PARSING DISTINCT ASPECTS OF THE ADDICTION PROCESS USING COCAINE SELF-ADMINISTRATION, BEHAVIORAL ECONOMICS, NEUROPHARMACOLOGY AND NEUROCHEMISTRY

By
Erik B. Oleson

A Dissertation Submitted to the Graduate Faculty of
WAKE FOREST UNIVERSITY GRADUATE SCHOOL OF ARTS AND SCIENCES
in Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY
Neuroscience Program -- Department of Physiology and Pharmacology
May 2010
Winston-Salem, North Carolina
Approved by:

David C.S. Roberts, Ph.D., Advisor ________________________________
Examining Committee:
Thomas Martin, Ph.D., Chairman ________________________________
Allyn Howlett, Ph.D. ____________________________________________
Sara Jones, Ph.D. ______________________________________________
Wayne Pratt, Ph.D. ____________________________________________
ACKNOWLEDGEMENTS

I would like to thank my primary advisor, Dr. David C.S. Roberts, who has done everything in his power to provide me with the resources necessary to thrive over the last five years. Dave provided me with not only financial and technical support, but also the freedom necessary for me to carry out many different scientific endeavors as a graduate student. While some of these collaborative efforts and technically challenging projects did not succeed, many of our experimental attempts were very fruitful. And in fact, I think that I learned more from my failures and struggles than my successes. In retrospect, I think Dave set me up so that I always had a safe project that would work while maintaining “several irons in the fire” that were high-risk exciting projects. In this regard, I was always provided with a safety net that caught me when my ambitions got the best of me.

I would like to thank Dr. Sara Jones and Dr. Evgeny Budygin, who trained me in fast-scan cyclic voltammetry. I enjoyed learning this exciting technique in addition to the primary behavioral training I received in the Roberts’ lab, and I hope to build on our previous interactions and maintain friendly, collaborative relationships in the future.

I would like to thank Dr. Allyn Howlett for providing me with the opportunity to gain teaching experience in addition to proving me with countless hours of instruction and advice on teaching techniques and career advancement.

Finally, I would like to thank the entire Neuroscience Program for providing me with a unique PhD experience, my committee for their support and the National Institute on Drug Abuse for my funding (F31DA024525).
# Table of Contents

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Parsing the Addiction Phenomenon: A Comparison of Behavioral Training Procedure Designed to Model Specific Addiction Symptoms</td>
<td>1</td>
</tr>
<tr>
<td>II.</td>
<td>Behavioral Economic Assessment of Price and Cocaine Consumption Following Self-Administration Histories that Produce Escalation of Either Final Ratios or Intake</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td><em>Neuropsychopharmacology, 2009 Feb;34(3):796-804.</em></td>
<td></td>
</tr>
<tr>
<td>III.</td>
<td>Dissociating the Behavioral Economic Concepts of Consumption and Price Paid Using Neuropharmacology and Cocaine Self-Administration</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td><em>In Preparation for Submission to Psychopharmacology</em></td>
<td></td>
</tr>
<tr>
<td>IV.</td>
<td>Dopamine Uptake Changes Associated with Cocaine Self-Administration</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td><em>Neuropsychopharmacology 2009 Apr;34(5):1174-84.</em></td>
<td></td>
</tr>
<tr>
<td>V.</td>
<td>Increased Motivation to Self-Administer Cocaine is Determined by The Pattern of Responding Associated with Fluctuating Brain Cocaine Levels</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td><em>In Preparation for Submission to Neuropsychopharmacology</em></td>
<td></td>
</tr>
<tr>
<td>DISCUSSION</td>
<td></td>
<td>260</td>
</tr>
<tr>
<td>APPENDIX I</td>
<td></td>
<td>277</td>
</tr>
<tr>
<td>APPENDIX II</td>
<td></td>
<td>294</td>
</tr>
<tr>
<td>CURRICULUM VITAE</td>
<td></td>
<td>297</td>
</tr>
</tbody>
</table>
# List of Figures and Tables

<table>
<thead>
<tr>
<th>Chapter I</th>
<th>Table 1. DSM IV Criteria for Substance Dependence</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Figure 1A. Cumulative Records Showing Final-Ratios Increasing Over 14 Days on a Progressive Ratio Schedule (ie. PR Training)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Figure 1B. A Rapid Speed of Cocaine Onset is Necessary for Final Ratios to Increase Over Time</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Figure 1C. Providing Long-Access (eg. 6 hr Sessions) Under a Fixed-Ratio Schedule Results in Cocaine Intake Increasing Over Time (ie. LgA Training)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Figure 2. PR Trained Rats Show a Leftward Shift on a Progressive Ratio Dose-Effect Curve</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Figure 3A. PR Trained Rats Do Not Show a Sensitized Locomotor Response to a Cocaine Challenge</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Figure 3B. PR Trained Rats Do Not Show a Sensitized Neurochemical Response to a Cocaine Challenge</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Figure 4A. PR Trained Rats Show Increased Responding for Low Cocaine Doses in a Between Sessions Threshold Procedure</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Figure 4B. PR Trained Rats Maintain Cocaine Intake at Low Cocaine Doses in a Between Sessions Threshold Procedure</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Figure 4C. LgA Trained Rats Do Not Maintain Cocaine Intake at Low Doses in a Between Sessions Threshold Procedure</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Figure 5. A Pharmacological History of High Cocaine Intake Blocks the Increase in Final Ratios if it Precedes PR Training but Only Temporarily Suppresses Final Ratios After PR Training</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Figure 6A. Rats Show Increased Final Ratios for Cocaine Following Periods of Forced Abstinence from Cocaine</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Figure 6B. Rats Display Hyperactivity to a Cocaine Challenge for Following Periods of Forced Abstinence from Cocaine</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Figure 6C. Rats Show Increased Cue-Induced Reinstatement Responding After Periods of Forced Abstinence from Cocaine</td>
<td>41</td>
</tr>
</tbody>
</table>
CHAPTER II

Figure 1A. A Dose Response Function From a Between Sessions Threshold Procedure is Shown 62

Figure 1B. Parsing A Dose-Intake Function From a Between Sessions Threshold Procedure is Shown 63

Figure 1C. A Graphical Determination of an Individual Rat’s Maximal Price Paid (Pmax) for Cocaine is Shown 64

Figure 2A. Representative Cumulative Records from Three Doses in The Between Sessions Threshold Procedure 70

Figure 2B. A Cumulative Record from a PR Trained Rat Responding for a Low Dose of Cocaine in the Between Sessions Threshold Procedure 71

Figure 3A. LgA Trained Rats Respond for Cocaine at an Increased Rate Over 14 Days 74

Figure 3B. LgA Trained Rats Show Increased Cocaine Intake at High, but not Low, Cocaine Doses in the Between Sessions Threshold Procedure 75

Figure 4A. PR Trained Rats Show an Increase in Final Ratios for Cocaine Over 14 Days 78

Figure 4B. PR Trained Rats Maintain Cocaine Intake at Lower Cocaine Doses In Comparison to Rats Matched for Dose and Intake in the Between Sessions Threshold Procedure 79

Figure 5. PR Trained Rats Show an Increase in Pmax Whereas LgA Trained Rats Show a Decrease in Pmax for Cocaine in the Between Sessions Threshold Procedure 82

Figure S1. Cocaine Dosage Can be Successfully Manipulated by Altering Pump Duration 95

Figure S2. Graphically Determined Pmax Values are Equivalent to Mathematically Determined Pmax Values 97

Figure S3. PR Final Ratios from a Progressive Ratio Schedule Correlate With Pmax Values from the Between Sessions Threshold Procedure 99

CHAPTER III

Figure 1A. A Representative Cumulative Record From One Rat Responding in the Within Session Threshold Procedure 111
Figure 1B&C. The Same Rat’s Price-Intake (b) and Price Response (c) Functions Showing Pmax and Consumption Values  

Figure 2A. Haloperidol Decreased the Maximal Price Paid (Pmax) for Cocaine  

Figure 2B. Haloperidol Increased Cocaine Consumption  

Figure 3A. Amphetamine Increased the Maximal Price Paid (Pmax) for Cocaine  

Figure 3B. Amphetamine Did Not Change Cocaine Consumption  

Figure 4A. Fluoxetine Decreased the Maximal Price Paid (Pmax) for Cocaine  

Figure 4B. Fluoxetine Decreased Cocaine Consumption  

Figure 5A. Baclofen Decreased the Maximal Price Paid (Pmax) for Cocaine  

Figure 5B. Baclofen Did Not Change Cocaine Consumption  

CHAPTER IV  

Figure 1A&B. Representative Concentration-Time Functions of Dopamine Before and After Either Saline (a) or Cocaine (b)  

Figure 2A. A Single IV Cocaine Injection Dose-Dependently Increase Dopamine Uptake Inhibition (Apparent $K_m$)  

Figure 2B. A Single IV Cocaine Injection did not Change the Rate of Dopamine Uptake (Vmax)  

Figure 2C. A Single IV Cocaine Injection Dose-Dependently Increased Evoked Dopamine Release  

Figure 3. Fluctuations in Dopamine Uptake Inhibition and Evoked Dopamine Release (Inset) Occuring During Multiple IV Cocaine Injections as Occurs During Self-Administration on a Fixed Ratio Schedule  

Figure 4. A Mathematical Model of Dopamine Uptake Inhibition Describes and Predicts Fluctuating Levels of Dopamine Uptake Inhibition  

Figure 5. The Fluctuating Levels of Dopamine Uptake Inhibition Shift Upward with Response Rates Observed After LgA Training
Figure 6A. LgA Trained Rats Showed an Increased Rate of Dopamine Uptake (Vmax) 179

Figure 6B. LgA Trained Rats Did Not Show a Change in the Potency of Cocaine to Inhibit Dopamine Uptake (Apparent $K_m$) 180

CHAPTER V

Figure 1A. A representative individual rat’s cumulative record from a PR schedule superimposed over modeled brain cocaine concentrations from a group of rats responding under the same conditions 208

Figure 1B. A representative individual rat’s cumulative record from the within-session threshold procedure superimposed over modeled brain cocaine concentrations from a group of rats responding under the same conditions 209

Figure 1C A representative individual rat’s cumulative record from a hold-down schedule superimposed over modeled brain cocaine concentrations from a group of rats responding under the same conditions 210

Figure 2 Demonstration of how the behavioral economic measures Pmax, Omax and consumption are determined 213

Figure 3A PR training increased final ratios over 14 days 216

Figure 3B PR training increased cocaine intake over 14 days 217

Figure 4A Within-session threshold training did not increase Pmax over 14 days 220

Figure 4B Within-session threshold training did not increase cocaine intake over 14 days 221

Figure 5A Hold-down training increased the duration of lever hold-down times over 14 days 224

Figure 5B Hold-down training did not increase cocaine intake over 2-14 days 225

Figure 6A PR training increased final ratios in comparison to within-session threshold and hold-down training 228

Figure 6B PR training increased Pmax in comparison to within-session threshold and hold-down training 229

Figure 6C PR training increased Omax in comparison to within-session threshold and hold-down training 230
**Figure 6D** PR, within-session threshold, and hold-down training produced similar effects on cocaine consumption 231

**Figure 7A** During cocaine self-administration under three different conditions rats maintain an upper brain cocaine level, whereas a lower brain cocaine level is associated with the cessation of responding 235

**Figure 7B** During cocaine self-administration under three different conditions rats maintain an upper level of dopamine uptake inhibition, whereas a lower level of dopamine uptake inhibition is associated with the cessation of responding 236

**Figure S1.** Hold-down training decreased the rate of responding for cocaine over 14 days 251

**Figure S2.** Session response rate was similar on the first day of PR, within-session threshold and hold-down training 253

**Figure S3.** The dependent measures of reinforcement strength (final ratio, Pmax and Omax) are heterogeneously related 255

**Figure S4.** The dependent measures of reinforcement strength (final ratio, Pmax and Omax) are not related to consumption 257

**Figure S5.** Predicted fluctuations in dopamine uptake inhibition are similar to predicted changes in brain cocaine concentrations in three different self-administration procedures 259

**APPENDIX I**

**Figure 1.** Representative concentration-time functions of evoked dopamine concentrations before and after cocaine in a freely-moving mouse 284

**Figure 2.** Changes in dopamine uptake inhibition (Apparent $K_m$) paralleled changes in evoked dopamine release ($DA_p$) following a cocaine injection in a freely-moving mouse 286

