and 12 healthy controls per phase. Findings from Phase 1 would provide empirical effect sizes, so that the number of additional subjects required to achieve 80% power of achieving statistical significance could be determined. For this initial study, only male participants were tested, as men tend to have a higher rate of pathological gambling compared to women and to avoid response variability due to menstrual cycle. The PHARM session is a part of a positron emission tomography (PET) study to determine dopamine function (baseline dopamine D2/D3 receptor binding and amphetamine-induced dopamine release) in these subjects. However, PET receptor binding protocol/results will not be included in this dissertation.

Subjects were compensated $720 for successful completion of entire study (including PET Session) plus an additional $80 ‘bonus’ in lieu of winnings from the slot machine game. The study was carried out in accordance with ethical standards of the Helsinki Declaration (1975; updated 1989), and approved by the Center for Addiction and Mental Health Research Ethics Board (REB)
2.2 Recruitment

Subjects were recruited through Internet-based advertisements on Kijiji, Craigslist and the Problem Gambling.ca website. Recruitment advertisements can be found in Appendix A (two advertisements: healthy controls and pathological gamblers). Interested volunteers left a message on the study hotline, and were contacted by the experimenter who administered the telephone pre-screening assessment to determine eligibility. Eligible volunteers came to the Center for Addiction and Mental Health (CAMH) for an assessment and subsequently for their experimental test sessions. Figure 1 shows overall recruitment for the sample.

Figure 1: Flowchart of the study recruitment of Pathological Gamblers and Healthy Controls (February 2010-June 2011). PG, Pathological Gamblers; CON, Healthy Controls; VAS, Visual Analog Scale; ARCI, Addiction Research Center Inventory.
2.3 Screening

During the telephone pre-screening assessment, the subjects were evaluated based on gambling, drinking, smoking, substance use, and mood status. Inclusion criteria are shown in Table 1. To participate, subjects needed to be male, between the ages of 19 and 65 years, have no concurrent physical or mental health illness, not wish to refrain from gambling and be drug and medication free, with the exception of nicotine and alcohol. Subjects who smoked more than 20 cigarettes / day were excluded to minimize effects of nicotine withdrawal during the test phase. Subjects who drank more than 12 alcoholic drinks per week were also excluded to ensure against co-morbidity with alcohol disorders. In terms of past drug use history, subjects with any prior exposure to stimulants were excluded to preclude a sensitized response to amphetamine, and those who smoked more than 1 marijuana cigarette per month or used ecstasy or hallucinogens more than twice in their lives were also excluded. Additional requirements included fluency in English (Grade 7 proficiency) and normal or corrected-to-normal vision to ensure ability to read instructions and stimuli on the experimental tasks and questionnaires. To ensure against adverse response to amphetamine, respondents with a family history of schizophrenia or bipolar disorder were also excluded.

Those who met the preliminary inclusion criteria were then rated on 5 psychometrically validated scales: (1) South Oaks Gambling Screen and (2) DSM-IV Pathological Gambling Scale to determine gambling status; (3) Hamilton Depression Scale to rule out depression; (4) Alcohol Dependence Scale to assess alcohol status and (5) Wechsler Vocabulary Task. Eligible subjects scored < 9 on the Alcohol Dependence Scale (Skinner and Allen 1982) which corresponded to no alcohol dependence. To exclude those with clinically relevant depression, subjects needed to score ≤ 15 on the Hamilton Depression Scale (Hamilton 1960). To ensure
verbal comprehension, subjects needed to score greater than 18 out of 30 on the Wechsler Vocabulary Test (Wechsler 1981). To meet criteria for Healthy Control (CON) group, subjects needed to score 0 on the two gambling scales: South Oaks Gambling Screen and DSM-IV Pathological Gambling Scale; and to meet the criteria for the Pathological Gambling (PG) group, subjects needed to score $\geq 11$ on South Oaks Gambling Screen and score $\geq 5$ on the DSM-IV Pathological Gambling Scale.

Table 1: Inclusion Criteria: Pathological Gamblers (PG) and Healthy Controls (CON)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Healthy Controls (CON)</th>
<th>Pathological Gamblers (PG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>19-65</td>
<td></td>
</tr>
<tr>
<td>Cigarettes /day</td>
<td>$&lt;20$</td>
<td></td>
</tr>
<tr>
<td>Alcoholic drinks/week</td>
<td>$&lt;12$</td>
<td></td>
</tr>
<tr>
<td>Caffeinated beverages/day</td>
<td>$&lt;8$</td>
<td></td>
</tr>
<tr>
<td>ADS</td>
<td>$&lt;9$</td>
<td></td>
</tr>
<tr>
<td>HAM-D</td>
<td>$\leq 15$</td>
<td></td>
</tr>
<tr>
<td>WAIS- Vocab.</td>
<td>$&gt;18$</td>
<td></td>
</tr>
<tr>
<td>Drugs</td>
<td>No medications, no use of stimulants, $&lt;1$ marijuana cig/month, $&lt;2$ x ecstasy/hallucinogens use</td>
<td></td>
</tr>
<tr>
<td>SOGS</td>
<td>0</td>
<td>$\geq 11$</td>
</tr>
<tr>
<td>DSM-IV Pathological Gambling</td>
<td>0</td>
<td>$\geq 5$</td>
</tr>
</tbody>
</table>

ADS- Alcohol Dependence Scale; HAM-D- Hamilton Depression Scale; WAIS- Vocab -Wechsler Vocabulary Task; SOGS- South Oaks Gambling Screen; DSM-IV Pathological Gambling- Diagnostic and Statistical Manual for Problem Gamblers IV.
2.4 Subjects:

Eligible subjects based on their South Oaks Gambling Screen and DSM-IV Pathological Gambling scale scores were invited for an in-person assessment interview and a physical exam with a doctor to further rule out any psychiatric and medical problems. Prior to the physician exam, subjects underwent the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders-IV (SCID DSM-IV) to rule out Axis I psychiatric co-morbidity, and completed a battery of trait scales to characterize them on potential moderators of performance. Twenty-three subjects, 12 pathological gamblers and 11 healthy controls have completed the study to date. The 12th control is in the process of completing the study at the time of writing and his results will not be included in this thesis.

2.5 Apparatus/Materials

A) Breathalyser:

A handheld J4X-ALERT (Alcohol Countermeasures Inc., Mississauga, ON, Canada) breathalyser measured blood alcohol concentrations (BAC) at the beginning of each test session to verify abstinence (BAC=0) from alcohol.

B) Heart Rate/Blood Pressure Monitor:

i) BEH Session: During the BEH test session, an unobtrusive wrist cuff (HEM-601; Omron, Vernon Hills, IL, USA) attached on the subject’s left wrist monitored heart rate (HR) and Blood Pressure (BP) at scheduled time intervals including before and after slot machine game and at dismissal. Subjects could not see the readings from the Heart Rate/Blood Pressure device to preclude possible reactivity to this information (Zack and Poulos 2004).
ii) **PHARM Session**: During the PHARM test session, a blood pressure cuff (Propaq® CS Monitor 242; Welch Allyn Protocol Inc. Beaverton, Oregon, USA) attached on the subject’s left arm monitored HR and BP at baseline, then at 15 minute intervals post-Amphetamine dosing until dismissal at 3.5 hours post-dose. This Propaq blood pressure cuff was designed to work in the PET scanner.

C) **Micro Experimental Laboratories Professional Version 2.01 with Integrated Microphone**

The Rapid Reading Task (RRT) was administered using a computer equipped with Micro Experimental Laboratories Software (v.2.01; Psychology Software Tools Inc., Pittsburgh, Pennsylvania). A microphone, held in place by a gooseneck clamp, was used to record speed of subject’s vocal responses. A serial response box (Psychology Software Tools Inc., Pittsburgh, Pennsylvania) was used by the experimenter to record the accuracy of vocal responses during this task.

D) **Dextro-Amphetamine**:

On the PHARM Session, subjects were given 0.4mg/kg oral dextro-amphetamine sulphate (Dexedrine®). The dose was rounded up to the nearest 5mg increment and the maximum dose, regardless of bodyweight, was set at 40-mg to help ensure no adverse effects.

2.6 **Questionnaires**

2.6.1 **Screening Instruments**

A) **South Oaks Gambling Screen (Lesieur and Blume 1987)**: This is a psychometrically validated 16-item self-report questionnaire used to identify pathological gambling status. It was derived from the Gamblers Anonymous Questionnaire and the third edition of the Diagnostic and Statistical Manual for Mental Disorders (DSM-III; American Psychiatric
Association 1980). Eleven out of the 16-items are scored and the maximum score is 20. Subjects who scored 0 were characterised as Healthy Controls and those who scored ≥ 11 were characterised as Pathological Gamblers. The score ≥ 11 inclusion criterion corresponds to the mean score for this scale for treatment-seeking pathological gambling subjects, thus ensuring clinically relevant levels of pathological gambling severity (Stinchfield 2002). Because subjects were required to play a slot machine, gamblers who wished to abstain from gambling were excluded. This scale was administered twice: orally during the telephone screening and on the Assessment Day as part of the Questionnaire Package.

B) Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM-IV) based problem gambling questionnaire (DSM-IV Pathological Gambling; questionnaire (Beaudoin and Cox 1999): To provide further validity of gambling status, the 10-item DSM-IV Pathological Gambling scale was also administered. This scale defines the formal diagnostic criteria for pathological gambling. The gamblers had to score ≥ 5 and the controls were required to score 0 to be eligible. This scale was administered twice: orally during the telephone screening and as part of the questionnaire package on Assessment Day.

C) Alcohol Dependence Scale (Skinner and Allen 1982): This 25-item scale provided a quantitative assessment of Alcohol Abuse status over the past 12 months. This scale was consistent with the Diagnostic Statistical Manual (DSM-III) for alcohol dependence syndrome. Subjects who scored greater than 9 were considered ineligible to ensure no comorbidity with alcohol dependence. This scale was administered twice: orally during the telephone screening and as part of the questionnaire package on Assessment Day.
D) **Hamilton Depression Scale (Hamilton 1960):** This scale evaluated mood states over the past two weeks. Respondents who scored ≥15 or reported suicidal ideation were excluded from the study and were referred to the Mood and Anxiety Clinic at the Center of Addiction and Mental Health for follow-up. This scale was administered on the telephone screening and served as an initial index of mood status.

E) **Wechsler Vocabulary Task- Shortened Version (Wechsler 1981):** This scale assessed verbal comprehension by determining the degree to which a subject can orally define common English words of increasing difficulty. This task was important to ensure full comprehension of the cognitive tasks and subjective measures. Score (0-2) per word was based on sophistication of definition. During the telephone screening, the subject was asked to define 15 words; this represents half of the full list. The full version with 30 vocabulary words was administered during the BEH Session to confirm results.

### 2.6.2 Trait Scales

All trait scales were administered on the Assessment Day in the form of a questionnaire package.

A) **Eysenck Personality Inventory (Eysenck and Eysenck 1963):** This self-report inventory assessed 3 dimensions: Extraversion, Neuroticism, and Lie Scale. The Lie (falsification) scale quantified the tendency to respond in a socially acceptable manner instead of truthfully. The overall inventory was composed of 57 ‘yes’ or ‘no’ questions. Extraversion scale scores range from 0-24; Neuroticism scale scores ranged from 0-24; and the Lie scale scores ranged from 0-9.
B) **Eysenck Impulsiveness Questionnaire (Eysenck et al. 1985):** This 54-item inventory scale assessed impulsivity, venturesomeness and empathy. The scale was also composed of ‘yes’ or ‘no’ questions. Impulsiveness scale scores ranged from 0-16; Venturesomeness scale score ranged from 0-16; Empathy scale score ranged from 0-19.

C) **Gambler’s Beliefs Questionnaire (Steenbergh et al. 2002):** This 21-item self-report scale measured erroneous beliefs and distortions of gamblers. The questionnaire consisted of 2 closely related factors: Luck/Perseverance and Illusion of Control. Examples of cognitive distortions identified in gamblers included the belief that one can influence the outcome of a chance-determined event and the Gambler’s Fallacy, which is the belief that future outcomes can be predicted on the basis of past outcomes. Subjects rated items on a 7 point scale [1(Strongly Agree) → 4(Neutral) → 7(Strongly Disagree)]

D) **Fagerström Nicotine Dependence (Heatherton et al. 1991):** This scale consisted of 6-items used to assess the subject’s level of nicotine dependence. A score of 1-2 implied ‘very low dependence’; a score of 3 implied ‘low to moderate dependence’; a score of 4 implied ‘moderate dependence’ and a score of 5 or more implied ‘high dependence’.

E) **Timeline Follow back (Sobell and Sobell 1992)**

i) **Alcohol:** Subjects were asked to provide a complete a history of their drinking behaviour over the last 90 days. It involved recording daily standard alcohol drink consumption starting with the previous day and working backwards. This served to confirm the drinking levels reported in the initial phone screening with the validated written version of the scale.
ii) **Nicotine**: Smokers were asked to report the history of their cigarette use over the last 7 days to confirm smoking status. Gamblers and controls were then matched on number of cigarettes smoked per day.

**F) Drug Abuse Screening Test (Skinner 1982)**: This 20-item ‘yes or no’ self-report scale assessed degree of drug use. The total score was computed by adding all the ‘Yes’ answers (indicating increased drug problems) except for two items which were keyed for a ‘No’ response: Subjects who scored $\geq 4$ were excluded from the study to ensure no co-morbidity with drug abuse.

**G) Beck Depression Inventory Short Form (Beck and Beck 1972)**: The 13-item self-report scale assessed the severity of depressive symptoms in the 2 weeks prior to the screening interview. Subjects who scored $> 10$ overall or $>1$ on suicide item (i.e., any evidence of attempts or plans) were excluded and referred to the Mood and Anxiety Clinic at Center for Addiction and Mental Health for follow-up.

### 2.6.3 Experimental Self-Report Measures

During the BEH Session, the Profile of Mood States-Short Form and Visual Analog Scales for gambling and alcohol (see below) were administered at several intervals including Baseline, pre-Slot Machine, and post-Slot Machine.

During the PHARM session, Profile of Mood States-Short Form, Addiction Research Center Inventory and Visual Analog Scales for gambling and alcohol (see below) were administered at 3 intervals: Baseline, 90 and 225 minutes post-Amphetamine. The Visual Analog Scales for drug effects were administered at 90, 120 and 225 minutes post-Amphetamine dose.
A) Visual Analog Scale (Fischman and Foltin 1991): These scales measured the intensity of current subjective effects or attitudes toward gambling, alcohol, as well as drug and slot machine effects. Subjects reported the extent to which they agreed with a series of statements, at baseline and again at post-administration of cognitive tasks and amphetamine. The scale ranged from 0–10 (with ½ gradient increments); Not at All–Extremely, unless otherwise specified.

- **Gamble:** The two gambling statements were: (1) Right now, I would like to go gambling and (2) Right now I am confident I could resist going gambling (if there was a casino across the street).

- **Alcohol:** The two alcohol related statements were: (1) Right now, I would like to drink some alcohol and (2) Right now I am confident I can resist drinking alcohol (if there was a bar across the street). This was used to assess selective priming effects in response to the slot machine and amphetamine.

- **Slot Machine Effects:** Subjects rated Enjoyment, Excitement, Involvement, High/Buzz and Difficulty Stopping at the end of the 15-minute slot machine game. The statements were: (A) I enjoyed playing the VLT slot machine game. (B) I found playing the VLT slot machine exciting. (C) While playing the VLT slot machine game, I found myself engaged or involved. (D) While playing the slot machine game, I felt a ‘buzz’ or a ‘high’. (E) When the VLT slot machine was over, I had difficulty stopping play.
Drug Effects: This Visual Analog Scale assessed the Good Effects, Liking and Desire to Take Amphetamine Again. The 4 statements were: (A) Right now, I would rate my liking of the drug as: [ -10 to 0 to +10; Dislike a lot - Neutral - Like a lot] (B) Right now, I am feeling the good effects of the drug. (C) Right now I am feeling the bad effects of the drug. (D) I would like to take this drug again.

B) Profile of Mood States- Short Form (Shacham 1983): The Profile of Mood States-Short Form assessed the transient effects of gambling and amphetamine on the subject’s mood at different time-points. The 37-mood-related adjectives were rated on a scale from 0-4; Not At All - Extremely). Six mood-related factors were then derived and scored: (1) Tension-Anxiety, (2) Depression-Dejection, (3) Anger-Hostility, (4) Fatigue-Inertia, (5) Vigour-Activity and (6) Confusion-Bewilderment.

C) Addiction Research Center Inventory (Haertzen 1965): This 49-item ‘true-false’ self-reported questionnaire was used to assess subjective effects post d-amphetamine administration. It contained five sub-scales: (1) the Amphetamine (AMPH) sub-scale for Amphetamine specific effects; (2) Morphine-Benzedrine group (MBG) for the measurement of euphoria, (3) the Pentobarbital-Chlorpromazine-Alcohol group (PCAG) for sedation; (4) the Lysergic Acid Diethylamide (LSD) scale for dysphoria and, (5) the Benzedrine group (BG) which is a stimulant-sensitive scale.

