GENDER AS A MODERATOR OF THE EFFECTS OF D2 AND D1-D2 DOPAMINE ANTAGONISTS ON GAMBLING AND AMPHETAMINE REINFORCEMENT IN PATHOLOGICAL GAMBLERS AND HEALTHY CONTROLS

By

Shenghao Fang

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

© Copyright by Shenghao Fang 2014
Gender as A Moderator of the Effects of D2 and D1-D2 Dopamine Antagonists on Gambling and Amphetamine Reinforcement in Pathological Gamblers and Healthy Controls

Shenghao Fang
Master of Science
Department of Pharmacology and Toxicology
University of Toronto
2014

This study investigated the roles of D1 and D2 dopamine receptors in gambling and amphetamine reinforcement and possible moderating effect of subject’s gender in 24 pathological gamblers and 26 controls. Subjects received the D2R antagonist, haloperidol (3-mg), or D1-D2R antagonist, fluphenazine (3-mg) prior to a 15-minute slot machine game or 20-mg amphetamine in a placebo-controlled, counterbalanced, design. Haloperidol alone increased subjective positive states but decreased rewarding effects of gambling and amphetamine. Fluphenazine tended to decrease positive states but increased rewarding effects of the reinforcers. Effects were more pronounced in females and gamblers. The differential effects of antagonists suggest that D1R mediates gambling, and to lesser extent, psychostimulant reinforcement. The pattern of effects suggested an inverted-U relation between D1R stimulation and subjective effects, whereby antagonists increased or decreased reward depending on whether they promoted optimal, supra-optimal, or sub-optimal D1R signaling. This interpretation indicated low baseline D1R availability in females and gamblers.
ACKNOWLEDGMENTS

I would like to sincerely my supervisor Dr. Martin Zack for the continuous support of my study and research. His great expertise and guidance, as well as his invaluable patience and assistance, have been very important to me over the past two years, and have made it a very thoughtful and rewarding journey.

My sincere thanks also go to my advisor Dr. Susan George for providing very much needed encouragement, direction, and insights.

I would like to express my sincere gratitude to my previous colleagues Aditi Kalia, Daniel Tatone and Kelly Smart for their previous work on this project, and important guidance. I also thank my current colleagues Heidy Morales, Candice Biback, Jennifer Parlee and Jackson Wong for their practical assistance, insightful comments and stimulating discussions.

I would also like to thank Dr. Daniela Lobo, Dr. Dan DiGiacomo and Dr. Wiebe for their expertise and time, without which this project would not have been successfully completed.

Last but not least, I would like to acknowledge the CAMH pharmacy, CAMH Addiction Medicine Clinic, CAMH Clinical Laboratory, Women’s Inpatient Program and CAMH Quality Assurance Office for their help and contribution to this study.
TABLE OF CONTENTS

1. INTRODUCTION ............................................................................................................1
   1.1 Study Overview ........................................................................................................1
       1.1.1 Importance and Purpose ..................................................................................1
       1.1.2 Study Objectives ............................................................................................2
   1.2 Background and Rationale .......................................................................................2
       1.2.1 Neurochemistry of PG ..................................................................................2
           1.2.1.1 Serotonin ................................................................................................2
           1.2.1.2 Norepinephrine .....................................................................................3
           1.2.1.3 Dopamine ...............................................................................................3
           1.2.1.4 Psychostimulant Addiction as a Model for PG .......................................4
       1.2.2 Overview of the DA System .............................................................................4
           1.2.2.1 DA Receptor Subtypes ............................................................................5
           1.2.2.2 DA Signaling and Reward .......................................................................6
           1.2.2.3 Hedonic Impact and Inventive Salience ....................................................8
       1.2.3 DA System in PG .............................................................................................8
           1.2.3.1 Deficient DA Activation to Monetary Reward .........................................8
           1.2.3.2 Evidence for Sensitization in PG ..............................................................9
       1.2.4 D1 and D2 Receptors in Stimulation Addiction .................................................10
           1.2.4.1 Differential Effects of D1R and D2R .......................................................10
           1.2.4.2 D1R Activation in Reward .....................................................................11
           1.2.4.3 Deficits in D1R and D2R Availability and Function ...............................13
       1.2.5 D1R and D2R in Gambling Reinforcement and PG .......................................13
       1.2.6 Gender Differences in DA System and Reinforcement ..................................15
   1.3 Objectives ............................................................................................................17
   1.4 Hypotheses ............................................................................................................17

2. MATERIALS AND METHODS ....................................................................................19
   2.1 Study Design ........................................................................................................19
   2.2 Study Medications ...............................................................................................19
       2.2.1 Haloperidol ....................................................................................................19
       2.2.2 Fluphenazine .................................................................................................20
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2.3 Rationale for Drug Selection</td>
<td>20</td>
</tr>
<tr>
<td>2.2.4 Dextroamphetamine sulfate</td>
<td>20</td>
</tr>
<tr>
<td>2.2.5 Diphenhydramine</td>
<td>21</td>
</tr>
<tr>
<td>2.3 Participants</td>
<td>21</td>
</tr>
<tr>
<td>2.3.1 Inclusion/Exclusion Criteria</td>
<td>21</td>
</tr>
<tr>
<td>2.3.2 Screening Instruments</td>
<td>21</td>
</tr>
<tr>
<td>2.3.3 Group Matching</td>
<td>26</td>
</tr>
<tr>
<td>2.3.4 Participant Safety</td>
<td>26</td>
</tr>
<tr>
<td>2.3.5 Time commitment and Study Payment</td>
<td>26</td>
</tr>
<tr>
<td>2.4 Apparatus</td>
<td>27</td>
</tr>
<tr>
<td>2.4.1 Experimental Self-Report Scales</td>
<td>27</td>
</tr>
<tr>
<td>2.4.2 Devices</td>
<td>28</td>
</tr>
<tr>
<td>2.4.3 Slot Machine</td>
<td>28</td>
</tr>
<tr>
<td>2.4.4 Rapid Reading Task (RRT)</td>
<td>29</td>
</tr>
<tr>
<td>2.4.5 Exploratory Tasks</td>
<td>30</td>
</tr>
<tr>
<td>2.5 Procedure</td>
<td>31</td>
</tr>
<tr>
<td>2.5.1 Telephone Screening</td>
<td>31</td>
</tr>
<tr>
<td>2.5.2 Interview Screening</td>
<td>31</td>
</tr>
<tr>
<td>2.5.3 Physician’s Exam</td>
<td>32</td>
</tr>
<tr>
<td>2.5.4 Test Day Procedure</td>
<td>32</td>
</tr>
<tr>
<td>3. RESULTS</td>
<td>36</td>
</tr>
<tr>
<td>3.1 Subject Characteristics</td>
<td>36</td>
</tr>
<tr>
<td>3.2 Results for Visual Analog Scales</td>
<td>38</td>
</tr>
<tr>
<td>3.2.1 Desire to Gamble</td>
<td>38</td>
</tr>
<tr>
<td>3.2.1.1 Effect of Slot Machine</td>
<td>38</td>
</tr>
<tr>
<td>3.2.1.2 Effect of AMPH</td>
<td>42</td>
</tr>
<tr>
<td>3.2.2 Desire for Alcohol</td>
<td>45</td>
</tr>
<tr>
<td>3.2.2.1 Effect of Slot Machine</td>
<td>45</td>
</tr>
<tr>
<td>3.2.2.2 Effect of AMPH</td>
<td>48</td>
</tr>
<tr>
<td>3.2.3 Pleasurable Effects</td>
<td>51</td>
</tr>
<tr>
<td>3.2.3.1 Effect of Slot Machine</td>
<td>51</td>
</tr>
<tr>
<td>3.2.3.2 Effect of AMPH</td>
<td>54</td>
</tr>
</tbody>
</table>
3.3 ARCI Subjective Drug-Like Effects

3.3.1 ARCI-MBG (Euphoria) Subscale

3.3.1.1 Effect of Slot Machine

3.3.1.2 Effect of AMPH

3.3.2 ARCI-AMP (Psychomotor Stimulation) Subscale

3.3.2.1 Effect of Slot Machine

3.3.2.2 Effect of AMPH

3.3.3 ARCI-LSD (Dysphoria) Subscale

3.3.3.1 Effect of Slot Machine

3.3.3.2 Effect of AMPH

3.4 Results for Profile of Mood States

3.4.1 Vigor Subscale

3.4.1.1 Effect of Slot Machine

3.4.1.2 Effect of AMPH

3.5 Betting Behavior

3.5.1 Lines Selected Per Spin

3.5.2 Credits Wagered Per Line on Each Spin

3.5.3 Total Spins Per Session

3.5.4 Winnings (Final Credit Tally)

4. DISCUSSION

4.1 Subject Characteristics

4.2 Subjective Effects

4.2.1 Motivational Effects

4.2.1.1 Desire to Gamble

4.2.1.2 Desire for Alcohol

4.2.2 Pleasurable Effects

4.2.2.1 VAS-Pleasurable Effects

4.2.2.2 ARCI-MBG: Drug-like euphoric effects

4.2.2.3 ARCI-LSD: Dysphoria

4.2.3 Other Effects

4.2.3.1 ARCI-AMP: Psychomotor stimulation

4.2.3.2 POMS-Vigor: Energy and activation
4.3 Betting Behavior ........................................................................................................... 100
4.4 General Discussion ...................................................................................................... 101
4.5 Limitations ................................................................................................................ 104
4.6 Future Directions ....................................................................................................... 105
4.7 Conclusions ............................................................................................................... 105

REFERENCES ................................................................................................................... 107

APPENDICES ..................................................................................................................... 116
LIST OF TABLES

Table 1  Summary of inclusion criteria………………………………………………..25
Table 2  Summary of test session procedure…………………………………………33
Table 3  Mean (SD) background characteristics and trait scores of subjects in each subgroup………………………………………………………………………36
Table 4  Mean (SD) scores on personality traits of subjects in each subgroup……37
Table 5  Mean (SD) scores for cognitive tasks of subjects in each subgroup……38
Table 6  Summary of the effects of HAL and FLU on gambling and AMPH reinforcement…………………………………………………………………………92
LIST OF FIGURES

1. Proposed inverted U relationship between D1 receptor activation and stimulant reinforcement ................................................................. 12

2. Proposed inverted U relationship between D1 receptor activation and gambling reinforcement, and predicted effects of DA antagonists .................................................. 15

3. Changes in Mean VAS Desire to Gamble scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level) with pre-treatment of HAL vs. placebo ................................................................. 40

4. Changes in Mean VAS Desire to Gamble scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level) with pre-treatment of FLU vs. placebo ................................................................. 41

5. Changes in Mean VAS Desire to Gamble scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level) with pre-treatment of HAL vs. placebo ................................................................. 43

6. Changes in Mean VAS Desire to Gamble scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level) with pre-treatment of FLU vs. placebo ................................................................. 44

7. Changes in Mean VAS Desire for Alcohol scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level) with pre-treatment of HAL vs. placebo ................................................................. 46

8. Changes in Mean VAS Desire for Alcohol scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level) with pre-treatment of FLU vs. placebo ................................................................. 47

9. Changes in Mean VAS Desire for Alcohol scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level) with pre-treatment of HAL vs. placebo ................................................................. 49

10. Changes in Mean VAS Desire for Alcohol scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level with pre-treatment of FLU vs. placebo ................................................................. 50

11. Mean VAS Pleasurable Effects scores of slot machine with pre-treatment of HAL vs. placebo ........................................................................................................ 52

12. Mean VAS Pleasurable Effects scores of slot machine with pre-treatment of FLU vs. placebo ........................................................................................................ 53
13. Mean VAS Pleasurable Effects scores of AMPH with pre-treatment of HAL vs. placebo ................................................................. 55

14. Mean VAS Pleasurable Effects scores of AMPH with pre-treatment of FLU vs. placebo ................................................................. 56

15. Changes in Mean ARCI MBG scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level) with pre-treatment of HAL vs. placebo ................................................................. 58

16. Changes in Mean ARCI MBG scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level) with pre-treatment of FLU vs. placebo ................................................................. 59

17. Changes in Mean ARCI MBG scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level) with pre-treatment of HAL vs. placebo ................................................................. 61

18. Changes in Mean ARCI MBG scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level) with pre-treatment of FLU vs. placebo ................................................................. 62

19. Changes in Mean ARCI AMP scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level with pre-treatment of HAL vs. placebo ................................................................. 64

20. Changes in Mean ARCI AMP scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level with pre-treatment of FLU vs. placebo ................................................................. 65

21. Changes in Mean ARCI AMP scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level with pre-treatment of FLU vs. placebo ................................................................. 67

22. Changes in Mean ARCI AMP scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level with pre-treatment of FLU vs. placebo ................................................................. 68

23. Changes in Mean ARCI LSD scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level with pre-treatment of HAL vs. placebo ................................................................. 70

24. Changes in Mean ARCI LSD scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level with pre-treatment of FLU vs. placebo ................................................................. 71
25. Changes in Mean ARCI LSD scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level) with pre-treatment of HAL vs. placebo .................................................................................................................. 73

26. Changes in Mean ARCI LSD scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level) with pre-treatment of FLU vs. placebo .................................................................................................................. 74

27. Changes in Mean POMS Vigor scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level) with pre-treatment of HAL vs. placebo .................................................................................................................. 76

28. Changes in Mean POMS Vigor scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level) with pre-treatment of FLU vs. placebo .................................................................................................................. 77

29. Changes in Mean POMS Vigor scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level) with pre-treatment of HAL vs. placebo .................................................................................................................. 79

30. Changes in Mean POMS Vigor scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level) with pre-treatment of FLU vs. placebo .................................................................................................................. 80

31. Mean number of lines selected per spin on a slot machine game with pretreatment of HAL vs. placebo .................................................................................................................. 82

32. Mean number of lines selected per spin on a slot machine game with pretreatment of FLU vs. placebo .................................................................................................................. 82

33. Mean number of credits bet per line on a slot machine game with pretreatment of HAL vs. placebo .................................................................................................................. 84

34. Mean number of credits bet per line on a slot machine game with pretreatment of FLU vs. placebo .................................................................................................................. 84

35. Mean total spins per 15-min on the slot machine with pretreatment of HAL vs. placebo .................................................................................................................. 86

36. Mean total spins per 15-min on the slot machine with pretreatment of FLU vs. placebo .................................................................................................................. 86

37. Mean winning credits (final tally after 15-min) on a slot machine game with pretreatment of HAL vs. placebo .................................................................................................................. 88

38. Mean winning credits (final tally after 15-min) on a slot machine game with pretreatment of FLU vs. placebo .................................................................................................................. 88
LIST OF ABBREVIATIONS

5-HT  Serotonin
AC    Adenylyl cyclase
ADS   Alcohol Dependence Scale
AMPH  d-amphetamine
ARCI  Addiction Research Clinical Inventory
ARCI-AMP ARCI Amphetamine subscale
ARCI-BG ARCI Benzodrine Group subscale
ARCI-LSD ARCI Lysergic Acid Diethylamine subscale
ARCI-MBG ARCI Morphine/Benzodrine subscale
ARCI-PCAG ARCI Pentobarbital/Chlorpromazine/Alcohol subscale
BDI   Beck Depression Inventory
cAMP  Cyclic adenosine monophosphate
CCE   Capsule contents evaluation
CS    Conditioned stimulus
DA    Dopamine
DAST  Drug Abuse Screening Test
DSM   Diagnostic and Statistical Manual of Mental Disorder
EIS   Eysenck Impulsiveness Scale
EPI   Eysenck Personality Inventory
fMRI  Functional magnetic resonance imaging
FTND  Fagerström Test for Nicotine Dependence
GBQ   Gambling Beliefs Questionnaire
GDT   Game of Dice Task
GPCR  G-protein coupled receptor
HC    Healthy Control
NAc   Nucleus accumbens
NE    Norepinephrine
PET   Positron emission tomography
PG    Pathological Gambling
PIM   Pimzoide
POMS  Profile of Moods State
PPI   Pre-pulse inhibition
RRT   Rapid Reading Task
SCID  Structured Clinical Interview for the DSM-IV
SOGS  South Oaks Gambling Screen
SST   Stop Signal Task
TDL   Temporal difference learning
UCS   Unconditioned stimulus
VAS   Visual Analogue Scale
VLT   Video Lottery Terminal
VTA   Ventral tegmental area
WAIS  Wechsler Adult Intelligence Scale
WCST  Wisconsin Card Sort Task
LIST OF APPENDICES

Appendix A: Binding profiles of haloperidol and fluphenazine……………………………………..116
   Table A-i. Receptor binding of haloperidol and fluphenazine at D2 receptors ………117
   Table A-ii. Receptor binding of haloperidol and fluphenazine at D1 receptors……..118
   Table A-iii. Receptor binding of haloperidol and fluphenazine at D2, D3, and D4
                receptors…………………………………………………………………………………119
   Table A-iv. Receptor binding of haloperidol and fluphenazine at serotonin
                receptors…………………………………………………………………………………120
   Table A-v. Receptor binding of haloperidol and fluphenazine at muscarinic
              acetylcholine receptors………………………………………………………………121
   Table A-vi. Receptor binding of haloperidol and fluphenazine at histamine H1
              receptors………………………………………………………………………………121
   Table A-vii. Receptor binding of haloperidol and fluphenazine at α1 adrenergic
               receptors………………………………………………………………………………121
   Table A-viii. Receptor binding of haloperidol and fluphenazine at α2 adrenergic
                receptors……………………………………………………………………………121

Appendix B: SCID Inclusion/Exclusion Criteria……………………………………………………..122

Appendix C: Recruitment ads for HC and PG subjects…………………………………………..124
   Healthy Volunteers………………………………………………………………………………126
   Pathological Gamblers………………………………………………………………………126

Appendix D: Informed Consent Form………………………………………………………………..127

Appendix E: Physical Exam Inclusion/Exclusion Criteria…………………………………………136

Appendix F: Flow Chart of Subject Recruitment, Eligibility and Group Assignment………..139
1. INTRODUCTION

1.1 Study Overview

1.1.1 Importance and Purpose

Pathological gambling (PG) afflicts ~3% of Canadians (el-Guebaly et al. 2006). With increased availability after liberalization and through proliferation of Internet gambling sites, as well as its association with adverse personal and social consequences, PG is becoming one of the major public health issues in Canada (Ferguson, 2011).

Due to the complexity and variation in its etiology and symptoms, and the frequent presence of co-morbidities (e.g., alcohol abuse, depression, anxiety disorder), pharmacotherapy of PG is a challenge. Although some promising candidates exist, a recent meta-analysis found no clinically significant benefits of any medications tested to date for the treatment of PG (Bartley and Bloch, 2013). As a result, there is no formally approved medication for PG. A better understanding of the neurochemistry of PG could facilitate medication development.

Due to the lack of well-defined and validated animal models of the PG phenotype, studies with human PG subjects are currently preferred.

PG was recently re-classified as a behavioral addiction, reflecting the similar symptom profile of PG and substance dependence (Holden 2001, Potenza 2008). Dopamine (DA) plays a pivotal role in substance dependence and, by implication, in PG, as well (Potenza 2008, Zack and Poulos 2009). The importance of DA in PG has been shown in genetic, neurochemical, and behavioral studies. In fact, DA dysregulation has been implicated in PG, and indirect evidence points to possible DA sensitization (i.e., hyper-reactivity due to repeated DA activation) in PG subjects (Meyer et al. 2004, Stojanov et al. 2003). Alterations in DA system reactivity may be especially important for understanding heterogeneity in the etiology and clinical presentation of PG. One critical source of this heterogeneity is gender.¹ A substantial body of research with healthy animals and humans has shown that females are generally more susceptible to sensitization (Brown et al. 2012, Becker 1999). Given the putative role of sensitization in the escalation and relapse to addictive behavior (Vanderschuren and Ahmed 2013), gender-based differences in sensitization may contribute to ‘telescoping’ (rapid escalation to severe problems) in female PG subjects (Fattore et al. 2014, Ceylan-Isik et al. 2010).

The DA D1 receptor (D1R) codes conditioned aspects of reward (cue reactivity) and mediates development of sensitization (DA hyper-responsiveness) in the case of psychostimulant drugs (Vanderschuren et al 1999, Wolf 2004). Indirect evidence suggests

¹ Throughout this thesis, the term ‘gender’ will be used when referring to humans, and the term ‘sex’ will be used when referring to animals or to both humans and animals.
functional anomalies in D1R in PG subjects, and these may be more pronounced in female PG subjects. Variations in the availability and/or function of D1R could be one of the key factors in the development of PG. Therefore, characterizing the roles of D1 receptors in gambling reinforcement could provide important insight for the development of pharmacotherapy for PG. If D1R function is deficient in PG, medications that restore D1R signaling may decrease sensitization or its consequences (Fletcher PJ et al, 2005; Shuto et al 2006, 2008) and associated PG symptoms. If D1R deficits are greater in female PG subjects, gender-based D1R pharmacotherapy may be warranted.

1.1.2 Study Objectives

The specific objectives of this study are

(a) To begin to clarify the role of D1R in mediating the rewarding aspects of gambling (slot machine game)

(b) To compare D1R mediated slot machine effects with those of the DA releaser, d-amphetamine (AMPH)

(c) To compare and contrast D1R mediated effects of gambling and AMPH effects in PG and healthy control (HC) subjects

(d) To investigate possible gender differences in D1R-mediated responses to these two reinforcers.

1.2 Background and Rationale

1.2.1 Neurochemistry of PG

The etiology of PG is complex and heterogeneous, and multiple neurotransmitter systems have been shown to be involved in the development of the disorder (Blaszczyński and Nower 2002). Among them, serotonin (5-hydroxytryptamine; 5-HT), norepinephrine (NE) and DA have been studied most extensively, and their genes have been found to contribute equally to the risk for PG (Comings et al. 2001).

1.2.1.1 Serotonin (5-HT)

In the central nervous system 5-HT functions critically in the regulation of mood, and in memory and learning processes (Miszkiel et al. 2011). Studies have found disturbances in 5-HT metabolism in PG subjects, with reduced activity of monoamine oxidase, a primary 5-HT
metabolizing enzyme (Blanco et al. 1996); decreased levels of 5-HT itself and its precursor, 5-hydroxytryptophan; and increased level of its main metabolite, 5-hydroxyindoleacetic acid in cerebrospinal fluid (Nordin and Sjödin 2006). Evidence also suggests a role for several 5-HT receptor subtypes in PG. An association between a specific genotype of 5-HT$_{2A}$ (C/C at T102C) receptor and PG phenotype has been identified (Wilson et al. 2013), and PG severity has been linked to increased levels of 5-HT$_{1B}$ receptors (Potenza et al. 2013), both suggesting 5-HT receptor anomalies as a vulnerability factor for PG. Furthermore, hyper-reactivity to 5-HT challenge and hypo-reactivity to 5-HT transporter inhibitors has been found in PG subjects, indicating 5-HT dysregulation, with greater dysregulation correlated with greater symptom severity (Pallanti et al. 2006).