**Figure 3.** Levels of dopamine uptake inhibition (Apparent $K_m$) temporally correlated with concentrations of evoked dopamine release ($DA_p$) in the presence of cocaine 288

**APPENDIX II**

**Figure 1.** The hypocretin 1 receptor antagonist SB334867 decreased the maximal price paid (Pmax) for cocaine without changing consumption 296
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>Silver</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AP</td>
<td>Anterior posterior</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Rate of change in consumption, also referred to as 'essential value' in literature</td>
</tr>
<tr>
<td>BL</td>
<td>Baseline</td>
</tr>
<tr>
<td>C</td>
<td>Celsius</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>C57 black 6</td>
</tr>
<tr>
<td>Calc</td>
<td>Pmax values calculated mathematically</td>
</tr>
<tr>
<td>Cl</td>
<td>Chloride</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>$\text{DA}_p$</td>
<td>Evoked dopamine per stimulus pulse</td>
</tr>
<tr>
<td>DAT</td>
<td>Dopamine transporter</td>
</tr>
<tr>
<td>DOPAC</td>
<td>3,4-dihydroxyphenylacetic acid</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>Diagnostic and statistical manual of mental disorders (4th edition)</td>
</tr>
<tr>
<td>DV</td>
<td>Dorsal ventral</td>
</tr>
<tr>
<td>eg</td>
<td>For example (exempli gratia)</td>
</tr>
<tr>
<td>$f$</td>
<td>Stimulation frequency</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>FR</td>
<td>Fixed ratio</td>
</tr>
<tr>
<td>FSCV</td>
<td>Fast scan cyclic voltammetry</td>
</tr>
<tr>
<td>GABA</td>
<td>(\gamma)-aminobutyric acid</td>
</tr>
<tr>
<td>Graph</td>
<td>Pmax values calculated graphically</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HD</td>
<td>Hold down schedule</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>ie</td>
<td>That is (id est)</td>
</tr>
<tr>
<td>Inf</td>
<td>Infusion</td>
</tr>
<tr>
<td>Inj</td>
<td>Injection</td>
</tr>
<tr>
<td>IP</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Km</td>
<td>The Michaelis constant (In this case an estimation of the affinity of dopamine for the dopamine transporter)</td>
</tr>
<tr>
<td>LgA</td>
<td>Long-Access</td>
</tr>
<tr>
<td>Match</td>
<td>Matched dose and intake group</td>
</tr>
<tr>
<td>ML</td>
<td>Medial lateral</td>
</tr>
<tr>
<td>(\mu)M</td>
<td>Micromole</td>
</tr>
<tr>
<td>nM</td>
<td>Nanomole</td>
</tr>
<tr>
<td>NAc</td>
<td>Nucleus accumbens</td>
</tr>
<tr>
<td>NIDA</td>
<td>National institute of drug abuse</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>Omax</td>
<td>Maximal response output</td>
</tr>
<tr>
<td>PR</td>
<td>Progressive ratio</td>
</tr>
<tr>
<td>Pmax</td>
<td>Maximal price paid</td>
</tr>
<tr>
<td>Q₀</td>
<td>Predicted consumption at price 0</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SN</td>
<td>Substantia nigra pars compacta</td>
</tr>
<tr>
<td>ShA</td>
<td>Short-Access</td>
</tr>
<tr>
<td>TTWYAGTTT</td>
<td>“Tell them what you’re going to tell them” - Dave Roberts</td>
</tr>
<tr>
<td>V</td>
<td>Volts</td>
</tr>
<tr>
<td>Veh</td>
<td>Vehicle</td>
</tr>
<tr>
<td>Vmax</td>
<td>Maximum velocity of dopamine uptake</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
</tr>
<tr>
<td>W/IN</td>
<td>Within session threshold procedure</td>
</tr>
</tbody>
</table>
**ABSTRACT**

ERIK B. OLESON

**PARSING DISTINCT ASPECTS OF THE ADDICTION PROCESS USING COCAINE SELF-ADMINISTRATION, BEHAVIORAL ECONOMICS, NEUROPHARMACOLOGY AND NEUROCHEMISTRY**

Dissertation under the direction of David C.S. Roberts, Ph.D., Professor of Physiology and Pharmacology

In order to develop a better understanding of the neurobiological basis of cocaine addiction, scientists have begun to investigate the neural adaptations occurring in association with changes in behavior observed in animal models of addiction. Exactly how these behavioral changes relate to the addiction process, however, remains unclear.

Two behavioral changes that are well documented to occur as the addiction process progresses are increased cocaine intake over time and increased time and energy devoted to obtain cocaine. The relationship between these two concepts can be addressed experimentally by performing behavioral economic analyses on cocaine self-administration data. Using behavioral economic theory, changes in cocaine intake can be investigated by studying changes in consumption when the unit-price of cocaine is relatively cheap; changes in the time and energy devoted to obtain cocaine can be studied by assessing changes in the maximal price paid for drug when the unit-price of cocaine is relatively high.

The current series of studies was designed to investigate the relationship between cocaine consumption and price paid, and to further identify the neurobiological factors that cause these two distinct concepts to change over time. In the first set of studies it was discovered that changes in cocaine consumption and the price paid for cocaine can increase independently, suggesting that these two concepts are dissociable. The relationship between cocaine consumption and price paid was then addressed using neuropharmacology. It was found that drug-pretreatments can affect cocaine consumption and price paid independently, further supporting the conclusion that these two aspects of addiction are dissociable. Next, the neuropharmacological regulation of cocaine consumption was addressed. It was found that the rate and pattern of cocaine intake is tightly associated with levels of dopamine uptake inhibition which shift upward following an escalation of cocaine intake. In the final chapter, it was found that an interaction between large brain cocaine fluctuations and patterns of high-rate responding for cocaine is necessary to produce an increase in the price paid for cocaine over time.
Overall, these studies suggest that cocaine consumption and price paid are dissociable phenomena that are regulated by distinct neural mechanisms.
All chapters presented herein concern the regulation of cocaine consumption, the behavioral price paid for cocaine, or the interaction between these two concepts within the context of the cocaine addiction phenomenon. A fundamental question addressed throughout these chapters is: how are the concepts of consumption of price paid for cocaine related?

**Chapter 1:** A review of the literature on animal models of cocaine addiction is presented. In this review I argue that some of the most commonly used addiction models address distinct aspects of the addiction phenomenon. Two self-administration training procedures are emphasized. I argue that the progressive ratio training procedure specifically models an increase in the time and energy devoted to obtain cocaine (i.e. increased motivation); whereas, the long-access training procedure specifically models an increase in cocaine consumption.

**Chapter 2:** A research article comparing these two self-administration training procedures is presented. It was found that progressive ratio training produced animals that paid a higher behavioral price for cocaine but did not show increased cocaine consumption. By contrast, long-access training produced animals that paid a lower behavioral price for cocaine but showed an increase in cocaine consumption.

**Chapter 3:** A research article investigating the neuropharmacological interaction between cocaine consumption and price paid for cocaine in presented. It was found that drug pre-treatments can similarly affect (e.g. fluoxetine), inversely affect (e.g. haloperidol) or independently affect (e.g. baclofen) cocaine consumption and the price paid for cocaine.
Chapter 4: A research article characterizing the role of dopamine uptake inhibition in the regulation of cocaine consumption is presented. It was found that the rate and pattern of cocaine consumption under continuous access conditions is closely associated with tightly-regulated levels of dopamine uptake inhibition, and that these levels shift upward following long-access training.

Chapter 5: A research article investigating the role of brain cocaine fluctuations during different patterns of responding for cocaine is presented. It was found that a critical characteristic of the progressive ratio schedule that contributes to the behavioral price paid for cocaine increasing over time is the interaction between fluctuating brain cocaine levels and periods of maximal behavioral responding for cocaine. The co-occurrence of large brain cocaine fluctuations during periods of maximal responding for cocaine was necessary to produce an increase in the price paid for cocaine, but did not result in an increase in cocaine consumption.

Overall Conclusion: Taken together, these studies demonstrate that the concepts of cocaine consumption and price paid for cocaine are dissociable. While an increase in one of these concepts might co-occur with an increase in the other, this is not necessarily the case. The implication of this dissociation is that distinct aspects of the cocaine addiction phenomenon can develop independently, and their development involves separate neural adaptations.
CHAPTER I

PARSING THE ADDICTION PHENOMENON: A COMPARISON OF BEHAVIORAL TRAINING PROCEDURES DESIGNED TO MODEL SPECIFIC ADDICTION SYMPTOMS.

Erik B. Oleson and David C.S. Roberts

This review focuses on several procedures that have been developed to model specific aspects of drug addiction using experimental animals. The phenomena being addressed include increased motivation to seek and take drugs, increased consumption over time, incubation of craving and behavioral sensitization.

**ABSTRACT**

Investigators who study drug addiction are fortunate to have access to excellent animal models. Such models will be invaluable in the assessment of factors involved in the progression of drug addiction. The relevance of these findings, however, will depend on the general understanding of how each model is related to drug addiction. The present review focuses on several procedures that were designed to model the addiction process and questions whether these models are tapping into the same underlying process or whether each is addressing a unique feature. Furthermore, various factors (e.g., rate of drug onset, dose magnitude, early drug history, periods of abstinence) influencing the progression of these addiction-like changes in behavior are discussed.

*Keywords: Cocaine, Escalation, Motivation, Sensitization, Tolerance, Self Administration*
**INTRODUCTION**

A better understanding of the neurobiology of drug addiction is beginning to emerge through the analysis of brain changes in individuals addicted to drugs. Recent advances in neuroimaging have shown activation of specific brain regions associated with drug craving (see reviews, (Gatley, et al 2005; Magalhaes, 2005). Chronic drug abuse has also been shown to cause changes in brain structure and function (see reviews, Goldstein and Volkow, 2002; Borne, et al 2005; Rojas et al., 2005). Additional advancements in the application of molecular biology techniques has provided important post-mortem analyses of brain tissue from addicts (see reviews, Bannon, et al 2005; Hemby, 2006).

There are, however, many limitations and challenges associated with studying drug addiction in human subjects. Various confounding factors exist within samples of drug addicts that may be difficult to parse apart, such as differences in environment, genetics, poly-drug use and route of administration. Attrition is also a concern due to the general social instability associated with drug addicts. Moreover, various ethical dilemmas may restrict the experimental design of longitudinal studies and the administration of pharmacological treatments to illicit drug users.

Animal studies offer an opportunity to investigate specific aspects of the addiction process without the confounding factors associated with human studies. As this special issue of *Drug Discovery Today* attests, a variety of animal models have been developed which can be used to address fundamental issues related to drug abuse and dependence. Such models will be invaluable in the assessment of genetic
influences, neurochemical changes, pharmacological variables and epigenetic influences involved in the addiction process. The relevance of these findings, however, will depend on the general understanding of how each model is related to drug addiction.

This review will focus on several procedures that have been developed to examine specific aspects of cocaine self-administration in rats. One fundamental question is whether each of the models is tapping into the same underlying process or whether each is addressing a unique feature. A continuing theme throughout this review will be the examination of whether the phenomena exposed by different procedures and models are correlated or dissociable.