2.6.4 Additional scales

A) Digit Span Task (Wechsler 1981): This task assessed short-term working memory, attention and concentration. It consisted of 2 parts: Digits Forward and Digits Backward. For Digits-Forward, the subject was required to repeat aloud a series of one-digit numbers in the same sequence as presented by the experimenter (e.g.: 1, 5,
3). The length of sequence of numbers (up to 9 digits per sequence) increased over trials leading to greater demand on the subject’s short term memory. The experimenter stopped the task after 2 successive inaccurate responses. During the Digits-Backward phase, the subject was required to repeat the numbers presented by the experimenter in the reverse sequence. For example, if the experimenter said 1, 3, 5 the subject was expected to say 5, 3, 1. The length of the sequence of numbers again increased over trials and the task was stopped after 2 successive inaccurate responses. This portion evaluated working memory.

B) Wechsler Coding Task (Wechsler 1981): This paper-and-pencil task measured memory, psychomotor speed and coordination. The subject was asked to match a series of numbers (1-9) to corresponding symbols shown at the top of the sheet as quickly and accurately as possible in 60 seconds. The number of correctly matched symbols within the time allotted was scored.

2.7 Experimental/Cognitive Tasks

2.7.1 Pre-Pulse Inhibition Task (PPI; Stojanov et al. 2003)

This task assessed the degree of sensory-motor gating in an individual through measurement of the acoustic startle reflex to sudden, intense noise. Auditory stimuli were administered through headphones. Throughout the task, a background white noise was present at 70 dB Sound Pressure Level (SPL). The task consisted of 70 acoustic startle stimuli (white noise, 115 dB SPL, 40 ms duration) presented with or without a pre-pulse (1 kHz tone, 86 dB SPL, 20 ms duration) with an inter-startle-interval ranging from 9 to 18 seconds (Stojanov et al. 2003). Five of the startle stimuli were presented consecutively at the beginning of the task to
acclimatize the subject to the stimuli, and five were presented consecutively at the end of the
task to provide a reference standard for the test trials. Responses on these reference trials were
not included in the analysis. Of the remaining 60 startle stimuli, 48 were preceded by a pre-
pulse, with 12 trials at each of 4 lead times: 60 ms, 120 ms, 240 ms, and 2000 ms; 12 trials
were presented with no pre-pulse. Pre-pulse condition was randomized over trials. The subjects
were asked to stay as still as possible and to listen to the stimuli while keeping their eyes fixed
on a cross on the wall in front of them.

Bipolar Ag/AgCl electrodes were positioned above the subject’s left eyebrow and below the
left eye to measure the acoustic startle response from the orbicularis oculi muscle. Impedances
were less than 5 kOhms throughout the experiment. The data were collected at a 256 Hz
sampling rate using a Refa 8 amplifier from TMS International B.V. and recorded using the
ASA 4.7.3.1 software by Advanced Neuro Technology (ANT; The Netherlands). After the
task, each subject’s electro-myographic recording was high-pass filtered with a cut-off of 1 Hz
and slope of 24 dB. The data were then sorted into epochs from 50 ms before startle stimulus
onset to 200 ms after stimulus onset. A Hilbert transformation was applied, and then each
subject’s data were averaged across epochs (Stojanov et al. 2003). These averages were then
exported for further analysis. The mean startle amplitude of each condition was calculated
using an averaging window over 100 to 180 ms after the onset of the startle stimulus. Mean
amplitude was used as it has been determined to be less susceptible to noise compared to peak
amplitude measures (Luck 2005). Prepulse inhibition was then calculated as a percentage
change score to compare the startle amplitudes following each prepulse-lead interval to the
startle amplitude in the pulse-only condition.
2.7.2 Stop Signal Task (SST; Logan et al. 1997)

This task measured inhibitory control of a pre-potent psychomotor response. The subject was required to press a key – ‘z’ or ‘/’ – with the left or right index finger respectively, as quickly and accurately as possible when the visual ‘GO’ stimuli (‘x’ or ‘o’) appeared on the screen. The visual stimuli were presented on the screen for 1000 ms and preceded by a 500 ms fixation point (‘+’) also presented in the center of the screen. On a random 25% of the trials, a stop signal (1000Hz tone) was presented for 100 ms shortly after the GO stimulus, indicating that the subject should not press either key on that trial. The stop signals were evenly split between ‘x’ and ‘o’ trials. The task involved 2 practice blocks followed by 256 test trials split into 3 blocks with 40 second breaks. The stop-signal delay, the interval between the Go and Stop stimuli, was set at 250 ms initially and then adjusted depending on the subject’s performance. If the subject inhibited successfully, the delay was increased by 50 ms, making it harder to inhibit on the next stop signal trial. However, when the subject failed to inhibit on a stop trial the delay was decreased by 50 ms to make it easier for the subject to inhibit the next stop signal trial. Over trials, this adjustment procedure was designed to result in 50% successful inhibition, and the value of the delay reflected mean inhibitory efficiency. By subtracting the mean stop signal delay from the mean Go-response time, it was possible to determine the average time required to inhibit the response. This difference was the stop signal reaction time (SSRT). Faster Go reaction time denoted greater psychomotor fluency while faster SSRT denoted greater inhibitory efficiency or decreased impulsivity.
2.7.3 Wisconsin Card Sort Task (WCST; Heaton 2003)

This task assessed ability to shift cognitive strategy in response to changing environmental demands. Subjects were required to match one card at a time to one of 4 different stimulus cards, which differ on three dimensions: colour, shape and number. The computer determined which one of the three dimensions was the criterion dimension for a particular series of trials. The subject had to determine the correct dimension by using the feedback received from the computer (i.e.: right or wrong). After 10 cards were selected correctly, the computer changed the criterion dimension without informing the subject. After the first incorrect response under the new response rule, the subject would have to change his response to discover the new criterion dimension. The test continued until 6 categories were correctly identified.

The computer recorded the number of trials taken for the subject to learn the new criterion dimension. Failure to adopt the new response rule was termed ‘perseveration,’ which indicated cognitive rigidity. This test measured the ability to learn from mistakes and look for alternative solutions, which together permitted efficient ‘set-shifting.’
2.7.4 Slot Machine Game

The slot machine (‘Cash Crop’, WMS Gambling, Chicago) is a commercial device that has been in use in Ontario casinos. Subjects played for 15 minutes and started with 400 cash credits equivalent to $100 (25 cent per credit). Subjects were informed that they would receive a monetary bonus proportional to their final credit tally in the game, which would be paid at the end of the study. This provided an incentive for the subjects to play the game as they normally would (i.e.: to maximize winnings). To enhance the external validity, the slot machine was played in a mock bar laboratory and without supervision.

The object of the slot machine game was to get as many of the same symbols on a single line as possible. Subjects used a touch screen to select any combination of horizontal, diagonal, or vertical lines up to a maximum of 9 lines on any one spin. They could also bet anywhere from 1 through 5 credits per line making the maximum bet per trial 45 credits. The more credits they chose per trial, the higher the probability of winning, but also the larger the loss if none of the lines paid off. One box on the screen showed the total credits available (cumulative winnings) which was updated after every spin, while another box showed the payoff, if any, on each spin.

The number of credits wagered and won on every trial was recorded electronically by a cable feed from the slot machine into another room. Subjects were not aware that their betting pattern was recorded, to ensure that it would not interfere with the way they played the game. They were informed of the recording during the debriefing and were given an opportunity to exclude their betting data on an additional informed consent form (See Appendix BII).
2.7.5 Rapid Reading Task (RRT; Zack and Poulos 2004)

This task was used to assess the incentive salience of target stimuli by measuring the time and accuracy of the subject’s vocal response to words shown on a computer screen. This task was administered in MS-DOS and programmed in Micro Experimental Laboratories Software. A microphone attached to the computer measured the vocal reading speed and the computer recorded the subject’s responses with millisecond accuracy. The serial response box was used by the experimenter to manually code response accuracy. Prior to the words appearing on the screen, a series of ampersands (i.e.: & & & & ) appeared on the screen for 350 ms to orient attention to the location of the target stimulus. The focus stimulus was followed by a blank screen for 200 ms after which the target stimulus appeared in the same location. The subject was then to read the word aloud as quickly as possible, and the experimenter coded the subject’s response accuracy (i.e.: correct, misread, or discard). The target remained on the screen until the subject responded and the interval between target offset and onset of the next target stimuli was 550 milliseconds. The task administered 20 practice trials and 150 test trials (5 categories with 30 stimuli per category).

The stimuli were words representing the categories: (1) gambling-related (e.g. wager, bet), (2) alcohol-related (e.g., whisky, drink), (3) positive affect (e.g., happy, excited) (4) negative affect (e.g., upset, angry) and (5) neutral (e.g., window, chair). Categories and items were randomized over the course of the 150 trials. To enhance priming effects, target stimuli were degraded with asterisks (e.g., w*a*g*e*r) (Neely 1991). This task provided an index of priming effects that occur involuntary and are relatively insensitive to experimental demand (Zack and Poulos 2004). This complemented the self-report measures, which are more susceptible to demand characteristics.
2.7.6 Game of Dice Task (GDT; Brand et al. 2005)

This task assessed the individual’s risk-taking behaviours in a probabilistic outcome decision-making situation. On the computer screen, a virtual die was thrown 18 times and before each trial, the subject had to guess which number (between 1 and 6) would be thrown. The subject could select a single number or combinations of different numbers depending on their risk-taking behaviours. The reward was based on the probability of the outcome chosen. i.e.: if the subject chose one possible outcome (maximal risk; probability is 1/6), they would earn $1000 if that number was thrown, but also lose $1000 if that number was not thrown. If two possible outcomes were chosen (probability of win is 2/6), and one of the numbers was thrown, the subject would earn $500 otherwise they would lose the $500. For the combination of 3 outcomes, the subject would earn or lose $200 and for the combination of 4 outcomes (minimal risk) the payoff was $100. Therefore, risk-taking was operationally defined by the number of possible outcomes selected, where 1 = maximal risk and 4 = minimal risk. If the subject won the trial, the corresponding amount would be credited to the current balance appearing on the computer screen. Losses were registered in the same way after each trial. The starting balance was $1000.

Subjects were informed that they should play as though they were playing for real money and that they could also lose more money than they had. The combination chosen, the actual outcome, the gain or loss on that trial and the current balance were recorded for each trial. The Game of Dice Task was administered during the Assessment Day to assess baseline measurement and again during the BEH Session after the slot machine game to assess possible priming effects.
2.8 Procedure
2.8.1 Assessment Day Procedure (Table 2)

Subjects deemed eligible after the telephone screening were invited to the Center for Addiction and Mental Health for a 3 hour interview and comprehensive assessment. As shown in Table 2, the subject was briefed on the study and asked to sign a consent form (Appendix BI: Study Consent Form). A breathalyser test was performed to ensure absence of blood alcohol and Heart Rate/Blood Pressure were measured to ensure normal cardiovascular function.

The experimenter then conducted the Structured Clinical Interview for DSM-IV (SCID) to ensure no co-morbid psychiatric disorders. Potential gamblers also completed an additional evaluation with the physician of record on the study (Qualified Investigator) to verify their pathological gambling status. Once the subject passed the assessment, he performed the Game of Dice Task followed by a Questionnaire package including the trait scales.

The subject was then escorted to the Clinical Lab at the Center for Addiction and Mental Health for blood, urine and electrocardiogram test. After the results of the assays had been transmitted to the Addiction Medicine Clinic, subjects were scheduled for their physician’s exam to ensure they were fit to undergo all elements of the test protocol. Subjects were provided with transit tokens for both assessment and physician’s examination and were provided with a copy of their consent form. This concluded the Screening Phase. Ineligible subjects were provided with compensation for time spent. If there were any abnormalities determined during physician’s exam or lab results, they were also asked to consult their family physician.
Table 2: Assessment Session Timeline

<table>
<thead>
<tr>
<th>Time-mins</th>
<th>Activity</th>
</tr>
</thead>
</table>
| 0         | Subject briefed on Study  
Subject signs consent form  
Breathalyser test (to ensure BAC=0)  
**Blood Pressure/Heart Rate Measurement** |
| 30        | **Structured Clinical Interview for DSM Disorders.**  
*For Pathological Gambling candidates only - Interview with study physician to verify Pathological Gambling status. |
| 90        | **Baseline Game of Dice Task** |
| 105       | **Screening Questionnaire Package** (SOGS, DSM-IV-PG, ADS, BDI, EIQ, EPI, DAST, GBQ, TLFB (alcohol), and FTND and TLFB (nicotine) for smokers only. |
| 150       | **Blood Test** (Routine Chemistry, Blood Glucose, Liver Enzyme Test)  
**Urine Screen** (Urinalysis, Urine Drug Toxicology)  
**Electrocardiogram** (ECG) |
| 180       | Transit Tokens  
Dismissal |

**Notes:**  
BAC - Blood Alcohol Concentration; SOGS - South Oaks Gambling Screen; DSM-IV Pathological Gambling; Diagnostic and Statistical Manual for Mental Disorders IV; ADS - Alcohol Dependence Scale; BDI - Beck Depression Inventory; EIQ - Eysenck Impulsiveness Questionnaire; EPI - Eysenck Personality Inventory; DAST - Drug Abuse Screening Test; GBQ - Gamblers Beliefs Questionnaire; TLFB - Timeline Follow back; FTND - Fagerström Nicotine Dependence.
2.8.2 Behavioural Slot Machine (BEH) Session Procedure (Table 3)

Upon arrival at the lab at 8:30AM, the subject was provided with a brief overview of the study. As shown in Table 3, he was given a breathalyser test followed by a measurement of baseline Heart Rate/Blood Pressure. The subject was then asked to complete the baseline subjective measures (Package A): Visual Analog Scales for gambling/alcohol, and Profile of Mood States-Short Form.

The Pre-Pulse Inhibition task was then conducted using the 64-lead cap and bipolar electrodes placed close to the left eye. The cap was fitted and gel was inserted into the electrode wells followed by the task. A pilot task, (30-min; not reported here) was then performed to measure reaction time to numeric target stimuli. The cap was taken off and the subject washed the gel out of his hair and proceeded with the rest of the tasks. After the Pre-Pulse Inhibition task, the subject completed a second set of Visual Analog Scales and Profile of Mood States-Short Form (Package B). The Digit Span and Vocabulary task were then administered. Following this, the Digit Symbol / Coding Task was completed by the subject and Heart Rate/Blood Pressure values were taken again. This was followed by the Stop Signal and Wisconsin Card Sort Tasks. Heart Rate/Blood Pressure measurements were taken again followed by a third set of Visual Analog Scales and the Profile of Mood States-Short Form (Package C).

The subject then played the slot machine game in the mock-bar for 15 minutes or until his credits ran out. Immediately after the slot machine game, cardiovascular measurements were taken in the mock-bar followed by a fourth set of questionnaires (Package D): Profile of Mood States-Short Form and Visual Analog Scales: Desire to Gamble, Desire for Alcohol, and Slot Machine Enjoyment, Excitement, Involvement, ‘High’ and Difficulty Stopping. Next, the
Rapid Reading Task was performed to assess incentive salience. The last task was the Game of Dice Task. Following this, subjects remained at the lab for 1.5 hours to allow any residual priming effects of the slot machine to dissipate. They were provided with lunch and a taxi ride home.

Table 3: Behavioural Session Timeline

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Activity</th>
</tr>
</thead>
</table>
| 0           | S briefed on Session Timeline  
|             | S re-consents  
|             | Breathalyser test  
|             | **BP/HR #1 (Baseline)**  
|             | **Package A** (VAS-G, VAS-A, POMS-sf)  
| 30          | Set-up PPI (EEG cap, electrodes, gelling process)  
| 60          | **BP/HR #2**  
|             | **Task#1**: Pre-Pulse Inhibition  
| 90          | **BP/HR#3**  
|             | **Task#2**: Pilot Task- Number Task  
| 120         | Wash gel, Washroom Break  
|             | **BP/HR#4**  
|             | **Package B** (VAS-G, VAS-A, POMS-sf)  
|             | **Verbal Tasks**: Digit Span, Vocabulary Task, Digit Substitution Task  
| 150         | **Task #3**: Conventional Stop Signal Task  
|             | **Task#4**: Wisconsin Card Sorting Task  
|             | **BP/HR#5**  
|             | **Package C** (VAS-G,VAS-A, POMS-sf)  
| 180         | **Task#5**: Slot Machine Game (15 minutes)  
|             | **BP/HR#6** in mock-bar  
|             | **Package D** (VAS-Slot Machine, VAS-G,VAS-A, POMS-sf)  
| 210         | **Task#6**: Rapid Reading Task  
|             | **Task#7**: Game of Dice Task  
| 240         | **BP/HR#7**  
|             | **Package E** (VAS-G,VAS-A, POMS-sf)  
| 270         | Lunch  
| 390         | **HR/BP #8**  
|             | Dismissal (Taxi)  

BP/HR- Blood Pressure/Heart Rate; VAS G- Visual Analog Scale- Gamble; VAS A- Visual Analog Scale-Alcohol; POMS-sf- Profile of Mood States; PPI- Prepulse Inhibition
2.8.3 Amphetamine (PHARM) Session Procedure (Table 4)

Subjects arrived at the PET Centre (CAMH- College St) 30 minutes prior to scan time. As shown in Table 4, they were provided with a quick overview, followed by a breathalyser test, Heart Rate/Blood Pressure measurement and a urine drug screen. They then filled out the baseline questionnaire package for Profile of Mood States-Short Form, Addiction Research Center Inventory, and Visual Analog Scales for gambling and alcohol. The PET technician inserted an IV in the subject’s left arm and drew one vial of blood (baseline).