1.2.1.2 Norepinephrine (NE)

The NE system is involved in mediating alertness, arousal and the responses of the reward system (Sofuoglu and Sewell 2009). Similar to 5-HT, evidence indicates disturbances of NE metabolism in PG subjects. Increased central noradrenergic activity has been suggested in PG subjects, with increased urinary levels of NE and its end stage metabolite, vanillylmandelic acid, as well as increased central and plasma levels of 3-methoxy-4-hydroxyphenylglycol, another major NE metabolite (Roy et al. 1988). In addition, highly significant correlations between such elevations and scores on the Extraversion scale of Eysenck Personality Questionnaire have been identified in PG subjects. PG subjects also demonstrate greater increase in their plasma NE levels in response to gambling as compared to HC subjects (Meyer et al. 2004).

1.2.1.3 Dopamine (DA)

DA is critical for cognition, motivation, and reward, among other functions, and it plays pivotal roles in both substance and behavioral addiction (Callahan et al. 1991, Baik 2013). DA dysregulation has been implicated in PG subjects in multiple studies. Studies have demonstrated greater increases in central and plasma levels of DA and its metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, in response to gambling in PG as compared to HC subjects (Bergh et al. 1997). Polymorphisms in DRD1 and DRD2 genes have been linked to PG in multiple studies, providing indirect evidence that DA receptor variations contribute to PG (Comings et al. 1996, Comings et al. 1997, Lobo et al. 2010).
Furthermore, studies have shown that DA D2/3R agonist medications can induce PG in a subset of patients with Parkinson’s disease (Voon et al. 2011a, Potenza et al. 2013, Santangelo et al. 2013), further indicating an important role for DA in PG.

1.2.1.4 Psychostimulant Addiction as a Model for PG

Historically, PG has been classified as an Impulse Control Disorder (DSM-III-R, DSM-IV; APA, 1987, 1994), but was re-classified as a behavioral addiction in the category shared with substance dependence disorders in the latest edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (APA, 2013). This reflects important commonalities between PG and substance addictions, including craving, impulsivity, sensation seeking, cue reactivity, withdrawal syndrome and high rates of relapse (Tavares et al. 2005). A variety of evidence suggests that PG may be especially similar to psychostimulant addiction from the perspective of neurochemistry, and in particular, with regard to the role of DA. Whereas other drugs of abuse may depend on systems other than DA for their reinforcing effects, DA has been shown to be both necessary and sufficient for psychostimulant reinforcement (Pierce and Kumaresan, 2006). Based on a range of clinical and preclinical evidence, Zack and Poulos (2009) argued that DA plays a similar fundamental role in PG. In psychostimulant addiction, chronic exposure to the drug leads to repeated episodes of intense DA activation. In the case of amphetamine, this DA activation plays a pivotal role in sensitization (Robinson and Berridge 2000). Sensitization is considered to be a core pathological process that mediates increased incentive salience for the target drug and corresponding compulsive drug seeking behaviors (Robinson and Berridge 2001). It has been suggested that sensitization is not unique to stimulant drugs, but could also be produced by environmental stimuli that modulate the DA system (Robinson and Becker 1986). If so, chronic exposure to environmental reinforcers like gambling could conceivably cause sensitization. Accordingly, this study focused on the role of DA and possible sensitization-like changes in individuals with PG.

1.2.2 Overview of the DA System

There are multiple distinct DA systems in the brain, which play critical roles in functions like motor control, cognition, motivation, and reward (Schultz 2001). The largest group of DA neurons in human brain is located in the ventral tegmental area (VTA), from which they project to numerous brain areas. The two largest projections are the mesolimbic
pathway, targeting nucleus accumbens (NAc) and limbic structures, and the mesocortical pathway, targeting the frontal cortex. These two dopaminergic pathways are especially important in reward processing of the brain (Tzschentke et al. 2000). Natural rewards such as food and sex, as well as drugs of abuse, stimulate DA release, which associates these stimuli with the experience of reward (Schultz et al. 1997).

1.2.2.1 DA Receptor Subtypes

There are five established DA receptor subtypes, which are categorized into two major families: D1-like family, including D1R and D5R subtypes, and D2-like family, including D2R, D3R and D4R subtypes.

D1-like family receptors are excitatory G-protein-coupled receptors (GPCRs). Upon activation, Gs alpha subunit activates adenylyl cyclase (AC), increasing the level of the second messenger cyclic AMP (cAMP) and leading to a variety of downstream signaling cascades. D1R is the most abundant DA receptor subtype, expressed throughout the brain (e.g., prefrontal cortex, striatum, and substantia nigra) (Wamsley et al., 1992), with greater density in the mesolimbic, mesocortical and nigrostriatal areas, and found primarily post-synaptically. D1Rs are involved in locomotor activity, reward and reinforcement mechanisms (Beaulieu and Gainetdinov 2011), as well as memory and learning processes (Castner and Williams 2007). In addition, D1R also mediates the development of sensitization, which is believed to be one of the core pathological processes in addiction (Vanderschuren and Pierce 2010). D1R has lower affinity for DA as compared to other subtypes, and therefore responds primarily to phasic firing, composed of DA bursts that occur when an unexpected reward is delivered (Dreyer et al. 2010). D5 receptors are found at much lower levels in prefrontal cortex, substantia nigra, hypothalamus and the hippocampus (Beaulieu and Gainetdinov 2011). Evidence suggests they also play roles in cognitive functions such as learning and memory (Missale et al. 1998).

The D2-like family of receptors is inhibitory with respect to GPCRs, and upon binding, Gi alpha subunit inhibits AC activity leading to decreases in cAMP level. D2Rs are expressed in many brain regions, with greatest density in striatum, NAc, and the olfactory tubercle (Beaulieu and Gainetdinov 2011). They are located both pre- and post-synaptically. Presynaptic D2 auto-receptors primarily regulate the synthesis and release of DA via a negative feedback mechanism. Postsynaptic D2Rs are involved in the control of locomotor activity, impulsivity, and emotionality as well as various cognitive functions (e.g., reversal learning).
Because D2Rs have high affinity for DA, they mainly respond to basal or tonic DA transmission and are sensitive to pauses in DA transmission that occur when an expected reward is omitted (Schultz et al. 1997). D3Rs are mostly found in limbic areas, and involved in reward and reinforcement mechanisms, as well as locomotor activity especially in response to cues and when the response demands considerable effort (Beaulieu and Gainetdinov 2011, Sokoloff et al. 2006). D4 receptors have very limited expression in the brain, and are involved in specific aspects of cognitive function as well as regulation of DA release (Missale et al. 1998, Sokoloff et al. 2006, Rondou et al. 2010).

**1.2.2.2 DA Signaling and Reward**

As noted above, due to differences in their affinity, D1R and D2R respond primarily to phasic and tonic DA signal, respectively. These two different types of DA signals have been considered to be very important in reward processing and reinforcement. Phasic DA signaling is composed of spike-dependent/burst DA releases in response to a stimulus which leads to transient high levels of DA in the synaptic cleft. Phasic DA is considered to encode the primary reward signal, and often modulated by substances of abuse that cause reward-seeking actions (Ostlund et al. 2014). A substantial amount of evidence indicates that, after initial encounters with a rewarding stimulus, phasic DA release follows after the delivery of the cue for reward (e.g., the sight, smell or thought of palatable food) rather than the delivery of the reward itself (e.g., the consumption of the palatable food) (Redish 2004). The term “temporal difference learning” (TDL) is used to describe the advance in timing of phasic DA release to cues for reward that facilitates timely responding to the reward itself (Redish 2004). Many studies have indicated that the phasic DA release does not encode reward itself, but the reward prediction error – that is, the discrepancy between an organism’s expectation of reward and the reward that is actually delivered (Fiorillo et al. 2003). The more reliably a cue (conditioned stimulus; CS) predicts a reward (unconditioned stimulus; UCS), the lower the reward prediction error and hence the lower the post-reward phasic DA release. Conversely, rewards that are novel or unpredicted cause a substantial reward prediction error and correspondingly large DA spike.

DA neurons also appear to code another dimension of reward aside from its familiarity and whether or not it is expected. Specifically, DA neurons have been found to code the uncertainty of reward delivery in the presence of a cue that signals the potential for reward but
does not confirm if in fact the reward will be delivered. This type of uncertain reward signaling would seem to have special importance in the case of gambling where cues are abundant, and rewards are expected but never fully predicted. To characterize this process, Fiorillo et al (2003) conducted a study where monkeys were exposed to 25%, 50%, 75% and 100% conditioned (variable) reward schedules, where the cue was an icon, the reward was a sip of juice, and the dependent measure was midbrain DA cell firing as recorded by an electrode. The investigators found that the activity of DA neurons was lowest in the 100% condition in which the cue always predicted reward delivery, and highest in the 50% condition (predictability equivalent to chance alone) in which the cue provided essentially no information about whether reward would be delivered or not on a given trial (Fiorillo et al. 2003). This is noteworthy given its close correspondence to the actual frequency of reward delivery over thousands of trials (46%) on a commercial slot machine (Tremblay et al. 2011). Collectively the evidence shows that phasic DA release after the UCS codes a reward prediction error that mediates reinforcement (the likelihood of repeating a behavior); this signal migrates to the CS after prior exposure, and elicits reward-seeking behaviors (approach); and a slower, less intense but escalating series of DA bursts between the CS and the USC codes the uncertainty of reward delivery under conditions of variable reward.

Tonic DA signaling is conveyed by basal low level DA transmission, and is thought to modulate the intensity of the phasic response to the primary reward signal through inhibitory pre-synaptic auto-receptors (Dreyer et al. 2010). Studies have suggested that the increase in tonic dopamine level after chronic exposure to a reinforcer (e.g., repeated drug administration) opposes phasic DA signaling via the stimulation of auto-receptors, leading to the requirement of a rewarding stimulus with higher intensity (i.e., increased dose or frequency of drug administration) to restore the phasic DA signal (Grace, 2000). In this way, sensitization of tonic DA transmission may promote tolerance to the rewarding effects of a drug by inhibiting the phasic DA response to drug administration. Although tolerance and sensitization denote opposite processes, the fact that sensitization is mediated by D2R (which is inhibitory) could account for why elevated signaling at this receptor site translates into tolerance – i.e., reduced phasic signal at excitatory D1R.
1.2.2.3 Hedonic Impact and Incentive Salience

It has been suggested that there is a distinction between the hedonic impact (the pleasure, or “liking”) of a rewarding stimulus, and its incentive salience (the motivation, or “wanting”). Considerable evidence indicates that the DA system primarily mediates incentive salience, but not hedonic impact (Berridge 2004, 2007) – although psychostimulant drugs may be an exception to this (Sofuogolu et al. 2008). For example, animals with lesions in their VTA DA system show reduced motivation for reward as they do not seek food (Berridge 2004), but experience pleasure assessed by facial expressions when food is placed in their mouths (Peciña 1997). In addition, in Parkinson’s disease patients with DA agonist medication dependence, VTA DA transmission levels are correlated with self-report score of “wanting” but not “liking” (Evans et al. 2006). It is unclear what role DA might play in the hedonic vs. incentive motivational aspects of gambling, and whether its role differs in PG subjects versus healthy controls.

1.2.3 DA System in PG
1.2.3.1 Deficient DA Activation to Monetary Reward

Results from functional magnetic resonance imaging (fMRI) studies show significant deficits of DA activation in response to monetary reward (a core element of gambling) in PG subjects as compared to healthy subjects (Knutson et al. 2001, Reuter et al. 2005). The deficits were primarily observed in the ventral striatum, a key targeting area of mesolimbic dopaminergic pathway and critically involved in reward reinforcement. In addition, there is a positive correlation between the degree of deficits in DA activation and the severity of PG symptoms.

Reduced striatal activation in response to reward has been considered as one of the key characteristics in psychostimulant addiction (Volkow et al. 1997, Volkow et al. 2002). Cocaine abusers show significant reduction in methylphenidate-induced striatal DA release and also report decreases in the pleasurable effects of stimulants. Furthermore, positron emission tomography (PET) studies have demonstrated a direct correlation between the degree of stimulant-induced striatal DA release and the subjective reinforcing effects (e.g., “high”) of stimulants (Drevets et al. 2001, Volkow et al. 1999).
If PG and psychostimulant addiction are neurochemically similar syndromes, DA manipulations may have similar effects on responses to gambling-related stimuli as they do for psychostimulant drugs.

### 1.2.3.2 Evidence for Sensitization in PG

Psychostimulant sensitization is the process whereby chronic exposure to stimulant drugs induces neuroplasticity that gives rise to increased DA response to signals for reward and to pharmacological DA challenge (Wolf et al. 2004). This manifests as hyper-locomotor response and enhanced DA release in the ventral striatum to a low dose of a stimulant drug after prior exposure to stimulants. In both animal and human studies, chronic administration of stimulant drugs such as AMPH and cocaine has been demonstrated to reliably produce robust DA sensitization (Robinson and Becker 1986, Boileau et al. 2006). Based on the hypothesis of similar DA disruptions in psychostimulant addiction and PG, it has been suggested that chronic exposure to gambling-related stimuli might induce sensitization in PG.

This has recently been demonstrated in a study with rats, in which chronic exposure to a gambling-like regimen - cues (a light) for uncertain reward (a sip of sucrose solution under 50% variable ratio reward schedule) enhanced locomotor response to a subsequent low-dose AMPH challenge compared with chronic exposure to cues that reliably predicted sucrose reward (a regimen unlike gambling) (Zack et al. 2014). Given the reliable linkage between striatal DA release and locomotor response to AMPH, the findings indicate that a gambling-like reward schedule, characterized by uncertainty of reward delivery in the presence of cues for reward, appeared to promote enhanced DA response to AMPH, an outcome that would be expected following chronic exposure to AMPH itself.

In humans, a range of indirect evidence suggests the possibility of DA sensitization in (Bergh et al. 1997, Zack and Poulos 2004). The most direct evidence for this comes from a recent PET study, in which stimulant-naïve PG subjects demonstrated increased AMPH-induced DA release in the dorsal striatum as compared to HC subjects (Boileau et al. 2013). In ventral striatum, the AMPH-induced DA release correlated directly with severity of PG symptoms on the South Oaks Gambling Screen (SOGS; Lesieur and Blume 1987). The latter result is noteworthy, given that a PET study of PG subjects in another lab, found a similar correlation between PG severity on the SOGS and ventral striatal DA release in response to large monetary rewards on a slot machine game (Joutsa et al 2012). Thus, AMPH and
gambling recruit a common pattern of severity-related DA response in PG subjects that is consistent with progressive sensitization of DA pathways. The PET data from these two studies directly support the psychostimulant model of PG (Zack and Poulos 2004). The rat study on cross-sensitization to AMPH following chronic exposure to cues plus uncertain reward further suggests that chronic exposure to gambling may have played a causal role in the elevated DA responses found in the PET studies of human PG subjects.

These findings extend the indirect evidence which pointed to possible sensitization in PG on the basis of deficient pre-pulse inhibition (PPI), an electrophysiological index of habituation (Stojanov et al. 2003), and cognitive rigidity in PG subjects (Marazziti et al. 2008), both of which are reliably observed in AMPH-sensitized rats and mediated by DA. Together the evidence strongly supports the existence of a sensitization-like syndrome in individuals with PG.

1.2.4 D1 and D2 Receptors in Stimulant Addiction

1.2.4.1 Differential Effects of D1R and D2R

D1R plays a pivotal role in stimulant reward via its preferential response to phasic DA release. In animal studies, “conditioned place preference”, a measure of the learned motivational aspect of reward (approaching a context previously linked with pleasure), has been reliably disrupted by D1R blockade (Baker et al. 1998, Cervo and Samanin 1995). In studies of cocaine self-administration, D1R blockade leads to an increase in cocaine self-administration which is considered as compensation for reduced rewarding effect of cocaine per unit (Quinlan et al. 2004). In cocaine addicts, the D1R antagonist ecopipam dose-dependently reduces the self-reported euphoric effects and craving following acute cocaine treatment, supporting an important role for D1R in human stimulant reward, from the perspectives of both pleasure and incentive motivation (Romach et al. 1999). However, an 8-day regimen of ecopipam has been found to significantly increase cocaine self-administration as well as the self-reported ‘good effects’ and ‘high’ of cocaine in cocaine addicted subjects (Haney et al. 2001). These findings suggest that D1R directly mediates the incentive motivational and hedonic effects of cocaine, and that increased availability or sensitivity of D1R (in response to chronic blockade) is associated with greater cocaine reward.

D2R has been suggested to primarily play a modulatory role in psychostimulant reward via a negative feedback mechanism. Evidence shows that chronic cocaine exposure leads to
increases in tonic dopamine levels (in animals) which oppose phasic dopamine release by activating pre-synaptic D2 auto-receptors (Grace 2000). This process is thought to cause increased drug administration in order to restore phasic DA and D1R stimulation (Self 1998, Grace 2000). Based on electrophysiological studies (Shi et al. 1997, Goto and Grace 2005), D2R blockade has been suggested to restore functions of hippocampus and prefrontal cortex by preferentially enhancing D1R stimulation in subjects with cocaine sensitization. In a PET study of healthy subjects, the availability of D2R has been inversely correlated to subjective rewarding effects of stimulants including amphetamine and methylphenidate (Drevets et al. 1999; Volkow et al 2002).

In general, these findings indicate important but differential roles of D1R and D2R in the subjective rewarding and incentive motivational effects of psychostimulants. Based on the hypothesized similarities between gambling and stimulant-related effects, we expect D1R and D2R to play similar roles in gambling and AMPH reinforcement in PG subjects.

1.2.4.2 D1R Activation in Reward

The relationship between D1R activation and stimulant reward has been studied extensively in animals and to a lesser extent in humans. In a series of studies with healthy volunteers, H. de Wit et al. examined the effects of D2R antagonists with varying affinities for D1Rs on subjective effects of AMPH (Brauer and de Wit 1996, Wachtel et al. 2002, Brauer and de Wit 1995). The antagonists used were pimzoide (PIM) a selective, high affinity D2R antagonist, haloperidol (HAL), a preferential D2R antagonist with moderate D1R affinity, and fluphenazine (FLU), a mixed D1-D2 antagonist with high affinity for both receptors. Healthy subjects were pretreated with one of the three antagonists and then received either AMPH or met-amphetamine. Subjective reward was measured using the Morphine-Benzedrine (MBG) sub-scale of the Addiction Research Centre Inventory (ARCI) which reflects drug-induced ‘euphoria.’ In response to 10-mg AMPH, 1-mg PIM enhanced euphoric effects relative to both 0-mg PIM (placebo) and 2-mg PIM. This result may reflect increased AMPH-induced DA signaling at D1R during blockade of inhibitory pre-synaptic D2 auto-receptors by PIM. The relatively lower euphoric response with 2-mg PIM + 10 mg AMPH is suggestive of possible supra-optimal D1R activation in healthy volunteers with near-optimal drug-free D1R signaling. In the 20-mg AMPH treatment group, PIM dose-dependently reduced euphoric effects, consistent with supra-optimal D1R activation at a higher AMPH dose. These findings suggest
an inverted U relationship (bell-shaped) between D1R stimulation and stimulant reward, where
the rewarding effects increase as D1R activation increases until an optimal level is reached,
and further D1R activation causes adverse subjective effects (Figure 1). The inverted U pattern
has been demonstrated in numerous studies examining the relationship between cortical D1R
activation and “on-line” cognitive processing – like working memory (Seamans and Yang
2004).

Figure 1. Proposed inverted U relationship between D1 receptor activation and stimulant
reinforcement (Seamans and Yang 2004). 10-mg AMPH plus 1-mg PIM enhanced euphoric effects,
reflect increased AMPH-induced DA signaling at D1R during blockade of inhibitory pre-synaptic D2
auto-receptors by PIM. The relatively lower euphoric response with 2-mg PIM + 10 mg AMPH is
suggestive of possible supra-optimal D1R activation in healthy volunteers with near-optimal drug-free
D1R signaling. In the 20-mg AMPH treatment group, PIM dose-dependently reduced euphoric effects,
consistent with supra-optimal D1R activation at a higher AMPH dose.

The possibility of an inverted U relationship between D1R stimulation and AMPH-
induced euphoria is also supported by results from a study on the D1-D2 antagonist FLU
(Brauer and de Wit 1995). In response to 20-mg AMPH, 3-mg FLU enhanced euphoric effects
relative to both 0-mg FLU (placebo) and 6-mg FLU, a similar pattern to the effects of PIM in
10-mg AMPH treatment group. This further supports the idea of optimal D1R activation,
where in the case of FLU, enhanced D1R stimulation (higher AMPH dose) together with
partial D1R blockade (but not extensive D1R blockade) yields the optimal outcome (maximal
euphoria).
HAL has similar affinity to D2R but only 1/20th the affinity for D1R as FLU. This would lead to similar increase in DA release via D2 auto-receptor blockade but greater D1R activation under HAL at the same dose. The study with 3-mg HAL found no change in the euphoric effect of 20-mg meth-amphetamine compared to placebo. The lack of enhancement may reflect a change from slightly sub-optimal to slightly supra-optimal D1R activation, with no net change in euphoric effects.

The dose-response pattern of AMPH and methamphetamine effects following pre-treatment with PIM, FLU and HAL supports the inverted U relationship between D1R activation and subjective stimulant reinforcement. This relationship also suggests that the rewarding effects of a stimulant may partially depend on baseline D1R function. For example, the same dose of stimulant could have highly pleasurable effects in subjects with low baseline D1R function (sub-optimal baseline), as it optimizes D1R activation; but adverse effects in subjects with high baseline D1R function (near-optimal), as it causes excessive D1R activation.

1.2.4.3 Deficits in D1R and D2R Availability and Function

Due to their differences in affinities and locations, D1R and D2R respond primarily for stimulus-induced DA release and basal low level of DA, respectively. In healthy individuals, the interactions between these receptors involve both cooperative and countervailing effects (Shi et al. 1997). It has been shown that chronic DA activation following chronic exposure to stimulants disrupts D1-D2 interactions, which contribute to the process of sensitization and the development of substance dependence (Seeman 1989). In animals, studies have shown that chronic cocaine exposure decreases the binding at both D1R and D2R (Nikolaus et al. 2007), and chronic AMPH exposure reduces the availability of D2R (Chen et al. 1999, Ginovart et al. 1999). Post-mortem studies of met-amphetamine abusers show reduced ability to activate adenylyl cyclase through the D1R signaling cascade (Tong et al. 2003). These findings suggest an association between chronic stimulant exposure (chronic DA activation) and deficits in D1R and/or D2R function and availability. Insofar as PG resembles psychostimulant addiction, similar receptor anomalies may be present in subjects with PG.