Before describing the rat models, it is important to briefly consider the condition under study. A logical point of reference for those attempting to model addiction-like symptoms is the current Diagnostic and Statistical Manual (2000). Table 1 lists several criteria from the DSM-IV associated with substance dependence. Whereas some of these criteria (i.e., those listed above the dotted line in table 1) might be difficult to address using experimental animals, others have been successfully modeled. Note that this is a broad classification system which is used to diagnose individuals who are dependent on a variety of substances including alcohol, opiates and stimulants. Each criterion may not apply equally across all drug categories. For example, the degree of physical dependence associated with cocaine is an issue that has been debated over the years.
<table>
<thead>
<tr>
<th>DSM IV Criteria for Substance Dependence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent desire, or unsuccessful attempts, to limit use</td>
</tr>
<tr>
<td>Important social, occupational, or recreational activities are reduced</td>
</tr>
<tr>
<td>Continued use despite knowledge of adverse health consequences</td>
</tr>
<tr>
<td>Increased Motivation: a great deal of time and energy expended to obtain substance</td>
</tr>
<tr>
<td>Increased Consumption: substance is taken in larger amounts over longer periods of time</td>
</tr>
<tr>
<td>Withdrawal: substance is taken to relieve withdrawal symptoms</td>
</tr>
<tr>
<td>Tolerance: a need for increased amounts of substance to achieve desired effects; diminished effects with continued use</td>
</tr>
</tbody>
</table>

DSM IV criteria for substance dependence.
PROGRESSIVE RATIO TRAINING AND FINAL RATIO ESCALATION

Our lab has focused on modeling changes in the motivation to self-administer cocaine. A progressive ratio (PR) schedule has been developed for the study of cocaine self-administration in rats which essentially asks the question, “How hard is an animal willing to work to gain access to the next injection?” Figure 1A shows an example of the pattern of cocaine self-administration on a PR schedule. The first response of the session results in an intravenous cocaine infusion; thereafter the delivery of this reinforcer is contingent upon an increasing number of responses incremented through the following series: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc. (Richardson and Roberts, 1996). Note that the pattern of self-administration is characterized by evenly spaced post-infusion pauses followed by a period of relatively rapid responding for the requisite number of responses. Responding ceases at some point in the session, presumably when the response cost exceeds the reinforcing value of the drug. The dependent measure is the ‘break point’ or final ratio, defined as the response requirement corresponding to the final drug infusion. We have argued (Arnold and Roberts, 1997) that the final ratio can be considered as an index of an animal’s motivation to self-administer psychostimulant drugs.

A number of factors affect whether final ratios remain stable or escalate over time. When the PR schedule has been employed to evaluate the effects of lesions, hormonal manipulations or drug pretreatments on the reinforcing efficacy of cocaine (Roberts, et al 1996; Roberts, et al 1989; Loh and Roberts, 1990), training procedures were used that produced very steady final ratios under baseline
conditions. However, as our interest turned toward the addiction process, we sought to identify conditions which would produce an increase in final ratios over time – as might be expected if there were an addiction process affecting drug taking behavior. Figures 1A and 1B show an example of an increase in final ratios over time. The cumulative records in Figure 1A show final ratios increasing from an initial value of 118 on day 1 to a value of 328 on day 14. Clearly, the amount of time and energy devoted to drug taking increases in this animal over a relatively brief period. This effect occurs in the majority, albeit not all animals, and is only seen under specific conditions. The unit injection dose of cocaine is a critical factor affecting the escalation of final ratios over time. Morgan and colleagues (Morgan et al., 2006a) showed that final ratios were more likely to increase at higher unit doses, with 1.5 mg/kg/inj being optimal. Note that the 1.5 mg/kg/inj cocaine dose occurs at the top of the PR dose effect curve, as illustrated in figure 2, (Ward, et al 2005; Morgan, et al 2005; Morgan, et al 2006) and is selected over lower doses (0.75 mg/kg/inj) in choice experiments (Ward, et al 2005). Thus the dose which is most likely to push the addiction process forward is also the most reinforcing dose and the one animals are most drawn toward if given a choice.

Liu and colleagues (Liu, et al 2005a) demonstrated that the speed of drug infusion has an important influence on the escalation of final ratios. Three groups of rats were given access to the same unit dose of cocaine (1.5 mg/kg/inj) on a PR schedule; in one group the injections were delivered relatively quickly (i.e. 5 sec duration), while the remaining two groups received their injections over a longer period (25 and 50 sec). As illustrated in Figure 1B, no significant difference was
observed between the three groups at the start of the two-week testing period. However, as testing continued the 5 sec group showed a significant increase in final ratios whereas the groups that received slower cocaine infusions (i.e., 25 or 50 sec) did not show this increase across sessions (Liu, et al 2005a).
Final ratios or drug intake can escalate over time during cocaine self-administration. A). (Morgan, et al. 2006) Two cumulative records show responding on the first experimental day (left) and the final experimental day (right) of PR training. Note the escalation of responses expended (vertical lines) to receive a final injection (diagonal ticks) increased across sessions. B). (Liu, et al 2005) Speed of injection effects the escalation of final ratios. Animals show increased final ratios during PR training when a high cocaine dose (1.5 mg/kg/inf IV) is delivered over 5-s (circles) but not 25-s (triangles) or 50-s (squares). C). (Ahmed and Koob, 1998) LgA training results in an escalation in cocaine intake. LgA rats (open circles) show an increased rate of cocaine intake across daily 6-hr sessions; whereas ShA rats (filled circles) show stable responding across daily 2-hr sessions.
FIGURE 1B
**Figure 1C**

Graph showing the number of infusions per session over FR experimental sessions (days) for ShA and LgA groups.
The escalation in final ratios represents a leftward and upward shift in the cocaine dose-response curve. The ascending limbs of two dose-response curves are shown in Figure 2. The data clearly show that higher unit injection doses of cocaine support higher final ratios. The lower curve is typical of many studies using training procedures which produce stable final ratios. The upper curve represents animals that were tested for 2 weeks using conditions that produced an escalation of final ratios; that is, they were given access to cocaine on a PR schedule reinforced with a high unit dose of cocaine (1.5 mg/kg/inj) delivered relatively rapidly (~5 sec). When other doses were subsequently tested, it was apparent that the escalating effects carried over to other doses causing an upward and leftward shift in the dose response curve (Morgan, et al 2006).
**FIGURE 2**

PR training produces sensitization to the reinforcing effects of cocaine. (Morgan, *et al* 2006) Two groups of rats were given access to 4 doses of cocaine under a PR schedule following different behavioral histories. One group of rats (filled squares) was tested after a self-administration history (see PR training) that results in an escalation of final ratios over 2 weeks. A second group of rats (open triangles) was tested after a history that produces stable final ratios. The augmentation of the reinforcing effects of cocaine following PR training was observed at all tested doses. Note the upward and leftward dose effect curve shift.
Figure 2

Final Ratio

PR trained

Stable Final Ratio

Cocaine (mg/kg/inf)

Sal 0.19 0.38 0.75 1.5 3.0


An upward and leftward shift in a dose-response curve is referred to as ‘reverse tolerance’ or ‘sensitization’. Note that the term sensitization describes a change in a very specific drug response and is not a conclusion regarding a more global modification. That is, the data illustrated in Figure 2 only show that the behavioral response for self-administered cocaine is sensitized – not that the animal is sensitized. It is possible that other behavioral and neurochemical responses to cocaine might remain unchanged or even show changes in the opposite direction.

**Behavioral Sensitization**

How does sensitization to the reinforcing effects of cocaine relate to the literature on ‘behavioral and neurochemical sensitization’? It has long been known that a variety of specific behavioral responses to psychostimulant drugs can become augmented with repeated drug administrations (Tatum and Seevers, 1929; Downs and Eddy, 1932; Segal and Kuczenski, 1987). This general phenomenon, commonly referred to as ‘behavioral’ sensitization, has been linked at a theoretical level with the addiction process (Robinson and Berridge, 1993; Robinson and Becker, 1986; Robinson and Berridge, 2000). Many detailed reviews have addressed specific issues, for example, the neurobiological (Vanderschuren and Kalivas, 2000) and external conditional factors (Robinson, *et al* 1998) that influence the development of behavioral sensitization. This phenomenon is paralleled by the development of neurochemical sensitization (i.e., increased drug-induced extracellular dopamine in the ventral striatum), which is considered to be of fundamental importance in the expression of behavioral sensitization (Robinson and Berridge, 2000).
Behavioral and neurochemical sensitization have also been reported to occur in the human literature. Multiple investigators have reported that drug-associated behaviors in human subjects become sensitized following repeated amphetamine administrations (Strakowski, et al 1996; Boileau, et al 2006), see review (Sax and Strakowski, 2001). In addition, Boileau and colleagues (Boileau, et al 2006) have demonstrated that amphetamine-induced neurochemical sensitization can be observed in the striatum of human subjects.

There is strong evidence linking behavioral and neurochemical sensitization with an augmentation of the reinforcing effects of psychostimulant drugs. Regimens of IP amphetamine administration that result in behavioral sensitization (Vezina, et al 1999; Lorrain, et al 2000) and neurochemical sensitization (Lorrain, et al 2000; Vezina, et al 2002) also result in increased final ratios on a PR schedule reinforced by IV amphetamine (Lorrain, et al 2000; Vezina, et al 2002) or cocaine (Suto, et al 2002; Suto, et al 2003). Although these data appear to strongly support the conclusion that behavioral and neurochemical sensitization is sufficient to produce an augmentation of the reinforcing effects of cocaine and amphetamine, the question remains whether behavioral and neurochemical sensitization is necessary to achieve this result. A conclusion suggesting that behavioral and neurochemical sensitization is sufficient but not necessary would imply that other important addiction processes exist.

If sensitization of the dopamine system is the only process involved, then it would be predicted that animals showing an escalation in the reinforcing effects of
cocaine in self-administration procedures should also show a sensitized dopamine response. Contrary to this hypothesis, Läck, *et al* (2008) showed that the cocaine-induced psychomotor and neurochemical responses in fact showed tolerance in animals in which the reinforcing effects of cocaine were enhanced. Animals were provided access to cocaine under a PR schedule using conditions which produced an escalation in final ratios over 14 days. Upon the cessation of training, measurements for cocaine-induced psychomotor activation (Figure 3A) and extracellular dopamine in the ventral striatum (Figure 3B) were taken following either one-day (early withdrawal) or 14-days (late withdrawal) of forced abstinence. As illustrated in Figure 3A, it was found that the psychomotor activating effects of cocaine were reduced following a history of PR training in comparison to naïve animals. In addition, no apparent neurochemical sensitization to a cocaine challenge was observed in the ventral striatum. That is, cocaine-induced extracellular dopamine levels were shown to be identical between all groups in the nucleus accumbens core. In fact, as illustrated in Figure 3B, extracellular dopamine levels in the nucleus accumbens shell were significantly decreased following 1-day of withdrawal from PR training in comparison to naïve animals. However, this reduced dopamine effect was found to recover following 14 days of withdrawal. These data suggest that the sensitizing effects (i.e., increased final ratios) produced by PR training are dissociable from the development of behavioral and neurochemical sensitization. We conclude that neurochemical sensitization may be sufficient, but is not necessary for the development of an increased motivation to self-administer
cocaine; thereby suggesting that more than one addiction process should be considered.
FIGURE 3

PR training does not produce behavioral and neurochemical sensitization. (Lack, et al 2006) A). PR training results in tolerance to the psychomotor activating effects of cocaine. PR trained rats (open squares) showed a tolerant psychomotor response to a cocaine challenge (15 mg/kg IP) in comparison to naïve rats (filled circles). B). PR trained animals show a tolerant cocaine-induced dopamine response in the nucleus accumbens shell. Administration of cocaine (15 mg/kg IP) resulted in decreased extracellular dopamine levels following both 1-day (open squares) and 14-days of withdrawal after PR training in comparison to naïve rats (filled circles).
**Figure 3A**
**Figure 3B**

Diagram showing the percentage of baseline (DA) over time (minutes) for different conditions: Naive, Early Withdrawal, Late Withdrawal. The graph indicates a rise and peak at approximately 120 minutes, followed by a decline.
**Long-access Training and Intake Escalation**

Long-access training is another procedure that is used to model the addiction process. The phenomenon of long-access escalation (LgA), which models the increased drug intake observed in human addicts, has been clearly demonstrated by Ahmed and Koob (1998). These investigators have repeatedly shown that providing extended access (i.e., at least 6 hours) during daily Fixed-Ratio1 (FR1) self-administration sessions results in an increase, or ‘escalation,’ in the rate of drug intake over weeks (Ahmed and Koob, 1998, 1999). As illustrated in figure 1C (Ahmed and Koob, 1998), the daily number of infusions that are self-administered by animals given short-access (i.e., 1 hour; ShA) to cocaine remained stable over 12 days (filled circles); whereas, when given long access (i.e., 6 hours) to cocaine the daily number of self-administered infusions drastically increased over 12 days (open circles). The observation of escalated rates of drug-intake after long-access training is a highly replicable phenomenon that has been documented to occur for several drugs of abuse including cocaine (Ahmed and Koob, 1998), methamphetamine (Kitamura, et al 2006) and heroin (Lenoir and Ahmed, 2008).