After a drug-free scan to establish baseline binding of the radio-tracer, a registered nurse practitioner administered the Dexedrine (d-amphetamine) tablets (0.4mg/kg) and measured heart rate/blood pressure every 15 minutes until dismissal time. When the subject was not in the scanner, he watched television or read in the waiting room. Blood draws were taken at baseline, 60, 120, 165, and 210 minutes post-Amphetamine dose to measure plasma amphetamine level changes over time. At 90 minutes after dosing, at which time the subjective–behavioural effects of oral Amphetamine are maximal (Brauer et al. 1996), subjects completed another questionnaire package with Profile of Mood States-Short Form, Addiction Research Center Inventory, Visual Analog Scales- gamble and alcohol; and Visual Analog Scale for subjective effects of amphetamine. At 120 minutes post-Amphetamine, the subject orally completed the Visual Analog Scale to assess drug effects again while in the PET scanner. The subject filled out his last Profile of Mood States-Short Form, Addiction Research Center Inventory, and Visual Analog Scales package at 225 minutes post-Amphetamine.

To ensure no residual effects of Amphetamine, the nurse assessed final Heart Rate/Blood Pressure and completed a discharge form. Before departing, the subject was debriefed, provided with the consent form of his slot machine data (see Appendix BII: Slot
Machine Consent Form), given a wallet card stating the medication he received and emergency contact information. He was provided with pamphlets for the Problem Gambling Helpline and sent home by taxi. The cheque for participation was mailed 2-3 weeks later.

**Table 4: Amphetamine Session Timeline**

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
</table>
| -30  | S briefed on Session Timeline  
      | S re-consents  
      | Breathalyser test  
      | Drug Test (Urine)  
      | **Package A** (VAS-G, VAS-A, Profile of Mood States-sf; ARCI)  
      | **BP/HR #1** (Baseline) |
| 0    | d-Amphetamine Administration (0.4mg/kg)  
      | *BP/HR #2-7 every 15 minutes |
| 90   | **Package B** (VAS-G, VAS-A, Drug Effects; POMS-sf; ARCI)  
      | *BP/HR #8-10 every 15 minutes |
| 120  | **Package C** (Drug Effects)  
      | *BP/HR #10-16 every 15 minutes |
| 225  | **Package D** (VAS-G, VAS-A, Drug Effects; POMS-sf; ARCI) |
| 240  | **BP/HR Dismissal** (Taxi) |

BP/HR- Blood Pressure/Heart Rate; VAS-G- Visual Analog Scale- Gamble; VAS A- Visual Analog Scale-Alcohol; POMS-sf- Profile of Mood States; ARCI- Addiction Research Center Inventory.
2.9 Data Analysis

All statistical analyses were conducted using SPSS (v. 15, Chicago, IL). Repeated Measures Analyses of Variance (ANOVA) assessed the effects of group and challenge (slot machine/amphetamine) on subjective, cognitive-behavioural and physiological outcome measures. Simple effects analyses were used to identify the source of significant interactions (Winer 1971). In the case of the primary outcome measures (e.g.: Visual Analog Scale- Desire to Gamble) simple effects for planned comparisons were also performed when no significant group interactions were observed (Winer 1971). Analyses of covariance (ANCOVA) were used to control for baseline between-group variation (e.g. Visual Analog Scale- Desire to Gamble), where necessary. Multiple linear regression analyses were used to assess the relationship between prepulse inhibition and slot machine motivation and physiological indices on BEH test session and amphetamine-related responses on the PHARM session. In cases where more than one index assessed a particular dimension (e.g. Visual Analog Scale for Slot Machine Effects) Bonferroni correction was applied to control for inflation of family-wise alpha. Because the study was powered to detect effects in PET, the sample represents half the number of subjects needed to achieve 80% power on cognitive and self-report measures. Therefore in cases where there no statistical significance was achieved, the partial effect size was reported. Additionally, the magnitude of the effect size was also reported based on conventions for small, medium or large effects (small partial eta squared \( \eta^2 \) > = .05, medium \( > = .125 \) or large \( > = .20 \)) (Cohen 1988).
3. RESULTS
3.1 Background Characteristics
3.1.1 Subject Eligibility
Over a 17-month period, a total of 546 potential subjects responded to advertisements and expressed interest in the study (Refer to Figure 1, Material and Method Section). Of these, 345 subjects were contacted successfully, out of which 49 were eligible. The most common reasons for exclusion during the telephone screening included: prior stimulant use, South Oaks Gambling Screen score between 1 and 10, DSM-IV Pathological Gambling Scale score between 1 and 4, Hamilton Depression Scale score greater than 15, suicidal ideation, and drinks per week >12. Thirty-four subjects attended the Assessment Interview, out of which 24 passed the Structured Clinical Interview (SCID) and physical exam. Common medical exclusions included abnormal electrocardiogram reports, abnormal kidney function, increased heart rate, and positive urine drug screen. The final eligible sample was 13 Pathological Gamblers (PG) and 11 Healthy Controls (CON). One gambler dropped out due to claustrophobia in PET scanner. Therefore, the final completed sample consisted of 12 gamblers and 11 controls.

Table 5 reports the mean (SD) scores on background characteristics in Healthy Controls and Pathological Gamblers as determined during the telephone screening. Independent samples t-tests were performed and yielded no significant between-group differences in average age and body mass index (p’s>0.5). The mean (SD) age was 34.1(11.7) in controls and 32.7(8.9) in gamblers. As anticipated, the mean (SD) South Oaks Gambling Screen [12(3.5)] and DSM-IV-Pathological Gambling Scale scores [13.1 (5.3)] in gamblers was significantly greater than controls [0(0)] scores even after Bonferroni corrections, indicative of substantial gambling
problems in the gamblers. A significant group difference (after Bonferroni Correction) was also observed on the depression scales (Beck Depression Inventory and Hamilton Depression Scale); with the mean (SD) scores significantly greater in the gamblers compared to controls although well below the cut-off for clinical depression (Table 5). No significant difference in Alcohol Dependence Scale score was observed (p>0.06).

Table 5: Mean (SD) background characteristics in Pathological Gamblers (PG) and Healthy Controls (CON)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Controls</th>
<th>Pathological Gamblers</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Age</td>
<td>34.09(11.72)</td>
<td>32.67(8.89)</td>
</tr>
<tr>
<td>BMI</td>
<td>25.92 (3.22)</td>
<td>26.95 (3.91)</td>
</tr>
<tr>
<td>SOGS***</td>
<td>0(0)</td>
<td>12 (3.49)</td>
</tr>
<tr>
<td>DSM-IV PG***</td>
<td>0(0)</td>
<td>13.08 (5.25)</td>
</tr>
<tr>
<td>ADS</td>
<td>1.00 (1.41)</td>
<td>2.58 (2.35)</td>
</tr>
<tr>
<td>BDI**</td>
<td>0.91(1.45)</td>
<td>5.83 (4.88)</td>
</tr>
<tr>
<td>HAM-D**</td>
<td>1 (1.73)</td>
<td>6.50 (5.28)</td>
</tr>
<tr>
<td>WAIS- Vocabulary (out of 30)</td>
<td>27.36 (2.78)</td>
<td>26.75 (2.83)</td>
</tr>
<tr>
<td># of Smokers</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

BMI, Body Mass Index; SOGS, South Oaks Gambling Screen; DSM IV-PG, Diagnostic and Statistical Manual Pathological Gambling Scale; ADS, Alcohol Dependence Scale; BDI-sf, Beck Depression Inventory- Short form; HAM-D, Hamilton Depression Scale; WAIS-Vocabulary, Wechsler Adult Intelligence Scale. Significant Group difference, *p<0.05, **p<0.01 *** p<0.001.
3.1.2 Subject Characteristics and Procedural Checks

3.1.2.1 Trait Questionnaires

Table 6 reports the mean (SD) scores of gamblers and controls on the various trait characteristics. Independent samples t-tests yielded main effects of Group (gamblers > controls) on Eysenck Impulsiveness Questionnaire- Impulsiveness and Eysenck Personality Inventory- Neuroticism scales. In addition, the two smoker-gamblers exhibited increased nicotine dependence scores (FTND) compared to the three smoker controls. However these group effects did not remain significant after Bonferroni corrections (p>.004). Gamblers also scored significantly less on the Gambler’s Beliefs Questionnaire Total and its subscales, Illusion of Control and Luck/ Perseverance, indicative of more severe cognitive distortion, which remained significant after controlling for multiple comparisons (Bonferroni; p<0.004). No other significant effects were observed. Therefore, pathological gamblers reported greater Impulsiveness and Neuroticism and significantly increased cognitive distortion related to gambling.
Table 6: Mean (SD) trait characteristics in Pathological Gamblers (n=12) and Healthy Controls (n=11)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Controls</th>
<th>Pathological Gamblers</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIQ - Impulsiveness**</td>
<td>3.45(1.69)</td>
<td>8.08(4.89)</td>
</tr>
<tr>
<td>EPI – Neuroticism*</td>
<td>4.00(3.00)</td>
<td>8.42(5.04)</td>
</tr>
<tr>
<td>EPI- Lie</td>
<td>4.09(1.30)</td>
<td>3.00(2.52)</td>
</tr>
<tr>
<td>EPI- Extroversion</td>
<td>10.82 (3.68)</td>
<td>13.42(4.10)</td>
</tr>
<tr>
<td>GBQ –Luck/perseverance**</td>
<td>76.27(9.41)</td>
<td>46.50(14.24)</td>
</tr>
<tr>
<td>GBQ- Illusion of control***</td>
<td>38.18(12.53)</td>
<td>20.75(5.22)</td>
</tr>
<tr>
<td>GBQ- Total Score***</td>
<td>114.45 (21.34)</td>
<td>67.25 (18.27)</td>
</tr>
<tr>
<td>TLFB-drinks/week</td>
<td>1.34(1.56)</td>
<td>2.65(3.54)</td>
</tr>
<tr>
<td>TLFB- cigarettes/day</td>
<td>4.22(1.11; n=2)</td>
<td>8.67 (5.00; n=3)</td>
</tr>
<tr>
<td>FTND*</td>
<td>1.50(0.71; n=2)</td>
<td>4.00(0.00; n=3)</td>
</tr>
<tr>
<td>DAST</td>
<td>0.27(0.65)</td>
<td>0.08(0.29)</td>
</tr>
</tbody>
</table>

EIQ, Eysenck Impulsiveness Questionnaire- Impulsiveness subscale ; EPI, Eysenck Personality Inventory- Extraversion, Neuroticism, Lie subscale ; GBQ, Gamblers beliefs questionnaire -Luck/perseverance, illusion of control subscale; TLFB, Timeline Follow-Back of number of standard alcoholic drinks per month for preceding 90 days; FTND, Fagerström Nicotine Dependence; DAST, Drug Abuse Screening Test

Significant Group difference, *p<0.05, **p<0.01 *** p<0.001.
3.1.2.2 Basic Cognitive Function

Table 7 reports mean Wechsler Intelligence Scale Scores for Pathological Gambling and Healthy Control groups. Independent samples t-tests revealed no significant group effects in Digit Span, Vocabulary and Digit Symbol Substitution Tasks (p>0.05). The lack of group effects indicates no overall difference in, verbal proficiency, short term memory, or working memory as a function of gambling status.

Table 7: Mean(SD) score on Wechsler Intelligence Scales(Digit Span, Digit Symbol Substitution, Vocabulary scale ) in Groups Pathological Gamblers (n=12) and Healthy Controls (n=11)

<table>
<thead>
<tr>
<th>Wechsler Test</th>
<th>Healthy Controls</th>
<th>Pathological Gamblers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vocabulary (out of 70)</td>
<td>59.55 (10.05)</td>
<td>54.75 (8.70)</td>
</tr>
<tr>
<td>Digit Span (out of 28)</td>
<td>16.45 (5.13)</td>
<td>15.83 (4.09)</td>
</tr>
<tr>
<td>Digit Symbol Substitution(out of 100 points in 60 seconds)</td>
<td>51.45 (13.06)</td>
<td>47.33 (8.10)</td>
</tr>
</tbody>
</table>

No significant group differences observed, p>.05
3.2 Slot Machine and Amphetamine Effects
3.2.1 Subjective Measures
3.2.1.1 Visual Analog Scale: Gamble

Repeated Measures ANOVAs were conducted on the subjective modified-Visual Analog Scale (m-VAS) ratings of 1) Desire to Gamble; and 2) Confidence to Refrain from Gambling in Groups Pathological Gamblers (PG) and Healthy Controls (CON) on the BEH (Slot Machine) and PHARM (Amphetamine) sessions.

1. Desire to Gamble:

Gamblers’ baseline Desire to Gamble scores were significantly higher than that of healthy controls across both sessions: BEH, \( t(21) = -4.38, p = .001 \), and PHARM, \( t(21) = -3.01, p = .007 \). To control for this extra-experimental variance and isolate the reinforcer effect, baseline Desire to Gamble scores for each session were entered as covariates in an analysis of covariance (ANCOVA). Individual differences in the final credit tally on the slot machine (which varied randomly) could impact the subjective-motivational response to the game. Therefore, final credit tally was also entered as a covariate in the BEH session. Figure 2 illustrates the mean desire to gamble scores across different time points on BEH and PHARM session with and without controlling for covariates. Bars depict raw means and diamonds indicate covariate-adjusted means.

On both sessions, without controlling for baseline variation and final credit tally, gamblers reported significantly greater desire to gamble at all time points compared to controls as denoted in Figure 2 (\( p < 0.01 \)). During the BEH session, comparing baseline scores to post-slot machine demonstrated that unadjusted mean desire to gamble post-slot machine increased in controls by 0.95 of a possible 10 points (\( p > 0.05 \)) and increased in gamblers by 1.21 out of
10 from baseline (p < 0.05). In the PHARM session, without controlling for covariates, both groups reported a trend of increased desire to gamble after exposure to amphetamine compared to baseline. No other significant effects were observed. Thus, playing the slot machine significantly increased (primed) desire to gamble in gamblers, whereas the priming effect of the game in controls and the effects of amphetamine were not significant.

On the BEH session, the 2(Group) x 2(time: post-slot machine], post-Game of Dice Task) ANCOVA yielded a significant Group effect, F (1, 19) =6.528, p=.019, and a marginally significant Group x Time interaction, F (1, 19) =3.81, p= .066; [partial η2] = .167 (medium effect size). Simple effects analysis demonstrated that, after controlling for final credits won and baseline desire scores, gamblers reported significantly greater desire to gamble compared to controls after the Slot Machine: t(21)= 6.208 p<0.001; and after the Game of Dice Task: t(21) =4.614,  p<0.001). Therefore, group differences in primed desire to gamble were not attributable to group differences in baseline desire or to group differences in winnings on the slot machine (i.e. uncontrolled variance in the strength of the prime).

On the PHARM session, the 2 x 2(time: 90 minutes and 225 minutes after Amphetamine dose) ANCOVA yielded no significant effects; [partial η2] =.011(negligible effect size). Upon inspection of Figure 2 (see diamonds- covariate-adjusted means), a trend toward increased subjective desire to gamble in both groups 90 minutes after Amphetamine dose was observed with a greater but non-significant increase observed in gamblers than controls; t(21)= .89, p>.05.Simple effects analysis indicated that significantly greater desire to gamble was observed in the gamblers after 225 minutes compared to controls; t(21)= 2.08, p=.05) Thus, when pre-capsule baseline differences in desire to gamble were controlled, amphetamine primed desire to gamble significantly more in gamblers than controls, and this
effect was evident at the expected time of the peak drug effect and became more pronounced later in the course of the dose.

In summary when trait differences and random variation in prime magnitude were controlled both slot machine and amphetamine led to significant increases in desire to gamble in gamblers but not in controls.
Figure 2: Mean (SE) visual analog scale ratings of Desire to Gamble (0-10; Not at All-Extremely) before and after the slot machine game (BEH Session) and amphetamine dose (PHARM Session) in Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12).

Without controlling for co-variates, *p < .05, two-tailed for significant group difference of Visual Analog Scale scores. #p<.05, two tailed significant effect of time on Visual Analog Scale scores. After controlling for baseline and final slot machine credit tally, + p<.05, two tailed significant group difference.

CON ANCOVA adjusted mean Visual Analog Scale ♦ PG ANCOVA adjusted mean Visual Analog Scale.
SM, Slot Machine; GDT, Game of Dice Task; AMPH, Amphetamine
2. **Confidence to refrain from gambling:**

Mean (SE) Visual Analog Scale Confidence to Refrain Scores are reported in Table 8. Gamblers reported significantly lower unadjusted mean confidence to refrain from gambling scores compared to controls during both sessions (p<.01). Both groups also reported decreased confidence to refrain from gambling post slot machine (p > .05), with gamblers reporting a greater decrease than controls. During the PHARM Session, gamblers reported less confidence to refrain from gambling after amphetamine relative to baseline while controls reported no substantial change (p > .05).

As gamblers’ baseline confidence to refrain from gambling was significantly less than controls in BEH Session [t (21) =4.483, p<.001)] and PHARM Session [t (21) =3.537, p=.002], baseline confidence scores were entered as covariates in ANCOVAs for each session. As with desire to gamble, final slot machine credits were also entered as a covariate for BEH Session analysis. No significant effects were observed in either ANCOVA (p > .05). (BEH Session: [partial η2] = .045 (negligible effect size); PHARM Session: [partial η2] = .020(negligible effect size)).