1.2.5 D1R and D2R in Gambling Reinforcement and PG

Genetic studies have shown that genetic polymorphisms associated with hypo-functional D1R and D2R are over-represented in PG subjects (Comings et al. 1996, Comings et
al. 1997). However, PET studies have found similar D2R availability in PG vs. HC subjects (Boileau et al. 2013, Clark et al. 2012), suggesting that differences at the level of D1R function may play a more important role in PG subjects differential response to gambling or psychostimulant reinforcers. The model of H. de Wit’s study has been used to examine the effects of a D2 antagonist with low affinity for D1R on responses to gambling in PG vs HC subjects (Zack and Poulos 2007). In this study, exposure to a slot machine was applied instead of administration of AMPH or met-amphetamine. The study demonstrated that 3-mg HAL significantly enhanced the euphoric effects of gambling and desire to gamble in PG subjects, but not HC subjects, after playing a commercial slot machine. This is consistent with the finding that HAL did not enhance the euphoric effects of met-amphetamine in HC subjects.

As noted earlier, FLU (3-mg), increased AMPH reinforcement in HC subjects (Brauer and de Wit 1995), just as HAL increased gambling reinforcement in PG subjects, suggesting functional similarities between 3-mg FLU in HC subjects and 3-mg HAL in PG subjects. FLU would have reduced the availability of D1Rs compared to HAL, suggesting that deficits in baseline D1R function and/or availability in PG may result in a comparable response to reward-related DA activation under 3-mg HAL as HC subjects displayed under 3-mg FLU (i.e., moderate D1R blockade by FLU in HC subjects may be functionally equivalent to low D1R blockade by HAL in PG subjects). Considering the inverted U relationship between D1R activation and rewarding effects noted earlier, HAL may have optimized D1R activation in PG subjects with low baseline D1R function, but led to a shift from slightly sub-optimal to slightly supra-optimal D1R activation in HC subjects, resulting in no net change in subjective reward (Figure 2).

This evidence indirectly suggests a role for D1R in gambling reinforcement and further implies that baseline differences in D1R function may have contributed to group differences in response to slot machine gambling under HAL.
Figure 2. Proposed inverted U relationship between D1R activation and gambling reinforcement, and predicted effects of DA antagonists (adapted from Seamans and Yang 2004). Dashed lines indicate the proposed D1 baseline activation in PG and HC subjects. Arrows denote the expected increases in D1R activation under HAL and FLU. Optimal D1R activation is achieved under HAL in PG subjects and under FLU in HC subjects. FLU induces sub-optimal D1 activation in PG subjects and HAL induces supra-optimal activation in HC subjects.

1.2.6 Gender Differences in DA System and Reinforcement

PG is a heterogeneous disorder and gender may be an important source of this heterogeneity. Although men display a higher prevalence of substance addiction, research has found that women show a faster progression and more severe profile of addictive symptoms than men do (Ceylan-Isik et al. 2010), a phenomenon described as the “telescoping effect”. A parallel pattern has been observed in PG (Grant et al. 2012). Multiple factors including genetic, physiological, social and environmental have been shown to contribute to this gender difference (Fattore 2014). Evidence from recent studies suggests that gender differences in DA system may significantly contribute the “telescoping effect” (Becker 1999, Ceylan-Isik et al. 2010). For example, healthy women have been found to have relatively lower DA levels and higher levels of DA metabolites such as homovanillic acid and 3,4-dihydroxyphenylacetic acid than healthy men, suggesting possible differences in DA synthesis and metabolism (Ceylan-Isik et al. 2010). An obvious factor to consider with respect to gender differences is
gonadal hormones. Endogenous DA levels are thought to be affected by gonadal steroid hormones, as they have been shown to fluctuate during the estrous cycle in rodents (Pohjalainen et al. 1998). Hormonal modulation of DA function in striatum and NAc has been postulated to have implications for gender differences in susceptibility to substance addiction (Becker 1999).

Sex differences in DA receptor levels have also been demonstrated in both animal and human studies. Striatal D2Rs in women display lower affinity compared to men, which is considered to play a role in the development of alcoholism (Pohjalainen et al. 1998). In animal studies, females display lower baseline D1R density than do males (Anderson et al. 1997, Anderson and Teicher 2000). In addition, female rats show lower levels of D1R binding after acute cocaine treatment (1hr) compared to male rats (Festa et al. 2006). In contrast, a selective decrease in D1R availability with longer cocaine exposure (24h) has been demonstrated in male rats, such that post-cocaine D1R availability in males drops to the levels seen at baseline in females, while females show no appreciable decline from baseline at 24-hr post-cocaine. It has been shown that cocaine-induced ambulatory and rearing activity are greater in female rats (Festa et al. 2006). Female rats also display greater sensitivity to D1R antagonist, SCH 23390, which reduces the locomotor activating effects of cocaine (Schindler and Carmona 2002). Thus, low initial levels of D1R in females may reflect increased DA signaling at this receptor site. Although this may deter initiation of substance use or gambling (relative to males), it may also encourage earlier onset of behaviors (e.g., compulsive reward seeking) associated with D1R down-regulation in males.

In humans, females are more sensitive to locomotor activating effects of AMPH and cocaine (DA hyper-responsivity), suggesting women may be more susceptible to DA sensitization (Dow-Edwards 2010). This evidence suggests that potential deficits in D1R (and possibly D2R) availability in females compared to males, may contribute to the “telescoping effect” in substance addiction and possibly PG.

Taken together, variations in the availability and/or function of D1R could be one of the key factors in the pathological process of PG. This study will begin to define the neurochemistry of PG by characterizing the roles of D1R and D2R in gambling and psychostimulant reinforcement, which could provide important insight to the development of pharmacotherapy for PG. Specifically, if D1R function is deficient in PG, medications that restore D1R signaling may decrease sensitization (Shuto et al 2006, 2008) and associated PG
symptoms. If D1R deficits are greater in female PG subjects, gender-based D1R pharmacotherapy may be warranted.

1.3 **Objective**

The primary goal of this study is to characterize the roles of D1R and D2R in gambling and psychostimulant reinforcement in male and female PG subjects and HCs subjects in order to better understand the neurochemistry of PG.

This objective will be addressed by comparing the effects of HAL, a preferential D2 antagonist, and FLU, a mixed D1 and D2 antagonist, on responses to a 15-minute slot machine game and AMPH, on separate sessions, in men and women with and without PG. The rationale for the selection of HAL and FLU, as well as the overall study design, is described in detail in later sections. Effects of the antagonists will be assessed with respect to intrinsic and reinforcer-induced (primed) incentive motivation (‘Wanting’), operationally defined by self-reported Desire to Gamble on Visual Analog Scales (VAS) and by betting behavior on the slot machine. Hedonic impact (‘Liking’) of the antagonists themselves and the reinforcers will be operationally defined by VAS Pleasurable Effects; by ARCI MBG and AMP subscales assessing drug-like euphoric and psychostimulant-like effects, respectively, and by more general energy or readiness to work as assessed by the Profile of Moods State (POMS) Vigor subscale.

1.4 **Hypotheses**

We expect deficits in baseline D1R function and/or availability in PG compared HC subjects, as well as in females compared to males. Based on the effect of HAL on gambling and methamphetamine reinforcement in PG and HC subjects, respectively, in previous studies, the following we hypothesize that:

a) Selective blockade of D2 auto-receptors (HAL) (3-mg) will increase rewarding effects of a 15-minute slot machine game and of a low-dose of AMPH (20-mg). (Antagonist Group x Pre-Treatment x Time)

b) Across groups, the effects of HAL on gambling and AMPH-reinforcement will be more pronounced in PG subjects than HC subjects, reflecting lower baseline D1R function in PG subjects. (Antagonist Group x Group x Pre-Treatment x Time)
c) Across groups, the effects of HAL on gambling and AMPH-reinforcement will be more pronounced in females than males, reflecting lower baseline D1R function in females. (Antagonist Group x Group x Gender x Pre-Treatment x Time)

d) Combined blockade of D1R by FLU (3-mg) should negate group differences in gambling and AMPH reward. (Antagonist Group x Pre-Treatment x Time)

These hypotheses will be tested by comparing effects of HAL and FLU on separate sessions that administer a slot machine game or AMPH to groups of PG and HC subjects matched on age, gender and smoker status.
2. MATERIALS AND METHODS

2.1 Study Design

This study employed a 2 Antagonist Group (HAL / FLU) x 2 Group (PG / HC) x 2 Gender (male / female) x 2 Pre-Treatment (active antagonist / placebo) x 2 Phase (slot machine / AMPH) repeated measures between-within subjects design. Each of 50 subjects was assigned to either one of the two antagonists. Drug treatment was double-blind and counterbalanced across all subjects. Prior to inclusion, subjects underwent a three-step screening process consisting of a telephone screening, an in-person interview screening and a physician’s exam. Eligible subjects attended 4 test sessions, each separated by a one-week washout period. The first and second test sessions (Phase I) assessed the effects of the antagonists on gambling reinforcement, and the third and fourth test sessions (Phase II) assessed the effects of the antagonists on AMPH reinforcement. Gambling effects were assessed with a 15-min standard session of play on a Video Lottery Terminal (VLT)-style slot machine, whereas AMPH effects were assessed with 20-mg oral d-amphetamine (Dexedrine®). In Phase II, we included the slot machine game after all AMPH-related tests had been completed, in order to preclude possible carryover effects that could influence response to AMPH. Data from slot machine session in Phase II were exploratory and do not form part of the analysis of this project.

2.2 Study Medications

2.2.1 Haloperidol

Haloperidol (HAL) is a typical antipsychotic primarily indicated for management of psychotic disorders (e.g., schizophrenia). It functions as a dopamine receptor antagonist, with high affinity to D2 receptors (K_i = 0.6) and only moderate affinity D1 receptors (K_i = 17). HAL also has high affinity for D3 receptors (K_i = 0.2), moderate affinity for D4 receptors (K_i = 22) and low affinity for D5 receptors (K_i = 169). In addition to dopamine receptors, it also shows moderate affinity for α1 adrenergic receptors and low affinity to 5-HT2 receptors (Appendix A, Binding Profiles of HAL and FLU).

The bioavailability of oral HAL ranges from 60-70%, with peak plasma concentration achieved in 2.75 hours and an elimination half-life of approximately 20 hours. HAL is metabolized mainly in the liver by CYP3A4, which it also inhibits. It is also a substrate of CYP2D6.
2.2.2 Fluphenazine

Fluphenazine (FLU) is a typical antipsychotic used for the symptomatic treatment of psychotic disorders including schizophrenia. It functions as a dopamine receptor antagonist, with high affinity for both D1 ($K_i = 0.85$) and D2 ($K_i = 0.4$) receptors. It also shows moderate affinity for D3 ($K_i = 1.4$) and D4 ($K_i = 7.1$) receptors, as well as low affinity for D5 receptors ($K_i = 54$). FLU also moderately antagonizes $\alpha_1$ adrenergic receptors and histamine H1 receptors (Appendix A, Binding Profiles of HAL and FLU).

FLU has an oral bioavailability of 2-5%, with peak plasma concentration achieved in 2 hours and elimination half-life from 13 to 33 hours. It is metabolized primarily by CYP3A4, and is also a substrate and inhibitor of CYP1A2 and CYP2D6.

2.2.3 Rationale for Drug Selection

HAL is the most selective D2 antagonist available for human use in Canada, which allows us to assess effects of D2 blockade and identify the role of D1 receptors in gambling reinforcement. Theoretically, a selective D1 antagonist would be ideal for this study. However, in Canada, no selective D1 antagonist is available for non-clinical testing in humans.

Using HAL and FLU also allows us to replicate prior findings for gambling and AMPH reinforcement, respectively, and to achieve statistically significant effects by obtaining data from more subjects.

Beside their differences in D1 affinity, HAL and FLU show very similar binding profiles, not only for other DA receptor subtypes (e.g., D2, D3, D4, D5) but also for non-DA receptors, including $\alpha_1$, $\alpha_2$ adrenergic receptors and muscarinic acetylcholine receptors. Therefore, these two antagonists are good comparators that isolate, and thus permit inferences about, the role of D1 in mediating gambling and AMPH reinforcement.

2.2.4 Dextroamphetamine sulfate

Dextroamphetamine sulfate (AMPH) is a DA releaser and psychostimulant indicated for attention deficit hyperactivity disorder (ADHD). It is a potent full agonist of trace amine-associated receptor 1 (TAAR1), which upon activation, increases cAMP production, inhibits DA transporters and induces monoamine neurotransmitter release. Consequently, it causes increased synaptic DA levels and produces central nervous system stimulation. The oral bioavailability of AMPH is over 75%, with peak plasma concentration achieved in 1-2 hours.
and the elimination half-life of 9-11 hours. It is metabolized by CYP2D6, dopamine β-hydroxylase, and flavin-containing monoxygenase.

2.2.5 Diphenhydramine

Diphenhydramine (Benadryl®) is a first-generation antihistamine mainly used to treat allergies. It is also commonly used for the management of extrapyramidal side effects related to first-generation antipsychotics. Participants were provided with 2 capsules of 25 mg diphenhydramine at the end of each test session, and were instructed to take them only if they felt delayed adverse effects (e.g., dystonia or akathisia) to the study medications.

2.3 Participants

2.3.1 Inclusion/Exclusion Criteria

This study included 50 subjects, 26 HCs and 24 PGs, 34 males and 16 females, between 19 and 65 years of age. HCs were social gamblers who had played a slot machine at least 5 times in their lifetime, which helped to minimize the impact of learning effects from session 1 to subsequent sessions in the present design. PGs were otherwise healthy individuals without co-morbidities, in order to minimize ambiguous attribution of group differences to other disease status. PGs who were treatment-seeking or wished to be abstinent were excluded due to exposure to slot machine during the study.

Participants with prior exposure to a psychostimulant, regular recreational drug use, and heavy nicotine use were ineligible. Participants with a first-degree relative with schizophrenia or bipolar disorder were also excluded, to avoid possible psychotic effect of AMPH. Women who were pregnant, trying to become pregnant, or breastfeeding were excluded to avoid potential exposure of fetuses to study medications.

Details of screening instruments are provided below, and the inclusion/exclusion criteria are summarized in Table 1.

2.3.2 Screening Instruments

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

SOGS is the most common and cited instrument used to screen for PG status. There are 16 items in the scale, 11 of which are scored, with a maximum score of 20. Participants who scored 0 or ≥5 were eligible as HCs or PGs, respectively. SOGS was first administered during
the telephone screening, and repeated during the interview screening by self-report (HCs) or in an interview with the study psychiatrist (PGs) designed to confirm PG status.

**DSM-IV PG Questionnaire (Beaudoin and Cox 1999)**

The DSM-IV PG questionnaire is based on the DSM-IV criteria for PG. It consists of 10 items, and each item was scored from 0 to 3 based on the time when the symptoms occurred (past week, past month, past year, never). Participants who scored 0 were eligible as HCs, and those who scored ≥5 and endorsed 5 or more symptoms were eligible as PGs. This scale was also administered during both telephone screening and interview screening in the same forms as SOGS.

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

The BDI is one of the most widely used instruments for measuring the severity of depression. The BDI short form consists of 13 items, and each item was scored from 0-3 depending on symptom severity. Participants who scored <10 and 0 on the suicide item were eligible for the study. It was administered during the telephone screening, and repeated in the interview screening as a written measure.

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

The ADS is one of the most widely used clinical tools for measuring the severity of alcohol dependence. It consists of 25 items, with a maximum score of 47. Participants with an ADS score <13 (1st quartile) were eligible for the study. The ADS was administered during the telephone screening, and repeated in the interview screening as a written measure.

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

The FTND is a standard instrument for assessing the intensity of addiction to nicotine. It is a 6-item scale, with a maximum score of 10. It was administered during the interview screening. The FTND score was not one of the inclusion/exclusion criteria, but one of the factors for matching PG and HC groups as well as drug subgroups.

**Alcohol Timeline Follow-back (Sobell and Sobell 1992)**
A 90-day Timeline Follow-Back was used to assess regular alcohol use of each participant. Participants provided retrospective estimates of the number of alcoholic drinks taken each day, starting with the preceding day and working backwards for 90 days prior to that. Participants who consumed > 20 (men) or > 15 (women) alcoholic drinks per week on average, indicating possible problem drinking (Sanchez-Craig), were ineligible for the study. The scale was administered during the interview screening.

**Nicotine Timeline Follow-back**

A 7-day nicotine Timeline Follow-Back (based on the validated alcohol scale) was given to assess typical nicotine use of each participant. Participants who smoked > 20 cigarettes daily on average were ineligible for the study, in order to minimize possible impact of withdrawal effects during the active test phase of every experimental session. It was administered during the interview screening.

**Drug Abuse Screening Test (DAST) (Skinner 1982)**

The DAST is a valid and sensitive screening instrument for drug abuse. It consists of 20 yes-or-no questions, with a maximum score of 20. Participants with who scored ≤ 4 were eligible for inclusion. It was administered on the interview screening to confirm lack of regular drug use.

**Structured Clinical Interview for the DSM-IV (SCID) (First et al. 1995)**

The SCID is a diagnostic instrument used to determine DSM-IV Axis I disorders in psychiatric patients and non-patient research subjects. It is a semi-structured interview during which the questions are usually open-ended. A series of pre-determined follow-up questions are used to achieve a comprehensive assessment of patient or participant’s psychiatric profile.

For the purpose of this study, modules covered included: mood episodes (current and past depressive and manic episodes), anxiety disorders, alcohol use, substance use and psychotic symptoms. Participants with current or past depressive or manic episodes (eligible if only one past depressive episode >1 year prior), current or past anxiety disorders, current or past alcohol dependence, alcohol abuse (eligible if >1 year prior), or presence of psychotic symptoms were excluded (Appendix B, SCID Inclusion/Exclusion Criteria). SCID was
administered during the interview screening by the experimenter and the study psychiatrist to HCs and PGs, respectively.

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

The EPI is a self-report questionnaire used to assess the personality traits including Extraversion (score 0-24), Neuroticism (score 0-24), and Lie Scale (score 0-9) of each participant. It consists of 57 yes-or-no questions, and was administered during the interview screening. The Lie Scale measures the likelihood that a participant would modify their self-report responses in order to meet perceived experimental requirements, and therefore served as an index of validity of other self-report questionnaires.

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

The EIS is a self-report questionnaire used to assess the personality traits of Impulsivity (score 0-16), Venturesomeness (score 0-16), and Empathy (score 0-19) of each participant. It consists of 54 yes-or-no questions and was administered during the interview screening. The EIS was selected because it has been used in multiple studies with PG subjects and found to discriminate between subtypes of PG – with and without substance use disorders, to predict performance on laboratory indices of impulsive behavior and to predict the response to DA enhancing medications in PG subjects (Alessi and Petry 2003, Zack and Poulos 2009).

**Gamblers’ Belief Questionnaire (Steenbergh et al. 2002)**

The GBQ is a self-report measure of gamblers’ cognitive distortions in the 2-factor model: Luck/Perserverance (Factor 1) and Illusion of Control (Factor 2). It consists of 21 items and each item is scored from 1 (Strongly Agree) to 7 (Strongly Disagree). Thus, lower scores indicate greater cognitive distortions. It was administered during the interview screening.

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

Selected sub-tests from the WAIS-R were administered during the interview screening to assess subjects’ basic cognitive function.

**WAIS Digit Span** The Wechsler Digit Span sub-test assesses attention, concentration and mental control. Forward Subscale measures short-term rote memory and Backward Subscale measures working memory. For the Forward Subscale, the experimenter read aloud
14 number sequences with increasing length (3 to 9 digits, 2 series for each; one digit per second), and subjects repeated each series in the same order. For the Backward Subscale, the experimenter read aloud another 14 number sequences (2 to 8 digits, 2 series for each) and subjects repeated each series in the reverse order. Each correct response scored 1 point, with a maximum score of 14 for each subscale, and a combined maximum score of 28.

**WAIS Digit Symbol-Coding** The Wechsler Digit Symbol-coding sub-test assesses visual perception/analysis, working memory, scanning speed and motor coordination. Participants were asked to match symbols to digits (0-9) by writing down corresponding symbols in a box below each number. Participants completed as many of these as possible during the 60-second task. Each correct answer scored 1 point, with a maximum possible score of 92.

**WAIS Vocabulary Task** The Wechsler Vocabulary sub-test assesses the degree to which subjects can comprehend and verbally define a range of English words, to ensure full understanding of other self-report and cognitive/behavioral tasks in the study. Participants were asked to orally define 15 English language words of increasing difficulty (Half the entire list of items). Each definition was scored from 0 to 2 based on accuracy and completeness, with a maximum total score of 30. Participants who scored <18 were ineligible for the study.

| **Table 1. Summary of inclusion criteria.** |
|-------------------------------|-----------------|-----------------|
| **Criterion**                 | **HC**          | **PG**          |
| Age                           | 19 – 65         |                 |
| BMI (kg/m²)                   | < 35            |                 |
| SOGS                          | ≥ 5             | 0               |
| DSM-IV PG                     | ≥ 5             | 0               |
| BDI                           | < 10 (low depression) |          |
| ADS                           | < 13 (low dependence) |         |
| Alcoholic drinks / week       | ≤ 20 / 15 (men / women) |  |
| Cigarettes / day              | ≤ 20            |                 |
| Caffeinated beverages / day   | ≤ 8             |                 |
| DAST                          | ≤ 4 (no drug abuse) |            |
| WAIS-vocabulary               | > 18            |                 |
Wisconsin Card Sort Task (WCST) (Heaton 2003)

The WCST is a neuropsychological test, which assesses subjects’ learning and cognitive flexibility. The present version was validated for, and administered by, computer. Participants were asked to match a series of different test cards on the screen to one of four standard criterion cards, without knowing the rules for matching; however, subjects were told whether a particular match was correct or incorrect by means of on-screen feedback after every trial. Cards could be matched based on one of 3 domains (color, number, or shape of symbol), and the domain for matching would change over time without notice. PGs show impairment in WCST performance as compared to HCs (Goudriaan et al. 2006, Rugle and Melamed 1993).

2.3.3 Group Matching

PG and HC groups were matched on gender, age and smoker status (FTND). Within each group, pairs of subjects matched on PG severity (SOGS), gender, age, Impulsivity (EIQ), ethnicity, alcohol use (ADS), depressive symptoms (BDI), and nicotine dependence (FTND) were randomly assigned to HAL or FLU Drug Groups. The randomization code was developed by Principal Investigator in conjunction with CAMH Pharmacy. Experimenters were blind to pre-treatment condition (drug vs. placebo) on a given test day.

2.3.4 Participant Safety

Prior to enrollment, lab test results including ECG, blood and urine analysis were confirmed by the Qualified Investigator of the study to ensure subjects’ eligibility. Each participant underwent a comprehensive physical exam performed by a physician and a registered nurse at CAMH to further ensure they were physically healthy and suitable to receive the study medications. During the testing phase, blood pressure and heart rate were monitored regularly to ensure the values were within the normal range.

Prior to discharge at the end of each test day, a registered nurse or physician examined subjects to ensure normal blood pressure and heart rate, as well as the absence of adverse effects. Participants were then given the diphenhydramine (50mg) in case of delayed side effects, and a wallet card with vital medication information (drug name, dose, time of drug administration) and the contact number for the study’s Qualified Investigator. Participants were instructed to avoid driving or operating heavy machinery for 24 hours, and to abstain from all
drugs, medications, as well as alcohol for 72 hours to avoid possible interactions with the study medications.