Many different laboratories are beginning to use this long-access training procedure in order to study the neurobiological (Ferrario, et al 2005; Ben Shahar, et al 2007; Briand, et al 2008) and behavioral (Vanderschuren and Everitt, 2004; Knackstedt and Kalivas, 2007) consequences of a history of escalated cocaine intake. Although this phenomenon is briefly reviewed here, more encompassing reviews exist that cover concepts such as the behavioral (Ahmed, 2005) and neurobiological (Koob, et al 2004) factors associated with long-access escalation.
An escalation of final ratios on a PR schedule is fundamentally different from an escalation in intake on an FR1 schedule of reinforcement. Inspection of Figure 1 reveals a superficial similarity in time course for the two procedures. The escalation in final ratios shown in the PR group (Fig. 1B) shows approximately the same slope as the escalation in intake on an FR1 schedule shown by the LgA group (Fig 1C). It should be emphasized, however, that the two dependent measures are very different. Final ratios reflect some measure of the motivation to obtain drug, while rate of drug intake on an FR1 schedule of reinforcement reflects the blood or brain level at which an animal will titrate its intake (Lynch and Carroll, 2001; Oleson, et al 2009; Ahmed and Koob, 2005; Tsibulsky and Norman, 1999) but see also (Panlilio, et al 2003). These are very different concepts. In behavioral economic terms, one reflects “price” and the other “consumption”. It remains unclear whether self-administration histories that produce changes in consumption necessarily result in changes in the maximum price paid. A number of studies have reported that LgA training can result in an increase in final ratios for cocaine on a PR schedule (Paterson and Markou, 2003; Allen, et al 2007; Wee, et al 2008) and faster runway times for cocaine (Ben Shahar, et al 2008) in comparison to ShA rats. However this does not appear to be a consistent result. Liu et al. (Liu, et al 2005b) found that higher final ratios are not necessarily observed in LgA animals. Additionally, under certain conditions, escalating the rate of cocaine intake can suppress the development of final ratio escalation that occurs during PR training (Morgan, et al 2006). Therefore, it remains unclear whether LgA and PR training produce parallel
escalations in both drug intake and the effort an animal is willing to expend to obtain cocaine.

Recently we have explored the use of a self-administration protocol which provides a measure of both price and consumption. The procedure, adapted from a study by Zittel-Lazarini, et al (2007), uses a descending series of unit injection doses (237μg - 1 μg) to determine the threshold dose of cocaine. The term ‘threshold’ is defined as the lowest dose that maintains stable responding throughout the duration of the session. In their study, Zittel-Lazarini and colleagues (2007) demonstrated that very high rates of responding can be maintained under an FR1 schedule of reinforcement by cocaine doses as low as 8 μg/inf. Due to the observations that the rate of cocaine intake maintained under an FR1 schedule of reinforcement is titrated around some blood or brain level (Oleson, et al 2009; Lynch and Carroll, 2001; Pettit and Justice, 1989) and maximal responding occurs at the threshold, these authors (Zittel-Lazarini, et al 2007) first suggested that behavioral economics analyses could be applied to threshold data.

As shown in figure 4 B and C, a relatively stable level of cocaine consumption is maintained as the available dose is decreased until a threshold dose is reached. Due to the observation that maximal responding occurs at this threshold dose, it can be inferred that the maintenance of drug intake at threshold is influenced by the price (i.e., effort) an animal expends to ‘defend’ a relative level of drug intake. An example of the high rates of responding that are engendered at threshold doses is illustrated in figure 4A. Here, responding at each individual animal’s threshold,
which ranged from 23.7-2.4 μg/inf, occurred at a rate of 955 responses/hr ± 244 (SEM). In behavioral economics terms, the point at which maximal responding occurs in order to maintain a relative level of drug consumption is Pmax (i.e., maximal price; (Hursh, 1991). Although Pmax is normally assessed by increasing the response requirement necessary to receive a single injection of a constant drug dose (Cosgrove and Carroll, 2002; Wade-Galuska, et al 2007), it is also valid to decrease the dose of a drug while holding the FR requirement constant (Bickel, et al 1990; Bickel, et al 1993; Zittel-Lazarini, et al 2007). Given that the threshold procedure apparently provides information about consumption in the high dose range in addition to a behavioral economics index of price, we applied this procedure to investigate the effects of LgA and PR training on the reinforcing effects of cocaine.

LgA and PR training produced opposite behavioral effects on consumption and price in the threshold procedure. The effects of four different training histories on daily cocaine intake are shown in figure 4. As illustrated in figure 4B, it was found that PR trained animals did not maintain a higher level of cocaine consumption in comparison to a group of animals that were matched for intake and dose during training. The PR trained animals did, however, defend their rates of intake to lower unit doses in comparison to other groups. This maintenance of intake required a higher response output (955 responses/hr ± 244 SEM), the results of which are illustrated in figure 4A. Therefore, in behavioral economics terms, PR trained animals paid the highest behavioral price (Pmax) for cocaine in comparison to all other tested groups (Oleson and Roberts, 2009b). As expected, Figure 4C shows that
LgA trained animals maintained a higher level of cocaine consumption at high doses in comparison to ShA animals. However, the threshold of the LgA animals was significantly higher than that of the ShA animals, suggesting the development of tolerance to the reinforcing effectiveness of cocaine. Therefore, LgA animals paid the lowest behavioral price (Pmax) for low doses of cocaine in comparison to all other tested groups (Oleson and Roberts, 2009). In summary, LgA trained animals consumed more cocaine at high doses, but did not pay a high price to maintain consumption at low doses; by contrast, the PR trained animals did not consume more cocaine at high doses, but did expend a considerably higher behavioral price to maintain consumption at low doses. Overall, these findings suggest that LgA and PR training result in very unique behavioral phenotypes that exhibit distinct addiction-like behavioral manifestations. Again, these data suggest that more than one process may be involved in the development of addiction.
FIGURE 4

PR and LgA training produce opposite effects in the threshold procedure. (Oleson and Robers, 2009) Self-administration was assessed across a descending series of ten doses (237μg - 1 μg) under an FR1 schedule of reinforcement to measure consumption in the high dose range and thresholds in the low dose range. A) PR trained rats (filled circles) showed a higher response output (955 responses/hr ± 244 (SEM)) for remarkably low threshold doses (23.7-2.4 μg/inf) in comparison to control rats (open circles). B) PR trained rats (filled circles) did not consume more cocaine at high doses in comparison to control rats (open circles), but maintained consumption at lower thresholds. Note that considerable effort is expended to maintain consumption as these low doses. C) LgA trained rats (filled triangles) consumed more cocaine at high doses but reached threshold doses prior to ShA rats (open triangles).
**Figure 4B**

A graph showing the relationship between intake (mg/session) and unit dose (μg/inf). The graph compares two conditions: PR and Match. The y-axis represents intake in a log scale, ranging from 0.01 to 100 mg/session, and the x-axis represents unit dose in a log scale, ranging from 1 to 1000 μg/inf. The data points for PR are shown with solid circles, and the data points for Match are shown with open circles. Error bars indicate variability in the data.
FIGURE 4C
An escalation of cocaine intake is also dissociable from the development of behavioral and neurochemical sensitization. The literature suggests that the time-course of LgA training does not promote, and may actually suppress, the development of drug-induced behavioral sensitization. For example, multiple investigators have reported that LgA trained animals do not show behavioral sensitization in comparison to ShA trained animals (Ben Shahar, et al 2004; Ben Shahar, et al 2005; Ahmed and Cador, 2006; Knackstedt and Kalivas, 2007). In fact, Ben Shahar and colleagues (Ben Shahar, et al 2004; Ben Shahar, et al 2005) reported that LgA training resulted in rats that showed a tolerant cocaine-induced psychomotor response in comparison to ShA trained animals. These latter reports are consistent with our findings that LgA training can produce tolerance to cocaine-associated behavioral effects (Oleson and Roberts, 2009). In addition, an important finding that appears to distinguish the effects of LgA training from neurochemical sensitization is that the dopamine response in the nucleus accumbens to a cocaine challenge is not increased in LgA trained rats (Ahmed and Koob, 2005). Therefore, these data suggest that LgA and behavioral/neurochemical sensitization are distinct processes.

**SEQUENCE OF PHARMACOLOGICAL EVENTS AND ABSTINENCE**

A sequence of pharmacological and behavioral events influences, in part, the emergence of observable tolerant or sensitized behavioral effects. The amount of drug exposure during initial training and the periods of abstinence following a history of drug administration greatly affect the expression of an escalation in the reinforcing effects of cocaine. Both of these considerations will be discussed
independently and – as with the rate of drug injection and the magnitude of the available dose – will be shown to affect the development of addiction-like behavioral changes.

A history of high drug intake suppresses the development of an increased motivation to self-administer cocaine, but only transiently suppress increased final ratios later in the sequence. Morgan, et al (Morgan, et al 2006) showed that final ratios generally increase as long as the quantity animals are permitted to self-administer during training is restricted. Figure 5 shows the expected escalation of final ratios with only a single day of training (filled circles). However, if animals are given access to high doses (1.5 mg/kg/inj) for several hours for 5 days, then the phenomenon of escalation is greatly attenuated (Figure 5, open circles). These data clearly demonstrate that greater drug access does not necessarily result in higher final ratios and in fact suggests that high cocaine intake retards the process which would result in an escalation of final ratios. It is important to emphasize that the sequence of events is critical. If higher levels of access are given after the final ratios have escalated, only transient suppression is seen. Figure 5 illustrates the effect of giving the escalated and non-escalated groups 5 days of high access (40 injections x 1.5 mg/kg/inj). The final ratios for the two groups temporarily converge, but surprisingly the two groups separate again after several days. We interpret these data to indicate that high access conditions, which can produce tolerance to the reinforcing effects of cocaine, prevent an escalation of final ratios. However, if animals are first exposed to a training procedure that fosters the escalation of final ratios, the presumed increased motivational state can survive periods of high intake.
Early exposure to high levels of cocaine intake attenuates the escalation of final ratios. (Morgan, et al 2006) The left pane shows the initial pharmacological history of two groups of animals. One group was given access to cocaine under an FR1 reinforcement schedule for 1 day (filled circles) and another for 5 days (open circles). The next pane shows the final ratios for both groups over 14 days. Note that only the group with a limited initial pharmacological history showed an increase in final ratios. The next pane illustrates that both groups then received access to cocaine for 5 days under an FR1 reinforcement schedule. Note that both groups showed an escalation in the rate of responding. The final pane illustrates that the effects of high cocaine intake only transiently suppressed final ratios in the group that originally showed an escalation in final ratios (filled circles).
FIGURE 5
Periods of abstinence can increase various drug-induced behavioral effects including final ratios, behavioral sensitization and cue-induced reinstatement responding. For example, we have repeatedly demonstrated that providing animals with extended access to cocaine (i.e., 4 trials/hr for 24 hrs/day) for at least 10 days followed by a deprivation period of 7 days produces animals that show increased final ratios when tested on a PR schedule (Morgan, et al 2002; Morgan, et al 2005); see review (Morgan and Roberts, 2004). As illustrated in figure 6A (Morgan, et al 2005), final ratios were significantly increased following this procedure in comparison to baseline final ratios (i.e., those before extended access and deprivation). Note that the deprivation period is a necessary component of this phenomenon. That is, animals that are given access to cocaine on an FR1 reinforcement schedule instead of undergoing a deprivation period following extended access conditions do not increase final ratios (Morgan, et al 2005). These data clearly demonstrate that periods of abstinence can produce increases in the reinforcing effects of cocaine. The expression of behavioral sensitization is another good example of the effects of abstinence periods. As illustrated in figure 6B (Kalivas and Duffy, 1993) the expression of behavioral sensitization is exacerbated by periods of forced abstinence. Here, Kalivas and Duffy (1993) treated rats with cocaine (30 mg/kg i.p.) for five consecutive days before measuring drug (15 mg/kg cocaine i.p.) induced psychomotor activation following various abstinence periods. It was shown that the magnitude of the psychomotor response became augmented along the course of abstinence. Likewise, these authors reported that the cocaine-
induced dopamine response in the ventral striatum increased over the abstinence period (Kalivas and Duffy, 1993).