In summary, the slot machine and amphetamine were each associated with a decline in confidence to refrain from gambling in gamblers but not controls. However, the negation of this effect in the ANCOVA indicates that confidence changes in response to each prime were due largely to trait-based differences in confidence or uncontrolled variance in prime magnitude, in the case of the slot machine.
Table 8: Mean (SE) Confidence to Refrain from Gambling subjective scores (0-10 Not at All - Extremely) before and after the Slot Machine Game (BEH Session) and Amphetamine dose (PHARM Session) in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12).

<table>
<thead>
<tr>
<th>Session</th>
<th>Condition</th>
<th>CON</th>
<th>PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEH</td>
<td>Baseline*</td>
<td>10 (0.0)</td>
<td>6.9 (0.7)</td>
</tr>
<tr>
<td></td>
<td>Post SM*</td>
<td>9.2 (0.9)</td>
<td>5.1 (0.9)</td>
</tr>
<tr>
<td></td>
<td>Post GDT*</td>
<td>9.4 (0.8)</td>
<td>6.2 (0.8)</td>
</tr>
<tr>
<td>PHARM</td>
<td>Baseline*</td>
<td>9.8 (0.2)</td>
<td>7.1 (0.7)</td>
</tr>
<tr>
<td></td>
<td>90 minutes Post AMPH*</td>
<td>9.5 (0.7)</td>
<td>6.9 (0.7)</td>
</tr>
<tr>
<td></td>
<td>225 minutes Post AMPH*</td>
<td>9.8 (0.7)</td>
<td>6.3 (0.6)</td>
</tr>
</tbody>
</table>

BEH, Behavioural Session; PHARM, Amphetamine Session; SM, Slot Machine; GDT, Game of Dice Task; AMPH, Amphetamine. *p<.05, two tailed significant group difference.
3.2.1.2 Visual Analog Scale: Alcohol

To control for non-specific addictive motivation, Visual Analog Scale ratings for Alcohol (a. Desire, b. Confidence to Refrain) were taken throughout BEH and PHARM sessions at the same time points as Visual Analog Scale Gamble. ANOVA analyses conducted for Visual Analog Scale alcohol scores were similar to that of Visual Analog Scale Gambling scores.

1. Desire for Alcohol

The group difference (Gamblers>Controls) in mean (SE) Baseline Visual Analog Scale Desire for Alcohol scores approached significance in both sessions: BEH, t (21) =-1.864, p =.076 and PHARM: t (21) = -1.678, p =.13. Therefore, baseline Desire for Alcohol scores were entered as a covariate in ANCOVAs for the BEH and PHARM sessions. The final slot machine credit tally was entered as a covariate for the BEH session.

On the BEH Session, the 2(Group) x 2 (Time: Post Slot Machine, Post Game of Dice Task) ANCOVA yielded a marginal Group x Time interaction, F (1, 19) = 3.459, p =.078; [partial η2] =.140 (medium effect size). On the PHARM session, the ANCOVA yielded no significant effects; [partial η2] =.021 (negligible effect size). Inspection of Table 9 indicates that both primes were associated with an increase in desire for alcohol relative to baseline. Baseline differences and uncontrolled variation in prime magnitude did not entirely account for differential priming effects of the slot machine, which dissipated rapidly after the game in controls but not gamblers. The lack of significant effects and negligible effect size on the ANCOVA of PHARM session scores indicate that trait differences in desire for alcohol accounted for differences in the priming effects of amphetamine.
Table 9: Mean (SE) Visual Analog Scale Desire for Alcohol subjective scores (0-10; Not at All- Extremely) before and after the slot machine game (BEH Session) and amphetamine dose (PHARM Session) in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12)

<table>
<thead>
<tr>
<th>Session</th>
<th>Condition</th>
<th>CON</th>
<th>PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEH</td>
<td>Baseline</td>
<td>0.3(0.1)</td>
<td>2.1(0.9)</td>
</tr>
<tr>
<td></td>
<td>Post SM</td>
<td>1.5(0.6)</td>
<td>3.8(1.1)</td>
</tr>
<tr>
<td></td>
<td>Post GDT</td>
<td>0.8(0.5)</td>
<td>3.9(1.1)</td>
</tr>
<tr>
<td>PHARM</td>
<td>Baseline</td>
<td>0.4(0.3)</td>
<td>2.0(1.0)</td>
</tr>
<tr>
<td></td>
<td>90 minutes post AMPH</td>
<td>1.8(0.9)</td>
<td>2.8(0.9)</td>
</tr>
<tr>
<td></td>
<td>225 minutes post AMPH</td>
<td>1.7(1.0)</td>
<td>2.1(0.9)</td>
</tr>
</tbody>
</table>

BEH, Behavioural Session; PHARM, Amphetamine Session; SM, Slot Machine; GDT, Game of Dice Task; AMPH, Amphetamine. *p<.05, two tailed significant group difference

2. Confidence to Refrain from Alcohol.

As with Visual Analog Scale desire for alcohol scores, baseline confidence to refrain from alcohol differed at marginally significant levels between controls and gamblers on BEH session (t (21) =1.948, p=.065) and PHARM session (t (21) =1.786, p=.089). ANCOVA analyses were conducted for both sessions controlling for baseline Visual Analog Scale Confidence to Refrain from Alcohol and final credits from slot machine game as an additional covariate in BEH session. No significant effects were observed in either analysis (p > .05) [BEH session: [partial η2] < .022(negligible effect size); PHARM Session: [partial η2] <.040(negligible effect size)]. Inspection of Table 10 indicates a trend towards lower confidence scores after each prime with no clear difference between the groups (Table 9). The negligible effect sizes suggest that low statistical power did not account for the lack of significant effects in confidence to refrain from alcohol.
Table 10: Mean (SE) Visual Analog Scale Confidence to Refrain from Alcohol subjective scores (0-10 Not at All- Extremely) before and after the slot machine game (BEH Session) and amphetamine dose (PHARM Session) in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12)

<table>
<thead>
<tr>
<th>Session</th>
<th>Condition</th>
<th>CON</th>
<th>PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEH</td>
<td>Baseline</td>
<td>9.9(0.1)</td>
<td>8.4(0.7)</td>
</tr>
<tr>
<td></td>
<td>Post SM*</td>
<td>9.5(0.8)</td>
<td>6.9(0.7)</td>
</tr>
<tr>
<td></td>
<td>Post GDT*</td>
<td>9.7(0.6)</td>
<td>6.9(0.6)</td>
</tr>
<tr>
<td>PHARM</td>
<td>Baseline</td>
<td>9.9(0.1)</td>
<td>8.5(0.7)</td>
</tr>
<tr>
<td></td>
<td>90 minutes post AMPH</td>
<td>9.5(0.6)</td>
<td>7.8(0.6)</td>
</tr>
<tr>
<td></td>
<td>225 minutes Post AMPH</td>
<td>9.4(0.6)</td>
<td>8.3(0.6)</td>
</tr>
</tbody>
</table>

BEH, Behavioural Session; PHARM, Amphetamine Session; SM, Slot Machine; GDT, Game of Dice Task; AMPH, Amphetamine. *p<.05, two tailed significant group difference
### 3.2.1.3 Visual Analog Scale: Subjective Effects of Reinforcers

#### 3.2.1.3.1 Slot Machine

Figure 3 shows the mean (SE) subjective reinforcing effects of the 15 minute slot machine game. A 2 (Group) x 5 (Subscale: Enjoyment, Excitement, Engaged, Buzz/High, and Difficulty Stopping the game) ANCOVA of ratings was performed, controlling for final slot machine credit tally. This yielded main effects of Subscale $F(4, 80) = 6.829 \ p < .001$, and Group, $F(1, 20) = 9.126 \ p = .007$, but no significant interaction ($p > .05$); $[\text{partial } \eta^2] = .107 \text{(small effect size)}$. Simple effects analyses revealed that gamblers reported significantly greater ($p$'s $< .05$) Enjoyment, Involvement, “Buzz”/“High,” and Difficulty Stopping after the game than controls did and all effects remained significant after Bonferroni corrections except Enjoyment ($p > .01$). Gamblers also reported marginally more excitement from the game than controls ($p = .06$).

![Figure 3: Mean(SE) Subjective Effects of Slot Machine Prime (0-10; Not at All - Extremely) post 15 minute Slot Machine Game while controlling for final slot machine credit tally in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12). *p<.05, two tailed significant group differences.](image-url)
3.2.1.3.2 Amphetamine

Figure 4 shows the mean (SE) subjective reinforcing effects of amphetamine at 90, 120 and 225 minutes post dose. A 2(Group) x 4(Subscale: Liking, Good Effects, Bad Effects, Desire to take Drug Again) x 3(Time: 90, 120 and 225 minutes post-Amphetamine dose) ANOVA yielded a main effect of subscale, $F(3, 63) = 4.28 \ p = .008$. No significant group or interaction effects were observed, $p > .05$ (Group x Subscale x Time: [partial $\eta^2$] = .042(negligible effect size); Group x Subscale: [partial $\eta^2$] = .080(medium effect size). Simple effects analyses found that gamblers reported significantly lower Bad Effects than controls at 120 min, $t(21) = 2.81 \ p = .011$.

In summary, gamblers reported significantly weaker bad effects from the drug compared to controls at the 120 minute mark. Although gamblers displayed greater mean scores than controls on all positive effect scales, apart from Good Effects at 90 minutes, these trends did not reach significance. The large error bars suggest that within-group variation may have obscured between-group differences.
Figure 4: Mean (SE) Subjective Effects of Amphetamine (0-10; Not At All - Extremely) at 90, 120 and 225 minutes after Amphetamine Dose in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12). Effects include Liking of Drug, Good Effects, Bad Effects of Amphetamine and Desire to take the drug again. *p<.05, two tailed significant group difference.
3.2.1.4 Profile of Mood States

Figure 5 Panels A-F show mean subscale scores for the Profile of Mood States at post Slot Machine and post-Amphetamine during the BEH and PHARM sessions. Mean baseline gambler and control scores on all subscales were not significantly different (p > .05). Therefore ANCOVA was not required and 2(Group) x 6(Subscale) x 3(Time) ANOVAs were performed for BEH and PHARM sessions.

For BEH session, the analysis yielded a main effect of Subscale F (5,105) =30.65, p<.001 and a Time x Subscale interaction F (10,210) =2.162, p=.02, but no significant effects involving Group, p > .05 (Group x Subscale x Time: [partial η²] =.440(large effect size); Group x Subscale: [partial η²] =.183(medium effect size). Similar results were observed for PHARM Session, where the ANOVA of scores at baseline, 90 min post-Amphetamine, and 225 min post-Amphetamine yielded a main effect of Subscale, F(6,126) =55.339, p<.001 and Time x Subscale interaction, F(12,252) = 2.963, p<.001. No significant group interactions were observed, p > .05; [Group x Subscale x Time: [partial η²] =.306(large effect size); Group x Subscale: [partial η²] =.207(large effect size)]. Simple effects analysis showed a significant difference between Baseline and 90 minutes post-Amphetamine for Vigour-Activity [combined p=.05; in gamblers p >.05, and controls p < .05.] However, none of the other sub-scales differed significantly as a function of Time or Group, p>.05.

In summary, overall gamblers tended to report higher scores on the negative mood scales (Depression-Dejection, Fatigue-Inertia, Confusion-Bewilderment, and Anger-Hostility) and lower scores on the Vigour-Activity scale. Both groups reported more positive effects and fewer negative effects after exposure to both reinforcers, with no significant difference.
between groups. Thus, apart from an increase in Vigour-Activity after amphetamine, neither reinforcer reliably altered mood state in either group.

Figure 5: Mean (SE) Scores on Profile of Mood States- short form Subscale: (A) Vigour-Activity (B) Depression-Dejection (C) Tension-Anxiety  (D) Confusion-Bewilderment (E) Fatigue-Inertia and (F) Anger-Hostility post Slot Machine (BEH Session) and Amphetamine (PHARM Session) in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; 68)
Figure 5: Mean (SE) Scores on Profile of Mood States- short form Subscale: (A) Vigour-Activity (B) Depression-Dejection (C) Tension-Anxiety (D) Confusion-Bewilderment (E) Fatigue-Inertia and (F) Anger-Hostility post Slot Machine (BEH Session) and Amphetamine (PHARM Session) in Groups Healthy Controls (CON; n=11) and Pathological Gamblers;
Figure 5: Mean (SE) Scores on Profile of Mood States- short form Subscale: (A) Vigour-Activity (B) Depression-Dejection (C) Tension-Anxiety (D) Confusion-Bewilderment (E) Fatigue-Inertia and (F) Anger-Hostility post Slot Machine (BEH Session) and Amphetamine (PHARM Session) in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12).
3.2.1.5  Addiction Research Inventory Scale

Baseline Addiction Research Center Inventory scores did not differ significantly between groups during the PHARM session, p > .05. Therefore ANCOVA analysis was not performed. A 2(GroupName) x 3(Subscale: AMPH (Amphetamine), MBG (Morphine-Benzedrine Group; Euphoria), LSD (Dysphoria)) x 3(Time point: Baseline, 90 minutes and 225 minutes post-Ampetamine) ANOVA yielded an interaction of Time x Subscale F (4, 84) =2.63, p=.04. However, there were no significant effects involving Group, p > .05 [Group x Subscale x Time: [partial η2] =.200(large effect size); Group x Subscale: [partial η2] =.057(small effect size)]. Simple effects analyses showed a significant within-group increase from baseline to 225 minutes post-Amphetamine for MBG (Euphoria) scale in gamblers (p<.01), however no other significant effects were observed involving group p >.05.

In sum, a similar pattern of drug effects was seen for both groups, and only gamblers exhibited a significant increase in euphoria under amphetamine.
Figure 6: Mean (SE) Addiction Research Center Inventory (ARCI) Subscale Effects: (A) Amphetamine (AMPH), (B) Morphine- Benzodrine (MBG; Euphoria), (C) LSD (Dysphoria) on PHARM Session at different time points in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12).

# p<.05, two tailed significant effect of time.
Figure 6: Mean (SE) Addiction Research Center Inventory (ARCI) Subscale Effects: (A) Amphetamine (AMPH), (B) Morphine- Benzedrine (MBG; Euphoria), (C) LSD (Dysphoria) on PHARM Session at different time points in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12). # p<.05, two tailed significant effect of time.
3.2.2 Physiological Measures

3.2.2.1 Heart Rate

Figure 7 reports the mean (SE) heart rate measures for gamblers and controls across BEH and PHARM session. There were no differences in baseline Heart Rate, therefore ANCOVA was not required.

In BEH Session, the 2 x 5(Time: baseline, post-Prepulse Inhibition, pre-Slot Machine, post-Slot Machine, post Game of Dice Task) ANOVA yielded no significant effects, \( p > .05; \) [partial \( \eta^2 \) = .170(medium effect size)]. Inspection of Figure 7A showed only modest variation over the course of the session.

In PHARM session, a 2 x 16 (time point: baseline and 15 minute intervals post Amphetamine dose) ANOVA yielded a main effect of Time, \( F(14, 352) = 3.44, p < .001, \) along with a Group x Time interaction for the Linear Trend, \( F(1, 18) = 4.81, p = .042; \) [partial \( \eta^2 \) = .210(large effect size)]. Inspection of the Figure 7B reveals that the interaction denoted a divergence between the groups after 90-min post-capsule, with gamblers exhibiting a marked decline and controls exhibiting more consistent elevation. Simple effects analyses found significant group differences at each time point from 120 – 210 minutes post-dose, \( p < .05, \) however not significant after Bonferroni corrected (Bonferroni \( p > .003; \) [partial \( \eta^2 \) > .210 (large effect size)]. Thus, amphetamine significantly increased heart rate in each group, and gamblers exhibited recovery relative to controls after expected peak drug effect at 90 minutes.
Figure 7: Mean (SE) Heart Rate across different time points in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12. (A) BEH Session (B) PHARM Session. *p<.05, two tailed significant group difference. PPI, Pre-pulse Inhibition; SM; Slot Machine; GDT, Game of Dice Task
3.2.2.2 Systolic Blood Pressure

Figure 8 reports the mean systolic blood pressure measures for gamblers and controls across BEH and PHARM session. There were no differences in baseline systolic blood pressure, therefore ANCOVA was not required

On the BEH Session, a 2x 5 (Time Point: baseline, post Prepulse Inhibition, pre-Slot Machine, post-Slot Machine, post Game of Dice Task) ANOVA yielded a main effect of Time, F (4, 84) = 4.048, p = .005, and no other significant effects, p > .05; [partial η2]=.038 (negligible effect size). Inspection of Figure 8A shows that blood pressure increased similarly in both groups from baseline to performance of the Prepulse Inhibition task and from post-slot machine to performance of the Game of Dice Task. Simple effects analyses confirmed that the increase for each of these intervals was significant in gamblers and controls (p < .05), but not after Bonferroni correction (p>.01)

On the PHARM session, a 2 x 15 (Time Point: baseline and 15 minute intervals post Amphetamine dose) ANOVA yielded a significant Group x Time interaction F (15, 315) =2.09, p=.01; [partial η2] =.110 (small effect size). Simple effects analyses comparing group means at different time points post amphetamine revealed that gamblers had significantly lower systolic blood pressure than controls at each time point during rising drug blood levels from 45 thought 90 minute post-dose, p < .05 (Figure 8B). However, effect did not remain significant after Bonferroni correction, p > .003; [partial η2] >.211(large effect size).