2.3.5 Time commitment and Study Payment

The total time commitment upon completion of the entire study was about 42 hours including interview screening (3 hours), physician’s exam (1 hour), 4 test sessions (8 hours each) and commute time (1 hour for each visit). Participants who completed the study received $920 for participation and a standard $80 bonus for the ‘winnings’ from the slot machine sessions, with a total payment of $1000 which worked out to an hourly pay rate of $23.80. Participants received their payment by cheque approximately 2-3 weeks after their last test day.

2.4 Apparatus

2.4.1 Experimental Self-Report Scales

Self-report questionnaire packages were administered at multiple time points of each test session: pre-capsule baseline, peak blood drug levels for all drugs (i.e., peak antagonist, followed 90 min later at peak AMPH), and right before/after the slot machine.

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

This study employed modified VAS (0 to 10 with ½ -point gradations), which assessed participant’s subjective motivation and pleasure in response to gambling and AMPH. In the case of slot machine game, the VAS was derived from the initial study on HAL (Zack and Poulos 2007), and assessed Desire to Gamble, along with the Enjoyment, Excitement, Involvement and perceived “High” from the slot machine game. In the case of AMPH, the VAS was derived form the initial study on AMPH (Zack and Poulos 2004), and assessed the High, Good effects, Bad effects, Liking, and Desire to take (Capsule) again for AMPH. Desire for Alcohol was also assessed to determine whether the various manipulations selectively affected gambling vs. other types of addictive reinforcement. Previous research has demonstrated the validity and utility of VAS in assessing subjective effects of drugs (Fischman and Foltin 1991).

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

The POMS was used to assess mood states at different time points of each test session. The scale consists of 37 mood adjectives which were rated from 0 (Not at all) to 4 (Extremely).
The POMS classifies moods into six categories: Tension-Anxiety (0-24), Depression-Dejection (0-32), Anger-Hostility (0-28), Fatigue-Inertia (0-24), Vigor-Activity (0-20), and Confusion-Bewilderment (0-20).

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

The ARCI consists of 49 true-or-false statements. It is a well-validated subjective drug effects measure that covers 6 drug effect domains: (1) Amphetamine (AMP), measuring amphetamine-specific effects (0-9), (2) Morphine/Benzedrine Group (MBG) for euphoria (0-16), (3) Lysergic Acid Diethylamine (LSD) for dysphoria (0-14), (4) Benzedrine Group (BG) for stimulant effects (0-13), and (5) Pentobarbital-Chlorpromazine-Alcohol Group (PCAG) for sedation (0-15). Previous research has demonstrated the utility and validity of ARCI in assessing subjective effects of drugs, including AMPH (Chait et al. 1986).

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

A Side Effect Checklist, validated for medication studies, was administered at the end of each test session to assess the presence and severity (from 0 (Absent) to 5 (Needs intervention)) of 24 potential adverse drug effects (e.g., headache, blurred vision).

**Capsule Contents Evaluation (CCE)**

The CCE was administered at the end of the second (Phase I) and fourth (Phase II) test session. Participants wrote down which capsule(s) they believed contained active drug on previous test sessions.

### 2.4.2 Devices

**Breathalyzer**

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

**Blood Pressure/Heart Rate Monitors**

An HEM-601 (OMRON, Vernon Hills, IL) wrist cuff instrument was used to measure blood pressure/heart rate regularly during each test session.
2.4.3 Slot Machine

On each test session (at peak blood antagonist level in Phase I and after a verbal cue salience task – 20 min post-peak AMPH in Phase II), subjects played a VLT-style commercial slot machine (‘Cash Crop’, WMS Gaming; Detroit MI) for 15 minutes or until 400 pre-loaded credits ($100) ran out, whichever came first. The slot machine session took place in a mock-bar room, to mimic the actual casino environment. To further encourage subjects’ spontaneous betting behavior, they were informed that they would receive a cash bonus proportional to the remaining credits at the end of each slot machine session. Participants were not permitted to add their own money to the machine.

The object of the game is to get as many of the same symbol on a line as possible. For each trial, subjects selected the number of lines to bet on, with a maximum of 9 lines, and the number of credits to bet on each line, with a maximum of 5 credits per line. Therefore, subjects were allowed to bet from 1 to 45 credits on any spin. There were two boxes onscreen that showed the total number of credits remaining and the payoff on the last spin. The bet size, line selection and payoff for each spin were recorded electronically. Lines and bet size were selected and spins initiated by touching icons on the screen or pushing buttons on the console. To encourage spontaneous patterns of play, subjects were not aware their betting behavior was monitored by computer until debriefing. In keeping with the principle of informed consent, at debriefing subjects were given the option of having their slot machine data (obtained without informed consent) omitted.

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

The RRT took place after the slot machine game and at peak blood AMPH level in Phase I and II, respectively. The task was administered on a computer with MicroExperimental Laboratory (MEL, v. 2.01; Psychology Software Tools, Pittsburgh, PA) software. During the RRT, a series words came up on the computer screen one at a time. All Words appeared with asterisks between each letter (e.g. p*e*n*c*i*l*). Participants were asked to report each word aloud as quickly and accurately as possible. A warning signal (‘&&&&&’) appeared before each trial to focus subjects’ attention to the target location. A microphone attached to the computer measured the vocal response time with millisecond precision. During the task, the experimenter coded response accuracy (correct, incorrect or invalid response) after each trial with a serial response button box (Psychology Software Tools, Pittsburgh, PA). Events on each
trial were the same: warning signal (350ms) → blank (250ms) → target word → participant response → experimenter coded response accuracy → inter-trial interval (550ms).

The words were drawn from 5 categories: Gambling-Related (e.g. blackjack), Alcohol-Related (e.g. martini), Positive Affect (e.g. hopeful), Negative Affect (e.g. sad), and Neutral (e.g. lattice). Participants performed 20 practice trials plus 150 test trials (30 from each category), with categories and items presented in random order.

Faster response time to Gambling-Related words compared to Neutral words is considered to be an indication of increased incentive salience of gambling stimuli.

2.4.5 Exploratory Tasks

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

The SST was administered during the interview screening and on each test session (after RRT in Phase I and after slot machine game in Phase II) to assess subjects’ motor speed and ability to inhibit a pre-potent manual response. Participants made a series of 2-choice key press (‘z’ or ‘/’ with their left or right index fingers, respectively) responses to visual (‘go’) stimuli (‘a’ / ‘b’ [interview, test session 1 and 3] or ‘c’ / ‘d’ [test session 2 and 4]) on the computer screen, but to inhibit their key press when a tone (stop signal) sounded on a random 25% of trials. On each test session, subjects performed two sets of practice trials before the test trial during which there were two brief breaks.

By manipulating the time interval between the onset of the visual stimulus and the onset of the stop signal tone, we could determine the delay at which success on stop-signal trials is 50%. Other things being equal, the longer the delay after the onset of the visual stimulus, the further the ‘go’ process will have progressed to completion, and the harder it is to pre-empt. The difference between this delay and the go response time yielded a measure of Stop-Signal Response Time (SSRT), with greater values indicating poorer inhibitory efficiency (i.e., longer time required for stop process to overtake the already initiated ‘go’ process).

Game of Dice Task (GDT) (Brand et al. 2005)

The GDT was administered to assess reward sensitivity and risk-taking behaviors during the screening interview and after all other tasks on each test session. In the GDT, a computer rolled a virtual die 18 times. Participants bet on the outcome of each throw by choosing either a single number (1-6) or a combination of two, three, or four possible
outcomes. Betting on a single number was maximally risky (odds = 1/6) whereas betting on four possible numbers (odds = 4/6) was minimally risky. Thus, low response selection values indicate more risky behavior. When the thrown number was the one selected or among the numbers in selected combination, subjects won designated amount of money shown next to the selected alternative, otherwise subjects lost the same amount of money. Bet size was $1000, $500, $200, and $100 for a single number, a combination of two, three and four numbers, respectively. Participants started with $1000 and were allowed to continue with negative balances. The aim was to maximize winnings or minimizes losses. The average outcomes selected per trial were calculated for early (trials: 1-6), middle (trials: 7-12) and late (trials: 13-18) stages of the game.

### 2.5 Procedure

#### 2.5.1 Telephone Screening

Participants were recruited from the communities in Greater Toronto Area via online advertisement posted mainly on Craigslist.com and Kijiji.ca (Appendix C, Recruitment Advertisements). Potential subjects called the CAMH study line and were given a brief overview of study objective, requirement and procedure. They were informed about the study medications: Haldol, Prolixin and Dexedrine as well as possible side effects. If interested, a standard 20-minute telephone questionnaire was then administered to assess eligibility. It included questions covering demographics, health status, and current/past substance use, as well as SOGS, DSM-IV PG questionnaire, BDI, ADS, EIS and WAIS-Vocabulary Task (if deemed necessary). An interview screening was scheduled at CAMH if subjects were eligible based on above measures.

#### 2.5.2 Interview Screening

At the beginning of the interview screening, subjects read and signed the informed consent form (Appendix D, Informed Consent Form). Then, subjects’ blood alcohol level (0 required), heart rate, blood pressure, weight and height were measured. A urine pregnancy test was given to all female subjects. The experimenter then administered the SCID to determine current and past psychiatric profile. Potential PG subjects underwent an additional interview with a study psychiatrist to verify PG status and severity, during which the SOGS and DSM-IV PG questionnaire were administered and a detailed history of the individual’s gambling habits.
was obtained. This interview was designed to identify and exclude individuals who falsely reported PG symptoms to enter the study simply for the sizeable compensation.

Eligible subjects then completed written interview screening questionnaire package which included SOGS and DSM-IV PG questionnaire (for HCs only), BDI, ADS, FTND, DAST, Alcohol and Nicotine Timeline Follow-backs, EIS, EPI, and GBQ. Experimenter then administered the WAIS Digit Span, WAIS Digit Symbol-Coding, and WAIS Vocabulary. Eligible subjects then proceeded to computer tasks including WCST, SST and GDT.

Lastly, subjects were brought to the CAMH Clinical Laboratory to complete blood and urine analysis, as well as an electrocardiogram (ECG). Blood samples were drawn from their arm by a CAMH nurse and were enough to fill 1-2 finger-length tubes. Urine samples were obtained to confirm lack of recent drug use. ECGs were done to confirm the absence of any heart problems. Lab test results were sent to the study’s Qualified Investigator to confirm eligibility of each participant. A physician’s exam was then scheduled (approximately one week after interview screening) if subjects were eligible based on lab test results.

### 2.5.3 Physician’s Exam

Eligible subjects underwent a standard physical examination by a doctor and nurse at the CAMH Addiction Medicine Clinic to further confirm the suitability to receive the study medications (Appendix E, Physical Exam Inclusion/Exclusion Criteria). If subjects were considered eligible after the physical, they moved on to the test sessions.

### 2.5.4 Test Day Procedure

There were four test sessions (two phases) with a one-week washout period between each session. Test days started at 8:30am. Upon subjects’ arrival, the experimenter measured their blood alcohol level (0 required), baseline heart rate and blood pressure. Participants were then provided with a standard breakfast and followed by the first questionnaire package (VAS for gambling and alcohol, POMS, and ARCI) for completion. Urine pregnancy tests were then given to all female subjects. Cigarette break was provided at this time if necessary and only one cigarette was permitted.

Participants were then escorted to the detox room. The first dose of study medication was administered in the form of 3 capsules, each of which contained either 1mg of HAL/FLU, or visually identical placebos. It took 2 hours 45 minutes and 2 hours for HAL and FLU to
reach blood drug peak level, respectively. During the waiting period, blood pressure and heart rate were recorded every 30 minutes. A second questionnaire package was administered 15 minutes before the peak blood drug level was reached. The second dose was administered at peak drug level in the form of 4 visually identical capsules, each of which contained placebo in Phase I or 5mg of AMPH in Phase II. In Phase II, subjects waited for another 90 minutes for AMPH to reach peak level, during which blood pressure and heart rate were measured every 15 minutes by a registered nurse.

Table 2. Summary of test session procedures.

<table>
<thead>
<tr>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Baseline physiological ratings (HR/BP, blood alcohol level)</td>
<td>• Capsule 2 (AMPH) administered</td>
</tr>
<tr>
<td>• Breakfast</td>
<td>• Waiting period: 90min</td>
</tr>
<tr>
<td>• Package A administered (VAS-Desire to Gamble/Drink Alcohol; POMS; ARCI)</td>
<td>• HR/BP measured</td>
</tr>
<tr>
<td>• HR/BP measured pre-capsule 1</td>
<td>• RRT</td>
</tr>
<tr>
<td>• Capsule 1 administered (HAL/FLU vs. placebo)</td>
<td>• HR/BP measured</td>
</tr>
<tr>
<td>• Waiting period: 2hr 45min (HAL); 2hr (FLU)</td>
<td>• Package C administered (VAS-Desire to Gamble/Drink Alcohol, VAS-High/Good effects/Bad effects/Desire to take again for AMPH; POMS; ARCI)</td>
</tr>
<tr>
<td>HR/BP measured every 30 minutes</td>
<td>• 15-min Slot machine game (in mock bar)</td>
</tr>
<tr>
<td>• Package B administered (VAS-Desire to Gamble/Drink Alcohol; POMS; ARCI)</td>
<td>• HR/BP measured</td>
</tr>
<tr>
<td>• Capsule 2 (placebo) administered</td>
<td>• Package C administered (VAS-Desire to Gamble/Drink Alcohol; POMS; ARCI)</td>
</tr>
<tr>
<td>• 15-min Slot machine game (in mock bar)</td>
<td>• Lunch</td>
</tr>
<tr>
<td>• HR/BP measured (in mock bar)</td>
<td>• Discharge by registered nurse</td>
</tr>
<tr>
<td>• Package C administered (VAS-Desire to Gamble/Drink Alcohol, VAS-Enjoyment/Excitement/Engagement/High from slot machine game; POMS; ARCI)</td>
<td>• Package E administered (VAS-Desire to Gamble/Drink Alcohol; POMS; ARCI; Symptom Side Effect Checklist)</td>
</tr>
<tr>
<td>• RRT</td>
<td>• CCE administered (session 2 and 4)</td>
</tr>
<tr>
<td>• HR/BP measured</td>
<td>• Lunch</td>
</tr>
<tr>
<td>• Package D administered (VAS-Desire to Gamble/Drink Alcohol; POMS; ARCI)</td>
<td>• Discharge by registered nurse</td>
</tr>
</tbody>
</table>
In Phase I, subjects were escorted to a mock-bar room and played the slot machine for 15 minutes. After that, they completed a third questionnaire package in the mock-bar environment. Participants proceeded to the RRT, followed by a fourth questionnaire package.

In Phase II, subjects proceeded to the RRT (at peak blood levels for AMPH), followed by a third questionnaire package. After that, they played the slot machine for 15 minutes and completed a fourth questionnaire package in the mock-bar room.

In each phase, when both the slot machine game and RRT were completed subjects proceeded to SST and GDT, followed by the last questionnaire package (including the Side Effect Checklist). The CCE was administered at the end of the second and fourth test sessions. Blood pressure and heart rate were recorded after the slot machine session as well as after every computer task. The testing phase of the session was completed at this time point.

Participants were then provided with lunch and allowed to watch videos and read during the lunch break. After a detoxification period, a registered nurse or the Qualified Investigator assessed subjects to blood pressure and heart rate had declined to meet discharge criteria, and confirmed the absence of adverse effects. Participants were then given diphenhydramine (50mg) in a sealed labelled bottle in case of delayed side effects, and a wallet card with study medication information and emergency contact number. Participants were sent home by prepaid taxi.

### 2.6 Data Analysis

This study employed a 2 Antagonist Group (HAL / FLU) x 2 Group (PG / HC) x 2 Gender (male / female) x 2 Pre-Treatment (active antagonist / placebo) x 2 Phase (slot machine / AMPH) repeated measures between-within subjects design. Data analysis was performed using SPSS (v. 15, Chicago IL). This is a preliminary analysis of a study with a projected final sample size of 80 Ss. Therefore, marginal significant effects with p values between 0.05 and 0.10, which could achieve significance with full sample, will be included in the current analysis.

**Subject Characteristics** Background characteristics, Gender and Smoker Status, were analyzed using 4 (Sub-group: PG-HAL, PG-FLU, HC-HAL, HC-FLU) x 2 (Gender / Smoker Status) Chi square analysis to identify differences in ration between groups. Other background characteristics, trait scores, and scores for cognitive function tasks were analyzed using 2
Subjective Effects: Data were analyzed separately for Phase I and Phase II in order to identify the patterns of the response to slot machine game and AMPH, respectively. For measures taken at multiple time points in each session, including VAS-gambling and alcohol, POMS and ARCI subscales, differences scores were computed for consecutive time points in order to isolate the effects of the antagonists (time 2 [Peak Antagonist] – time 1 [Pre-Capsule Baseline]) and the reinforcers (time 3 [Post-Slot Machine/Peak AMPH] – time 2 [Peak Antagonist]). 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time: Peak Antagonist, Post-Slot Machine/AMPH) repeated measures ANCOVA were used. For measures taken at a single time during each session, including VAS pleasurable effects of the slot machine and AMPH, raw mean scores were analyzed using 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time: Lines, Credits, and Spins) multivariate ANCOVA.

Betting Behavior: Mean number of lines selected per line (lines; 1-9), mean credits wagered per line on each (credits; 1-5), and mean total spins per 15-min on the slot machine (spins) were analyzed using 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 3 (Measure: Lines, Credits, and Spins) multivariate ANCOVA.

Simple Effects: Within-subjects t tests were performed to identify simple effects of Pre-Treatment in each Antagonist Group, Group, Gender and Time condition. These tests were performed when significant and marginal (0.05 < p < 0.10) effects or interactions emerged on the ANCOVA. For each outcome measure, simple effects tests used mean square error for the effect in question from ANCOVA outcome, incorporating variance form all points (Winer 1971).
3. RESULTS
3.1 Subject Characteristics

In total, 50 participants, 26 HCs and 24 PGs, 34 males and 16 females have completed the study (Appendix F, Flow Chart of Subject Recruitment, Eligibility and Group Assignment). Table 3 shows the background characteristics and trait scores for each of the four subgroups (PG-HAL, PG-FLU, HC-HAL, HC-FLU). Based on a 2 (Gender) x 4 (Sub-group) and a 2 (Smoker Status) x 4 (Sub-group) Chi square analysis and a 2 (Group) x 2 (Antagonist Group) x 2 (Gender) x 8 (Measure: Age, SOGS, DSM-IV PG, BDI, ADS, Mean drinks/week, and DAST) MANOVA, PGs had significantly higher SOGS and DSM-IV PG scores, which are two scales for gambling assessment. In addition, PGs also displayed higher BDI scores, which measures the severity of depression. However, all BDI scores were below clinical thresholds as per study requirement. There were no other differences presented among subgroups.

Table 3. Mean (SD) background characteristics and trait scores of subjects in each subgroup.

<table>
<thead>
<tr>
<th></th>
<th>PG</th>
<th></th>
<th>HC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAL</td>
<td>FLU</td>
<td>HAL</td>
<td>FLU</td>
</tr>
<tr>
<td>Subject number (n)</td>
<td>12</td>
<td>12</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Age [mean (SD)]</td>
<td>33.9 (7.8)</td>
<td>32.3 (10.1)</td>
<td>36.2 (12.2)</td>
<td>37.3 (14.4)</td>
</tr>
<tr>
<td>Gender (n male : n female)</td>
<td>9 : 3</td>
<td>8 : 4</td>
<td>10 : 4</td>
<td>7 : 5</td>
</tr>
<tr>
<td>Smokers : Non-smokers (n)</td>
<td>1 : 11</td>
<td>1 : 11</td>
<td>1 : 11</td>
<td>0 : 12</td>
</tr>
<tr>
<td>SOGS [mean (SD)] *</td>
<td>10.5 (5.1)</td>
<td>10.6 (5.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>DSM-IV PG [mean (SD)] *</td>
<td>13.5 (5.6)</td>
<td>15.3 (5.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>BDI [mean (SD)] *</td>
<td>2.2 (2.6)</td>
<td>2.9 (3.5)</td>
<td>0.21 (0.43)</td>
<td>0.58 (1.0)</td>
</tr>
<tr>
<td>ADS [mean (SD)]</td>
<td>0.83 (1.2)</td>
<td>1.4 (2.4)</td>
<td>0.71 (1.4)</td>
<td>0.33 (0.78)</td>
</tr>
<tr>
<td>Mean drinks/week</td>
<td>2.2 (2.4)</td>
<td>2.2 (2.9)</td>
<td>1.1 (1.1)</td>
<td>0.91 (0.91)</td>
</tr>
<tr>
<td>DAST</td>
<td>0.25 (0.62)</td>
<td>0.33 (0.49)</td>
<td>0.50 (0.65)</td>
<td>0.42 (0.79)</td>
</tr>
</tbody>
</table>

SOGS = South Oaks Gambling Screen; DSM-IV PG = DSM-IV Pathological Gambling Questionnaire; BDI = Beck Depression Inventory; ADS = Alcohol Dependence Scale; DAST = Drug Abuse Screening Test; * = main effect of Group (PG, HC), p<0.05.

Table 4 shows scores on personality traits based on different scales in each subgroup. According to a 2 (Group) x 2 (Antagonist Group) x 2 (Gender) x 8 (Subscale: EPI-Extraversion, EPI-Neuroticism, EPI-Lie, EIS-Impulsiveness, EIS-Venturesomeness, EIS-Empathy, GBQ-Luck/Perseverance and GBQ-Illusion of control) MANOVA, PGs displayed
significantly higher scores on the Extraversion subscale of EPI, as well as Impulsiveness subscale of EIS. PGs also had significantly lower scores on Luck/Perseverance and Illusion of control subscales of GBQ, indicating greater cognitive distortions. Scores on the Lie scale of the EPI are below the normative population mean (3.9) in PG subjects, and interestingly, slightly higher in the HC group. These findings support the validity of the other self-report measures. The EIS-Impulsiveness scores, although higher in PG vs. HC subjects, are still below the normative mean of 9, indicating that, despite their PG status, the subjects are not more impulsive than the general population.

Scores on the GBQ subscales indicate that, relative to HCs, PGs report a greater belief in luck and the importance of continuing to gamble when faced with losses, as well as a greater belief that their behavior (e.g., skills, experience, strategies) can influence the outcome of their gambling.