**INCUBATION OF CRAVING**

Another related behavioral procedure used to model the addiction process has been termed ‘incubation of craving.’ This phenomenon of incubation refers to the observation that the amount of cue-induced responding observed during a reinstatement self-administration session increases in relation to the duration of the abstinence period (Grimm, et al 2001; Grimm, et al 2003). As illustrated in figure 6C (Grimm, et al 2001), the amount of cue-reinforced responding increases, or incubates, over periods of abstinence. In this study (Grimm, et al 2001), animals self-administered cocaine under an FR1 schedule of reinforcement, and then underwent various periods of abstinence before being returned to operant chambers. Upon meeting extinction criteria (i.e., 15 responses/hr on a lever not paired with any cue), cue-induced reinstatement responding was assessed by reintroducing a conditioned tone-light cue in response to each lever press. The amount of tone-cue maintained responding increased following progressively longer periods of abstinence. The incubation of craving phenomenon has been reported to increase over the first three months of abstinence but wanes after six months (Lu, et al 2004). Taken together, these studies suggest that periods of abstinence may contribute to the development of certain addiction-like behavioral effects.
**FIGURE 6**

Periods of abstinence can increase final ratios, behavioral sensitization and cue-induced reinstatement. A). (Morgan, et al 2004) Final ratios increase following abstinence periods. Seven days of abstinence following extended access (24-hr/day; 4 trials/hr; 10 days) conditions during cocaine self-administration results in rats that show increased final ratios (filled diamonds) in comparison to baseline final ratios (open circles). It should be noted that the abstinence period is necessary for the increase in final ratios. B). (Kalivas and Duffy, 1993) Behavioral sensitization increases following abstinence periods. Cocaine-induced (15 mg/kg IP) psychomotor activation progressively increased over extended periods of abstinence following a sensitizing drug-treatment (30 mg/kg IP cocaine x 5 days). C). (Grimm, et al 2001) Cue-reinforced responding increases, or incubates, over periods of abstinence. Following various periods of abstinence from cocaine self-administration animals were returned to operant chambers. Upon meeting extinction criteria (i.e., 15 responses/hr on a lever not paired with any cue), cue-induced reinstatement responding was assessed by reintroducing a conditioned tone-light cue in response to each lever press. The amount of tone-cue maintained responding increased following progressively longer periods of abstinence.
FIGURE 6A

[Diagram showing a graph with two lines representing 'Baseline' and 'Extended Access+7d off', with the x-axis labeled 'Dose (mg/kg/inf)' and the y-axis labeled 'Final Ratio'].

Legend: 
- □ Baseline
- ● Extended Access+7d off
**Figure 6B**

[Bar chart showing Photocell Counts (120 min) over different withdrawal periods (days)].
Figure 6C

Cues Available

Baseline

Responses (1 hr)

Withdrawal Period (days)
Increased reinstatement responding can be dissociated from the development of behavioral sensitization. Many parallels linking behavioral sensitization to changes in drug-self-administration have been documented (see review, (Vezina, 2004). In fact, it has been hypothesized that the development of behavioral sensitization to the acute effects of drugs may facilitate the reinstatement of drug-seeking behavior (Robinson and Berridge, 1993) in addition to promoting drug-taking behavior (Vezina, 2004). In support of this hypothesis, it has been demonstrated that the expression of behavioral sensitization coincides with the expression of drug-induced reinstatement responding (De Vries, et al 1998). That is, following periods of abstinence, animals that show increased psychomotor sensitization also show increased reinstatement responding (De Vries, et al 1998). However, other observations suggest that an animal’s propensity to reinstate does not depend upon the development of behavioral sensitization. For example, LgA animals show increased reinstatement responding but do not exhibit increased behavioral sensitization in comparison to ShA animals (Ahmed and Cador, 2006; Knackstedt and Kalivas, 2007). Thus, sensitization may be sufficient, but is not necessary for the expression of increased reinstatement responding. This dissociation provides yet another example that independent processes exist.

**CONCLUSIONS**

How many addiction processes are there? If we are seeking the most parsimonious explanation we should start with as few assumptions as possible and only when confronted with a need should we add more complexity to the theory. The simplest
idea is that there is a single process which is manifest in a variety of ways in the clinical population. If animal models demonstrate that fundamental behavioral characteristics are always correlated it would imply that a single process is involved. If, however, critical features can be dissociated then it would not only suggest multiple processes but also guide the identification of core aspects of the addiction process. Robust behavioral changes that develop over time and appear to be symptomatic of an addiction process have been modeled using distinct training procedures. In the present review we provide several examples showing that many of the changes resulting from these procedures are dissociable. While it may be difficult to determine whether each of these behavioral changes models an exact DSM criterion several clear associations have emerged. For example, the escalation in drug intake observed after LgA training clearly models increased consumption over time. Similarly the increased final ratios observed after PR training appears to address the DSM-IV criterion of increased time and energy expended to obtain cocaine. Because the phenomena discussed in this review are dissociable, they can be viewed as distinct components of drug addiction. This idea suggests the possibility that each of these behavioral phenomena have discrete neurobiological substrates. Overall, we conclude that drug addiction is too complex a disorder to be explained as a single process or alteration in the function of one particular transmitter; however, the identification of specific sub-processes may actually enhance our ability to isolate neural mechanisms that are unique to various animal models. The challenge for the field will be to identify and distinguish between a yet
unidentified number of addiction processes. Progress will likely be made through further development of animal models that address DSM-IV symptomatology.

**ACKNOWLEDGEMENTS**

The authors would like to thank Keri Chiodo for helpful comments in the preparation of this review.
REFERENCES


Lack CM, Jones SR, Roberts DC (2008) Increased breakpoints on a progressive ratio schedule reinforced by IV cocaine are associated with reduced locomotor activation
and reduced dopamine efflux in nucleus accumbens shell in rats. Psychopharmacology (Berl) 195: 517-525.


Oleson EB and Roberts DC (2009) Behavioral economic assessment of price and cocaine consumption following self-administration histories which produce escalation of either final ratios or intake. Neuropsychopharmacology 34: 796-804.


CHAPTER II

BEHAVIORAL ECONOMIC ASSESSMENT OF PRICE AND COCAINE CONSUMPTION FOLLOWING SELF-ADMINISTRATION HISTORIES THAT PRODUCE ESCALATION OF EITHER FINAL RATIOS OR INTAKE.

Erik B. Oleson and David C.S. Roberts

The following manuscript was published by *Neuropsychopharmacology* 34(3):796-804, February 2009.


**ABSTRACT**

Various self-administration procedures are being developed to model specific aspects of the addiction process. For example, 'increased cocaine intake over time' has been modeled by providing long-access (LgA) to cocaine during daily self-administration sessions under a fixed-ratio (FR1) reinforcement schedule. Additionally, 'increased time and energy devoted to acquire cocaine' has been modeled by providing access to cocaine during daily self-administration sessions under a progressive-ratio (PR) schedule. To investigate the distinctiveness of these models, the behavioral economics variables of consumption and price were applied to cocaine self-administration data. To assess changes in consumption and price, cocaine self-administration was tested across a descending series of doses (0.237 – 0.001 mg/inj) under an FR1 reinforcement schedule in order to measure drug intake in the high dose range and thresholds in the low range. Cocaine consumption remained relatively stable across doses until a threshold was reached, at which maximal responding was observed. It was found that a history of LgA training produced an increase in cocaine consumption; whereas a history of PR training produced an increase in the maximal price (Pmax) expended for cocaine. Importantly, the concepts of consumption and price were found to be dissociable. That is, LgA training produced an increase in consumption but a decrease in Pmax, whereas PR training produced an increase in Pmax without increasing consumption. These results suggest that distinct aspects of the addiction process can be parsed using self-administration models, thereby facilitating the investigation of specific neurobiological adaptations that occur through the addiction process.

**KEYWORDS:** Cocaine, self-administration, behavioral economics, progressive ratio, price, consumption
INTRODUCTION

A number of investigators are developing self-administration procedures to model specific aspects of the cocaine addiction process. The current Diagnostic and Statistical Manual (DSM-IV) describes many different symptoms associated with drug dependence in general and cocaine dependence in particular. Some of these might be difficult to model in rats – such as “unsuccessful attempts to reduce drug use” or “abandonment of social or occupational activities” – however other features have successfully been addressed. For example, a growing literature has focused on relapse, which seeks to understand how environmental or interoceptive events cause drug-seeking (see reviews, Lu, et al 2004; Schmidt, et al 2005; Epstein, et al 2006). Others have focused on continued drug taking in the face of adverse consequences (Deroche-Gamonet et al 2004; Vanderschuren and Everitt, 2004) or changes in attention or impulsivity after extended cocaine intake (Dalley, et al 2005; Perry and Carroll, 2008). Each of these approaches has demonstrated that addiction-like symptoms change over time.

Two aspects which appear to be fundamental to the addiction process are “increased intake over time” and “increased time and energy devoted to acquiring the drug” (DSM-IV). In behavioral economic terms, the first aspect is concerned with consumption whereas the second is concerned with price (i.e., effort expended). Increased cocaine intake over time has been clearly demonstrated by Ahmed and Koob, (1998). This highly cited work has taken advantage of the fact that access to cocaine on a Fixed Ratio 1 (FR1) reinforcement schedule during relatively long daily self-administration sessions (e.g., 6 h/day) results in an increased rate of drug
intake after multiple sessions (Ahmed and Koob, 1998; 1999). A second aspect that focuses on increases in time and energy devoted to acquiring the drug has been explored using a progressive ratio (PR) schedule. Depending on the unit injection dose and speed of injection, the behavioral cost an animal might pay for a cocaine injection can dramatically increase with time (Liu, et al 2005b; Morgan, et al 2006).

It remains unclear whether self-administration histories that produce changes in consumption necessarily result in changes in maximum price paid (cf. Paterson and Markou, 2003; Liu, et al 2005a). The present study sought to examine the relationship between consumption and price using the two different cocaine self-administration histories that have been specifically designed to increase either drug intake or price paid for cocaine.

The procedure used here was adapted from a study by Zittel-Lazarini, et al (2007) in which a descending series of unit injection doses were used to determine the threshold dose of cocaine. The concept of ‘threshold’ implies that there is a dose that is minimally effective in supporting self-administration behavior. However, in determining this threshold, it should be noted that animals must respond at very high rates on an FR1 reinforcement schedule in order to maintain a constant level of drug intake. For example, Zittel-Lazarini, et al (2007) report that a cocaine dose as low as 8 μg/inf can support stable patterns of responding, engendering rates as high as 251 responses per hour. It is likely, therefore, that the threshold determined on an FR1 reinforcement schedule must be influenced by the relatively high ‘price’ observed at very low doses. In fact, Zittel-Lazarini, et al (2007) suggested that concepts of behavioral economics could be used to advantage in the analysis of
threshold determinations. Since the threshold procedure yields information concerning both consumption (at high doses), as well as a behavioral economic index of price (Pmax), this procedure was used here to assess the consequences of self-administration histories suggested to model important aspects of the addiction process.

**MATERIALS AND METHODS**

**ANIMALS, SURGERY, AND HOUSING**

Male Sprague-Dawley rats (Harlan, Indianapolis, Ind., USA), weighing approximately 350 g at the start of each experiment, were used as subjects. Upon acquisition, 57 rats were used for these experiments. A primarily cannula-related attrition resulted in complete data being collected from 45 rats. Throughout the experiments, rats were maintained on a reverse 12 hour light/dark cycle (lights on at 3 pm) with food and water available *ad libitum*. Prior to the beginning of the study, rats were anesthetized with an IP injection of ketamine (100 mg/kg) and xylazine (8 mg/kg) and implanted with a chronically indwelling Silastic® cannula (CamCaths, Cambridgeshire, UK) into the right jugular vein. The cannula exited through the skin on the dorsal surface in the region of the scapulae (Roberts and Goeders, 1989). Animals were then housed individually in 30 x 30 x 30 cm experimental chambers. The cannula was connected with Tygon® tubing (through a stainless steel tether) to a counterbalanced fluid swivel (Instech Laboratories, Inc., Plymouth Meeting, Pa., USA) mounted above the chamber. The swivel was connected to an infusion pump.
(Razel Scientific Instruments, Inc., Stamford, Conn., USA). Cannulae were flushed daily with heparinized saline to help maintain patency. All experiments were approved by the Wake Forest University Institutional Animal Care and Use Committee.