Over all, gamblers displayed weaker pressor effects of amphetamine than controls did. These differences emerged during rising blood levels, whereas gamblers relative insensitivity to the cardiac effects of amphetamine emerged after the expected peak.
Figure 8: Mean (SE) Systolic Blood Pressure across different time points in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12). (A) BEH Session (B) PHARM Session. *p<.05, two tailed significant group difference. Prepulse Inhibition, Pre-pulse Inhibition; SM; Slot Machine; GDT, Game of Dice Task
3.2.3 Amphetamine Plasma Levels

Figure 9 reports mean (SE) Amphetamine levels in Plasma of gamblers and controls after amphetamine dose. A 2 x 5 (Time: baseline, 60, 120, 165, 210 minutes after Amphetamine dose) ANOVA yielded a main time effect, $F(4, 80) = 103.566$, $p<.001$, and a Time x Group interaction, $F(4, 80) = 6.252$, $p<.001$. Simple effects analyses comparing group means at different time points revealed that Gamblers had significantly lower plasma Amphetamine level 60 minutes after drug administration, $F(1,21)= 11.161$, $p=.003$, even after Bonferroni correction. However no group differences were observed by 90 minutes after amphetamine dose (peak subject effects). Thus, group differences in subjective or physiological effects of amphetamine that occurred at 90 minutes or thereafter are not attributable to different concentrations of the dose.

Figure 9: Mean (SE) Plasma Amphetamine (ng/ml) across different time points after amphetamine dose in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12). *$p<.05$, two tailed significant group difference.
3.3 Cognitive- Behavioural Indices

3.3.1 Pre-Pulse Inhibition

Pre-Pulse Inhibition (PPI) was calculated for each pre-pulse lead interval (time between pre-pulse onset and pulse onset) as % change from the pulse only condition (Stojanov et al. 2003).

Startle amplitude to the pulse only condition did not differ significantly between controls [76.24(13.71)] and gamblers [52.01(7.39)]; t (21) =1.593, p>.12. Figure 10 shows the % Prepulse Inhibition scores – with greater negative scores indicating greater pre-pulse inhibition; positive scores indicate increased startle in the presence of the pre-pulse compared to no pre-pulse. Across all lead intervals (stimulus onset asynchrony; SOA), %Prepulse Inhibition appeared to be greater for Controls (-39.67% change) compared to Gamblers (-4.86% change).

A 2(Group) x 4(lead interval: 60, 120, 240, 2000milliseconds) ANOVA analyses yielded a significant group effect, F (1, 21) =4.20 p=.05 but no interaction, p > .05; [partial η²] =.064(small effect size). Simple effects analyses demonstrated a significantly greater Prepulse Inhibition in controls compared to gamblers at lead interval: 120 millisecond (p=.02), [partial η²] =.265(large effect size) but not the other intervals, p > .05, [partial η²] >.026 (negligible effect size). In summary, gamblers displayed a consistent deficit in Prepulse Inhibition compared to controls at all lead intervals, with the most pronounced difference observed at 120 millisecond before pulse where gamblers reported a greater startle response to prepulse compared to pulse.

Inspection of scores for individual subjects revealed four scores with 2 greater than 100 and 2 greater than 0 at the 120 millisecond lead in gamblers that contributed to the group’s overall mean facilitation of inhibition at this pre-pulse. However, none of the cases was a statistical outlier, so there is no basis to exclude them from the analysis.
Figure 10: Pre-Pulse Inhibition. Mean (SE) Pre-Pulse Inhibition % of acoustic startle relative to baseline at pre-pulse intervals (SOA) 60, 120, 240, 2000ms in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12) on BEH Session. SOA; stimulus onset asynchrony. *p<.05, two tailed significant group difference.
3.3.2 Game of Dice Task

The Game of Dice Task (GDT) assessed risk taking behaviour in both groups. This task was administered at baseline and post-slot machine game to assess slot machine priming effects. Figure 10 reports the mean (SE) combination (1-4; more risky to less risky) chosen by the subject. Each block represents 6 throws, with 3 blocks in each game.

A 2(Group) x 3(Trial Block) x 2(Session: Interview [no-prime], BEH [slot machine prime]) ANOVA yielded main effects of Session, F (1, 21) =6.53, p=.018, and Block, F (2, 42) =3.684, p=.034 and no other significant effects [Group x Block: [partial η2]=.102(small effect size); Group x Block x Session: [partial η2]=.101(small effect size)]. Low scores indicate riskier selections. Figure 11 shows that both groups exhibited less risky decisions overall during the BEH ‘test’ session (after the slot machine) than during the Screening Interview (baseline). In addition, decisions tended to become more risky as the game progressed across blocks. Thus, both gamblers and controls exhibited greater risk-taking during the initial screening phase than during the test session, i.e., the slot machine did not prime risk-taking on this task in either group.
Figure 11: Game of Dice Task. Mean (SE) combination of outcomes selected for virtual die (1-6); Block 1 (tosses 1-6), Block 2 (tosses 7-12), Block #3 (tosses 3-18) during Game of Dice Task at baseline and post slot machine (SM) game in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12).
3.3.3 Wisconsin Card Sort Task

The Wisconsin Card Sort Task assessed cognitive rigidity and failure to maintain set in terms of Perseverative and Non-Perseverative errors, respectively, in Gamblers and Healthy Controls. A 2(Group) x 2(Error Type) ANOVA yielded a marginal effect of Error Type, $F(1, 21) = 2.98$, $p = .099$; $[\text{partial } \eta^2] = .125$ (medium effect size) and no other significant group effects or trends, $p > .05$; $[\text{partial } \eta^2] = .020$ (negligible effect size). Inspection of Figure 12 shows a trend towards relatively more perseverative errors (more cognitive rigidity) than Non-Perseverative errors in both groups.

![Graph showing mean (SE) Perseverative and Non-Perseverative Errors in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12) during BEH Session.]

Figure 12: Wisconsin Card Sort Task: Mean (SE) Perseverative and Non-Perseverative Errors in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12) during BEH Session.
3.3.4 Stop Signal Task

The Stop Signal Task assessed Go and Stop reaction time and error rate to motivationally neutral visual stimuli in gamblers and controls.

A 2(Group) x 2 (Response Type: Go, Stop) ANOVA of Reaction Time (RT) scores yielded a main effect of Response Type, $F(1, 21) = 84.34, p < .001$, and no other significant group effects, $p > .50$; $\text{partial } \eta^2 = .017$ (negligible effect size) (Figure 13). A parallel 2(Group) x 2(Error Type: Go, Stop) ANOVA of error rates yielded a significant effect of Error Type, $F(1, 21) = 241.01, p < .001$, and no other significant group effects, $p > .50$; $\text{partial } \eta^2 = .012$ (negligible effect size) (Figure 14).

As usual, Stop Reaction Time was faster than Go Reaction Time, and Stop errors were more numerous than Go errors. The pattern of effects did not differ in the two groups.

![Figure 13: Stop Signal Task. Mean (SE) reaction time for visual Go and auditory Stop Signals on the Conventional Stop Signal Task in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12) during Behavioural Slot Machine (BEH) Session.](image-url)
Figure 14: Stop Signal Task. Mean (SE) Go and No-Go Errors on the Conventional Stop Signal Task in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12) during BEH Session.
3.3.5 Slot Machine

A MANOVA assessed slot machine betting patterns and payoffs during the 15 minute gambling episode (Table 11). The analysis yielded no significant effects, $p > .05$. However, gamblers tended toward a faster rate of play (higher number of spins); $\text{partial } \eta^2 = .070$ (small effect size) and higher bet per spin (max 45 credits); $\text{partial } \eta^2 = .020$ (negligible effect size) than controls. Controls also tended to win more credits per spin and accumulate more credits at the end of the game; $\text{partial } \eta^2 = .020$ (negligible effect size). The Group difference in final credit tally was marginal, $F(1, 21) = 2.90$, $p = .103$; $\text{partial } \eta^2 = .100$ (small effect size).

Table 11: Slot Machine Betting Pattern and Payoff in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12) during BEH Session

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>PG</th>
<th>F test (1,21)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td># of spins</td>
<td>55(8.96)</td>
<td>72.33 (8.58)</td>
<td>1.952</td>
<td>0.18</td>
</tr>
<tr>
<td>Total Bet/Spin</td>
<td>12.78(3.22)</td>
<td>16.21(3.09)</td>
<td>0.590</td>
<td>0.45</td>
</tr>
<tr>
<td>Total Pay/Spin</td>
<td>15.58(4.38)</td>
<td>11.90(4.20)</td>
<td>0.368</td>
<td>0.55</td>
</tr>
<tr>
<td># of bonus games</td>
<td>1(0.286)</td>
<td>0.58(0.27)</td>
<td>1.106</td>
<td>0.31</td>
</tr>
<tr>
<td># of final credits</td>
<td>618.09(162.35)</td>
<td>235.42(155.44)</td>
<td>2.899</td>
<td>0.10</td>
</tr>
</tbody>
</table>
3.3.5 Rapid Reading Task

The Rapid Reading Task was administered immediately after the slot machine game on the BEH session.

A 2\( (\text{group}) \times 5 \) (word type: gambling, alcohol, positive, negative, neutral) ANOVA was conducted for the Response Time (RT) scores of the Rapid Reading Task. The analysis yielded a main effect of Word Type, \( F(4, 84) = 4.739, p = .002 \) and no significant group effects; \([\text{partial } \eta^2] = .033\) (negligible effect size). Figure 15 shows Response Time to all motivationally relevant words was faster than Response Time to Neutral words, with gamblers reporting slower response time compared to controls. The degree of priming was comparable in both groups with substantial variability within subjects.

![Figure 15: Rapid Reading Task. Mean(SE) vocal response time(ms) to word stimuli on the Rapid Reading Task following the slot machine game in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12)]](image)
3.4 Relationship between Prepulse Inhibition and motivational-reinforcing effects of Slot Machine and Amphetamine

Slot Machine Effects vs. Pre-Pulse Inhibition:

Regression Analyses yielded no significant relations (For Analyses, see Appendix C Table I-III) for Prepulse Inhibition with Visual Analog Scale-Desire to Gamble (Table I), Heart Rate (Table II) or Systolic Blood Pressure post-Slot Machine (Table III), p’s > .05. Therefore, sensorimotor gating was not related to the motivational priming or physiological effects of gambling.

Amphetamine Effects vs. Pre-Pulse Inhibition

Visual Analog Scale- Desire to Gamble:

Table 12 shows the regression analyses between Prepulse Inhibition and Visual Analog Scale-Desire to Gamble at 90 minutes post-Amphetamine. The greater the reported desire to gamble post-Amphetamine after controlling for baseline, the greater the drug-free startle magnitude (i.e., poorer sensorimotor gating) at lead interval 120 milliseconds.

Inspection of the scatter plot of regression residuals revealed that the positive correlation between Prepulse Inhibition and Desire to Gamble involved a continuous distribution of scores and was not simply due to a bimodal distribution with high startle scores in gamblers and low startle scores in controls, nor was it attributable to a small number of extreme scores.
Table 12: Linear Regression Analysis of relation between Desire to Gamble 90 minutes after Amphetamine dose and Pre-Pulse Inhibition at different lead intervals while controlling for Baseline Desire to Gamble score in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12).

**ANOVA (b)**

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>162.342</td>
<td>5</td>
<td>32.468</td>
<td>8.961</td>
<td>.000(a)</td>
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<tr>
<td>Residual</td>
<td>61.593</td>
<td>17</td>
<td>3.623</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>223.935</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Predictors: (Constant): Visual Analog Scale- Desire to Gamble (baseline), %Prepulse Inhibition 60ms, %Prepulse Inhibition 120ms, %Prepulse Inhibition 240ms, %Prepulse Inhibition 2000ms

b Dependent Variable: Visual Analog Scale-Desire to Gamble (90 minutes post-AMPH).

**Coefficients (a)**

<table>
<thead>
<tr>
<th></th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>1.045</td>
<td>.555</td>
<td>1.883</td>
<td>.077</td>
</tr>
<tr>
<td>%Prepulse Inhibition 60ms</td>
<td>.010</td>
<td>.010</td>
<td>.170</td>
<td>.999</td>
</tr>
<tr>
<td>%Prepulse Inhibition 120ms*</td>
<td>.021</td>
<td>.007</td>
<td>.436</td>
<td>2.843</td>
</tr>
<tr>
<td>%Prepulse Inhibition 240ms</td>
<td>-.019</td>
<td>.011</td>
<td>-.291</td>
<td>-1.783</td>
</tr>
<tr>
<td>%Prepulse Inhibition 2000ms</td>
<td>-.008</td>
<td>.006</td>
<td>-.181</td>
<td>-1.320</td>
</tr>
<tr>
<td>Visual Analog Scale- Desire to</td>
<td>.808</td>
<td>.157</td>
<td>.667</td>
<td>5.156</td>
</tr>
<tr>
<td>Gamble (baseline)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Dependent Variable: Visual Analog Scale-Desire to Gamble (90 minutes post-AMPH)

*p<.05; Significant regression relation.
Cardiovascular Response:

Systolic BP: Regression Analysis for Systolic Blood Pressure shows no significant relations between Prepulse Inhibition and Systolic Blood Pressure after Amphetamine, p > .05 (For Analyses, see Appendix C Table IV).

Heart Rate: Table 13 shows the regression analysis between Prepulse Inhibition and mean Heart Rate after Amphetamine dose. At 120 minutes post-Amphetamine dose, the lower the Heart Rate response the weaker the Prepulse Inhibition (i.e.: the greater the startle) at lead interval 120 milliseconds (p=.036). Thus, the greater the recovery from the cardiac effects of amphetamine, the greater the deficits in drug-free sensorimotor gating.

Table 13: Linear Regression Analysis of relation between Cardiovascular Response (Heart Rate) at 120 minutes after Amphetamine dose and Pre-Pulse Inhibition while controlling for Baseline Heart Rate in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12).

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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<tr>
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<td>258.359</td>
<td>3.602</td>
<td>.021(a)</td>
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<tr>
<td>Residual</td>
<td>1219.422</td>
<td>17</td>
<td>71.731</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2511.217</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Predictors: (Constant), Heart rate (Baseline, Pre-Amphetamine), %Prepulse Inhibition 60ms, %Prepulse Inhibition 120ms, %Prepulse Inhibition 240ms, %Prepulse Inhibition 2000ms

ANOVA (b)

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Constant)</td>
<td>47.352</td>
<td>14.652</td>
<td>3.232</td>
<td>.005</td>
</tr>
<tr>
<td>%Prepulse Inhibition 60ms</td>
<td>-.055</td>
<td>.045</td>
<td>-.271</td>
<td>-1.201</td>
</tr>
<tr>
<td>%Prepulse Inhibition 120ms*</td>
<td>-.073</td>
<td>.032</td>
<td>-.463</td>
<td>-2.272</td>
</tr>
<tr>
<td>%Prepulse Inhibition 240ms</td>
<td>-.009</td>
<td>.047</td>
<td>-.044</td>
<td>-.201</td>
</tr>
<tr>
<td>%Prepulse Inhibition 2000ms</td>
<td>.043</td>
<td>.028</td>
<td>.279</td>
<td>1.534</td>
</tr>
<tr>
<td>Heart rate (Baseline, Pre-AMPH)</td>
<td>.289</td>
<td>.205</td>
<td>.242</td>
<td>1.410</td>
</tr>
</tbody>
</table>

a Dependent Variable: Heart rate (120 minutes post-Amphetamine)

*p<.05; significant regression relation
4. DISCUSSION

Previous research suggested that amphetamine and gambling may exert parallel effects in pathological gambling, and that sensitization of the dopamine (DA) pathways may contribute to this commonality. Reward uncertainty is a defining feature of gambling. Highest levels of midbrain dopamine activation occur when the probability of reward delivery is at maximum uncertainty (50%) (Fiorillo et al. 2003) which corresponds very closely to the actual payoff rate (47%) on a commercial slot machine (Tremblay et al. 2011). Therefore, participation in gambling should result in exposure to repeated bouts of robust dopamine release. Moreover, activation of dopamine is both necessary and sufficient for psychostimulant reinforcement (Pierce and Kumaresan 2006). Therefore, dopamine would appear to play a pivotal role in acute and chronic responses to gambling and amphetamine. This study aimed to provide direct evidence for a correspondence between gambling and psychostimulant reinforcement by assessing responses to a slot machine and to d-amphetamine in the same subjects, who were either pathological gamblers or controls.

Specifically, we hypothesized that an episode of slot machine gambling and a dose of d-amphetamine should result in parallel subjective pleasurable (e.g., enjoyment), priming (desire to gamble) and physiological (heart rate/blood pressure) effects in pathological gambling subjects. Moreover, if the patterns seen in gamblers are due in part to disturbances in neurobiological function such as sensitization, healthy controls should exhibit overall less robust priming effects from both reinforcers than pathological gamblers do. Lastly, if pathological gamblers have undergone sensitization they should display decreased pre-pulse inhibition (PPI), an electrophysiological correlate of hyper-dopaminergic function, and this
deficit should be associated with greater slot machine and amphetamine reinforcement. The results discussed below address these hypotheses.

4.1. Slot Machine and Amphetamine Reinforcement

4.1.1. Subjective Effects

Self report scales assessed the influence of slot machine play and amphetamine on subjective motivation for gambling and alcohol (i.e. ‘priming’), mood states and drug effects.