**Table 4.** Mean (SD) scores on personality traits of subjects in each subgroup.

<table>
<thead>
<tr>
<th></th>
<th>PG</th>
<th></th>
<th>HC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAL</td>
<td>FLU</td>
<td>HAL</td>
<td>FLU</td>
</tr>
<tr>
<td>EPI – Extraversion *</td>
<td>13.5 (4.2)</td>
<td>13.8 (3.0)</td>
<td>9.9 (3.7)</td>
<td>11.6 (3.6)</td>
</tr>
<tr>
<td>EPI – Neuroticism</td>
<td>5.8 (3.2)</td>
<td>5.9 (5.0)</td>
<td>4.0 (2.9)</td>
<td>3.1 (3.6)</td>
</tr>
<tr>
<td>EPI - Lie</td>
<td>3.3 (1.5)</td>
<td>3.8 (2.2)</td>
<td>4.4 (2.0)</td>
<td>4.5 (1.8)</td>
</tr>
<tr>
<td>EIS – Impulsiveness *</td>
<td>7.9 (5.1)</td>
<td>7.6 (4.9)</td>
<td>2.7 (2.8)</td>
<td>2.6 (3.1)</td>
</tr>
<tr>
<td>EIS – Venturesomeness</td>
<td>11.5 (4.8)</td>
<td>12.8 (8.7)</td>
<td>12.5 (7.3)</td>
<td>12.1 (8.3)</td>
</tr>
<tr>
<td>EIS – Empathy</td>
<td>8.1 (6.7)</td>
<td>7.1 (6.3)</td>
<td>8.2 (6.1)</td>
<td>7.8 (6.2)</td>
</tr>
<tr>
<td>GBQ – Luck/Perseverance *</td>
<td>49.9 (18.6)</td>
<td>46.5 (16.0)</td>
<td>84.6 (5.9)</td>
<td>82.6 (9.2)</td>
</tr>
<tr>
<td>GBQ – Illusion of control *</td>
<td>22.8 (8.2)</td>
<td>23.1 (8.3)</td>
<td>46.4 (7.6)</td>
<td>45.0 (7.7)</td>
</tr>
</tbody>
</table>

* = main effect of group, p<0.05

Table 5 shows the scores for cognitive function tasks in each subgroup. Based on a 2 (Group) x 2 (Antagonist Group) x 2 (Gender) x 6 (Measure: WAIS-Vocabulary, WAIS-Digit Span, WAIS-Digit Symbol/Coding, WCST-Completed Trials, WCST-Perseverative Errors, and WCST-Non-Perseverative Errors) MANOVA, no significant differences existed among subgroups. This is noteworthy, given that PG subjects often exhibit impairment on
neuropsychological tests. The present sample would therefore appear to be relatively high functioning.

**Table 5.** Mean (SD) scores for cognitive function tasks of subjects in each subgroup.

<table>
<thead>
<tr>
<th></th>
<th>PGs</th>
<th></th>
<th>HCs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAL</td>
<td>FLU</td>
<td>HAL</td>
<td>FLU</td>
</tr>
<tr>
<td>WAIS – Vocabulary</td>
<td>28.3 (2.4)</td>
<td>27.5 (2.5)</td>
<td>29.1 (1.6)</td>
<td>28.7 (2.1)</td>
</tr>
<tr>
<td>WAIS – Digit Span</td>
<td>24.4 (12.7)</td>
<td>19.3 (10.4)</td>
<td>18.1 (4.3)</td>
<td>19.9 (6.8)</td>
</tr>
<tr>
<td>WAIS – Digit Symbol Coding</td>
<td>43.9 (20.1)</td>
<td>40.7 (2.5)</td>
<td>42.4 (9.3)</td>
<td>36.5 (7.1)</td>
</tr>
<tr>
<td>WCST – Completed Trials</td>
<td>80.3 (11.2)</td>
<td>90.2 (21.7)</td>
<td>81.4 (13.8)</td>
<td>82.8 (13.8)</td>
</tr>
<tr>
<td>WCST – Perseverative Errors</td>
<td>6.8 (3.3)</td>
<td>12.1 (12.4)</td>
<td>8.0 (7.6)</td>
<td>10.4 (9.1)</td>
</tr>
<tr>
<td>WCST – Non-Perseverative Errors</td>
<td>6.0 (2.8)</td>
<td>8.7 (8.4)</td>
<td>6.3 (3.9)</td>
<td>8.3 (5.0)</td>
</tr>
</tbody>
</table>

*WAIS = Wechsler Adult Intelligence Scale; WCST = Wisconsin Card Sort Task.*

### 3.2 Results for Visual Analog Scales

#### 3.2.1 Desire to Gamble

As would be expected, baseline scores on the measures of motivation to gamble differed significantly for PG vs. HC subjects. To isolate the effects of the antagonists and the reinforcers (slot machine, AMPH), difference scores were computed for consecutive time points during each test session. The difference (time 2 [Peak Antagonist] – time 1 [Pre-Capsule Baseline]) indicates the effects of the antagonist *per se*. The difference (time 3 [Post-Slot Machine/Peak AMPH] – time 2 [Peak Antagonist]) indicates the effects of the reinforcer. Difference scores retain within-group variance in their respective components (e.g., time 2 and time 1) and therefore do not artificially inflate mean effects. Although not recommended for use in correlational/regression analyses, difference scores are valid and appropriate for use in analyses of mean effects.

#### 3.2.1.1 Effect of Slot Machine

Figures 3 and 4 show the mean change in Desire to Gamble between Peak Antagonist level and Pre-capsule Baseline, and between Post-slot machine and Peak Antagonist level in response to pre-treatment of HAL and FLU, respectively. Panels a, b, c, and d show the results for each subgroup, HC-male, HC-female, PG-male and PG-female, respectively (This arrangement is consistently applied to all figures throughout the thesis). Error bars show the
standard error of the mean (SEM). Based on results from simple effects tests (Winer 1971), "*" indicates significant within-subject difference (p<0.05) in mean change in Desire to Gamble under antagonist vs. placebo.

A 2 (Antagonist Group: HAL, FLU) x 2 (Group: PG, HC) x 2 (Gender) x 2 (Pre-Treatment: Active Antagonist, Placebo) x 2 (Time: Peak Antagonist, Post-Slot Machine) ANCOVA, controlling for credits won on the slot machine, yielded significant main effects of Group [F(1,40)=5.152, p=0.029], Pre-Treatment [F(1,40)=6.660, p<0.014] and Time (i.e. pre-vs. post-slot machine) [F(1,40)=10.778, p=0.002]. In addition, significant Antagonist Group x Gender x Pre-treatment [F(1,40)=4.556, p=0.039], Group x Time [F(1,40)=7.647, p=0.009], Antagonist Group x Pre-Treatment x Time [F(1,40)=11.276, p=0.002], and Antagonist Group x Gender x Pre-Treatment x Time [F(1,40)=8.978, p=0.005] interactions were found. The highest order significant effect for a given factor, or combination of factors, will be interpreted.

Inspection of the figures reveals that the 4-way interaction reflected a consistent decline in post-slots Desire to Gamble under HAL in females but not males of both groups (Figure 3, panels a-d), whereas under FLU the effect primarily reflected a consistent increase in post-slots Desire to Gamble in females but not males of both groups (Figure 4, panels a-d). The pattern of post-slots effects was largely congruent across groups but more pronounced in PGs, which explains the lack of moderating effect of Group on the 4-way interaction. The generally inconsistent pre-slots pattern of scores vs. the consistent post-slots pattern of scores explains the significant moderating effect of Time in the 4-way interaction.
Haloperidol and Pre-post Slot Machine Desire to Gamble in Healthy Controls and Pathological Gamblers

Figure 3(a-d). Changes in Mean VAS Desire to Gamble scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level, with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
Fluphenazine and Pre-post Slot Machine Desire to Gamble in Healthy Controls and Pathological Gamblers

Figure 4(a-d). Changes in Mean VAS Desire to Gamble scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level, with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
3.2.1.2 Effect of AMPH

Figures 5 and 6 show the mean change in Desire to Gamble between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level in response to pre-treatment of HAL and FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time: Peak Antagonist, Post-AMPH) ANCOVA yielded significant main effects of Group [F(1,42)=33.011, p<0.001], Gender [F(42)=22.546, p=0.001], and Time (i.e., pre- vs. post-AMPH) [F(1,42)=24.090, p<0.001]. In addition, significant Antagonist Group x Gender [F(1,42)=8.009, p=0.038], Group x Gender [F(1,42)=15.189, p=0.00], Gender x Pre-Treatment [F(1,42)=6.634, p=0.014], Group x Time [F(1,42)=8.483, p=0.006] as well as Gender x Time [F(1,42)=6.548, p=0.014] interactions were found. The Gender x Pre-Treatment interaction was the key result with respect to the primary manipulation.

Inspection of the figures shows that this effect denoted a general reduction in Desire to Gamble under both Antagonists in females, with no appreciable effects in males of either group. The relatively stronger Gender-based difference for HAL would appear to explain the Antagonist Group x Gender interaction.

In summary, males showed limited response to both HAL and FLU. Neither antagonist changed males’ response to study reinforcers (slot machine and AMPH). In females, on the other hand, HAL consistently diminished the motivational effect of both slot machine and AMPH in females, whereas FLU displayed the opposite effects.
Haloperidol and Pre-post Amphetamine Desire to Gamble in Healthy Controls and Pathological Gamblers

**Figure 5(a-d).** Changes in Mean VAS Desire to Gamble scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level, with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
Fluphenazine and Pre-post Amphetamine Desire to Gamble in Healthy Controls and Pathological Gamblers

Figure 6(a-d). Changes in Mean VAS Desire to Gamble scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level, with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
3.2.2 Desire for Alcohol

The measure of Desire for Alcohol mainly serves as a positive control, which provides information about the specificity of the motivational effects.

3.2.2.1 Effect of Slot Machine

Figures 7 and 8 show the mean change in Desire for Alcohol between Peak antagonist level and pre-antagonist Baseline, and between Post-Slot Machine and Peak antagonist level in response to pre-treatment of HAL and FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time) ANCOVA, controlling for credits won on the slot machine, yielded a significant main effect of Time \([F(1,40)=5.473, p=0.024]\) and a significant Antagonist Group x Pre-Treatment x Time interaction \([F(1,40)=4.230, p=0.046]\).

Inspection of Figures 7 and 8 reveals that the 3-way interaction denoted a more pronounced effect of HAL at post-slots than pre-slots, whereas FLU effects were generally more modest and the within-session pattern was inconsistent. The lack of significant effects involving Group or Gender contrasts with Desire to Gamble and suggests that different processes mediate motivation to gamble vs. drink alcohol in subjects with no alcohol use disorder.
Haloperidol and Pre-post Slot Machine Desire for Alcohol in Healthy Controls and Pathological Gamblers

Figure 7(a-d). Changes in Mean VAS Desire for Alcohol scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level, with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
Fluphenazine and Pre-post Slot Machine Desire for Alcohol in Healthy Controls, Pathological Gamblers

**Figure 8(a-d).** Changes in Mean VAS Desire for Alcohol scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level) with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
3.2.2.2 Effect of AMPH

Figures 9 and 10 show the mean change in Desire for Alcohol between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level in response to pre-treatment of HAL and FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time) ANCOVA yielded no significant main effects or interactions. Inspection of the vertical axis shows that AMPH effects on motivation for alcohol were very modest.

In summary, changes in Desire for Alcohol were modest compared to Desire to Gamble scores, indicating a stronger association between study interventions (slot machine and AMPH) and motivation to gamble (vs. motivation to drink). Though significant within-subject differences (drug vs. placebo) were observed in several subgroups, there were no consistent effects and patterns observed across subgroups based on statistical analysis, suggesting the observed motivational effects of study interventions were specific to gambling.
Figure 9(a-d). Changes in Mean VAS Desire for Alcohol scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level, with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
Fluphenazine and Pre-post Amphetamine Desire for Alcohol in Healthy Controls, Pathological Gamblers

**Figure 10(a-d).** Changes in Mean VAS Desire for Alcohol scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level, with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
3.2.3 Pleasurable Effects

VAS pleasurable effects of the slot machine and AMPH were measured only once after the reinforcer during each test session. Therefore, the following figures represent the raw mean scores rather than mean change scores.

3.2.3.1 Effect of Slot Machine

Figures 11 and 12 show the mean scores in VAS pleasurable effects of the slot machine game (4 measures: Enjoyment, Excitement, Involvement, and High) with pre-treatment of HAL and FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 4 (Measure) ANCOVA, controlling for credits won on the slot machine, yielded significant main effects of Group \(F(1,40)=8.707, p=0.005\), Pre-Treatment \(F(1,40)=5.746, p=0.021\) and Measure \(F(3,120)=10.291, p<0.001\). In addition, a significant Antagonist Group x Group x Gender interaction \(F(1,40)=4.034, p=0.050\) was found.

The main effect of Pre-treatment reflected a more pronounced decline in scores under the active antagonist. The three-way interaction occurred because scores were more pronounced in Female HCs in the HAL group vs. Female PGs in the FLU group regardless of antagonist pretreatment.
Haloperidol and Post Slot Machine Pleasurable Effects in Healthy Controls and Pathological Gamblers

Figure 11(a-d). Mean VAS Pleasurable Effects scores of slot machine with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
Mean VAS Pleasurable Effects scores of slot machine with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
3.2.3.2 Effect of AMPH

Figures 13 and 14 show the mean scores for VAS pleasurable effects of AMPH (4 measures: High, Good effects, Bad effects and Desire to take again) with pre-treatment of HAL and FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 4 (Measure) ANCOVA yielded significant main effects of Measure \([F(3,126)=14.643, p<0.001]\). In addition, significant Antagonist Group x Group \([F(1,42)=4.175, p=0.047]\), Antagonist Group x Group x Measure \([F(3,126)=3.488, p=0.018]\), Pre-Treatment x Measure \([F(3,126)=3.343, p=0.021]\), as well as Antagonist Group x Group x Pre-Treatment x Measure \([F(3,126)=4.201, p=0.007]\) interactions were shown.

Inspection of Figures 13 and 14 reveals that the 4-way interaction denoted a more pronounced reduction of scores under HAL in HCs, but a more pronounced reduction of scores under FLU in PGs. The moderating effect of Measure indicated that, for a given combination of Antagonist Group x Group x Gender, the pattern of scores was directionally opposite for Bad effects compared to the other 3 subscales.

In summary, males again showed limited impact of both antagonists on pleasurable effects of study reinforcers (slot machine and AMPH). In females HAL reduced the pleasurable effects of AMPH in HCs; whereas FLU reduced pleasurable effects of AMPH in female PGs.
Haloperidol and Post Amphetamine Pleasurable Effects in Healthy Controls and Pathological Gamblers

Figure 13(a-d). Mean VAS Pleasurable Effects scores for AMPH with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
Figure 11(a-d). Mean VAS Pleasurable Effects scores of AMPH with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
3.3 ARCI Subjective Drug-Like Effects

3.3.1 ARCI-MBG (Euphoria) Subscale

3.3.1.1 Effect of Slot Machine

Figures 15 and 16 show the mean change in ARCI-MBG between Peak antagonist level and pre-antagonist Baseline, and between Post-Slot Machine and Peak antagonist level following pre-treatment with HAL or FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time) ANCOVA, controlling for credits won on the slot machine, yielded a significant main effect of Time \([F(1,40)=8.519, p=0.006]\) and a significant Antagonist Group x Pre-Treatment x Time \([F(1,40)=21.446, p<0.001]\) interaction. In addition, a marginal main effect of Group \([F(1,40)=3.836, p=0.057]\) was found.

The interactions among Antagonist Group, Pre-Treatment, and Group are shown in Figure 15 and 16. HAL largely reduced the post-slots increase in MBG euphoria in PGs and HCs, whereas FLU tended to enhance the post-slots increase in MBG euphoria scores in both groups.
Figure 15(a-d). Changes in Mean ARCI MBG scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level, with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
Fluphenazine and Pre-post Slot Machine Euphoria (MBG) in Healthy Controls and Pathological Gamblers

Figure 16(a-d). Changes in Mean ARCI MBG scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level, with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
3.3.1.2 Effect of AMPH

Figures 17 and 18 show the mean change in ARCI-MBG between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level in response to pre-treatment of HAL and FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time) ANCOVA yielded significant main effects of Pre-Treatment \[ F(1,42)=6.877, p=0.012 \] and Time \[ F(1,42)=42.122, p<0.001 \]. In addition, significant Group x Gender x Pre-Treatment \[ F(1,42)=4.789, p=0.034 \] and Antagonist Group x Gender x Pre-Treatment x Time \[ F(1,42)=6.249, p=0.016 \] interactions were shown. Moreover, marginal Antagonist Group x Group \[ F(1,42)=3.026, p=0.089 \], Antagonist Group x Gender \[ F(1,42)=3.594, p=0.065 \] and Gender x Pre-Treatment \[ F(1,42)=3.393, p=0.073 \] interactions were found.

Figure 17 and 18 show that the 3-way interaction reflected a consistent profile of Pre-treatment effects (Antagonist vs. Placebo) across Group and Gender for FLU, but an inconsistent Pre-treatment effect across Group and Gender for HAL. The 4-way interaction denoted a selective enhancement in post-AMPH MBG under HAL in male HCs, with no corresponding effects for FLU in any subgroup before or after AMPH.

In summary, the changes in MBG scores differed between the two reinforcers, i.e., slot machine and AMPH. HAL consistently decreased the euphoric effect of the slot machine but had relatively inconsistent effects on response to AMPH. FLU consistently increased the euphoric effect of the slot machine, but decreased euphoric effects of AMPH in females.
Haloperidol and Pre-post Amphetamine Euphoria (MBG) in Healthy Controls and Pathological Gamblers

Figure 17(a-d). Changes in Mean ARCI MBG scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level, with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
Figure 18(a-d). Changes in Mean ARCI MBG scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level, with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
3.3.2 ARCI-AMP (Psychomotor Stimulation) Subscale

3.3.2.1 Effect of Slot Machine

Figures 19 and 20 show the mean change in ARCI-AMP between Peak antagonist level and pre-antagonist Baseline, and between Post-Slot Machine and Peak antagonist level in response to pre-treatment of HAL and FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time) ANCOVA, controlling for credits won on the slot machine, yielded a significant main effect of Gender \[F(1,40)=6.649, p=0.014\]. In addition, significant Antagonist Group x Gender x Time \[F(1,40)=4.600, p=0.038\] and Antagonist Group x Pre-Treatment x Time \[F(1,40)=6.616, p=0.014\] interactions were found.

Figures 19 and 20 show that HAL uniformly reduced perceived psychomotor stimulation from the game, whereas FLU primarily ‘potentiated’ the psychomotor stimulant effects of the game that was absent under placebo. The interaction with Time denoted a lower overall score at pre-slots under placebo in the HAL group as against a lower overall score under the active antagonist at pre-slots in the FLU group.
Haloperidol and Pre-post Slot Machine Stimulation (AMP) in Healthy Controls and Pathological Gamblers

Figure 19(a-d). Changes in Mean ARCI AMP scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level, with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
Figure 20(a-d). Changes in Mean ARCI AMP scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level, with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
3.3.2.2 Effect of AMPH

Figures 21 and 22 show the mean change in ARCI-AMP between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level in response to pre-treatment of HAL and FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time) ANCOVA yielded a significant main effect of Time [F(1,42)=23.390, p<0.001]. However, no other significant effects or interactions were found.

As Figure 21 and 22 shows, the changes in ARCI-AMP scores were generally greater at Post-AMPH vs. Peak Antagonist, reflecting the main effect of Time.

In summary, HAL decreased the psychomotor stimulation of both reinforcers, whereas FLU increased the psychomotor stimulation of both reinforcers in females. In general, the pattern was more consistent in response to the slot machine game compared AMPH.
Figure 21(a-d). Changes in Mean ARCI AMP scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level, with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
Figure 22(a-d). Changes in Mean ARCI AMP scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
3.3.3 LSD (Dysphoria) Subscale

3.3.3.1 Effect of Slot Machine

Figures 23 and 24 show the mean change in ARCI-LSD scores between Peak antagonist level and pre-antagonist Baseline, and between Post-Slot Machine and Peak antagonist level in response to pre-treatment of HAL and FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time) ANCOVA controlling for credits won on the slot machine, yielded no significant main effects or interactions. Inspection of the vertical axes shows that the magnitude of the scores was extremely modest.
Haloperidol and Pre-post Slot Machine Dysphoria (LSD) in Healthy Controls and Pathological Gamblers

**Figure 23(a-d).** Changes in Mean ARCI LSD scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level, with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
Figure 24(a-d). Changes in Mean ARCI LSD scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level, with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
3.3.3.2 Effect of AMPH

Figures 25 and 26 show the mean change in ARCI-LSD between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level in response to pre-treatment of HAL and FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time) ANCOVA yielded a significant main effect of Time [F(1,42)=13.777, p=0.001] and a significant Antagonist Group x Group x Gender [F(1,42)=4.525, p=0.039] interaction as well as a marginal Group x Gender x Time [F(1,42)=3.595, p=0.065] interactions.

As Figure 25 and 26 shows, the increases in ARCI-LSD scores were generally greater at Post-AMPH (pair of bars on the right) vs. Peak Antagonist (pair of bars on the left), reflecting the main effect of Time.

In summary, the lack of significant effects involving Pre-treatment indicates that neither HAL nor FLU altered perceived dysphoria, which helps to rule out antagonist-induced dysphoria as an explanation for antagonist-induced reductions in the perceived positive subjective effects of the slot machine and AMPH.
Figure 25(a-d). Changes in Mean ARCI LSD scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level, with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
Fluphenazine and Pre-post Amphetamine Dysphoria (LSD) in Healthy Controls and Pathological Gamblers

Figure 26(a-d). Changes in Mean ARCI LSD scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level, with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
3.4 Results for Profile of Mood States
3.4.1 Vigor Subscale
3.4.1.1 Effects of Slot Machine

Figures 27 and 28 show the mean change in POMS-Vigor scores between Peak antagonist level and pre-antagonist Baseline, and between Post-Slot Machine and Peak antagonist level in response to pre-treatment of HAL and FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time) ANCOVA, controlling for credits won on the slot machine, yielded significant main effects of Group [F(1,40)=7.899, p=0.008] and Time [F(1,40)=13.596, p=0.001]. In addition, significant Gender x Time [F(1,40)=5.313, p=0.026] and Group x Gender x Time [F(1,40)=8.768, p=0.005] interactions, as well as marginal Antagonist Group x Pre-Treatment [F(1,40)=3.788, p=0.059] and Antagonist Group x Group x Gender x Pre-Treatment [F(1,40)=3.732, p=0.06] interactions were found.

Figure 27 shows that the four-way interaction arose because HAL generally reduced perceived Vigor relative to placebo across Gender and Group, whereas Figure 28 shows that FLU enhanced perceived Vigor before and after the slot machine in Female PG subjects.
Haloperidol and Pre-post Slot Machine Vigor in Healthy Controls and Pathological Gamblers

**Figure 27(a-d).** Changes in Mean POMS Vigor scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level, with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
Fluphenazine and Pre-post Slot Machine Vigor in Healthy Controls and Pathological Gamblers

Figure 28(a-d). Changes in Mean POMS Vigor scores between Peak antagonist level and pre-antagonist Baseline, and between Post-slot machine and Peak antagonist level, with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Means adjusted to control for variation in final credit tally (winnings) in slot machine game on each session. Vertical bars denote standard error of the mean.
3.4.1.2 Effects of AMPH

Figures 29 and 30 show the mean change in POMS-Vigor scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level in response to pre-treatment of HAL and FLU, respectively. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 2 (Time: Peak Antagonist, Post-AMPH) ANCOVA yielded significant main effects of Pre-Treatment [F(1,42)=9.899, p=0.003] and Time [F(1,42)=13.596, p=0.001].