**GENERAL SELF-ADMINISTRATION METHODS**

Following surgery, rats were allowed 3-5 days to recover before self-administration testing began. Self-administration sessions occurred 7 days per week, each beginning in the middle of the dark cycle. The start of a session was signaled by the extension of a lever into the experimental chamber. During acquisition and during FR and PR training sessions cocaine was infused as a bolus of about 0.1 ml of cocaine solution over approximately 4–5 seconds (adjusted according to body weight). Doses (0.75 and 1.5 mg/kg/inf) were manipulated by adjusting the concentration of cocaine solution (2.5 and 5.0 mg/ml, respectively). Following each response the lever was retracted and a light was illuminated for a 20-second timeout period. During threshold testing the dose was manipulated by adjusting the pump duration as described below.

**ACQUISITION PHASE**

During the acquisition phase all animals received access to cocaine (0.75 mg/kg/infusion) on an FR1 schedule of reinforcement during daily training sessions, which terminated after a maximum of 20 infusions or a period of 6 hours had elapsed. An animal was considered to have acquired stable self-administration if 20 injections were self-administered during a single session and the pattern displayed consistent post-infusion pauses between each of the injections.
**Test Group Used to Demonstrate Threshold Procedure and Analysis**

One group of rats (n=8) was used to demonstrate proof-of-principle of the threshold procedure and data analyses. Following acquisition, animals were given access to cocaine (0.75 mg/kg/infusion) during 1 hr sessions on an FR1 schedule of reinforcement for 14 consecutive days and then were tested using the threshold procedure describe below.

**Cocaine Self-Administration Threshold Procedure**

Rats were tested through a descending series of unit injection doses reinforced on an FR1 reinforcement schedule during daily 2 hr sessions. Threshold was defined as the lowest dose that maintained daily intake. The dose was manipulated by holding the concentration of the drug constant and manipulating the pump duration. Pump times were decreased each day along a quarter-log scale as follows: 3156, 1780, 1000, 562, 310, 178, 100, 56, 31, 18 and 10 msec. A time-out period corresponded only to the duration of the pump infusion. The calculated dose equivalents (5 mg/ml x 1.6 ml/min x pump duration) are as follows: 421, 237, 133, 75, 41, 24, 13, 7.5, 4.1, 2.4 and 1.3 μg/inf. These provided unit-doses corresponding to doses of (1120, 630, 350, 200, 110, 60, 30, 20, 10, 5 and 3 μg/kg/inf) for a rat weighing 375g. Only the final 10 unit-doses were used for group comparisons. It should also be noted that no significant differences in body weight were observed between groups.

An important consideration is whether turning an infusion pump on for only a few milliseconds is sufficient to deliver any amount of drug. This issue is addressed in the Supplemental Information (S1). The computer logged the cumulative pump durations across the sessions and drug volumes were recorded by
the experimenter before and after each session. It was verified that intermittent
delivery – even at very short intervals – resulted in the appropriate total volume of
drug being delivered. The correlation between expected and actual drug delivered
by this method was $r = 0.98$.

**Behavioral Economics**

$P_{\text{max}}$, defined as the unit-price at which maximal responding occurs (Hursh 1991),
can be determined two ways. A common method used to determine $P_{\text{max}}$ involves
calculating the unit-price at which maximal responding occurs on a demand curve
(Hursh, 1991; Campbell, *et al* 1999; Cosgrove and Carroll, 2002). Individual demand
curves were obtained by graphing daily intake (total drug in mg per 2 hr session) as
a function of unit-price (FR1/unit dose). $P_{\text{max}}$ values were first calculated by
analyzing the point at which the slope of a demand curve became elastic, which
theoretically represents the point at which maximal responding occurs (Hursh,
1991). Calculated $P_{\text{max}}$ values and representative demand curves that were curve-
fitted using equations described in greater detail by Hursh and Silberberg (2008)
are demonstrated in the Supplementary Information (S2). $P_{\text{max}}$ can also be
determined graphically by measuring the unit-price at which maximal responding
occurs by plotting daily responding as a function of unit-price (Greenwald and
Hursh, 2006; Lenoir and Ahmed, 2007). The apex of the price-response function
(white-circles; Figure 1C) was used to determine $P_{\text{max}}$. The relationship between
$P_{\text{max}}$ values calculated using a demand curve analysis and $P_{\text{max}}$ values that were
graphically determined is demonstrated in the Supplemental Information (S2) and
further described in the results section. All $P_{\text{max}}$ values reported in the current
study were determined graphically from individual animals, as demonstrated in Figure 1C.
**Figure 1**

Dose-effect and price-effect relationships derived from self-administration data using a threshold procedure. (A) Dose-response relationship from a group of animals (n=8) tested through a descending series of unit injection doses of cocaine during daily 2 h sessions. The dose of cocaine was reduced each day by adjusting the pump duration (top x-axis); the corresponding unit injection dose is shown on the bottom x-axis. Data are expressed as mean (±SEM) responses on an FR1 schedule. (B) The dose-intake relationship demonstrates that a relatively stable level of cocaine intake is maintained at the high end of the dose range. Data are expressed as mean (±SEM) daily intake. (C) An individual animal’s Pmax (maximal price) is graphically determined. The animal’s daily responses (right y-axis) and intake (left y-axis) are both plotted as a function of unit-price (FR1/unit-dose). The unit-price at which maximal responding occurs (Pmax), which is graphically distinguishable as the apex of the price-response function (white circles), coincides with the point before which daily intake (black triangles) rapidly declines in response to an increase in price.
**Figure 1A**

Graph showing the relationship between pump duration (msec) and responses (2 hr session) across different unit doses (µg/inf). The graph indicates an optimal pump duration for maximum responses, after which responses decrease with higher dose levels.
Figure 1B

![Graph showing intake (mg/2 hr session) vs. unit dose (μg/inf).]
Figure 1C
LONG-ACCESS AND SHORT-ACCESS TRAINING

Two groups of animals were used for this experiment. Animals in the long-access group (LgA; n=8) were given access to cocaine (0.75 mg/kg/infusion) on an FR1 schedule of reinforcement during daily 6-hour sessions for 14 consecutive days. Previous reports indicate that animals increase, or 'escalate,' cocaine intake over 14 days under long-access conditions (Ahmed and Koob, 1998; 1999). Animals in the short-access group (ShA; n=6) were given access to cocaine (0.75 mg/kg/infusion) on an FR1 schedule of reinforcement for 14 consecutive days during 2-hour sessions. It has previously been reported that three hours of access or less does not result in an escalation of cocaine intake across days (Wee, et al 2007). After completion of 14 days with either short or long access conditions, the two groups entered the threshold procedure. The independent variable in this experiment was the exposure to a pharmacological history of long versus short access to cocaine.

PROGRESSIVE-RATIO RESPONDING AND MATCHED INTAKE

Two groups of animals were used for this experiment. Animals in the PR group (n=8) were given access to cocaine (1.5 mg/kg/infusion) on a PR schedule of reinforcement for 14 consecutive days during 6-hour sessions. Under this PR schedule, delivery of intravenous cocaine injections (1.5mg/kg/infusion) was contingent upon an increasing number of responses incremented through the following progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603 (Richardson and Roberts, 1996). Final ratios were defined as the response requirement corresponding to the final drug infusion. Although, the session duration was 6 hrs, animals typically reached the final ratio
within 3 hrs. Previous reports have shown that, with this dose of cocaine, final ratios increase across days (Liu, et al 2005b; Morgan, et al 2006). A matched-intake group (Match; n=7) was given access to cocaine (1.5 mg/kg/inf) on an FR1 schedule of reinforcement for 14 consecutive days. The total number of injections available during each daily session corresponded to the mean number of daily infusions self-administered by the PR group, which gradually incremented over days. The number of daily infusions was incremented across days as follows (12, 14, 15, 14, 15, 16, 15, 18, 17, 17, 18, 17, 18). It should be noted that one animal from the Match cohort was excluded due to the observation that the animal’s mean data was a statistical outlier, occurring 3 standard deviations away from the group mean. Upon completion of training these two groups entered the threshold procedure. The independent variable in this experiment was the exposure to a behavioral history of responding on a PR schedule.

**Drug**

Cocaine HCl, obtained from the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, N.C., USA), was dissolved in a solution of sterilized 0.9% saline. The cocaine solution (5 mg/ml) was passed through a microfilter (0.45 μm pore size).

**Statistics**

All statistics were performed using SigmaPlot (Version 11). All dose-intake comparisons were performed using a repeated measures ANOVA with Holm-Sidak *post hoc* analyses. All Pmax and individual dose comparisons were performed using Student's t-tests. Correlations are reported as a Pearson's r.
**RESULTS**

Figure 1 illustrates the dose-response and dose-intake curves as well as a representative graph illustrating how the Pmax value was derived using the threshold procedure. Figure 1A shows the dose-response curve for a group of animals (n=8; test group used to demonstrate threshold procedure and analyses) that self-administered cocaine through a descending series of 11 unit-injection doses on an FR1 reinforcement schedule. Note that each pump-duration (top x-axis) corresponds to a unit-dose (bottom x-axis) as described in greater detail in the methods section (see cocaine self-administration threshold procedure). This method of data presentation, which is commonly used to depict the dose-effect relationship, shows an apparent ascending and descending limb. The same data are plotted in terms of dose versus intake (Figure 1B) in order to demonstrate that a relatively constant level of cocaine is maintained at the upper-end of the dose range. Although the log-log coordinates emphasize that intake is relatively stable at high doses before rapidly declining at some point, it should be noted that intake is actually gradually declining in response to decreases in the available unit-dose. However, it is not clear from either Figure 1A or 1B which point corresponds to the threshold dose that maintains a maximal rate of responding before intake rapidly declines.

An individual animal’s daily response (right y-axis) and intake (left y-axis) data, both plotted as a function of unit-price, are illustrated in Figure 1C. Note that the X-axis is transposed – relative to 1A and B – so that the lowest doses appear on the right, to represent the higher work cost required in order to maintain a relative
level of cocaine intake at the low doses. The unit-price at which maximal responding occurs ($P_{\text{max}}$), which is graphically distinguishable as the apex of the price-response function (white circles), coincides with the point at which daily intake (black triangles) rapidly falls off in response to an increase in price. The unit-price at which maximal responding occurs before intake rapidly decreases, or demand becomes elastic, can also be calculated from a demand function as demonstrated in the Supplementary Information (S2). $P_{\text{max}}$ values were first calculated using equations analyzing the slopes of individual demand curves (see S2). However, as previously reported by Greenwald and Hursh (2006), no significant difference was found between $P_{\text{max}}$ values calculated from demand curve analyses and graphically determined $P_{\text{max}}$ values ($t_{(7)} = -0.059$, n.s.; S2). Additionally, a high correlation was found between the calculated and graphically determined $P_{\text{max}}$ values reported in the Supplementary Information ($r = .99$).
**Figure 2**

Cumulative records from the threshold procedure demonstrate supra-, threshold, and sub-threshold responding. (A) Cumulative records from one animal responding at the 41 μg/inf dose (i), the 24 μg/inf dose (ii), and the 13 μg/inf dose (iii) are shown. These cumulative records represent supra-, threshold, and sub-threshold responding respectively. Note that responding is maintained throughout the duration of the session in each cumulative record, except at the sub-threshold dose (13 μg/inf; iii). (B) A cumulative record from one animal from the PR group responding at the 2.4 μg/inf dose is shown. In this case, cocaine (2.4 μg/inf) was found to sustain responding at a rate of 1334 responses/hr. Note that every 1000 responses the record of cumulative responses resets at zero.
**Figure 2A**

The figure shows a graph with responses (2 hr session) on the y-axis and time (min) on the x-axis. There are three sections labeled i, ii, and iii, each with different response rates:

- Section i: 1000 responses with a rate of 41 μg/inf
- Section ii: 800 responses with a rate of 24 μg/inf
- Section iii: 600 responses with a rate of 13 μg/inf
Figure 2B

[Graph showing the number of responses over time for a 2-hour session. The graph includes a vertical line at 2.4 μg/inf.]
Representative cumulative records from the threshold procedure are illustrated in Figure 2. Figure 2A shows cumulative records from one animal used in Figure 1 (i.e., the test group used to demonstrate threshold procedure and analyses) responding at the 41 μg/inf dose (i), the 24 μg/inf dose (ii), and the 13 μg/inf dose (iii). These cumulative records demonstrate responding at a suprathreshold dose (i), a threshold dose (ii), and a subthreshold dose (iii).