**Desire to Gamble:** Overall, as expected, pathological gamblers reported significantly higher desire to gamble than controls across all time points including baseline. In addition, compared to baseline the two groups reported a similar average increase in desire to gamble after the slot machine and after the amphetamine dose. However, when variation in baseline scores and slot machine credit tally (a random influence on reward/reinforcement) were controlled by ANCOVA, the adjusted means revealed that desire to gamble was significantly increased by the slot machine in gamblers but not in controls. Similarly, after controlling for baseline scores by ANCOVA, desire to gamble was also differentially primed by amphetamine, with gamblers reporting significantly greater desire to gamble at 225 minutes after the dose. Therefore, both slot machine and amphetamine primed desire to gamble to a greater extent in gamblers than controls when extra-experimental variation was controlled. In addition, the priming effects of amphetamine were less robust than those of the slot machine game both in controls and gamblers.

As expected, subjective confidence to refrain from gambling was inversely related to desire to gamble. However, neither gamblers nor controls reported appreciable change in response to the slot machine or amphetamine, and no significant differences were observed between the two groups.
**Desire for Alcohol:** Subjective measures of desire for alcohol were assessed to confirm selective priming for gambling. Gamblers reported higher baseline scores for desire for alcohol compared to controls. Although the scores on the Alcohol Dependence Scale did not significantly differ between the groups and were well below the cut-off for alcohol abuse, gamblers scored ~2.5 times higher on the Alcohol Dependence Scale compared to controls. This could have contributed to the difference in baseline scores between the two groups. Both groups reported a consistent increase in desire for alcohol and decrease in confidence to refrain from alcohol after slot machine play and amphetamine. When baseline variation was controlled, the priming effects of the slot machine persisted in gamblers, but not controls, and neither group exhibited priming of desire for alcohol or confidence to refrain from alcohol on the amphetamine session. These effects indicate some degree of generality in the incentive-motivational effects of gambling and alcohol in pathological gamblers, with no such commonality for amphetamine and alcohol in either group.

Studies have shown that problem gamblers frequently report drinking alcohol while engaging in a gambling episode (Baron and Dickerson 1999). Zack *et al* (2005) demonstrated that an implicit association exists in memory between gambling wins and alcohol concepts in pathological gamblers, and that more severe alcohol problems are associated with stronger gambling-alcohol associations. Thus, a cognitive linkage between gambling and alcohol may contribute to the priming effects of the slot machine on desire for alcohol in the present sample of gamblers.

Amphetamine and alcohol also share similar underlying mechanisms: A study by Holdstock and de Wit (2001) comparing amphetamine and alcohol in healthy subjects found that subjects who reported greater stimulant-like effects of alcohol also reported greater
stimulant like effects of amphetamine. However, the lack of cross-priming of desire for alcohol by amphetamine in the present study suggests that amphetamine does not readily substitute for alcohol in pathological gamblers or controls. The contrast between this null effect and cross-priming of desire for alcohol by the slot machine further suggests that cognitive rather than pharmacological factors mediated the linkage between gambling and drinking in pathological gamblers.

**Reinforcer Effects:** After exposure to slot machine and amphetamine, both groups completed scales to assess pleasurable effects. The Slot Machine led to moderate pleasurable effects (i.e.: Enjoyment, Excitement, Involved) in gamblers (~75% maximal rating) and healthy controls (~50% maximal rating), and the group difference was significant. These effects are consistent with previous findings (Zack and Poulos 2007). Gamblers also reported increased buzz or high from the game (~70% in pathological gamblers vs. ~30% healthy controls) and more difficulty stopping when it was over (~50% pathological gamblers vs. ~20% healthy controls) than the controls. These findings confirm that a brief episode of slot machine gambling can have subjective ‘intoxicating’ effects and can promote an urge to persist at gambling, with both of these effects differentiating between pathological gamblers and controls.

After amphetamine, pathological gamblers reported more of the pleasurable effects from amphetamine compared to healthy controls such as greater liking and increase desire to take the drug again. However, effect sizes were modest and the lack of group difference did not appear to reflect insufficient statistical power. In contrast, pathological gamblers reported weaker bad effects of amphetamine compared to controls, and this difference was statistically significant. This pattern is somewhat consistent with previous findings by Zack and Poulos (2004) who found *significantly* greater subjective pleasurable effects of 30 mg amphetamine in
pathological gamblers vs. controls. The lack of significance on pleasurable effects may be related to design and procedural factors. Subjective effects of amphetamine may differ when assessed inside a PET scanner vs. outside a scanner. In addition, the original study included a placebo comparison to control for group differences in the expected reinforcing effects of amphetamine, whereas the present study did not include a placebo condition, due to the prohibitive cost of the PET scans.

The subjective reinforcing effects of amphetamine were further assessed with the Addiction Research Center Inventory. The increase in the MBG (euphoria) scale of the Inventory from baseline to 225-min post-amphetamine was 66% (5.7 to 9.5) in gamblers, whereas the increase in healthy controls was 31% (6.5 to 8.5). The within-subjects increase was significant in gamblers but not controls. Therefore, amphetamine seemed to be more euphorigenic to pathological gamblers than to controls.

**Profile of Mood States:** Overall both groups reported more positive effects and less negative effects after both reinforcers, although no significant differences in groups or reinforcers were evident. Pathological gamblers tended to report overall higher scores for negative effects compared to healthy controls, this could be attributed to the significantly higher scores (although below cut-off) Hamilton Depression Scale and Beck Depression Inventory observed in the pathological gamblers compared to healthy controls.

**In summary,** the pattern of effects was largely consistent with – though less robust than – previous results and with the expected group difference in relative response to each reinforcer.
4.1.2 Physiological Effects

Physiological indices like heart rate (HR) and blood pressure (BP) provide an objective assessment of reactivity to the two reinforcers, which can cross-validate the subjective measures.

For both groups, significant increases in physiological function were only observed after amphetamine but not after the slot machine prime. Both groups exhibited an increase in blood pressure after the prepulse inhibition task and subsequent decrease after the slot machine game. This differs from the previous haloperidol study, where gamblers and controls exhibited an increase in blood pressure after the slot machine game (Zack and Poulos 2007). This difference may reflect the arousing effects of the random startle pulses on blood pressure during the prepulse inhibition task, with diminished subsequent sympathetic reactivity to the slot machine.

The groups’ response to amphetamine diverged on heart rate and blood pressure, but at different points in the course of the dose. For heart rate, the groups’ scores rose comparably up to expected peak (90-min) after which gamblers exhibited a marked decline, whereas healthy controls’ heart rate scores remained elevated, resulting significant group differences in heart rate from 90 through 225 minutes post-dose. For blood pressure, on the other hand, gamblers had significantly lower blood pressure than controls during rising drug blood levels, but were indistinguishable after the 90-minute mark. The biphasic and complementary profile of effects suggests a possible compensatory relationship between cardiac output and blood vessel tone in response to a Central Nervous System stimulant challenge in pathological gamblers but not in controls. The relatively weaker response on both indices relative to controls suggests that the
gamblers may have been more ‘tolerant’ to the cardiovascular effects of amphetamine than the controls despite never having previously been exposed to stimulants. This is analogous to endurance exercise training, where overstimulation of the peripheral nervous system over time leads to decreases in noradrenaline and thus improves endurance as observed through more blunted increases in heart rate and blood pressure over time (Meredith et al. 1991).

However, the source of this apparent tolerance is unclear. Pathological gamblers subjects also exhibited lower plasma levels of amphetamine between 60 and 120 min than healthy controls subjects did (47 vs. 30 ng/ml). Thus, pharmacodynamic or pharmacokinetic factors could account for the group differences in physiological reactivity to the dose. This is a novel result with no obvious explanation. Blood samples have been saved for genotyping and this may reveal differences in the genetic metabolic profile of amphetamine. Importantly, there were no group differences in plasma amphetamine after 120 minutes; therefore the relatively lower heart rate of gamblers throughout the latter third of the dose is not attributable to differences in drug concentration/functional dose.

It has been observed that pathological gamblers have lower plasma noradrenaline metabolites but higher cerebrospinal fluid metabolites compared to controls (Roy et al. 1988; Bergh et al. 1997). The cardiovascular effects of d-amphetamine are mediated by a noradrenergic mechanism (Nurnberger et al. 1984); Central alpha-2 adrenergic receptors exert a tonic inhibitory effect on noradrenaline release (McDonald et al. 1997). Deficits in these receptors (e.g., in transgenic mice) cause deficits in prepulse inhibition (Lähdesmäki et al. 2004). Like these transgenic mice, pathological gamblers have deficits in central alpha-2 receptor function as revealed by clonidine challenge (Pallanti et al. 2010). Furthermore, pathological gamblers show deficits in prepulse inhibition (Stojanov et al. 2003), a result
replicated in the present study. In addition, noradrenaline agonists lead to deficits in prepulse inhibition, but these are restricted to centrally rather than peripherally administered agonists (Alsene et al. 2006). Taken together, the findings suggest that an imbalance in central versus peripheral noradrenaline function in pathological gamblers may contribute to their cardiovascular hypo-reactivity to amphetamine.

4.2 Sensitization
4.2.1 Prepulse Inhibition and Wisconsin Card Sorting Task

Prepulse inhibition indicates ‘gating’ of redundant sensory information. Deficits in prepulse inhibition are associated with sensitization of the brain dopamine system (Braff et al. 2001, Stojanov et al. 2003). Deficits in prepulse inhibition have reliably been shown in animals exposed to dopamine agonists (Mansbach et al. 1988, Davis 1988; Geyer et al. 1999, Swerdlow et al. 1988); and this disruption is reversed by treatment with dopamine antagonists such as haloperidol (Mansbach et al. 1988). Therefore decreased prepulse inhibition is a hallmark feature of elevated dopamine tone. Previous research has found deficient prepulse inhibition in pathological gamblers suggestive of elevated dopamine tone (Stojanov et al. 2003). Decreased Pre-Pulse Inhibition was used as an indirect, electrophysiological index of sensitization in the present study.

Our analyses revealed that pathological gamblers exhibited deficits on all measures of prepulse inhibition with the most profound effect at the 120-milliseconds pre-pulse lead interval. In fact, at 120-milliseconds lead interval, the prepulse seemed to facilitate startle response to the pulse in the gamblers. Outlier analysis did not reveal any extreme scores at the 120 ms lead; however the scores were spread between the positive and negative ranges. Thus some gamblers experienced increased startle response relative to pulse alone (i.e.: facilitation),
while other gamblers experienced a diminished reduction in startle by the prepulse. In both cases, the direction of the effect is the same: The prepulse is not inhibiting reactivity to the pulse as it is expected to, and as it did in healthy controls.

This increased startle response in the presence of the prepulse is known as “prepulse facilitation” and usually occurs when the interval between the prepulse and the pulse is very short (less than 20 ms) or very long (greater than 1000 ms) (Graham 1975, Hoffman and Searle 1968). The prepulse facilitation seen in the present study occurred at an inter-pulse interval of 120 ms rather than at a very short or longer interval. Because absolute facilitation as opposed to decreased inhibition, relative to pulse alone, only emerged in a small group of subjects, the reliability of this effect is uncertain. More importantly, the emergence of this effect in the group as a whole cannot be attributed to those subjects who exhibited facilitation, because even when those individuals were removed, the prepulse inhibition was deficient in gamblers (-20%) relative to controls (-30%).

In addition to deficient pre-pulse inhibition, lack of set-shifting ability or perseveration is also considered a feature of sensitization. In the Wisconsin Card Sorting Task, pathological gamblers subjects tended to exhibit greater perseverative errors compared to healthy controls (20.6 vs. 16.8) implying ~23% decreased cognitive flexibility. This was not a generic deficit, as pathological gamblers subjects actually displayed ~22% fewer non-perseverative errors (failures to maintain set) than healthy controls (9.8 vs. 12.5). Marazziti (2008) demonstrated perseveration and increased difficulty in finding alternative solutions in pathological gamblers compared to healthy controls.
Fletcher and colleagues (2005) demonstrated that chronic exposure to amphetamine can induce perseveration in a set-shifting task in animals that is mediated by dopamine D1 receptors in the pre-frontal cortex. The combined deficits in prepulse inhibition and increased perseveration observed in pathological gamblers subjects are consistent with the pattern expected following chronic exposure to amphetamine. These are not generic effects of addictive drugs, as amphetamine reliably reduces prepulse inhibition in animal and human models (Swerdlow et al. 2003; Brunell and Spear 2006, Cadenhead et al. 1993; Hutchinson and Swift 1999) while alcohol shows inconsistent results (Grillon et al. 1994, Brunell and Spear 2006) and nicotine enhances prepulse inhibition (Kumari et al, 1996; Curzon et al, 1994). Although the pattern of effects is consistent with expectations, their magnitude and reliability was modest. Therefore, any conclusions with respect to sensitization based on these results alone are strictly provisional pending replication or augmentation (i.e., via subjects in study phase two).

4.2.2 Pre-Pulse Inhibition and Reinforcement of Slot Machine and Amphetamine.

Regression analyses demonstrated no significant relationship of pre-pulse inhibition with cardiovascular response or change in desire to gamble in response to the slot machine. However, deficits in pre-pulse inhibition at lead interval 120 ms were significantly associated with increased desire to gamble after amphetamine, and this result persisted after controlling for baseline variation, indicating that the relationship involved acute response to the drug rather than a generic motivation to gamble. Inspection of the scatter plot confirmed that the correlation was not due to a bimodal distribution in gamblers vs. controls, but rather a normal distribution of scores for the full sample, in which gamblers generally fell into the higher range.
(i.e. less inhibition of startle). Thus, deficits in sensorimotor gating are associated with primed motivation to gamble in response to amphetamine.

A second regression analysis revealed that deficits in Pre-pulse inhibition were associated with decreased heart rate in response to amphetamine at 120 minutes. Again, the relationship involved a continuous distribution of scores. This result is noteworthy given that the cardiovascular effects of amphetamine are known to be mediated by noradrenaline rather than dopamine (Nurnberger et al. 1984) whereas pre-pulse inhibition is mediated by noradrenaline as well as dopamine (Lähdesmäki et al. 2004, Alsene et al. 2006). Collectively, the findings raise the possibility of a functional relationship between central noradrenergic disturbance (e.g., deficits in alpha-2 noradrenaline receptor sensitivity; Pallanti et al. 2010) and sensitized central dopamine response (e.g., Schank et al. 2006; Yamashita et al. 2006).

4.3 Other Cognitive Effects

Both groups performed similarly on the Wechsler Intelligence Scales indicating comparable basic short-term and working memory and cognitive function. The lack of group differences ensured that any group differences in the other tasks are not readily attributed to deficits in general cognitive function.

The cognitive tasks served to further characterise pathological gamblers. Although no significant group differences were observed in any of the cognitive tasks, some patterns did emerge.

In the Game of Dice Task, contrary to expectation, the slot machine game did not prime risk-taking behaviour in either group. However, as the game progressed, betting
decisions tended to become more risky with gamblers slightly more risky than controls. In studies by Cavedini et al (2002) and Brand et al (2005), pathological gamblers displayed a preference for disadvantageous (riskier) options on the Game of Dice Task compared to controls. The present results are generally consistent with these prior data. However, this task appears to more sensitive to trait factors rather than state or situational factors.

The **Rapid Reading Task** measured incentive salience in terms of reaction time to motivationally relevant stimuli. The slot machine game served as the priming stimulus. Subjects in both groups responded faster to all motivationally relevant stimuli such as gambling, alcohol, positive and negative affect words, than to the neutral words. However, no differences were observed between both groups. Zack and Poulos (2004) demonstrated that in the presence of amphetamine, pathological gamblers reacted faster to gambling related stimuli compared non-gambling stimuli. If gambling was similar to psychostimulant use, then after the gambling reinforcement (slot machine), gamblers should have a faster reaction time to gambling related stimuli compared to controls. In contrast to the prior amphetamine study, absence of a placebo control (or motivationally neutral activity in lieu of the slot machine) in the present design made it difficult to isolate primed vs. un-primed incentive salience. In addition, outlier scores (i.e.: reaction time greater than 3 standard deviations above the mean) are common on reaction time tasks due to momentary lapses of attention, and these outliers are typically excluded from analyses of mean scores. With the small sample size, the standard deviation was likely not reliable, so there was no stable criterion to identify outliers. As a result extreme scores may have obscured modest differences in mean response time, further impeding detection of group or priming effects. Increasing the sample size may permit detection of
differential incentive salience between gambling and neutral words when the invalid scores are removed.

The conventional Stop Signal Task was administered to measure inhibitory control and response execution to motivationally neutral stimuli. As expected, the reaction time to the Stop Signal was much faster than to the Go stimuli and there were more numerous inhibitory errors (incorrect response commission) than key press errors (incorrect response selection). No obvious differences were seen between the two groups. Greater stop signal reaction time (SSRT), denoting poorer inhibitory control, has been shown to predict relapse in pathological gamblers (Goudriaan et al. 2008). In that case, the subjects were treatment seeking pathological gamblers and poorer inhibition (longer SSRT) predicted increased risk of relapse. Group differences in SSRT between non-treatment seeking pathological gamblers and controls do not appear to have been tested before.