Figures 29 and 30 show that both HAL and FLU were associated with a reduction in perceived Vigor and that this effect was more evident at peak AMPH than prior to receiving AMPH.

In summary, HAL consistently decreased the energy/activation level following slot machine game and AMPH. However, FLU had relatively inconsistent effects, as it increased the energy/activation following slot machine, but decreased energy/activation following AMPH in female PGs.
Figure 29(a-d). Changes in Mean POMS Vigor scores between Peak antagonist level and pre-antagonist Baseline and between Post-AMPH and Peak antagonist level, with pre-treatment of HAL vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
Fluphenazine and Pre-post Amphetamine Vigor in Healthy Controls and Pathological Gamblers

Figure 30(a-d). Changes in Mean POMS Vigor scores between Peak antagonist level and pre-antagonist Baseline, and between Post-AMPH and Peak antagonist level, with pre-treatment of FLU vs. placebo. Panel a: HC-male; panel b: HC-female; panel c: PG-male; panel d: PG-female. * = p<0.05. Vertical bars denote standard error of the mean.
3.5 Betting Behavior

Betting behavior, including average lines per spin (lines), average credits per line (credits) and total spins per session (spins), was only measured once during each of the two slot machine sessions. Therefore, the following figures present raw mean values instead of changes in means. A 2 (Antagonist Group) x 2 (Group) x 2 (Gender) x 2 (Pre-Treatment) x 3 (Measure: Lines, Credits, and Spins) multivariate ANCOVA was used.

3.5.1 Lines Selected Per Spin

Figures 31 and 32 show the average number of lines selected per spin on a slot machine game with pretreatment of HAL and FLU, respectively. According to ANOVA, a significant main effect of Antagonist Group [F(1,42)=7.45, p=0.009], and significant Antagonist Group x Gender [F(1,42)=6.074, p=0.018] as well as Antagonist Group x Group x Gender [F(1,42)=4.162, p=0.048] interactions were shown, reflecting fewer lines per spin in the PG-female HAL subgroup and greater number of lines per spin in PGs-female FLU subgroup, regardless of antagonist pre-treatment.

The absence of significant effect of Pre-Treatment-related effects indicates that neither antagonist reliably altered the number of lines selected per spin. Exploratory simple effects indicated that, in HCs both antagonists increased the number of lines selected in females but not males, whereas in PGs, both antagonists increased the number of lines selected in males but not females.
Haloperidol and Lines Selected per Spin on Slot Machine in Healthy Controls in Pathological Gamblers

**Figure 31(a-b)**. Mean number of lines selected per spin on a slot machine game with pretreatment of HAL vs. placebo. Panel a: HC; panel b: PG. * = p<0.05. Vertical bars denote standard error of the mean.

Fluphenazine and Lines Selected per Spin on Slot Machine in Healthy Controls in Pathological Gamblers

**Figure 32(a-b)**. Mean number of lines selected per spin on a slot machine game with pretreatment of FLU vs. placebo. Panel a: HC; panel b: PG. * = p<0.05. Vertical bars denote standard error of the mean.
3.5.2 Credits Wagered Per Line on Each Spin

Figures 33 and 34 show the average number of credits bet per line on a slot machine game with pretreatment of HAL and FLU, respectively. According to ANOVA, a marginal main effect of Antagonist Group [F(1,42)=3.648, p=0.063] was shown, reflecting slightly higher bets in FLU group regardless of antagonist pre-treatment.

The absence of significant effect of Pre-Treatment and significant interactions including Pre-Treatment indicates that neither antagonist reliably altered number of credits bet per line. However based on simple effects tests, FLU increased the bets in males regardless of Group, and had inconsistent effect on females: HCs (no effect) and PGs (decrease).
Figure 33(a-b). Mean number of credits bet per line on a slot machine game with pretreatment of HAL vs. placebo. Panel a: HC; panel b: PG. * = p<0.05. Vertical bars denote standard error of the mean.

Figure 34(a-b). Mean number of credits bet per line on a slot machine game with pretreatment of HAL vs. placebo. Panel a: HC; panel b: PG. * = p<0.05. Vertical bars denote standard error of the mean.
3.5.3 Total Spins Per Session

Figures 35 and 36 show mean total spins per 15-min on the slot machine (speed of play) under of HAL and FLU, respectively. According to ANOVA, a marginal Pre-Treatment x Antagonist Group x Group x Gender interaction \[F(1,42)=3.111, \, p=0.083\] emerged.

The figures show that, in HCs, HAL decreased and FLU increased spins in males but had little impact on females. In contrast, in PGs, HAL decreased and FLU increased total spins in females but had no consistent effects in males.
Haloperidol and Spins per 15-min Game on Slot Machine in Healthy Controls in Pathological Gamblers

Figure 35(a-b). Mean total spins per 15-min on the slot machine with pretreatment of HAL vs. placebo. Panel a: HC; panel b: PG. * = p<0.05. Vertical bars denote standard error of the mean.

Fluphenazine and Spins per 15-min Game on Slot Machine in Healthy Controls in Pathological Gamblers

Figure 36(a-b). Mean total spins per 15-min on the slot machine with pretreatment of FLU vs. placebo. Panel a: HC; panel b: PG. * = p<0.05. Vertical bars denote standard error of the mean.
3.5.4 Winnings (Final Credit Tally)

Figure 37 and 38 show the average winnings (final credit tally after 15-min) on a slot machine game under HAL and FLU, respectively. According to ANOVA, a significant main effect of Gender [F(1,42)=5.113, p=0.029] and a Group x Gender interaction [F(1,42)=6.074, p=0.018] were found, reflecting more winnings in males, in particular, HC-male subgroups, regardless of pre-treatment or antagonist group. Differences in winnings are due to random factors and not attributable to study interventions or group differences. However this measure may have affected subjective scores. Therefore, as noted above, winnings were included as covariate in the subjective effects analyses for slot machine sessions. The lack of significant Pre-treatment related effects ensures that credits did not mediate (account for) the effects of Pre-treatment in other analyses. The lack of significant Pre-treatment effects in HC males under FLU (Figure 38a) despite a considerable difference in mean winnings may be due the greater difficulty to detect a significant effect of only one condition out of many in ANOVA using the degrees of freedom for the error term distributed equally across all conditions (Howell 1992).
Haloperidol and Winnings (Final Credits) on Slot Machine in Healthy Controls and Pathological Gamblers

**Figure 37(a-b).** Mean winning credits (final tally after 15-min) on a slot machine game with pretreatment of HAL vs. placebo. Panel a: HC; panel b: PG. * = p<0.05. Vertical bars denote standard error of the mean.

Fluphenazine and Winnings (Final Credits) on Slot Machine in Healthy Controls in Pathological Gamblers

**Figure 38(a-b).** Mean winning credits (final tally after 15-min) on a slot machine game with pretreatment of FLU vs. placebo. Panel a: HC; panel b: PG. * = p<0.05. Vertical bars denote standard error of the mean.
4. DISCUSSION

Previous studies have demonstrated key similarities between PG and substance addiction, based on features such as craving, impulsivity, sensation seeking, as well as a withdrawal-like syndrome (Holden 2001, Tavares et al. 2005, Potenza 2008). A range of preclinical evidence further indicated a special correspondence between PG and psychostimulant dependence based on common neuronal circuits, and the fact that the DA system plays a pivotal role in conditioned reward, uncertainty and non-drug reinforcement (core elements of gambling) as it does in psychostimulant reward and reinforcement (Zack and Poulos 2009). In fact, similar disruptions of the DA system, including deficits in DA activation to monetary reward as well as DA sensitization to cues for reward have been shown in both stimulant addiction and PG (Reuter et al. 2005, Volkow et al. 1999, Boileau et al. 2013, van Holst et al. 2012). The present study therefore used stimulant reward as an index to investigate DA’s role in gambling reward in PG. In light of the critical roles for DA D1 and D2 receptors in stimulant reward, and evidence of D1R and D2R abnormalities in stimulant addiction (Quinlan et al. 2004, Grace 2000, Nikolaus et al. 2007, Chen et al. 1999, Tong et al. 2003), this study primarily aimed to investigate the function of D1R and D2R in the motivational (‘wanting;’) and pleasurable (‘liking’) aspect of gambling reinforcement (cf. Robinson and Berridge 2000). In addition, the study examined gender as a potential factor moderating D1R and D2R response to gambling and AMPH, based on evidence indicating important sex-based differences in acute and chronic response to AMPH and of D1R differences in animals exposed to stimulants (Dow-Edwards 2010, Anderson and Teicher 2000, Festa et al. 2006).

A previous study showed that D2-autoreceptor blockade by HAL (3-mg), a preferential D2R antagonist, enhanced the reinforcing effects of a slot machine game in PG but not HC subjects (Zack and Poulos 2007). HAL, at low doses, would be expected to block pre-synaptic D2 autoreceptors (Pucak and Grace 1994), which would remove negative feedback, and lead to increased DA release and post-synaptic D1R activation. The finding of increased gambling reward under HAL thus suggested a potential role for D1R anomalies in PG subjects’ maladaptive response to gambling. Specifically, based on the idea of an inverted-U relationship between D1R activation and reward, we suspected that PGs may experience lower baseline D1R activation, and that HAL might have reversed this deficit to promote optimal D1R signaling during the slot machine game.
D1R anomalies could well interact with gender. At the clinical level, the term, “telescoping” (later onset but more rapid progression to severe addiction) has been used to characterize the etiology of substance dependence in women, and a parallel pattern has been seen in PG. Given the important role of D1R in psychostimulant sensitization (Vanderschuren et al. 1999), we reasoned that gender differences in D1R function or availability may contribute to telescoping in females with PG. Both animal and human studies have found sex differences in DA system function, such as the hormonal modulation of DA function (Pohjalainen et al. 1998) and DA metabolism (Ceylan-Isik et al. 2010). At the receptor level, deficiencies in D1R function and availability have been suggested in females. For example, female animals display lower D1R density than do male animals (Anderson and Teicher 2000); they also show a lower level of D1R binding after cocaine treatment (Festa et al. 2006). These findings indicate that sex differences in D1R may moderate gambling reinforcement, which is the key question of this study.

To address this question, the study employed a 2 Group (PG / HC) x 2 (Gender) x 2 Antagonist (HAL / FLU) x 2 Treatment (drug / placebo) x 2 Reinforcer (slot machine / AMPH) repeated measures between-within subjects’ design. We focused primarily on subjective outcome measures, using a range of validated self-report scales at multiple time points before and after study interventions. Subjects in subgroups were matched on key background variables and randomly assigned to receive one of the two DA antagonists, HAL (3-mg) and FLU (3-mg). The binding profiles of the two antagonists were similar except for D1R, for which FLU has much greater affinity than HAL. In addition, at low doses, both antagonists primarily block pre-synaptic D2 autoreceptors, enhancing post-synaptic D1R stimulation through the inhibition of negative feedback. This design maximized the ability to attribute observed differences between HAL and FLU to the increase in D1R activation in the HAL Group.

Based on the hypothesized common neurophysiology of PG and psychostimulant addiction with respect to DA and our prior research findings, we predicted that: 1) HAL would increase the incentive motivational effects of a slot machine game and of a low-dose of AMPH; 2) these effects would be more pronounced in PGs compared to HCs, reflecting lower baseline D1R function in PGs; 3) the effects would be more pronounced in females than males reflecting lower baseline D1R availability in females; 4) no such effects would occur with FLU, which blocks both D1R and D2 autoreceptors.
4.1 **Subject Characteristics**

Subjects in each subgroup were matched on key background characteristics including age, gender, smoker status, drinking habits and drug use. PG subgroups displayed more severe depression symptoms based on BDI scales, as depression and anxiety are among the comorbidities commonly observed in PGs (Lorains et al. 2011). However, all BDI scores were within the normal range and below the clinical threshold (McKenzie and Marks 1999). PGs scored higher on Impulsivity and Extraversion subscales on the Eysenck scales. Impulsivity is a common correlate of PG (Tavares et al. 2005), which supports the external validity of our sample. Also as expected, PGs displayed more severe cognitive distortions on the Gamblers’ Beliefs Questionnaire, as they had greater belief in luck and the importance of continuing to gamble when losing, as well as a greater belief in their ability to control the outcome of gambling. However, subjects in each subgroup showed a comparable level of verbal IQ and cognitive functions, helping to ensure that group differences in cognitive effects of the antagonists did not account for their subjective effects. Of particular importance was the finding that neither group displayed a tendency to dissimulate on the Eysenck “Lie” scale, helping to ensure the validity of the self-report data.

4.2 **Subjective Effects**

The self-report indices tapped different aspects of subjective reinforcement including Incentive Motivation – what Robinson and Berridge (2000) refer to as “Wanting” of the addictive reinforcer; and Hedonic Impact or Pleasurable effects – what Robinson and Berridge (2000) refer to as “Liking.” Although these authors argued that DA primarily mediates “Wanting,” a range of evidence suggests that, in the case of psychostimulants, DA may mediate aspects of Liking as well (Sofuooglu et al. 2008, Volkow et al. 1999). To the extent that psychostimulant effects correspond to those of gambling, DA may also play a role in the pleasurable aspects of slot machine gambling.
Table 6. Summary of the effects of HAL and FLU on gambling and AMPH reinforcement

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Reinforcer</th>
<th>Antagonist Group</th>
<th>Group</th>
<th>Gender</th>
<th>Antagonist Alone</th>
<th>Response to reinforcer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desire to Gamble</td>
<td>Slot machine</td>
<td>HAL</td>
<td>PG</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FLU</td>
<td>PG</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>AMPH</td>
<td>HAL</td>
<td>PG</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td>FLU</td>
<td>PG</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td>VAS-Pleasurable Effects</td>
<td>Slot machine</td>
<td>HAL</td>
<td>PG</td>
<td>M</td>
<td>N/A</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td>FLU</td>
<td>PG</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td>AMPH</td>
<td>HAL</td>
<td>PG</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td>FLU</td>
<td>PG</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td>ARCI-MBG</td>
<td>Slot machine</td>
<td>HAL</td>
<td>PG</td>
<td>M</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
<td>ộ</td>
</tr>
<tr>
<td></td>
<td>FLU</td>
<td>PG</td>
<td>M</td>
<td>ộ</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td>ộ</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>ộ</td>
<td>ộ</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Dependent Variable</td>
<td>Reinforcer</td>
<td>Antagonist Group</td>
<td>Group</td>
<td>Gender</td>
<td>Antagonist Alone</td>
<td>Response to reinforcer</td>
</tr>
<tr>
<td>--------------------</td>
<td>------------</td>
<td>------------------</td>
<td>-------</td>
<td>--------</td>
<td>-----------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>ARCI-MBG</td>
<td>AMPH</td>
<td>HAL</td>
<td>PG</td>
<td>M</td>
<td>↑</td>
<td>∅</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FLU</td>
<td>PG</td>
<td></td>
<td>∅</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FLU</td>
<td>PG</td>
<td></td>
<td>∅</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARCI-AMPH</td>
<td>Slot machine</td>
<td>HAL</td>
<td>PG</td>
<td>M</td>
<td></td>
<td>∅</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FLU</td>
<td>PG</td>
<td></td>
<td>∅</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AMPH</td>
<td>HAL</td>
<td>PG</td>
<td>M</td>
<td></td>
<td>∅</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FLU</td>
<td>PG</td>
<td></td>
<td>∅</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POMS-Vigor</td>
<td>Slot machine</td>
<td>HAL</td>
<td>PG</td>
<td>M</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FLU</td>
<td>PG</td>
<td></td>
<td>∅</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AMPH</td>
<td>HAL</td>
<td>PG</td>
<td>M</td>
<td></td>
<td>∅</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FLU</td>
<td>PG</td>
<td></td>
<td>∅</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HC</td>
<td>M</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Motivation was assessed by Desire to Gamble (and as a positive control, Desire for Alcohol). Pleasurable effects were more varied qualitatively, and ranged from drug-like euphoria (ARCI-MBG scale) to specific effects of the slot machine (e.g., Excitement/Involvement) and AMPH (Good/Bad Effects, High), and finally more global feelings, like POMS Vigor. The pattern of significant effects across the various indices is summarized in Table 6. Unless otherwise stated, the reported effects of HAL and FLU per se and on responses to the reinforcers, are defined relative to placebo pre-treatment.

4.2.1 Motivational Effects

4.2.1.1 Desire to Gamble:

Slot Machine: The topmost cells in Table 6 report the effects of Antagonists on Desire to Gamble before and after the slot machine and AMPH. The Table shows that HAL alone selectively increased pre-game desire in PG females. With respect to the priming effects of the slot machine, HAL had no significant effects in males, regardless of PG status, but diminished the priming effects of the game in HC females and to a somewhat greater degree, in PG females.

In the case of FLU, the antagonist alone reduced pre-game Desire to Gamble in male and female PGs. In males, FLU did not significantly alter the priming effects of the game. In contrast, FLU significantly enhanced post-game Desire to Gamble in females from both groups.

These findings indicate that preferential blockade of D2 autoreceptors under HAL had no effect in males, but was associated with a decreased priming effect of the game in females. Combined blockade of D1R and D2 autoreceptors under FLU reduced baseline desire to gamble in PGs, but selectively enhanced the priming effects of the game in females, regardless of PG status.

AMPH: HAL had no intrinsic motivational effects prior to the AMPH dose, nor did it alter the priming effects of AMPH in males. In females - both PG and HC - HAL diminished the priming effects of AMPH on Desire to Gamble, as it had in the case of the slot machine, and this effect was more pronounced in PG.

FLU also had few intrinsic motivational effects, with the exception of female PGs, who reported decreased motivation to gamble under FLU, as they had in phase I. However, in contrast to its effects on slots-induced priming, FLU did not alter the priming effects of AMPH in either gender or group.
These results indicate that preferential blockade of D2 autoreceptors by HAL again had no effect in males, but decreased the priming effect of AMPH in females, particularly with PG. In contrast, combined blockade of D1R and D2 autoreceptors selectively reduced intrinsic motivation to gamble in female PGs, but had no effect on AMPH-induced priming, regardless of Gender or Group.

The inhibitory effect of HAL on priming by the slot machine and by AMPH is consistent with the hypothesis that preferential blockade of D2 autoreceptors would have parallel effects on gambling and psychostimulant-induced incentive motivation. The emergence of this effect in females but not males is also consistent with our hypothesis of increased sensitivity to a manipulation that augments D1R signaling in females.

The enhancing effect of FLU on priming by slots and AMPH further supports the hypothesis of parallel effects of DA receptor manipulation on incentive motivational effects of gambling and a psychostimulant. The emergence of this effect in a separate group of subjects (i.e., along with HAL group) supports the reliability of the correspondence in DA’s role in gambling and psychostimulant reinforcement. The selective emergence of this effect in females but not males is also consistent with our hypothesis of increased sensitivity to D1R manipulations in females. The directionally opposite pattern of HAL and FLU effects implies a critical role for D1R. Lastly, the consistent inhibitory effect of FLU on baseline motivation to gamble in phase I and phase II in females with PG suggests an interaction between gender and PG status in terms of the role of D1R in desire to gamble, in line with a possible relationship between D1R anomalies and telescoping in females with PG.

Despite the internally consistent pattern of effects, there were several key inconsistencies: First, based on our previous study (Zack and Poulos, 2007), HAL was expected to increase post-slots Desire to Gamble in PGs but not HCs. This was not observed. Second, in females, HAL was expected to increase and FLU was expected to decrease post-slots Desire to Gamble. Instead the direction of effects was exactly reversed such that HAL decreased and FLU increased the priming effects of the game. The lack of increased slots-induced priming in PG subjects under HAL indirectly suggests a possible methodological inconsistency between the current study and the previous study. This possibility is explored later. The directionally opposite effects of HAL and FLU on slots-induced priming in females raises two key possibilities: Either females have heightened D1R sensitivity, and this mediated the opposite motivational effects of HAL vs. FLU, or D1R alone does not mediate the priming
effects of slots or AMPH. With regard to the latter possibility, 3-mg doses of HAL and FLU would both be expected to increase DA release and thereby increase signaling at post-synaptic D2 receptors. It is conceivable therefore, that D1R influences motivation to gamble by negatively modulating post-synaptic D2R signaling. To see which of these possible explanations better explains the data, we will consider the impact of HAL and FLU on the other self-report indices.

4.2.1.2 Desire for Alcohol (Results not shown in Table 6)

The ratings of Desire for Alcohol served as a positive control to determine whether the motivational effects of the slot machine game and AMPH are generic (to a range of addictive reinforcers) or specific to gambling. Both the slot machine game and AMPH increased the motivation to drink alcohol, reflecting the association between gambling and alcohol use. However, the magnitude of Desire for Alcohol scores was much smaller (absolute scores ~1 out of 10) as compared to Desire to Gamble. This indicates that both reinforcers had much weaker impact on motivation for alcohol compared to gambling. In addition, neither antagonist displayed consistent effects based on group or gender, further suggesting that different processes mediate motivation to gamble vs. motivation for alcohol in subjects with no alcohol use disorder.

4.2.2 Pleasurable Effects

4.2.2.1 VAS-Pleasurable Effects

Slot machine: Table 6, second panel from the top reports the overall pattern of pleasurable effects on the visual analogue scales for the slot machine (Enjoyment, Excitement, Involvement, High) and AMPH (Good/Bad Effects, High, Take Again). There were no baseline measures for these indices (i.e., no control for group or individual differences). HAL had no effects in males, regardless of PG status. In females, HAL displayed inconsistent effects in PGs, but decreased the pleasurable effects in HCs. FLU also had no significant effects in males, regardless of PG status. In females, FLU displayed directionally opposite effects as a function of PG status: increasing scores in HC and decreasing them in PG.

AMPH: HAL had no effects in males, regardless of PG status. In females, HAL had no effects in PGs, but decreased the pleasurable effects in HCs. FLU also had no significant effect
In males, regardless of PG status. In females, FLU displayed no effects in HCs, but decreased the pleasurable effects in PGs.

In sum, neither preferential blockade of D2 autoreceptors by HAL nor combined blockade of D1R and D2R by FLU altered the pleasurable effects of the game or AMPH in males. In females, preferential blockade of D2 autoreceptors decreased the pleasurable effects of both the slot machine and AMPH; and interestingly, combined blockade of D1R and D2R also decreased the pleasurable effects of both the slot machine and AMPH in PGs, but not HCs.

The pleasurable effects data reveal a congruent effect of HAL and FLU across both reinforcers, with males exhibiting no significant effects and females displaying a consistent reduction, in the case of PGs. These findings suggest that D2R (either pre- or post-synaptic), which HAL and FLU block with similar affinity, may mediate the hedonic impact of slots and AMPH, whereas group differences in D1R may account for the different profile of effects for HAL and FLU in female PGs vs. female HCs. The consistency of the findings for the slot machine and AMPH indicates that the pleasurable effects – or Liking – of the two reinforcers are mediated by the DA system (contrary to Robinson and Berridge, 2000), and that D1R and D2R each play a role in these effects.