The results of LgA and ShA training on responding for cocaine over 10 doses are illustrated in figure 3. Figure 3A shows the escalation of cocaine reinforced responding across sessions observed in animals given access to cocaine for 6h/day (LgA), in comparison to the relatively constant responding across sessions in animals given access to cocaine for 2h/day (ShA). As expected, the LgA training procedure resulted in a significant increase in responding across 14 sessions \( (F_{(13, 91)} = 6.794, p < 0.01) \). Figure 3B shows cocaine intake as a function of dose between the LgA and ShA groups. Repeated measures ANOVA revealed a significant effect of Dose \( (F_{(9,108)} = 67.0, p < 0.001) \), no significant effect of Group \( (F < 1) \) and a significant Dose x Group interaction \( (F_{(9,108)} = 7.2, p < 0.001) \). Holm-Sidak post hoc analysis revealed the LgA group consumed significantly more cocaine at two higher doses \( (237 \mu g/inf: \ t_{(12)} = 2.85, p < 0.05; \ 133 \mu g/inf: \ t_{(12)} = 2.80, p < 0.05) \) and significant less cocaine at a lower dose \( (24 \mu g/inf: \ t_{(12)} = -2.98, p = 0.05) \).
FIGURE 3

LgA training results in an increase in cocaine consumption. (A) LgA training (black triangles) results in an increased rate of cocaine intake across daily six hour sessions; whereas ShA training (white triangles) results in stable responding across daily two hour sessions. Data are expressed as mean (±SEM) daily responses on an FR1 schedule. (B) LgA trained animals (black triangles) consume more cocaine at the 237 and 133 μg/inf doses and less cocaine at the 24 μg/inf dose in comparison to the ShA group. Data are expressed as mean (±SEM) daily intake and asterisks indicate significant differences between groups (p < 0.05).
Figure 3A

![Graph showing responses over days for ShA and LgA groups.](image)
Figure 3B

The graph shows the intake (mg/2 hr session) against the unit dose (μg/inf) for two groups: ShA and LgA. The data points are marked with error bars, and the graph includes an inset highlighting specific intake values.
The results of a behavioral history of PR training on responding for cocaine over 10 doses are illustrated in Figure 4. Figure 4A shows the expected escalation of final ratios over 14 days ($F_{(13,91)} = 2.892, p < 0.01$). Figure 4B shows cocaine intake as a function of dose between PR trained animals and animals matched for intake and dose (Match). Repeated measures ANOVA revealed a significant effect of Dose ($F_{(9,108)} = 34.3, p < 0.001$), no significant effect of Group ($F < 1$) and a significant Dose x Group interaction ($F_{(9,108)} = 5.8, p < 0.001$). Holm-Sidak post hoc analysis revealed the PR group consumed significantly more cocaine at two lower doses (7.5 μg/inf: $t_{(12)} = 3.45, p < 0.05$; 4.1 μg/inf: $t_{(12)} = 3.63, p < 0.05$) The mean rate of responding for the PR group at threshold (apex for the dose response curve for each animal) was 955 responses/hr ± 244 (SEM). Figure 2B shows a cumulative record from one animal from the PR group responding at an extremely low dose of cocaine (2.4 μg/inf dose). This record was chosen to illustrate that high rates of responding could be maintained throughout the 2 h session. In this case, cocaine was found to sustain responding at a rate of 1334 responses/hr. Note that every 1000 responses the record of cumulative responses resets at zero.
**FIGURE 4**

PR training increases the reinforcing strength of cocaine. (A) PR training (black circles) results in increased final ratios across daily sessions. Data are expressed as the mean (±SEM) final ratio. (B) PR trained animals (black circles) consume more cocaine at the 4.1 and 7.5 μg/inf doses in comparison to the Match group. Data are expressed as mean (±SEM) daily intake and asterisks indicate significant differences between groups (p < 0.05).
FIGURE 4B

The graph shows the intake (mg/2 hr session) as a function of unit dose (µg/inf). Two conditions are compared: PR (solid line) and Match (dashed line). The graph indicates that intake increases with increasing unit dose, with PR typically showing higher intake than Match. Significant differences are indicated by asterisks (*) on the graph.
The results of the behavioral economics variable Pmax determined from each experimental group are illustrated in figure 5. The left side of figure 5 shows Pmax values determined from the LgA and ShA groups. The LgA group is represented by black bars whereas the ShA group is represented by white bars. LgA trained animals showed a significantly reduced Pmax in comparison animals given ShA to cocaine ($F_{(1,12)}$ = 12.417, $p < 0.01$). The right side of figure 5 shows Pmax values determined from the PR group and the match group. The PR group is represented by black bars whereas the match group is represented by white bars. PR trained animals exhibited a significantly increased Pmax versus the match group ($F_{(1,12)}$ = 8.33, $p = 0.014$). Pmax values and final ratios were also compared within the same animal. A correlation was found between Pmax values established using the threshold procedure and final ratios established using a PR schedule ($r = 0.705$) as illustrated in the supplementary information (S3).
FIGURE 5

Effect of self-administration procedures which produce an escalation of consumption or final ratio on Pmax. Data are expressed as the mean (±SEM) Pmax. The left pair of bars illustrates Pmax values determined from animals that had been tested with access to cocaine during daily 6h (LgA) or 2 hr (ShA) sessions for 14 days. The right pair of bars illustrates Pmax values from groups of animals that had been tested for 14 days on a PR schedule (PR) or an FR1 schedule matched for total drug intake (Match). Asterisks indicate significant differences between groups (p < 0.05).
**Figure 5**

Bar graph showing the distribution of Pmax across different groups:
- LgA
- ShA
- Match
- PR

The graph indicates significant differences (* symbol) between certain groups.
**DISCUSSION**

In the present study, cocaine self-administration was tested across a descending series of doses in order to assess threshold and rate of drug intake. Ten unit injection doses (237 – 1.3 μg/inj) were examined between groups so as to allow for the evaluation of drug intake rates in the high dose range and thresholds in the low dose range. This experimental approach was used to examine how two different behavioral histories of cocaine self-administration – each suggested to model different aspects of the addiction process – affect the behavioral economic measures of price and consumption.

Consumption of cocaine on an FR1 reinforcement schedule appears to be tightly regulated. It has long been known that, at supra-threshold doses, the rate of self-injection is inversely related to unit injection dose (Pickens and Thompson, 1968; Wilson, *et al* 1971). Lower doses are self-administered more frequently than higher doses, thus resulting in hourly cocaine intake that remains relatively constant across a wide dose range. Figure 1B clearly shows that from 421 to 41 μg/inf, total cocaine intake per 2 hr session is relatively constant. While there is some debate as to whether cocaine intake is controlled by satiety mechanisms alone (Tsibulsky and Norman, 1999; Lynch and Carroll, 2001), or whether avoidance of aversive/toxic consequences of high blood levels may also suppress responding (Pettit and Justice, 1989; Lynch and Carroll, 2001; Roberts and Zito, 1987; Ettenberg, 2004), it is commonly assumed that animals titrate their cocaine intake around some preferred blood or brain level (Tsibulsky and Norman, 1999; Lynch and Carroll, 2001; Ahmed and Koob, 2005; but see also Panlilio, *et al* 2003). The
observation that animals alter response output in order to maintain cocaine intake has led to theories suggesting that self-administration continues until intake falls below a set-point (Ahmed and Koob, 1998), trigger-point (Wise, et al 1995) or satiety threshold (Tsibulsky and Norman, 1999).

At some point in the descending series of doses, responding sharply declines. The lowest dose that maintains consistent intake is defined as the threshold. It is important to note that as the supra-threshold dose approaches the threshold, animals must respond at very high rates in order to maintain a relatively constant level of drug intake. Figure 2B shows a remarkable example of an animal responding on the lever more than 2600 times during a 2 hr session, reinforced by an exceedingly small dose of cocaine (2.4 μg/inj). Clearly this animal is expending considerable time and energy in “defending” a particular drug level, and this response cost must necessarily be an important factor influencing responding near threshold. Behavioral economics provides a theoretical framework that can be used to examine data from threshold determinations for information concerning both price and consumption.

Central to behavioral economic analysis is unit-price, which is defined as the response requirement per unit injection dose (mg/inf). Since unit-price is a ratio, it can be altered by changing either the response requirement (numerator) or the dose (denominator); either way, the theoretical issues influencing unit-price remain the same (Bickel, 1990; 1993). Altering unit-price is more commonly accomplished by changing the FR requirement or by using a PR schedule (Cosgrove and Carroll,
Manipulating the dose and holding the response requirement constant (e.g., FR1) is also a valid method as illustrated by the Zittel-Lazarini, et al. (2007) and the present study. Analysis of the unit-price data yields $P_{\text{max}}$, which is defined as the unit-price at which maximum responding occurs (Hursh, 1991).

$P_{\text{max}}$ and final ratio have been suggested to measure similar aspects of reinforcement strength (Rodefer and Carroll, 1997; Cosgrove and Carroll, 2002). However, it was not clear if $P_{\text{max}}$ values established using threshold determinations would correlate with final ratios established at high doses. Obviously differences in dose, frequency of drug delivery, and fluctuations in blood/brain levels may influence $P_{\text{max}}$ and final ratio differently. This prompted us to examine the relationship between $P_{\text{max}}$ and final ratio within the same animal. We found a correlation between $P_{\text{max}}$ and final ratio as illustrated in the Supplementary Information ($r = 0.705$; S3), suggesting a relatively strong relationship between $P_{\text{max}}$ established from threshold determinations and final ratios established at a high dose (1.5 mg/kg/inf) of cocaine. These data suggest that threshold determinations may provide information pertaining to some aspect of reinforcement strength.

A behavioral economic analysis was used here to assess changes in $P_{\text{max}}$ and consumption following behavioral histories suggested to model different aspects of the addiction process. Ahmed and Koob, (1998; 1999) have shown that consumption of cocaine increases across sessions if animals are given extended
access to cocaine during daily 6 hr sessions in comparison to animals that are given short-access. This robust effect was replicated here; animals given daily 6 hr access (LgA) to cocaine on an FR1 reinforcement schedule showed a clear escalation in cocaine intake (Figure 3A). Previous reports have shown that the animals exhibiting increased responding at the 250 μg/inf dose also show increased responding at lower doses 250-31.25 μg/inf (Ahmed and Koob, 1998; 1999). Here we extended this analysis through a series of ten unit doses. As expected, the LgA group displayed significantly higher intake in the upper end of the dose range. By contrast, however, inspection of Figure 3B shows that intake was reduced at the lower end of the dose range compared to the ShA group. In effect, the LgA group reached a threshold at a higher dose in comparison to ShA animals (Figure 3B). A behavioral economic analysis revealed that the LgA group displayed a significantly lower Pmax than the ShA group. We interpret the observations that LgA escalation training produces animals exhibiting increased consumption of cocaine at high doses, but a diminished reinforcing effectiveness of low doses, to be indicative of the development of tolerance to the reinforcing effects of cocaine. According to the DSM-IV the development of tolerance, defined by either a need for markedly increased amounts of the substance to achieve intoxication or desired effects, or markedly diminished effects with continued use of the same amount of the substance, is an important component of the addiction process. We wish to emphasize that the LgA model, which produces an increase in cocaine consumption, captures a fundamental aspect of the addiction process.
We also examined a procedure that models a different aspect of cocaine addiction, i.e. “increased time and energy devoted to acquiring the drug.” Animals given the opportunity to self-administer cocaine on a PR schedule demonstrate increased final ratios over time. This effect is dose dependent (1.5 mg/kg/inj being the optimum dose), and is only seen with rapid drug infusions, suggesting that speed of drug onset is an important variable (Liu, et al 2005b; Morgan, et al 2006).

In the current study, we replicate the finding that final ratios escalate across daily PR sessions (Figure 4A) and extend the analysis to an examination of consumption and Pmax. Here we show that the PR group, which showed an escalation in final ratios, responded at higher rates in the lower dose range in comparison to animals that were matched for intake and dose during training (Figure 4B). That is, PR training produced animals that paid a higher behavioral price for low doses of cocaine versus comparative controls. Note that animals in the PR group escalated their final ratios when reinforced with a high dose of cocaine (1.5 mg/kg/inj). The observation that these animals show higher Pmax values calculated from threshold doses suggests that the augmentation of the reinforcing effects extends across the entire dose range.