Overall, the small sample size and considerable variability in subject response obscured variations in mean scores in the cognitive tasks. This was to be expected given that the sample included only half the subjects (phase I) based on power estimates for cognitive tasks. Increasing the sample size and removing potential outlier scores from response time tasks should increase the power and provide a clearer indication of the cognitive-behavioural and subjective-physiological effects of slot machine and amphetamine primes in pathological gamblers versus controls.
4.4. General Discussion

This study served to expand on previous findings by Zack and Poulos (2004) that amphetamine primes desire to gamble selectively in pathological gamblers which, together with other indirect evidence, led to the idea that psychostimulant addiction and pathological gambling may be mediated by common neurochemical processes. After controlling for baseline variation, both slot machine and amphetamine primed desire to gamble in both groups with a greater increase observed in the gamblers than controls. This effect is consistent although less pronounced than the findings from previous studies on amphetamine reinforcement and slot machine gambling (Zack and Poulos 2004, 2007). Gamblers also tolerated amphetamine better as they reported a pattern of more good effects (euphoria, liking), less bad effects and less of an amphetamine-induced cardiovascular response compared to controls. Therefore, although not all effects achieved statistical significance, the patterns – as reflected by effect size data ($\eta^2$) – did conform to expectations.

Understanding the overlapping neurochemical processes between these two disorders may be useful in guiding development of pharmacological interventions for pathological gambling. The priming data suggest a role for dopamine, which is pivotal to the incentive motivational properties of natural and drug reinforcers. That is, dopamine may have mediated group differences in the motivational effects of the slot machine and amphetamine.

The electrophysiological (prepulse inhibition) and physiological (heart rate/blood pressure) suggest a possible role for norepinephrine in the present results as well. Specifically, deficits in (drug-free) prepulse inhibition and decreased heart rate reactivity to amphetamine were each observed in gamblers. Given that pathological gamblers have been found to exhibit
decreased central alpha-2 noradrenergic sensitivity (Pallanti et al. 2010), and such deficits are associated with decreased prepulse inhibition in animals (Lähdesmäki et al. 2004, Swerdlow et al. 2006, Barr et al. 2006), it is conceivable that alpha-2 noradrenergic receptor deficits contributed to group differences in prepulse inhibition and physiological reactivity to amphetamine in the present study.

The Incentive Sensitization theory states that repeated drug use sensitizes the neural systems that mediate motivational processes involved in addictive appetite and seeking, (drug “wanting”) but not the neural systems that affect the pleasurable effects of drugs (drug “liking”) (Robinson and Berridge 2001). Therefore, as chronic exposure continues, the degree to which drugs are ‘wanted’ increases inversely or disproportionally to the degree to which they are ‘liked’. If pathological gambling is an addiction in the functional sense, sensitized brain reward pathways would increase appetite for and priming effects – wanting - of gambling but not necessarily “liking” of the game.

In the present study, amplification of wanting was manipulated by administration of the slot machine and amphetamine ‘primes.’ Both primes were effective, however amphetamine was less distinguishable between gamblers and controls. This could have been due to differences in the context of the two primes. Animal studies have shown that sensitization is powerfully modulated by the context where the drug is administered (Robinson et al. 1988). Therefore, sensitization may not manifest if effects are measured in a context where the drug or prime has never been administered. During this study, amphetamine was administered in the context of a PET scan, a novel context with no association to amphetamine or gambling which could have disturbed cross-priming effects. The slot machine in contrast was administered in a mock-bar context very similar to the one in which subjects likely experienced gambling.
previously. If the gambling and amphetamine primes were administered in the same context, this may have evoked the expected substantial increased desire to gamble after amphetamine dose, as seen when slot machine was administered and in the previous amphetamine study (Zack and Poulos 2004, 2007).

The self-report data also indicated that the slot machine and amphetamine were more pleasurable to gamblers than controls. This is not consistent with the Incentive Sensitization theory. One possible explanation is that multiple neurochemical anomalies exist in pathological gamblers, and that these anomalies played different roles in the incentive motivational versus subjective pleasurable effects of the slot machine and amphetamine. For example, sensitization of the dopamine system may have largely mediated group differences in primed desire to gamble whereas deficits in noradrenergic receptor sensitivity may have mediated group differences in ‘liking’ as indexed by increased self-reported enjoyment of the slot machine and increased euphoric response to amphetamine on the MBG scale of the Addiction Research Center Inventory.

At the same time, the regression data revealed that (drug-free) deficits in pre-pulse inhibition predicted post-amphetamine desire to gamble and deficits in heart rate response to amphetamine. These correlations controlled for baseline (i.e. trait) differences in desire to gamble, suggesting that they reflected differences in response to the drug. Increased noradrenergic transmission (via reuptake inhibition) can restore deficits in prepulse inhibition in dopamine transporter knockout (i.e. hyperdopaminergic) mice (Yamashita et al. 2006). Dopamine antagonists, like haloperidol, also restore deficits in prepulse inhibition in hyperdopaminergic (e.g., amphetamine-sensitized) animals. Furthermore, haloperidol enhances the priming effects of the slot machine game and this effect coincides with post-game
restoration of systolic blood pressure in pathological gamblers (but not controls) (Zack and Poulos 2007). Taken together, these findings indicate an intimate, possibly reciprocal, role of noradrenaline and dopamine in sensitization-like states, and raise the possibility that the correspondence between decreased prepulse inhibition, decreased heart rate response to amphetamine, and increased post-amphetamine priming of desire to gamble reflects a reciprocal interaction of dopamine and norepinephrine in pathological gamblers.

Recent evidence supports this possibility. In a study with rats, Park et al. (2011) reported that “An aversive stimulus, quinine, activated noradrenergic signalling but inhibited dopaminergic signalling [in the basal nucleus of the stria terminalis], whereas a palatable stimulus, sucrose, inhibited norepinephrine while causing dopamine release…[and concluded that] This reciprocal relationship, coupled with their different time courses, can provide integration of opposing hedonic states to influence response outputs appropriate for survival.” (Park et al. 2011 p. 1). The preferential reduction in the bad effects of amphetamine in the present group of gamblers is consistent with hyposensitivity to its noradrenergic (aversive) properties, and a possible disinhibition of its dopaminergic (i.e. priming) effects.

In light of the current findings, future studies should directly assess the respective roles of dopamine and norepinephrine in the present paradigm. The role of dopamine could be assessed by means of tyrosine depletion (Harmer et al. 2001), whereas the role of norepinephrine could be assessed with disulfiram, a preferential inhibitor of noradrenaline synthesis.
4.5 Limitations

A number of limitations exist with respect to the current study. The generalization of the study findings is limited by the small sample size. This was phase one of a two phase study which was adequately powered for a PET protocol rather than cognitive-behavioural or self-report indices. This limited our ability to detect statistically significant effects on these indices.

Secondly, as this was a pilot study, it involved only males; therefore gender effects were not taken into account. Overall, women display more dopamine release from a weight adjusted amphetamine dose than men, and women also report less “high” from amphetamine but a greater blood pressure increase than men do (Vansickle et al. 2007). In addition, there is variability in prepulse inhibition. Adult males have more robust prepulse inhibition compared to females (Swerdlow et al. 1993, Aasen et al. 2005). The level of prepulse inhibition also varies across the menstrual cycle (Swerdlow et al. 1997); therefore, using male subjects limits the generalizability of the results. Thirdly, the study employed a highly selective sample of subjects with severe gambling pathology and no co-morbidities. Although this permits clear attribution of group differences to pathological gamblers status rather than other psychiatric disturbance, pathological gambling is highly co morbid with other disorders such as clinical depression and alcohol dependence; this further limits the generalization of the results. As noted earlier, since amphetamine assessment was performed in the context of positron emission tomography (PET) study, there was no placebo control group. Therefore the relative contribution of expectancy-based vs. pharmacological effects of amphetamine cannot be established. However, within-session comparisons did serve to confirm that effects were a consequence of exposure to the slot machine or amphetamine rather than trait differences.
between groups. Lastly, gambling encompasses a wide range of activities. It is plausible that heterogeneity in subjects’ preferred gambling activity limited responses to the slot machine game. Subsequent studies should consider using pathological gamblers who are primarily slot machine players in order to maximize the priming influence of the game.

4.6 Future Directions

As a next step, the current study findings need to be reproduced in a larger sample to further validate the model and increase power to detect significant effects. Although correlations between amphetamine effects, sensitization and physiological tolerance were observed, it cannot be confirmed that dopamine or norepinephrine mediated these effects. Future studies, isolating dopamine or noradrenaline through the use of selective dopamine and noradrenaline depleting agents, could verify the respective roles of these two transmitters in incentive sensitization and physiological tolerance to amphetamine in gamblers. In addition, if pathological gambling and psychostimulant addiction are similar, then it would be prudent to repeat this protocol and include an additional group of psychostimulant dependent subjects to assess whether slot machine gambling also cross primes desire for amphetamine or cocaine in this addicted population. This study serves as a pilot whose data provide a link between sensitization, motivation to gamble, and response to a psychostimulant drug. As such it provides a solid basis for future research on the neurobiology of pathological gambling.
4.7 Conclusion

In conclusion, parallel subjective motivational effects to gambling and amphetamine reinforcement were observed in pathological gamblers, the difference between the gamblers and normal volunteers was not profound. In addition, deficits in pre-pulse inhibition, a hallmark of the sensitized state, were observed in gamblers and these deficits predicted greater priming effects of amphetamine. These findings are consistent with the possibility that a sensitized dopamine system mediates stimulant-induced “wanting” to gamble in pathological gamblers subjects. Conversely, deficient pre-pulse inhibition predicted lower cardiovascular response to amphetamine, suggestive of noradrenergic hypo-reactivity, or tolerance. The latter result suggests that sensitization and tolerance are functionally related aspects of problem gambling, and point to a possible disturbance in the reciprocal modulatory roles of dopamine and norepinephrine in pathological gamblers. These results, if replicated, are important for developing anti-gambling medications and suggest that both norepinephrine and dopamine are key targets for normalizing motivation and information processing in pathological gamblers.
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APPENDIX A:
Study Advertisements from February 2010 to June 2011
Do you gamble?

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

If you are:
Male
19-65 years of age
Drug- and Medication-Free
Available for Weekday Sessions

Call Bindiya: (416) 535-8501, ext. 6743

NOTE: This is not a treatment study.

Financial Compensation Is Provided

All Information Will Remain Confidential
To The Extent Allowed By Law

To find out about treatments and services for mental illness or addiction problems, please call CAMH at: (416) 535-8501.
Healthy Volunteers

You may be eligible for a research study.

If you are:
Male
19-65 years of age
Drug- and Medication-Free
Available for Weekday Sessions

Call Bindiya: (416) 535-8501, ext. 6743

FINANCIAL COMPENSATION IS PROVIDED

All Information Will Remain Confidential To The Extent Allowed By Law

To find out about treatments and services for mental illness or addiction problems, please call CAMH at: (416) 535-8501.
APPENDIX B: Consent Forms
I. Study Consent Form
II. Slot Machine Consent Form
I. STUDY CONSENT FORM

Bio-behavioural study of mental processes and dopamine function in gamblers and non-gamblers

Principal Investigator: Martin Zack, PhD
Co-Investigators: Isabelle Boileau, PhD
Stephen Kish, PhD
Daniela Lobo, MD, PhD

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

Overview
You are invited to participate as a volunteer in a combined study of computer-based task performance and brain imaging. You qualify for this study because you (a) currently gamble regularly; or (b) rarely/never gamble. The purpose of this research project is to determine the relationship between participation in gambling and activity of the neurotransmitter dopamine (DA) in the brain.

DA is a molecule naturally produced by the brain that facilitates transmission between nerve cells and that has been implicated in motor control, in the regulation of emotions and in processes related to learning and attention. Dysfunctions in DA neurotransmission are associated with disorders such schizophrenia, Parkinson’s disease and drug addiction.

This research study involves two test sessions: Test session 1 administers several tasks on a computer and measures your responses – usually in terms of speed or accuracy. During this session, you will also be asked to play a commercial slot machine and will be asked some questions about your experience of the game. The money to play the slot machine will be provided by us, but you will have an opportunity to keep an amount proportional to the credits you win during the game. These winnings will be paid at the end of the study when you receive your participation fee.

The second test session involves undergoing a brain imaging procedure called PET imaging. PET imaging uses a radioactive agent to obtain pictures of the brain, the agent that we are using in this study is called [$^{11}$C]-propyl-hydroxy-naphthoxazine ($[^{11}$C]PHNO), and it will provide us information about DA in the brain. In order to investigate DA levels in the brain we will stimulate its release from DA cells by using a low dose of d-amphetamine (Dexedrine$: 0.4$ mg/kg given orally).

The full procedure involves one MRI and 2 PET scans as well as completion of a battery of neuropsychological tests. The MRI scan gives us information about the structure of the brain that helps us interpret the information from the PET scan.

In order to decide whether or not you wish to participate in this study, you should know about the risks and benefits to make an informed judgment. This sheet gives you detailed information about the study and you should feel free to ask us any other questions that you might have. Once you understand the study and its risks you may choose to participate by signing the attached form.

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Initials_____
**Information about Dexedrine**

Dexedrine (D-amphetamine) is a stimulant drug currently in clinical use in Canada for the management of attention deficit hyperactivity disorder (ADHD) and sleep-disorder (narcolepsy). Depending on your body weight you will receive between 25 and 40 mg of Dexedrine® orally. 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®. Sustained high doses of amphetamine (>100mg /day) can lead to dependence or cause psychosis but this is very rare at the dose you will receive. Subjects will be asked to remain in the PET unit for observation during these three hours. If an adverse response were to develop, the subject would be treated by one of the study’s physicians as deemed necessary. Because Dexedrine® can stimulate the heart and the blood vessels there is the rare possibility that the drug could overstimulate 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 causes these serious side effects.

Animal studies using high doses of Dexedrine have found that it may damage brain cells containing dopamine. However, the relevance of these studies to human beings is uncertain.

You should not take Dexedrine® if you have heart problems, high blood pressure, a family history of sudden cardiac death, glaucoma (elevated pressure inside the eye orbit), arteriosclerosis (a buildup on the inside of artery walls), or hyperthyroidism (overproduction of thyroid hormone).

Dexedrine® may impair the ability to engage in potentially hazardous activities such as operating machinery or vehicles. You should not drive or engage in strenuous exercise or activities on the day you take Dexedrine®.

You should avoid caffeinated beverages for 5 hours after taking Dexedrine®.

**Number of People Taking Part in the Study**

A total of 24 participants will complete this part of the study. You should not participate in the study if you have a past history of serious medical illness, alcohol abuse, current or prior use of illicit drugs, or exposure to other nuclear medicine scans (e.g., PET scans) which could exceed the maximum annual amount of radiation.

Description of Procedures (see table 1 for study calendar)

**Preliminary screening:** You will have a visit with a research staff member to ensure that you meet the requirements to take part in the study. In this visit we will ask you about your medical, psychiatric and drug history. It is very important that you provide us accurate, to the best of your knowledge, information in response to these questions. If this information is not accurate, we cannot be certain whether any changes that we might find are accurate or due to unreported aspects of your history. As a result, it is likely that we would provide misleading information in published reports of our findings. We will confirm that you have not used drugs or alcohol by analysing urine/blood samples and from breath samples on each day of the study.

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Initials____
**Treatment History:** As part of the screening process, to confirm you do not have significant mental or emotional concerns (apart from gambling), we check health records at CAMH to ensure that no participant has received treatment for these concerns.

You are free not to consent to have your treatment records reviewed. However, because this information is critical to ensure that your data can be meaningfully interpreted by us, if you choose not to consent to have your records reviewed, you are not eligible to participate in the study.

**Physician’s Exam:** As part of the screening procedures to ensure that it is safe for you to participate in this trial, a physician will perform a physical examination. You will be required to have an ECG (electrocardiogram or heart tracing) and to provide a blood sample (approximately one tablespoon) for complete blood cell count and routine chemistry and a urine sample for drug-screen and pregnancy test in females.

**Behavioural Testing:** The behavioural test session involves performance of a series of tasks run by a computer. Some of the tasks ask you to read words aloud; others require you to make rapid key presses in response to symbols on the screen, or to make decisions among various options described by the computer. You will also receive an assessment of brain wave activity similar to an EEG (or electroencephalogram). For this, you will wear a cap with wires connected to a computer and listen to some sounds or watch some symbols on a screen. There is no pain involved in this, but some clear (fully washable) gel will be applied to your scalp, which can be a bit uncomfortable and messy. For this reason it is best to wear very casual clothing on this day.

**MRI scan:** The MRI scan will be done at the Toronto General Hospital. In order to do the MRI scan, you will be placed on a scanning bed, such that your body will be within the camera. The MRI scan takes approximately one half-hour. You will be asked to make sure that there is no metal object in or on your body (e.g., decorative body piercing, dental implants, or metal pins or plates used to repair certain types of bone fractures), as metal objects may lead to injuries during the MRI scanning procedure.

**PET scans:** PET scanning is a technique of making pictures of the brain to give information about where chemicals in the brain are distributed and in what amounts. The PET scans are done by injecting a very small amount of radioactive substances into your vein and by taking pictures of your brain using a PET camera. This technique has been used for research and for clinical diagnosis in different centers around the world. We have also used this scanning technique in over 300 participants at the centre in recent years.

During this study you will participate in **two PET scans** on the same day (baseline and DA stimulation):

1) **Baseline:** You will be asked to not eat for 6 hours before the study, but you can drink water (though not excessively). Because it is very important to make sure that you keep still during the study we will make a head-holder out of styrofoam to minimize movements during the scan.