**4.2.2.2 ARCI-MBG: Drug-like (Morphine-Benzedrine; MBG) euphoric effects**

**Slot machine:** The middle panels of Table 6 show ratings of morphine-benzedrine-like (MBG; euphoric) effects. In male and female PGs and female HCs, HAL alone increased MBG ratings relative to session baseline. HAL had the precise opposite effect on euphoric response to the slot machine in these three sub-groups. FLU alone had no effect on self-reported euphoria in male or female PGs, but reduced euphoria in male and female HCs. Playing the slot machine reversed the effects of FLU in male and female HCs, such that self-reported euphoria increased, and a similar enhancement in post-game euphoria was seen in male and female PGs, who were insensitive to euphoric effects of FLU alone.

**AMPH:** In phase II, HAL alone reduced euphoria relative to session baseline in female PGs and HCs, which contrasted with HAL’s effects in these subjects in Phase I. However, male PGs continued to report increased euphoria from HAL as they had in Phase I. In terms of the response to AMPH, HAL had gender-based opposite effects in HCs (M increase, F decrease), which were also inconsistent with the respective responses of these subjects to the slot machine in Phase I.
In phase II, FLU had no intrinsic effects on euphoria in males or females of either group. In contrast, FLU consistently decreased AMPH-induced euphoria ratings in female PG and HCs, with no effect in males of either group. The effects of FLU in females are opposite to its effects on post-slots euphoria in these subjects.

Taken together, the MBG data indicate that preferential blockade of D2 autoreceptors *per se* under HAL consistently enhances perceived euphoric (‘dream-like’) feelings in male PGs, but has inconsistent effects in females with or without PG. This manipulation reduced the euphoric effects of the slot machine in all subjects aside from male HCs. Combined blockade of D1R and D2 autoreceptors *per se*, under FLU, had no consistent effects across both phases and had directionally opposite effects on euphoric responses to the slot machine (increase) vs. AMPH (decrease) in both female PGs and HCs. In the original HAL study, there were no significant effects on any ARCI sub-scale, which further suggests a potential methodological difference between the two studies. However, the pattern of MBG effects for FLU is generally consistent with the pattern seen for VAS pleasurable effects in the present study. This indicates that the change scores (MBG) were capturing a similar process as the raw post-reinforcer ratings of pleasurable effects, and that single-item VAS ratings yield similar results as a multi-item validated index of drug-like euphoria.

The differential effects on MBG euphoria after the slot machine under D2R blockade (decrease) due to HAL, versus the effects of combined D1R and D2R blockade (increase) due to FLU, suggest that D1R negatively modulates the euphoric effect of the slot machine. The general reduction in post-AMPH MBG scores under HAL and FLU suggests that D2R (pre- or post-synaptic), which is similarly affected by both antagonists, rather than D1R mediates the euphoric effects of AMPH.

Interestingly, the pre-game MBG effects under HAL were consistent with our hypothesis that preferential D2R blockade (which increases basal DA release and D1R signaling; Shi et al 1997) would increase euphoria in PGs, and the reversal of this effect after playing the game raises the possibility that HAL alone optimized D1R activation whereas HAL + slot machine led to supra-optimal D1R activation. This possibility is further supported by the time-dependent opposite effect of FLU (in separate subjects) on MBG scores in Phase I – i.e., pre-game reduction in euphoria but post-game enhancement in euphoria during combined D1R and D2R blockade. It should be noted that (as in Brauer and de Wit, 1995), 3-mg FLU would lead to partial D1R blockade but not saturation of these receptors.
**4.2.2.3 ARCI-LSD: Dysphoria** (Results not shown in Table 6)

Overall, the dysphoric effects were extremely modest and inconsistent. Neither preferential D2R blockade nor combined blockade of D1R and D2R caused any consistent or significant effects, which helps to rule out antagonist-induced dysphoria as a factor contributing to the reductions in perceived positive subjective effects of either reinforcer under HAL or FLU.

**4.2.3 Other Effects**

**4.2.3.1 ARCI-AMP: Psychomotor stimulation**

**Slot machine:** Table 6 shows that HAL alone increased AMP ratings in female PGs and HCs. These effects were reversed after playing the slot machine, and furthermore, post-slot AMP ratings were diminished by HAL in male PG and HCs as well. FLU alone diminished pre-game AMP ratings in female HCs, but enhanced post-game AMP ratings in female HCs as well as PGs.

**AMPH:** HAL alone had no intrinsic effects on AMP scores in phase II and reduced AMP scores in response to the AMPH dose in PG females and HC males. FLU had parallel effects on AMP ratings in response to the AMPH dose as it did on response to the slot machine in female HCs (with no effects in other subjects): a decrease in AMP ratings relative to baseline followed by increased AMP ratings after the AMPH dose.

These results indicate that preferential blockade of D2R by HAL alone elicited a psychomotor stimulant-like effect in females regardless of PG status, but reduced the stimulant-like effects of the slot machine – uniformly across groups - and of the AMPH dose in PG females and HC males. Combined blockade of D1R and D2R reduced psychomotor stimulation relative to baseline in females, particularly HCs, but enhanced the stimulant effects of the slot machine and the AMPH dose in these subjects and of the slot machine (but not the AMPH dose) in female PGs.

This profile of effects again suggests an optimization of D1R signaling by HAL and supra-optimal D1R signaling when HAL was combined with slots or AMPH, and the complementary pattern of effects for FLU at pre- vs. post-reinforcer supports this interpretation. The tendency for this effect to emerge in females suggests that gender moderates the role of D1R on psychostimulant-like states regardless of PG status. However, the differential effect of FLU on psychostimulant responses to slots (increase) vs. the AMPH dose (no effect) in female
PGs, suggests that the role of D1R may depend on the intrinsic value of the reinforcer (e.g., negative reinforcing value of gambling) in subjects who are ‘dependent’ on gambling.

4.2.3.2 POMS-Vigor: Energy and activation

In keeping with common practice in studies on the subjective effects of AMPH (cf. Brauer and de Wit 1996), we included a measure of Vigor, which captures a more general mood state as well as a willingness or ability to do work, as opposed solely to a sense of arousal or excitement.

**Slot machine:** The bottom panel in Table 6 shows that HAL alone selectively enhanced pre-game Vigor in male PGs but decreased post-game Vigor in all sub-groups except female HCs. FLU alone decreased pre-game Vigor in female PGs and HCs and increased post-game Vigor in female PGs only.

**AMPH:** HAL alone had no effect on Vigor prior to the AMPH dose and decreased post-game Vigor in female PGs and male HCs. FLU alone had no effects on Vigor prior to the AMPH dose but decreased post-AMPH Vigor in female PGs and HCs.

Thus, preferential blockade of D2 autoreceptors under HAL and combined blockade of D1R and D2R under FLU had opposing effects on Vigor before and after gambling but congruent effects after AMPH. These findings again suggest that D2R may play a more important role than D1R in the energizing effects of AMPH. However, combined blockade of D1R and D2R selectively increased the energizing effect of the game in female PGs, suggesting that (hyper-sensitive) D1R may mediate the reinforcing effects of gambling in individuals at risk for telescoping of PG symptoms.

4.3 Betting Behavior

**Lines:** Both HAL and FLU increased the lines selected per spin in HC females and PG males.

**Credits:** HAL did not change the average number of credits bet per line, whereas FLU increased the credits bet per line in males, but not females.

**Spins:** HAL decreased the total number of spins in HC males and PG females, whereas FLU increased the total number of spins in HC males and PG females. These effects were significant based on simple effects tests, although the overall F statistic was not significant in the MANOVA.
An increase in the number of lines selected can indicate a risk-averse strategy of play when combined with a reduction or no change in credits bet per spin. Neither drug caused this combined profile of effects. Increased lines selected combined with increased bet per spin indicates a more ‘profligate’ pattern of betting. FLU appeared to induce this effect in PG males. The number of spins, measured over a finite interval (15-min) reflects the speed of play. Thus, HAL appeared to decrease whereas FLU increased speed of play in both HC males and PG females. These opposing effects indicate that supra-optimal D1R stimulation inhibits speed of play in male social gamblers, perhaps reflecting relatively higher baseline D1R sensitivity in healthy males who are more likely to initiate gambling than healthy females, and also in female PGs who are more likely than male PGs to escalate rapidly to severe gambling problems.

4.4 General Discussion

The hypothesis that HAL would enhance the reinforcing effect of a slot machine game and of a low-dose of AMPH was not supported. In fact, an opposite effect to our prediction was consistently displayed.

In general, HAL displayed a very consistent negative impact on multiple aspects of subjective feelings, in particular in females and to a greater extent in PG subjects. The inhibitory effect of HAL on the reinforcing effects of both slots and AMPH is largely consistent with the hypothesis that preferential blockade of D2 autoreceptors would have parallel effects on gambling and psychostimulant reinforcement.

However, the reducing effect of HAL was contradictory to hypothesis 1 and our previous study (Zack and Poulos 2007) in which HAL enhanced the reinforcing effects of the game. As noted above, the difference might reflect a methodological inconsistency between the current study and the previous study. In this regard, the most salient difference between the current study and previous study was that the present study included the administration of a second (dummy) capsule prior to the slot machine session in phase I (sessions 1, 2). The second capsule in phase I was intended to serve as a control for administration of the second capsule (AMPH) in phase II.

However, it is conceivable that the (dummy) capsule itself led to the expectation of reward (placebo effect), which might have interacted with the effects of HAL. The increase in ARCI-MBG effects in PGs and female HCs under HAL alone in Phase I coupled with the reversal of this effect in these same groups following the slot machine fits with this possibility.
Subjects knew from the Consent Form that Dexedrine was one of the drugs they may receive, but were naïve to its subjective effects. Under these conditions, the capsule may have served as a cue for potential AMPH reward and this may have increased D1R signaling. This effect would be enhanced by HAL (cue + HAL = optimal D1R stimulation) but also may have led to supra-optimal D1R stimulation when combined with the slot machine. Consistent with this possibility, previous animal research has found that: “The response reinstatement and Fos expression induced by the cocaine S(D) [discriminative stimulus/cue for cocaine] were both reversed by selective dopamine D(1) receptor antagonists… results [that] implicate D(1)-dependent neural mechanisms within the medial prefrontal cortex and basolateral amygdala as substrates for cocaine-seeking behavior elicited by cocaine-predictive environmental stimuli” (Ciccocioppo et al. 2001)

This interpretation is further supported by the increase in euphoric effect (MBG) reported by FLU subjects after the slot machine (i.e., FLU may have counteracted supra-optimal D1R stimulation). The emergence of this effect in a separate group of subjects further suggests that the HAL results were not an anomaly in a specific set of subjects. This is discussed more below.

The tendency for the effects of HAL and FLU to emerge in females supports our hypothesis that gender moderates the role of D1R in gambling reinforcement. HAL had parallel effects (pre-game increase, post-game decrease in MBG) on HC females and PG subjects of either gender. Conversely, FLU selectively decreased pre-game AMP scores in HC females but increased post-game AMP in these subjects and PG females. These findings indicate increased sensitivity to partial D1R blockade in the absence of DA stimulation and a restorative effect of gambling on stimulant like subjective states under these conditions. This aligns with the assumption of lower baseline D1R availability in females and PG subjects.

On the other hand, FLU decreased the pleasurable effects of both the slot machine and AMPH in female PGs, and also decreased drug-like euphoric effects and post- AMPH energy/activation in females from both groups. The lack of effects of FLU before the reinforcers in these cases suggests that, for indices that are insensitive to D1R blockade per se, blockade of D1R can diminish the increase in positive effects normally conferred by gambling and AMPH, relative to placebo pre-treatment.
Collectively, the pattern of effects for HAL and FLU before and after the reinforcers is consistent with the idea of an inverted U-relation between D1R stimulation and gambling and stimulant reinforcement.

Effects that support this interpretation are as follows: (1) Increased D1R signaling under HAL increased subjective hedonic states (MBG, AMP) in male and female PGs and female HCs; and playing the slot machine under HAL decreased these states in both groups and both genders (consistent with supra-optimal post-game D1R signaling). (2) Decreased D1R signaling under FLU decreased euphoria (MBG) in HCs; and playing the slot machine under FLU increased this state in both groups and both genders (consistent with optimal D1R signaling). (3) Increased D1R signaling following AMPH reversed a pre-dose deficit in euphoria in HC females, but decreased post-AMPH euphoria in HC males (consistent with optimal D1R signaling in healthy females [low D1R availability] but supra-optimal D1R signaling in healthy males [high D1R availability]). (4) Decreased D1R signaling decreased post-AMPH euphoria and post-AMPH energy/activation in HC females and PG females (consistent with sub-optimal D1R signaling in females). (5) Increased D1R signaling increased pre-game Wanting (Desire to Gamble) in PG females; and playing the slot machine reduced Wanting in these subjects and in HC females (consistent with a link between optimal D1R signaling and desire to gamble, and a link between supra-optimal D1R stimulation and satiation of this desire). (6) Decreased D1R signaling decreased pre-game Wanting in male and female PGs, and playing the game reversed this effect in female PGs (consistent with a link between low D1R stimulation and weak motivation to gamble and a link between moderate D1R stimulation and strong motivation to gamble; this effect echoes the enhanced pre-game desire to gamble under HAL in a separate group of female PGs).

In sum, the data on subjective effects suggest that D1R mediates the pleasurable and incentive motivational effects of gambling; that the role of D1R is similar but less consistent with respect to AMPH reinforcement; that the role of D1R in gambling and stimulant reinforcement is more pronounced in females than in males (due possibly to lower baseline D1R availability in females), and that reversal of suboptimal D1R may explain the increased pleasurable and incentive motivational effects of gambling in PG subjects relative to controls. Gender differences in D1R in PG parallel the gender/sex differences in sensitization to cocaine and amphetamine (Dow-Edwards 2010), further supporting the hypothesized correspondence between PG and stimulant addiction.
The betting behavior data provide an objective complement to the self-reported effects and show that FLU increased risky betting (more lines per spin, more credits per line) in male PGs and increased speed of play but not betting behavior in female PGs. HAL primarily inhibited speed of play in female PGs and male HCs. The effects of FLU are consistent with a priming effect of moderate D1R stimulation and a satiating effect of high D1R stimulation under FLU and HAL, respectively.

4.5 Limitations

The study had a number of limitations. First, the sample size was modest, particularly for females. A second issue concerns the attribution of effects to D1R. Because the present doses of HAL and FLU both preferentially block pre-synaptic D2 autoreceptors, increased DA release under both antagonists will not only increase signaling at D1R, but will also increase signaling at post-synaptic D2R. Although this cannot explain differences in the pattern of effects for HAL vs. FLU, in cases where the antagonists had similar effects (e.g., POMS-Vigor), these may not be solely attributable to supra-optimal (HAL) vs. sub-optimal (FLU) D1R stimulation, but may also reflect an action of these drugs at post-synaptic D2R, which mediates reward-related choice behavior in humans (Eisenegger et al. 2014).

Further to point 2, the lack of a selective D1R antagonist for human use in Canada made it necessary for us to infer the role of D1R based comparisons between HAL and FLU. However, this design could not provide definite evidence on the role of D1R alone (in the absence of D2R blockade).

Differences in Body Mass Index between men and women could result in women receiving a higher functional dose, which might contribute to gender differences in subjective response to the different drugs. Genetic variations in pharmacokinetics or pharmacodynamics of study subjects may also have affected the functional drug doses or baseline receptor level, and therefore influenced the outcomes of this study. Although the subjects are being genotyped, these data will not be available until the sample is complete.

Another limitation concerns the self-report nature of the primary outcome measures, which may increase variability in interpretation (e.g., vigor, ‘high’). Major analyses were performed using difference scores to help minimize this effect. The final projected sample size of 80 subjects will significantly increase the power and reliability of the tests.
PG subjects in this study had moderate symptom severity, and HCs were social gamblers, which may have limited the ability to detect differences between the Drug Groups and Gender due to deficits in DA receptor function or availability. Subjects with other comorbidities were excluded in order to attribute between-group differences to PG status. Therefore, PG subjects in this study may not be representative of the general population of PGs with more severe conditions and common comorbidities.

Lastly, due to practical constraints, women in different sub-groups started testing at different time points in their menstrual cycle and were tested at weekly intervals throughout their cycle. Therefore hormonal variation both between and within-female subjects was not controlled. However, the emergence of consistent effects in females despite this uncontrolled variability attests to the strength of the drug-related effects.

### 4.6 Future Directions

Expanding the sample size will help to clarify differences in DA receptor function between PGs and HCs as well as explore the impact of factors such as age, gender, and ethnic background, which may strongly influence DA systems and responses.

Exploring genotypes for enzymes that metabolize DA (e.g., catechol-O-methyltransferase; COMT) (Grant et al. 2013) and DA antagonists (e.g., CYP3A4) will also help inform understanding of variation in responses to gambling and the drug effects.

Direct measurement of DA transmission and receptor binding (e.g., using PET) would help to confirm the association between changes in DA release and subjective-behavioral effects, as well as differences in these effects based on gender and PG status.

### 4.7 Conclusion

The major finding of this study is that D1R mediates the incentive motivational and pleasurable effects of gambling, and to a lesser degree, of a psychostimulant drug. An inverted-U relation between D1R signaling and subjective reinforcement accounts quite well for the pattern of effects. Female gender and presence of PG were each associated with increased sensitivity to D1R manipulations, consistent with the possibility of lower D1R availability (although not necessarily lower sensitivity) in these subgroups relative to their male, healthy counterparts. These results help to advance the understanding of the role of DA in gambling
reinforcement and may be beneficial to the development of targeted biomedical interventions for the treatment of women and men with PG.
REFERENCES


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


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

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Response</th>
<th>IC\textsubscript{50}</th>
<th>(K_i)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromperidol</td>
<td>-54 ± 6</td>
<td>2.1 ± 0.6</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>Spiperone</td>
<td>-49 ± 1</td>
<td>0.3 ± 0.1</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Fluspiridene</td>
<td>-48 ± 6</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Pimozide</td>
<td>-45 ± 14</td>
<td>0.5 ± 0.1</td>
<td>2.4 ± 1.3</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>-43 ± 16</td>
<td>0.2 ± 0.0</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>-43 ± 15</td>
<td>0.8 ± 0.2</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Trifluoperidol</td>
<td>-42 ± 12</td>
<td>0.2 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Cisflupenthixol</td>
<td>-41 ± 7</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Sulpiride</td>
<td>-41 ± 4</td>
<td>11 ± 9</td>
<td>8.6 ± 1.9</td>
</tr>
<tr>
<td>Chlorprothazine</td>
<td>-40 ± 14</td>
<td>10 ± 5</td>
<td>11 ± 5</td>
</tr>
<tr>
<td>Butaclamol</td>
<td>-40 ± 2</td>
<td>0.3 ± 0.3</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>Amoxapine</td>
<td>-40 ± 3</td>
<td>38 ± 6</td>
<td>3.6 ± 1.5</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>-39 ± 6</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Sertindole</td>
<td>-39 ± 1</td>
<td>0.1 ± 0.0</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>Omaperidone</td>
<td>-38 ± 11</td>
<td>0.3 ± 0.3</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Remoxipride</td>
<td>-37 ± 3</td>
<td>16 ± 6</td>
<td>105 ± 38</td>
</tr>
<tr>
<td>Tiapride</td>
<td>-35 ± 11</td>
<td>31 ± 13</td>
<td>226 ± 223</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>-34 ± 12</td>
<td>3.0 ± 1.1</td>
<td>5.7 ± 1.5</td>
</tr>
<tr>
<td>Raclopride</td>
<td>-34 ± 9</td>
<td>0.5 ± 0.3</td>
<td>2.4 ± 0.8</td>
</tr>
<tr>
<td>Mephenoxazine</td>
<td>-34 ± 7</td>
<td>1.0 ± 0.6</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Tefludazine</td>
<td>-33 ± 2</td>
<td>0.3 ± 0.2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Transflupenthixol</td>
<td>-32 ± 6</td>
<td>17 ± 6</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>Clozapine</td>
<td>-31 ± 10</td>
<td>71 ± 21</td>
<td>72 ± 20</td>
</tr>
<tr>
<td>Molindone</td>
<td>-30 ± 8</td>
<td>3.8 ± 1.5</td>
<td>20 ± 7</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>-29 ± 13</td>
<td>1.7 ± 1.1</td>
<td>8.1 ± 2.1</td>
</tr>
<tr>
<td>Sulpiridazine</td>
<td>-29 ± 11</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>9-OH-risperidone</td>
<td>-29 ± 13</td>
<td>1.0 ± 0.2</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>Thiothixene</td>
<td>-27 ± 15</td>
<td>0.2 ± 0.0</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Sultopride</td>
<td>-24 ± 6</td>
<td>4.5 ± 0.7</td>
<td>1.6 ± 1.0</td>
</tr>
<tr>
<td>Melperone</td>
<td>-24 ± 4</td>
<td>N.D.</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>Octoclothepin</td>
<td>-24 ± 6</td>
<td>0.2 ± 0.1</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Promazine</td>
<td>-18 ± 13</td>
<td>N.D.</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>-14 ± 10</td>
<td>N.D.</td>
<td>16 ± 12</td>
</tr>
<tr>
<td>Mesoridazine</td>
<td>-14 ± 9</td>
<td>N.D.</td>
<td>4.3 ± 1.4</td>
</tr>
<tr>
<td>N-Desmethylolanzapine</td>
<td>-14 ± 7</td>
<td>N.D.</td>
<td>32 ± 29</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>-13 ± 12</td>
<td>N.D.</td>
<td>106 ± 43</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>-12 ± 15</td>
<td>N.D.</td>
<td>21 ± 16</td>
</tr>
<tr>
<td>L-745,870</td>
<td>N.D.</td>
<td>N.D.</td>
<td>343 ± 297</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>194 ± 42</td>
<td>N.D.</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td>N-Desmethylclozapine</td>
<td>129 ± 21</td>
<td>N.D.</td>
<td>89 ± 26</td>
</tr>
</tbody>
</table>

N.D., not done; N.R., no response.