The first scan will be performed at baseline. Prior to scanning, a fine needle-catheter will be inserted into an arm vein for the administration of small amounts of the radioactive substance $[^{11}C]$PHNO. You will be asked to lie still on the couch in the scanner. The length of the scan is approximately two hours (90 minutes of scanning).

_I have read this page of the document_  

Initials____
2) **DA stimulation with Dexedrine:** The second PET scan will be similar to the first one except that we will give you Dexedrine (by capsule) 2 hours before the second PET scan starts. After the capsule we will ask you questions to assess your mood and thinking. The second PET scan will also last about 90 minutes.

During both scans, blood will be drawn for hormonal measurements via the catheter in your arm vein. The total amount of blood will be 28ml (2 tbsp) 7 ml per sample (1 ½ tsp) for each scan.

Electrodes will be placed on your skin in order to record your heart rate and muscle tone.

When the studies are done, you will be evaluated and discharged. A nurse will be available throughout the second scan and can answer questions you may have or help if you feel any discomfort.

**Psychological testing:** In addition to the behavioural test session, there will be some follow-up tests on the PET session. The complete set of tests will take 3-4 hours. We will test a number of things, including your memory, how you learn, think and plan, your attention, and reaction time. A number of these will be performed on a computer under certain standard rules.

**Risks and Inconveniences**

*From PET scanning:* You will be exposed to a small amount of radiation from a brief transmission scan to measure how much radiation is absorbed by your head. You will also receive 20 mCi $[^{11}\text{C}]\text{PHNO}$ for the 2 scans. The radiation dose given to you during a PET scan is comparable to other nuclear medicine scans and represents a very low risk. The radiation dose from each scan is 2 mSv for a total of 4 mSv, which is approximately the amount of radiation received from natural sources during one year (3mSv). The potential long-term risk from the radiation dose you will receive is uncertain, but these doses have never been associated with any definite adverse effects. While the radiation exposure from this PET scan is below the limits set by the PET Centre, you should notify the investigator of any other radiation exposures which you have received over the past year. The PET scan requires an injection; this injection will be combined with taking a small amount of blood. As with any injection or blood work, there is a small risk of bruising at the site of injection in your arm.

*From PHNO:* PHNO has been investigated as a medication for Parkinson’s disease in Canada, the U.S.A., and Europe. Since it was not as effective as initially thought, these studies were stopped. Side effects of PHNO observed in these studies were drowsiness, nausea, and sometimes vomiting. While the dose administered for a PET scan is lower than what has been used to treat Parkinson’s disease, it is still possible that after injection of PHNO you may experience mild and short-lasting (five to ten minutes) nausea or drowsiness. Please note that $[^{11}\text{C}]\text{PHNO}$ is an investigational positron emitting radiopharmaceutical used for research purposes and not yet marketed in Canada.

*From Dextro-amphetamine,* Dexedrine®: D-amphetamine is a stimulant drug currently in clinical use in Canada for the management of attention deficit hyperactivity disorder (ADHD) and sleep-disorder (narcolepsy). Dexedrine® has been used by several other studies conducted at CAMH. Depending on your body weight you will receive a dose of 25-30 or 35 mg of Dexedrine® orally. This dose is within the dose range given to children after the age of 6 (to treat ADHD).

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**Initials**
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®. Sustained high doses of amphetamine (>100mg /day) can lead to dependence or cause psychosis but this is very rare at the dose you will receive. Participants will be asked to remain in the PET unit for observation during these three hours. If an adverse response were to develop, the participant would be treated by one of the study’s physicians as deemed necessary. You should not take Dexedrine® if you have heart problems, high blood pressure, glaucoma, arteriosclerose, hyperthyroidism. Also to avoid serious drug-interaction you should not have taken any psychotropic medication or antidepressants (Parnate®, Manerix,® Moclobemide, Nardil®) for at least two weeks before the PET scan.

From MRI scanning: If you have metal objects or implants in or on your body (e.g., pacemaker, aneurysm clip, heart / vascular clip, prosthetic valve, metal prosthesis) you must not undertake MRI scanning. The scanner can cause movement and heating of the metal parts and this could cause serious injury to you. Apart from that, the only other known risk associated with an MRI scan is the feeling of nervousness that is experienced by some people because of the closed space of the MRI scanner (i.e.: claustrophobia).

From behavioural, neuropsychological testing and psychiatric interview: During the testing you may experience some emotional discomfort or frustration when answering some of the questions or performing some of the tasks. If any particular question makes you feel uncomfortable, you may discuss its importance with the specially trained interviewer. You may choose not to answer any question or test with which you still feel uncomfortable.

Women please note: Pregnant women should avoid radiation to minimize any risk to the fetus. You should not participate in this study if you are, could be, or plan to become pregnant during the study. Prior to your entering the study we will discuss with you the need to avoid becoming pregnant. If you change your mind about becoming pregnant during the study, please notify us immediately. It is possible for a woman to be pregnant without knowing it. For this reason, and to protect the welfare of the fetus, female participants must perform (under private conditions) a pregnancy self-test (urine dipstick) provided by us at the laboratory before proceeding to the PET scans.

Safety To ensure your safety the following precautions will be taken:

i) All adequate precautions and procedures will be explained to you.

ii) Support will be available to you for the entire duration of the study.

Benefits

By participating in this study you will not have any direct benefit. Your participation will contribute to scientific knowledge and possibly assist in development of medications to treat addictive behaviour.

I have read this page of the document

Initials_____
Reimbursement for time and inconvenience

Study participants will receive a fee to reimburse them for the time and inconvenience incurred during participation in this study. Incidental expenses such as travel and small meals directly related to participation in the study will also be reimbursed. The participants will be compensated as follows:

<table>
<thead>
<tr>
<th>Visit</th>
<th>Compensation</th>
<th>Time (max)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interview</td>
<td>$50</td>
<td>2-3 hours</td>
<td>Eligibility assessment, paper &amp; pencil tests, Blood/urine, ECG.</td>
</tr>
<tr>
<td>Physician’s Exam</td>
<td>$50$</td>
<td>1 hour</td>
<td>Comprehensive physical exam</td>
</tr>
<tr>
<td>Behavioural Testing</td>
<td>$200</td>
<td>4 hours</td>
<td>Computer tasks, brain wave assessment, slot machine game, paper and pencil questionnaires</td>
</tr>
<tr>
<td>MRI scan *</td>
<td>$ 70</td>
<td>1 hour</td>
<td>MRI scan</td>
</tr>
<tr>
<td>PET Scan</td>
<td>$ 350</td>
<td>4-5hrs</td>
<td>PET scan</td>
</tr>
<tr>
<td>Total</td>
<td>$ 720</td>
<td>14 hr + travel</td>
<td></td>
</tr>
</tbody>
</table>

*Note: The MRI scan may be completed at any time during the study period, depending on the availability of the MRI scanner.

No advance payment will be given, and participants will be reimbursed by cheque 2-3 weeks after completion of the study. However, if a participant drops out of the study at any time, the participant will be reimbursed for the visits, blood sampling, and imaging sessions that have been completed.

The payment you receive counts as income and therefore is subject to taxation. A T4A will be issued to you for declaration in your income tax return and we will need to obtain your Social Insurance Number for this purpose. You may choose to have your payment sent (by cheque) to you by mail. Alternatively, you may pick up your cheque at the CAMH cashier’s desk (2-3 weeks after study completion). In either case, your contact information will be available in the financial database at CAMH indicating that you have participated in a research study entitled, “Bio-behavioural study of mental processes and dopamine function in gamblers and non-gamblers.”

I have read this page of the document

Initials_____

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Confidentiality

In the event that any reports or publications result from this study, no information will be revealed that will permit readers to identify you. Blood samples taken from you during this study will be sent to an outside laboratory. These will only be labelled by a number and will be destroyed on completion of the analysis. As part of continuing review of the research, your study records may be assessed on behalf of the Research Ethics Board or if applicable by the Health Canada Therapeutic Products Program. A person from the research ethics team may contact you (if your contact information is available) to ask you questions about the study and your consent to participate. The person assessing your file or contacting you must maintain your confidentiality to the extent permitted by law. If you would like to know the results of the study or your individual results on any of the measures we would be happy to reveal them to you after the data have been fully analyzed. Under certain circumstances researchers are legally required to release confidential information by order of a court of law. Apart from such exceptional circumstances, all of the information obtained in this study will be kept confidential.

The T4A issued to you for income tax purposes will state that you were paid for participation in a research experiment. Your financial record at CAMH will show that the study was However, your data and all personal information will remain confidential as outlined above.

Voluntary Participation
You are free to choose not to participate. If you choose to participate you are free to withdraw from this study at any time without giving any reason.

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 (Behavioural Testing) at 416-535-8501 ext. 6052; Dr. Isabelle Boileau (PET/MRI scan) at 416- 535 8501 ext. 4918. 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.

I have read this page of the document

Initials_____
Consent Statement
Bio-behavioural study of mental processes and dopamine function in gamblers and non-gamblers

- The investigator or a member of the investigator’s staff has discussed with me the risks of participation in this study.
- I have not been exposed to other nuclear medicine scans (e.g., PET scans) in the last year.
- I have read all of the information in this 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 voluntarily agree to allow the investigators to review my health records to confirm that I have not attended a clinic or service to deal with a mental or emotional concern apart from a gambling-related problem.
- 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

__________________________________________
Participant’s Signature

__________________________________________
Signature of Individual Obtaining Consent

__________________________________________
Signature of Investigator
(If investigator did not obtained the consent)

__________________________________________
Date

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.

I have read this page of the document

Initials ______
II. SLOT MACHINE CONSENT FORM

Bio-behavioral study of mental processes and dopamine function in gamblers and non-gamblers.

Consent Statement – Use of Slot Machine Data

On the first test session in this study I played a slot machine game for about 15 minutes with credits provided by the study investigators. The plays (i.e., lines) I selected on the game, the bet size for each play, and the credits earned on each play were recorded electronically while I was playing. This information is coded numerically by participant number, is completely anonymous and will not be reported in any way that is identifiable to others. As such, my identity with respect to this data, as with all my other data, is protected to the extent allowed by law.

- I voluntarily agree to allow the investigators to use my (anonymous) slot machine data for scientific analysis only.

- 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)

Study Investigator (for questions about study procedure or use of data):
Martin Zack, PhD
Scientist
Clinical Neuroscience Section
Centre for Addiction and Mental Health
33 Russell Street
Toronto, ON M5S 2S1
Phone: (416) 535-8501, ext. 6052
APPENDIX D: Regression Analyses
Slot Machine Reinforcement and PPI

Desire to Gamble

Table I: Linear Regression Analysis of relation between Desire to Gamble after Slot Machine and Pre-Pulse Inhibition while controlling for Baseline Desire to Gamble in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12).

### ANOVA(b)

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>143.668</td>
<td>5</td>
<td>28.734</td>
<td>4.159</td>
<td>.012(a)</td>
</tr>
<tr>
<td>Residual</td>
<td>117.436</td>
<td>17</td>
<td>6.908</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>261.103</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Predictors: (Constant): VAS- Desire to Gamble (baseline), %PPI 60ms, %PPI 120ms, %PPI 240ms, %PPI 2000ms
b Dependent Variable: VAS-Desire to Gamble (Post-Slot Machine)

### Coefficients(a)

<table>
<thead>
<tr>
<th></th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>2.701</td>
<td>.899</td>
<td>3.005</td>
<td>.008</td>
</tr>
<tr>
<td>%PPI 60ms</td>
<td>.017</td>
<td>.014</td>
<td>.265</td>
<td>1.200</td>
</tr>
<tr>
<td>%PPI 120ms*</td>
<td>.014</td>
<td>.011</td>
<td>.271</td>
<td>1.291</td>
</tr>
<tr>
<td>%PPI 240ms</td>
<td>-.012</td>
<td>.015</td>
<td>-.176</td>
<td>-.841</td>
</tr>
<tr>
<td>%PPI 2000ms</td>
<td>-.004</td>
<td>.009</td>
<td>-.079</td>
<td>-.442</td>
</tr>
<tr>
<td>VAS- Desire to Gamble (baseline)</td>
<td>.685</td>
<td>.229</td>
<td>.557</td>
<td>2.994</td>
</tr>
</tbody>
</table>

d Dependent Variable: VAS-Desire to Gamble (Post-Slot Machine)
Cardiovascular Function

a) Blood Pressure:

Table II: Linear Regression Analysis of relation between Cardiovascular Response (Blood Pressure Systolic) after Slot Machine and Pre-Pulse Inhibition while controlling for Baseline Systolic Blood Pressure in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12).

ANOVA(b)

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>725.681</td>
<td>145.136</td>
<td>1.481</td>
<td>.248(a)</td>
</tr>
<tr>
<td>Residual</td>
<td>1666.145</td>
<td>98.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2391.826</td>
<td>22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a  Predictors: (Constant), Systolic Blood pressure(Baseline), %PPI 60ms, %PPI 120ms, %PPI 240ms, %PPI 2000ms
b  Dependent Variable: Systolic Blood pressure (Post Slot Machine)

Coefficients(a)

<table>
<thead>
<tr>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>99.403</td>
<td>22.465</td>
<td>4.425</td>
</tr>
<tr>
<td>%PPI 60ms</td>
<td>-.045</td>
<td>.055</td>
<td>-.230</td>
</tr>
<tr>
<td>%PPI 120ms*</td>
<td>-.037</td>
<td>.037</td>
<td>-.238</td>
</tr>
<tr>
<td>%PPI 240ms</td>
<td>-.027</td>
<td>.061</td>
<td>-.125</td>
</tr>
<tr>
<td>%PPI 2000ms</td>
<td>.049</td>
<td>.033</td>
<td>.321</td>
</tr>
<tr>
<td>Systolic Blood pressure (Baseline)</td>
<td>.154</td>
<td>.187</td>
<td>.186</td>
</tr>
</tbody>
</table>

a  Dependent Variable: Systolic Blood pressure (Post Slot Machine)
b) Heart Rate

Table III: Linear Regression Analysis of relation between Cardiovascular Response (Heart Rate) after Slot Machine and Pre-Pulse Inhibition while controlling for Baseline Heart Rate in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12).

ANOVA(b)

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>243.264</td>
<td>5</td>
<td>48.653</td>
<td>1.121</td>
<td>.387(a)</td>
</tr>
<tr>
<td>Residual</td>
<td>738.040</td>
<td>17</td>
<td>43.414</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>981.304</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Predictors: (Constant), Heart rate (Baseline), %PPI 60ms, %PPI 120ms, %PPI 240ms, %PPI 2000ms
b Dependent Variable: Heart rate (Post Slot Machine)

Coefficients(a)

<table>
<thead>
<tr>
<th></th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
</tr>
<tr>
<td>(Constant)</td>
<td>49.774</td>
<td>12.392</td>
<td></td>
</tr>
<tr>
<td>%PPI 60ms</td>
<td>-.041</td>
<td>.035</td>
<td>-.328</td>
</tr>
<tr>
<td>%PPI 120ms*</td>
<td>-.020</td>
<td>.025</td>
<td>-.199</td>
</tr>
<tr>
<td>%PPI 240ms</td>
<td>.009</td>
<td>.037</td>
<td>.068</td>
</tr>
<tr>
<td>%PPI 2000ms</td>
<td>.018</td>
<td>.022</td>
<td>.185</td>
</tr>
<tr>
<td>Heart rate (Baseline)</td>
<td>.194</td>
<td>.182</td>
<td>.235</td>
</tr>
</tbody>
</table>

a Dependent Variable: Heart rate (Post-Slot Machine)
Amphetamine Reinforcement and PPI

Blood Pressure (90 minutes post-AMPH dose)

Table IV: Linear Regression Analysis of relation between Cardiovascular Response (Blood Pressure Systolic) at 90 minutes after Amphetamine dose and Pre-Pulse Inhibition while controlling for Baseline Systolic Blood Pressure in Groups Healthy Controls (CON; n=11) and Pathological Gamblers (PG; n=12).

ANOVA(b)

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>766.691</td>
<td>5</td>
<td>153.338</td>
<td>.473</td>
<td>.791(a)</td>
</tr>
<tr>
<td>Residual</td>
<td>5509.744</td>
<td>17</td>
<td>324.103</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>6276.435</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Predictors: (Constant), Systolic Blood pressure: Baseline, Pre-AMPH, %PPI 60ms, %PPI 120ms, %PPI 240ms, %PPI 2000ms.
b Dependent Variable: Blood pressure: Systolic (90mins)

Coefficients(a)

<table>
<thead>
<tr>
<th></th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>120.266</td>
<td>53.470</td>
<td></td>
<td>.038</td>
</tr>
<tr>
<td>%PPI 60ms</td>
<td>-.099</td>
<td>.097</td>
<td>-.311</td>
<td>-1.024</td>
</tr>
<tr>
<td>%PPI 120ms</td>
<td>-.033</td>
<td>.071</td>
<td>-.134</td>
<td>-0.471</td>
</tr>
<tr>
<td>%PPI 240ms</td>
<td>.124</td>
<td>.107</td>
<td>.360</td>
<td>1.164</td>
</tr>
<tr>
<td>%PPI 2000ms</td>
<td>-.020</td>
<td>.065</td>
<td>-.083</td>
<td>-0.315</td>
</tr>
<tr>
<td>Blood pressure: Systolic: Baseline, Pre-AMPH</td>
<td>.101</td>
<td>.444</td>
<td>.064</td>
<td>.228</td>
</tr>
</tbody>
</table>

a Dependent Variable: Blood pressure: Systolic (90mins post-AMPH)