Table A-ii. Receptor binding of haloperidol and fluphenazine at D1 receptors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 nM</th>
<th>Ki nM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thioxanthenes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cis(Z)-chlorprothixene</td>
<td>3.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Trans(E)-chlorprothixene</td>
<td>270</td>
<td>130</td>
</tr>
<tr>
<td>Zuclopenthixol</td>
<td>1.4</td>
<td>0.66</td>
</tr>
<tr>
<td>Trans(E)-clopenthixol</td>
<td>110</td>
<td>52</td>
</tr>
<tr>
<td>Cis(Z)-flupentixol</td>
<td>2.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Trans(E)-flupentixol</td>
<td>130</td>
<td>62</td>
</tr>
<tr>
<td>Cis(Z)-piflutixol</td>
<td>0.41</td>
<td>0.19</td>
</tr>
<tr>
<td>Trans(E)-piflutixol</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>Teflutixol</td>
<td>5.8</td>
<td>2.7</td>
</tr>
<tr>
<td>Cis-thiothixene</td>
<td>13</td>
<td>6.2</td>
</tr>
<tr>
<td><strong>Phenothiazines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>1.8</td>
<td>0.85</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>8.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>7.8</td>
<td>3.7</td>
</tr>
<tr>
<td><strong>Butyrophenones + analogues</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bromperidol</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Domperidone</td>
<td>3,900</td>
<td>1,800</td>
</tr>
<tr>
<td>Droperidol</td>
<td>860</td>
<td>410</td>
</tr>
<tr>
<td>Halopemide</td>
<td>6,800</td>
<td>3,200</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>36</td>
<td>17</td>
</tr>
<tr>
<td>Pirenperone</td>
<td>470</td>
<td>220</td>
</tr>
<tr>
<td>Setoperone</td>
<td>560</td>
<td>270</td>
</tr>
<tr>
<td>Spiperone</td>
<td>210</td>
<td>100</td>
</tr>
<tr>
<td><strong>Diphenylbutylpiperidines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluspiridene</td>
<td>57</td>
<td>27</td>
</tr>
<tr>
<td>Penfuridol</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>Pimozide</td>
<td>530</td>
<td>250</td>
</tr>
<tr>
<td>Clopimozide</td>
<td>670</td>
<td>320</td>
</tr>
</tbody>
</table>

Table A-iii. Receptor binding of haloperidol and fluphenazine at D2, D3, and D4 receptors.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Response</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>K&lt;sub&gt;i&lt;/sub&gt;</th>
<th>%</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>K&lt;sub&gt;i&lt;/sub&gt;</th>
<th>%</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>K&lt;sub&gt;i&lt;/sub&gt;</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromperidol</td>
<td>-54 ± 6</td>
<td>2.1 ± 0.6</td>
<td>1.0 ± 0.6</td>
<td>54</td>
<td>3.1 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>34</td>
<td>7.0 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>12</td>
</tr>
<tr>
<td>Sipiperone</td>
<td>-49 ± 1</td>
<td>0.3 ± 0.1</td>
<td>0.03 ± 0.01</td>
<td>49</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>49</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>49</td>
</tr>
<tr>
<td>Fluspiridene</td>
<td>-48 ± 1</td>
<td>0.2 ± 0.1</td>
<td>0.02 ± 0.0</td>
<td>48</td>
<td>0.2 ± 0.1</td>
<td>0.02 ± 0.0</td>
<td>48</td>
<td>0.2 ± 0.1</td>
<td>0.02 ± 0.0</td>
<td>48</td>
</tr>
<tr>
<td>Pimozide</td>
<td>-47 ± 4</td>
<td>0.5 ± 0.1</td>
<td>1.3 ± 0.3</td>
<td>47</td>
<td>2.4 ± 0.1</td>
<td>7.8 ± 0.3</td>
<td>54</td>
<td>16 ± 0.8</td>
<td>12 ± 0.6</td>
<td>23</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>-43 ± 16</td>
<td>0.2 ± 0.1</td>
<td>1.1 ± 0.4</td>
<td>43</td>
<td>7.4 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>18</td>
<td>11 ± 0.3</td>
<td>4.4 ± 0.1</td>
<td>26</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>-43 ± 15</td>
<td>0.8 ± 0.2</td>
<td>0.6 ± 0.3</td>
<td>43</td>
<td>2.5 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>11</td>
<td>2.2 ± 0.1</td>
<td>1.9 ± 0.3</td>
<td>22</td>
</tr>
<tr>
<td>Trifluoperside</td>
<td>-42 ± 12</td>
<td>0.2 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>42</td>
<td>4.2 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>13</td>
<td>3 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>23</td>
</tr>
<tr>
<td>Clozapine</td>
<td>-41 ± 7</td>
<td>0.2 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>41</td>
<td>6.6 ± 0.1</td>
<td>0.8 ± 0.3</td>
<td>17</td>
<td>13 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>21</td>
</tr>
<tr>
<td>Sulfazine</td>
<td>-41 ± 4</td>
<td>11 ± 9</td>
<td>8.6 ± 1.9</td>
<td>41</td>
<td>8.2 ± 4.4</td>
<td>7.8 ± 3.8</td>
<td>18</td>
<td>6 ± 0.8</td>
<td>12 ± 0.8</td>
<td>25</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>-40 ± 14</td>
<td>10 ± 5</td>
<td>11 ± 5</td>
<td>40</td>
<td>18 ± 9</td>
<td>5.9 ± 1.9</td>
<td>25</td>
<td>23 ± 11</td>
<td>21 ± 11</td>
<td>21</td>
</tr>
<tr>
<td>Butaclamol</td>
<td>-40 ± 2</td>
<td>0.3 ± 0.3</td>
<td>0.4 ± 0.4</td>
<td>40</td>
<td>1.7 ± 1.5</td>
<td>1.7 ± 1.5</td>
<td>21</td>
<td>16 ± 7</td>
<td>17 ± 7</td>
<td>21</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>-39 ± 3</td>
<td>36 ± 6</td>
<td>3.6 ± 1.5</td>
<td>39</td>
<td>31 ± 5</td>
<td>11 ± 7</td>
<td>7</td>
<td>23 ± 2</td>
<td>17 ± 2</td>
<td>21</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>-36 ± 2</td>
<td>0.4 ± 0.2</td>
<td>0.18 ± 0.0</td>
<td>36</td>
<td>1.4 ± 0.8</td>
<td>3.1 ± 0.8</td>
<td>22</td>
<td>2 ± 0.1</td>
<td>9 ± 0.1</td>
<td>23</td>
</tr>
<tr>
<td>N-desmethylclozapine</td>
<td>-39 ± 4</td>
<td>0.7 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>39</td>
<td>1.0 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>14</td>
<td>27 ± 15</td>
<td>4.5 ± 2.2</td>
<td>23</td>
</tr>
</tbody>
</table>

Table A-iv. Receptor binding of haloperidol and fluphenazine at serotonin receptors.

### Table 2 Antipsychotic Medication Serotonin Receptor Kᵢ Values

<table>
<thead>
<tr>
<th>Drug</th>
<th>Clinically effective dose (mg)</th>
<th>Kᵢ Values (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antipyrine</td>
<td>5-30</td>
<td>5.6</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>300-900</td>
<td>3115</td>
</tr>
<tr>
<td>Chlorprothixene</td>
<td>50-100</td>
<td>0.43</td>
</tr>
<tr>
<td>Clozapine</td>
<td>300-900</td>
<td>105</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>2-15</td>
<td>145</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>2-15</td>
<td>1202</td>
</tr>
<tr>
<td>Loxapine</td>
<td>25-100</td>
<td>2456</td>
</tr>
<tr>
<td>Mesoridazine</td>
<td>100-400</td>
<td>157</td>
</tr>
<tr>
<td>Moltindone</td>
<td>20-100</td>
<td>157</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>10-20</td>
<td>105</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>8-64</td>
<td>421</td>
</tr>
<tr>
<td>Pimozide</td>
<td>2-10</td>
<td>650</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>250-800</td>
<td>431</td>
</tr>
<tr>
<td>Risperidone</td>
<td>2-8</td>
<td>427</td>
</tr>
<tr>
<td>Sertraline</td>
<td>12-24</td>
<td>280</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>200-800</td>
<td>108</td>
</tr>
<tr>
<td>Thiothixene</td>
<td>5-30</td>
<td>410</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>5-30</td>
<td>950</td>
</tr>
<tr>
<td>Zimelidine</td>
<td>80-160</td>
<td>76</td>
</tr>
</tbody>
</table>

Neil M Richand⁴, Jeffrey A Welge⁵,⁶, Aaron D Logue⁴,⁵, Paul E Keck Jr.⁴,⁶, Stephen M Strakowski⁵ and Robert K McNamara⁶

Dopamine and Serotonin Receptor Binding and Antipsychotic Efficacy

Neuropsychopharmacology (2007) 32, 1715-1725, dx.doi:10.1038/npp.2007.300; published online 21 January 2007
### Table A-v. Receptor binding of haloperidol and fluphenazine at muscarinic acetylcholine receptors.

<table>
<thead>
<tr>
<th>Neuroleptics</th>
<th>$K_D$ (nM) $^*$</th>
<th>Hill coefficient $^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine</td>
<td>$12 \pm 4$</td>
<td>0.95 $\pm 0.06$</td>
</tr>
<tr>
<td>Thoridazine</td>
<td>$18 \pm 3$</td>
<td>-</td>
</tr>
<tr>
<td>Mesoridazine</td>
<td>$69 \pm 2$</td>
<td>-</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>$70 \pm 6$</td>
<td>1.05 $\pm 0.01$</td>
</tr>
<tr>
<td>Promazine</td>
<td>$150 \pm 30$</td>
<td>-</td>
</tr>
<tr>
<td>Losapine</td>
<td>$450 \pm 80$</td>
<td>1.0 $\pm 0.2$</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>$540 \pm 120$</td>
<td>1.1 $\pm 0.2$</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>$660 \pm 40$</td>
<td>-</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>$3,500 \pm 30$</td>
<td>-</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>$1,900 \pm 500$</td>
<td>1.32 $\pm 0.08$</td>
</tr>
<tr>
<td>Sipiperone</td>
<td>$2,700 \pm 800$</td>
<td>1.01 $\pm 0.09$</td>
</tr>
<tr>
<td>cis-Thiostixine</td>
<td>$2,900 \pm 100$</td>
<td>1.2 $\pm 0.1$</td>
</tr>
<tr>
<td>d-Butaclamol</td>
<td>$12,000 \pm 3,000$</td>
<td>1.4 $\pm 0.2$</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>$24,000 \pm 9,000$</td>
<td>0.7 $\pm 0.1$</td>
</tr>
<tr>
<td>Molindone</td>
<td>$390,000 \pm 90,000$</td>
<td>1.3 $\pm 0.1$</td>
</tr>
</tbody>
</table>

### Table A-vi. Receptor binding of haloperidol and fluphenazine at histamine H1 receptors.

<table>
<thead>
<tr>
<th>Neuroleptics</th>
<th>$K_D$ (nM) $^*$</th>
<th>Hill coefficient $^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesoridazine</td>
<td>$1.8 \pm 0.1$</td>
<td>0.66 $\pm 0.09$</td>
</tr>
<tr>
<td>Promazine</td>
<td>$2.0 \pm 0.1$</td>
<td>0.7 $\pm 0.2$</td>
</tr>
<tr>
<td>Clozapine</td>
<td>$2.8 \pm 0.1$</td>
<td>1.03 $\pm 0.04$</td>
</tr>
<tr>
<td>Loxapine</td>
<td>$4.9 \pm 0.8$</td>
<td>0.71 $\pm 0.03$</td>
</tr>
<tr>
<td>cis-Thiostixine</td>
<td>$6 \pm 2$</td>
<td>0.9 $\pm 0.1$</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>$8 \pm 1$</td>
<td>1.2 $\pm 0.2$</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>$9 \pm 3$</td>
<td>1.0 $\pm 0.2$</td>
</tr>
<tr>
<td>Thoridazine</td>
<td>$16 \pm 3$</td>
<td>0.97 $\pm 0.06$</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>$19.0 \pm 0.2$</td>
<td>0.8 $\pm 0.1$</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>$21 \pm 4$</td>
<td>0.9 $\pm 0.2$</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>$62 \pm 7$</td>
<td>1.3 $\pm 0.3$</td>
</tr>
<tr>
<td>d-Butaclamol</td>
<td>$390 \pm 70$</td>
<td>1.03 $\pm 0.07$</td>
</tr>
<tr>
<td>Spiperone</td>
<td>$480 \pm 70$</td>
<td>1.08 $\pm 0.05$</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>$1,900 \pm 300$</td>
<td>0.77 $\pm 0.05$</td>
</tr>
<tr>
<td>Molindone</td>
<td>$124,000 \pm 12,000$</td>
<td>0.53 $\pm 0.07$</td>
</tr>
</tbody>
</table>

### Table A-vii. Receptor binding of haloperidol and fluphenazine at α1 adrenergic receptors.

<table>
<thead>
<tr>
<th>Neuroleptics</th>
<th>$K_D$ (nM) $^*$</th>
<th>Hill coefficient $^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiperone</td>
<td>$1.2 \pm 0.2$</td>
<td>0.82 $\pm 0.03$</td>
</tr>
<tr>
<td>Mesoridazine</td>
<td>$2.0 \pm 0.5$</td>
<td>1.1 $\pm 0.1$</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>$2.6 \pm 0.3$</td>
<td>0.97 $\pm 0.08$</td>
</tr>
<tr>
<td>Thoridazine</td>
<td>$5 \pm 1$</td>
<td>1.1 $\pm 0.1$</td>
</tr>
<tr>
<td>Promazine</td>
<td>$6 \pm 2$</td>
<td>0.82 $\pm 0.04$</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>$6.1 \pm 0.8$</td>
<td>0.83 $\pm 0.07$</td>
</tr>
<tr>
<td>Clozapine</td>
<td>$9 \pm 3$</td>
<td>0.90 $\pm 0.05$</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>$9 \pm 2$</td>
<td>1.02 $\pm 0.06$</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>$10 \pm 2$</td>
<td>1.10 $\pm 0.04$</td>
</tr>
<tr>
<td>cis-Thiostixine</td>
<td>$11 \pm 1$</td>
<td>0.9 $\pm 0.1$</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>$24 \pm 7$</td>
<td>1.10 $\pm 0.05$</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>$24 \pm 3$</td>
<td>1.0 $\pm 0.1$</td>
</tr>
<tr>
<td>Losapine</td>
<td>$28 \pm 6$</td>
<td>0.9 $\pm 0.1$</td>
</tr>
<tr>
<td>d-Butaclamol</td>
<td>$56 \pm 8$</td>
<td>0.9 $\pm 0.1$</td>
</tr>
<tr>
<td>Molindone</td>
<td>$2,500 \pm 600$</td>
<td>0.71 $\pm 0.07$</td>
</tr>
</tbody>
</table>

### Table A-viii. Receptor binding of haloperidol and fluphenazine at α2 adrenergic receptors.

<table>
<thead>
<tr>
<th>Neuroleptics</th>
<th>$K_D$ (nM) $^*$</th>
<th>Hill coefficient $^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine</td>
<td>$160 \pm 20$</td>
<td>0.98 $\pm 0.02$</td>
</tr>
<tr>
<td>cis-Thiostixine</td>
<td>$200 \pm 20$</td>
<td>1.1 $\pm 0.2$</td>
</tr>
<tr>
<td>d-Butaclamol</td>
<td>$310 \pm 40$</td>
<td>0.96 $\pm 0.06$</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>$510 \pm 20$</td>
<td>1.10 $\pm 0.08$</td>
</tr>
<tr>
<td>Molindone</td>
<td>$640 \pm 100$</td>
<td>0.91 $\pm 0.05$</td>
</tr>
<tr>
<td>Spiperone</td>
<td>$660 \pm 20$</td>
<td>0.89 $\pm 0.04$</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>$750 \pm 50$</td>
<td>1.33 $\pm 0.02$</td>
</tr>
<tr>
<td>Thoridazine</td>
<td>$900 \pm 100$</td>
<td>1.23 $\pm 0.06$</td>
</tr>
<tr>
<td>Promazine</td>
<td>$900 \pm 100$</td>
<td>1.00 $\pm 0.02$</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>$1,550 \pm 100$</td>
<td>0.86 $\pm 0.04$</td>
</tr>
<tr>
<td>Mesoridazine</td>
<td>$1,600 \pm 100$</td>
<td>0.70 $\pm 0.03$</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>$1,700 \pm 100$</td>
<td>0.97 $\pm 0.05$</td>
</tr>
<tr>
<td>Loxapine</td>
<td>$2,400 \pm 600$</td>
<td>0.91 $\pm 0.05$</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>$2,600 \pm 200$</td>
<td>1.33 $\pm 0.07$</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>$3,800 \pm 400$</td>
<td>1.33 $\pm 0.07$</td>
</tr>
</tbody>
</table>


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

Module F: Anxiety
PANIC DISORDER WITH AGORAPHOBIA (PDA) → ineligible
PANIC DIRODER (PD) WITHOUT AGORAPHOBIA → ineligible
AGORAPHOBIA WITHOUT PD (AWOPD) → ineligible
SOCIAL PHOBIA → if only public speaking (If age<18yrs, at least for 6 months) → eligible
SPECIFIC PHOBIA (If age<18yrs, at least for 6 months) → ineligible
OBSESSIVE-COMPULSIVE DISORDER → ineligible
POST-TRAUMATIC STRESS DISORDER (PTSD) (1 month) → ineligible
GENERALIZED ANXIETY DISORDER (GAD) (at least 6 months) → ineligible
ANXIETY DISORDER (NOS) → ineligible
ANXIETY DUE TO MEDICAL CONDITION (GMC)/SUBSTANCE USE → ineligible

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

Module B/C: Psychotic
DELUSIONS → ineligible
AUDITORY HALLUCINATIONS → ineligible
VISUAL HALLUCINATIONS → ineligible
OTHER HALLUCINATIONS → ineligible
SCHIZOPRENEIA → ineligible (plus NO Family history)
SCHIZOPHRENIFORM DISORDER (duration less than 6 months) → Ineligible
SCHIZOAFFECTIVE DISORDER → ineligible
DELUSIONAL DISORDER → ineligible
PSYCHOTIC DISORDER DUE TO GMC/SUBSTANCE-INDUCED → ineligible
APPENDIX C:
Recruitment Ads for HC and PG Subjects
Healthy Volunteers
You may be eligible for a medication research study.

If you are:

19-65 years of age
Drug- and Medication-Free
Available for Weekly Day-long Sessions (M – F)

Call Study Line: (416) 535-8501, ext. 36574

NOTE: This is not a treatment study.

- FINANCIAL COMPENSATION IS PROVIDED
- All Information Provided Will Remain Confidential To The Extent Allowed By Law.

CAMH provides treatment options for mental illness and addictions. For more information about programs and services at CAMH, visit www.camh.net or call (416) 535-8501, or 1-800-463-6273
Do you gamble?

You may be eligible for a medication research study.

**If you are:**

19-65 years of age

Drug- and Medication-Free

Available for Weekly Day-long Sessions (M – F)

Call Study Line: (416) 535-8501, ext. 36574

**NOTE:** This is not a treatment study.

- FINANCIAL COMPENSATION IS PROVIDED
- All Information Provided Will Remain Confidential To The Extent Allowed By Law.

CAMH provides treatment options for mental illness and addictions.

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

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

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

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

Introductory Statement

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

Conflict of Interest

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

Study Sponsor

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

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

Ethics, Confidentiality and Continuing Review:

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

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

Participant Enrolment:

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

Pregnancy and Breastfeeding:

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

Study Procedure:

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

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

3. Physician’s Exam. If the information from your interview shows that you meet the initial requirements for participation, you will be asked to undergo a physical examination by a doctor at CAMH. The purpose of the exam is to make sure you have no physical condition that would make it risky for you to receive any of the study medications. The exam will take ½ - 1 hour.

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

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

Details of Test Sessions:

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

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

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

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

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

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

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

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

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

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

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

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

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

**Study Requirements:**

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

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

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

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

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

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

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

8. The schedule of payment is as follows:

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

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

Haloperidol

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

Fluphenazine

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

Dexedrine

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

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

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

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

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

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

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

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

Benefits:

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

Payment, Conditions, and Confidentiality:

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

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

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

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

Safety

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

New Findings

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

v. 3 03/26/2014 I have read this page of the document Participant’s Initials _______ Page 6 of 8
Questions

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

Contact

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

If you have any questions about your rights as a participant in this study, you may contact Dr. Pdraig Darby, Chair, Research Ethics Board, Centre for Addiction and Mental Health, at 416 535 8501 ext. 6876.

Genetics Screen

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

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

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

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

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

<table>
<thead>
<tr>
<th>Print Participant’s Name</th>
<th>Participant’s Signature</th>
<th>Date (mm/dd/yyyy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Print Name of Individual Obtaining Consent</td>
<td>Signature of Individual Obtaining Consent</td>
<td>Date (mm/dd/yyyy)</td>
</tr>
</tbody>
</table>

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.

v. 3 03/26/2014 I have read this page of the document Participant’s Initials _______ Page 8 of 8
APPENDIX E:
Physical Exam Inclusion/Exclusion Criteria
**Physical Exam Inclusion/exclusion criteria:**

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

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

**Specific Medication-related Contraindications:**

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

**FLUPHENAZINE:**
- Volunteers must have no known sensitivity to fluphenazine nor can they be taking another phenothiazine.
- **Volunteers at risk for hypotension (the elderly > 65 years; or those who misuse alcohol), with abnormal EKG will be excluded.**
- Volunteers who may be exposed to extreme heat or cold will be alerted and must explicitly consent to NOT expose themselves to extreme heat or cold for 24 hours after each test session.
- Volunteers who would be screened positive for exposure to organophosphate insecticide must explicitly consent to NOT expose themselves to these insecticides for 24 hours after each test session.
• Volunteers taking any of the following medications will be excluded: medications that modify CYP2D6 will be excluded, CYP2D6 Inducers, CYP2D6 Inhibitors, Levodopa, Lithium, Metoclopramide, Antipsychotics, Anticonvulsants, Anticholinergics, Antidepressants, Tricyclic, Antihypertensives.

• Volunteers who have severe CNS depression due to CNS medications, with a history of head trauma with associated loss of consciousness, those with bone marrow depression and those who are comatose will be excluded.

• Volunteers with brain tumor, any abnormalities in hepatic function, with respiratory difficulties, agranulocytosis, hypocalcemia, obstruction or Reye’s syndrome, at risk for retinopathy, with either glaucoma or prostatic hypertrophy or suspicion of any of these syndromes will be excluded.

**DEXEDRINE®:**

• Advanced arteriosclerosis symptomatic cardiovascular disease, cardiomyopathy, moderate to severe hypertension, hyperthyroidism, myocardial infarction, ventricular arrhythmia, hypersensitivity or idiosyncrasy to sympathomimetic amines, history of drug abuse, glaucoma, diabetes, concomitant treatment with MAO inhibitors.

• Patients with known hypersensitivity to dextroamphetamine or to any ingredient in the formulation or component of the container.

• **Volunteers with history of allergy to Aspirin will be excluded.**

• Known interactions with amphetamines are as follows: Synergistic Interactions: tricyclic antidepressants, MAO inhibitors, meperidine, norepinephrine, phenobarbital, phenytoin, propoxyphene, acetazolamide, thiazides, gastrointestinal and urinary alkalinizing agents. Antagonistic Interactions: adrenergic blockers, antihistamines, antihypertensives, chlorpromazine, ethosuximide, guanethidine, haloperidol, lithium carbonate, methenamine, Veratrum alkaloids, gastrointestinal and urinary acidifying agents
APPENDIX F:
Flow Chart of Subject Recruitment, Eligibility and Group Assignment
750 phone calls received

60 eligible after telephone screening

14 failed to attend interview

46 attended interview screening

22 excluded at interview

24 attended physical exam

6 excluded after physical exam

32 participants from prior experimenters

18 participants enrolled

50 study participants

24 PG

12 HAL

12 FLU

26 HC

14 HAL

12 FLU