Some studies (Paterson and Markou, 2003; Allen, et al 2007; Wee, et al 2008) and theoretical arguments based upon the concept of opponent processes (Ahmed and Koob, 2005; Ahmed, 2005) suggest that a history of increased consumption should result in an increase in the price expended for cocaine. For example, it has been reported that LgA to cocaine on an FR1 reinforcement schedule can result in an increase in final ratios on a PR schedule (Paterson and Markou, 2003, Allen, et al
However, a great deal of evidence, including the data from the current study, suggests that the two phenomena can be dissociable. Liu, et al (2005b) have demonstrated that LgA does not necessarily result in higher final ratios. In fact, in some circumstances, extended access to cocaine can prevent the increase in final ratios seen in the PR model (Morgan, et al 2006). In addition, treating animals with a high level of cocaine (18-20 mg/kg i.v. every 8 hours for 7 days) resulted in an increased rate of cocaine intake on an FR1 reinforcement schedule (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby, et al 1993), but a decrease in the final ratio reached on a PR schedule (Li, et al 1994). These data lead us to conclude that a history of high cocaine consumption can be dissociated from an increase in the price an animal will expend for cocaine. Why, in some studies, high cocaine consumption can have a different effect on final ratios is presently unclear but may be related to factors such as strain differences, PR ratio requirements, housing conditions and drug abstinence periods.

We have argued elsewhere (Roberts, et al 2007) that there are likely many different aspects to the addiction process, each with their own time course. The development of tolerance is thought to emerge late in the addiction process. For example, the DSM-IV suggests that the development of cocaine dependence occurs in sequential stages. Specifically, the early stages of cocaine addiction are considered to involve the development of an increased desire, or motivation, to take cocaine whenever it is available, whereas the later stages involve the development of tolerance to various effects of cocaine (DSM-IV pg. 243). Indeed, we have shown that if animals are first exposed to procedures that increase final ratios on a PR
schedule, this apparent increase in motivation to self-administer cocaine will survive periods of high drug intake (Morgan, et al 2006) which would normally produce tolerance to the reinforcing effects of cocaine. Thus the paradox of why an addict might pay a high price for a drug that seems to have diminished reinforcing value might eventually be understood in the context of sequential addiction processes. The fact that ‘price’ and ‘consumption’ can be dissociated through procedures that address each process individually should make the task of parsing the addiction process into its component parts somewhat easier.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Steve Hursh for supplying spreadsheets containing modeling tools which we used to calculate Pmax values. The authors would also like to thank Leanne Thomas for providing technical assistance and Keri Chiodo for helpful comments in the preparation of this manuscript. This work was supported by National Institutes of Health Grants F31DA024525 (EBO); R01DA14030 (DCSR).
REFERENCES


Campbell UC, Rodefer JS, Carroll ME (1999). Effects of dopamine receptor antagonists (D1 and D2) on the demand for smoked cocaine base in rhesus monkeys. *Psychopharmacology (Berl)* **144**: 381-388.


SUPPLEMENTARY FIGURE S1

Manipulating pump duration provides a reliable method to decrease the dose delivered during self-administration. Empirically observed mls highly correlate with predicted mls across 11 doses ($r = 0.988$, df = 6; $p < 0.01$). Predicted mls were calculated by multiplying the number of infusions per session by the pump duration by the pump speed (1.6 mls/min). Due to the observation that the sum of observed mls highly correlates with predicted mls across all pump durations tested, we argue that unit-dose can be adequately manipulated by changing the pump duration per response.
SUPPLEMENTARY FIGURE S2

Pmax values were either calculated using a formula (right side; Calc) or graphically determined (left side; Graph) using animals from graph 1 (paper graph 1). Representative Pmax determinations from two animals are depicted in S1 A,B and S1 C,D respectively. To mathematically calculate Pmax values each animal’s dose-intake data were curve-fit using the function: \( \log(Q) = \log(Q_0) + k(e^{-\alpha Q_0 c} - 1) \). The value \( k \), which represents the range of the exponential from \( Q_0 \) to minimum, was constrained to 4.19 for all animals. \( Q_0 \), which represents initial consumption at minimal price, and \( \alpha \), which represents the acceleration of the function in response to changes in price, were manipulated to achieve best fit (\( R^2 \}; see table). Calc Pmax is determined to be the price at which the first derivative point slope of the function = -1. There was no significant difference between Calc Pmax (black bar) and Graph Pmax (gray bar) (\( t(7) = -0.059 \), n.s.).
Supplementary Figure S2

<table>
<thead>
<tr>
<th>ID #</th>
<th>Graph Pmax</th>
<th>Calc Pmax</th>
<th>Q_0</th>
<th>α</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>133.9</td>
<td>130</td>
<td>12.6</td>
<td>7.8x10^{-5}</td>
<td>0.92</td>
</tr>
<tr>
<td>2</td>
<td>42.1</td>
<td>43</td>
<td>10.5</td>
<td>2.8x10^{-5}</td>
<td>0.95</td>
</tr>
<tr>
<td>3</td>
<td>42.1</td>
<td>43</td>
<td>12.8</td>
<td>2.3x10^{-4}</td>
<td>0.91</td>
</tr>
<tr>
<td>4</td>
<td>42.1</td>
<td>36.4</td>
<td>13</td>
<td>2.7x10^{-4}</td>
<td>0.96</td>
</tr>
<tr>
<td>5</td>
<td>42.1</td>
<td>47</td>
<td>13.6</td>
<td>2.0x10^{-4}</td>
<td>0.9</td>
</tr>
<tr>
<td>6</td>
<td>24.2</td>
<td>33</td>
<td>16.2</td>
<td>2.4x10^{-4}</td>
<td>0.81</td>
</tr>
<tr>
<td>7</td>
<td>133.9</td>
<td>146</td>
<td>3.8</td>
<td>2.3x10^{-4}</td>
<td>0.91</td>
</tr>
<tr>
<td>8</td>
<td>241.9</td>
<td>221.1</td>
<td>9.8</td>
<td>5.9x10^{-5}</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Supplementary Figure 2
SUPPLEMENTARY FIGURE S3

Pmax values established using threshold determinations correlate with final ratios determined using a progressive ratio schedule. Median final ratios taken from 3 consecutive progressive ratio sessions determined either immediately before (n=8; PR group) or immediately after (n=22; FR1 trained) the threshold procedure were compared to graphically determined Pmax values. A significant within-subjects correlation was found between Pmax and final ratio ($r = 0.705$, $df = 28$, $P<0.01$).
CHAPTER III

DISSOCIATING THE BEHAVIORAL ECONOMIC CONCEPTS OF CONSUMPTION AND PRICE PAID USING NEUROPHARMACOLOGY AND COCAINE SELF-ADMINISTRATION.

Erik B. Oleson, Jasmine M. Richardson, and David C.S. Roberts

The following manuscript is in preparation for submission to Psychopharmacology.
ABSTRACT

Rationale: Cocaine consumption and price paid are important concepts in drug addiction and can be addressed pre-clinically by combining behavioral economics with self-administration. These two concepts are usually addressed independently using separate reinforcement schedules. For example, price variables are often assessed with a progressive ratio schedule whereas consumption is typically measured using a fixed ratio schedule. Objectives: Depending on the schedule used, it is often difficult to determine whether a particular drug pretreatment is affecting self-administration through an effect on consumption, price or perhaps both. In the present study we tested the effect of pretreating rats with compounds from a variety of drug classes on price and consumption. Materials and methods: We have recently developed a technique that provides an independent assessment of both price and consumption within the same experimental session. In this threshold self-administration procedure rats are offered a descending series of eleven unit doses (422–1.3µg/inj) during consecutive timed intervals under a fixed ratio schedule. An estimate of consumption can be determined from response rates associated with high unit doses; an estimate of price can be determined from response rates surrounding the threshold dose. Results: The dopamine receptor antagonist haloperidol increased cocaine consumption but decreased price paid. In contrast, the indirect dopamine agonist d-amphetamine increased the price paid for cocaine. The serotonin uptake inhibitor fluoxetine decreased consumption and price paid. Baclofen, a GABA<sub>B</sub> receptor agonist, selectively decreased price paid for cocaine. Conclusions: These data suggest that the neurobiological substrates of consumption and price paid involve separate mechanism.

Keywords: Cocaine, Self-Administration, Behavioral Economics, Dopamine, Serotonin, GABA
INTRODUCTION

Price and consumption are key concepts in both drug addiction and behavioral economics. In the context of cocaine self-administration, consumption refers to rate of drug intake on a simple schedule of reinforcement. Rate of intake under a fixed ratio 1 (FR1) schedule of reinforcement, for example, reflects the maintenance of cocaine concentrations in blood/brain within a preferred range (Ahmed and Koob, 1999; Lynch and Carroll, 2001). By contrast, the concept of price refers to the effort an animal expends to self-administer cocaine. The behavioral price an animal might pay for cocaine is usually measured with procedures such as a progressive ratio (PR) schedule (Rowlett, 2000; Chiodo and Roberts, 2009) or by performing behavioral economics analyses on an adapted FR schedule (Hursh, 1991; Bickel, et al.) It should be noted that the dependent measure of price (ie. Pmax) obtained using a behavioral economics approach correlates highly with the index of price (ie. break point or final ratio) obtained using a PR schedule (Rodefer and Carroll, 1997; Cosgrove and Carroll, 2002; Oleson and Roberts, 2009).

The pharmacological relationship between consumption and price paid for cocaine remains unclear. Certain drug pretreatments have been reported to oppositely affect cocaine consumption and price paid. For example, the indirect dopamine agonist amphetamine decreases the rate of cocaine consumption on an FR1 schedule of reinforcement (Barrett, et al. 2004) but increases the price paid for cocaine on a progressive ratio schedule (Läck, et al. 2006). In contrast, dopamine receptor antagonists increase the rate of cocaine consumption on an FR1 schedule of reinforcement but decrease the price paid for cocaine on a PR schedule of
reinforcement (Richardson, et al 1994; De Wit and Wise, 1977). However, other drug pretreatments affect the price paid for cocaine independently of consumption. For example, the hypocretin/orexin 1 receptor antagonist SB334867 does not change the rate of drug intake on an FR1 schedule of reinforcement (Smith, et al 2009) but decreases price paid on a PR schedule (Borgland, et al 2009). Similarly, the GABA$_B$ receptor agonist baclofen does not change the rate of cocaine consumption on an FR1 schedule but decreases price paid on a PR schedule (Brebner, et al 2000). Taken together, these data seem to suggest that the relationship between consumption and price varies depending on the pharmacological target. Unfortunately, however, it can be difficult to determine the exact relationship between consumption and price paid using data obtained by different labs using different schedules and experimental conditions. For example, it is well accepted that many variables – including environmental context (Caprioli, et al 2007), rat strain (Kosten, et al 2007), the experimenter imposed inter-injection interval (Martelle, et al 2008) and the pharmacological history of the animal (Orio, et al 2009) can influence the price that an animal might pay for cocaine.

There is a need for a technique which is theoretically grounded in behavioral economics that allows for the investigation of the interaction between consumption and price. We have recently developed a procedure that provides an independent assessment of both price and consumption across multiple self-administration sessions (Oleson and Roberts, 2009). The technique is an adaptation of a threshold procedure (see (Zittel-Lazarini, et al 2007). Rats are offered a descending series of eleven unit doses (422 – 1.3 µg) on an FR1 schedule of reinforcement during

103
consecutive daily 2-hour sessions. An estimate of cocaine consumption can be determined from response rates associated with high unit doses; an estimate of the maximal price (Pmax) paid for cocaine can be determined from response rates surrounding the threshold dose (Oleson and Roberts, 2009). However, this between-sessions approach is not useful for pharmacological studies because many drug injections would be required.

To study the pharmacological interaction between consumption and price paid we developed a within-session threshold procedure, which allows us to examine the effects of drug pretreatments on consumption and price paid within the same experimental session. Instead of offering rats a descending series of cocaine doses across sessions, all doses are presented consecutively within a single session in timed intervals. In the current study we combined this within-session threshold procedure with behavioral economics to investigate the pharmacological relationship between cocaine consumption and price paid for cocaine. A battery of drug pretreatments was used, including haloperidol, amphetamine, fluoxetine and baclofen.

**MATERIALS AND METHODS**

**ANIMALS, SURGERY, AND HOUSING**

Male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA), weighing approximately 350 g at the time of surgery were used. All experiments were approved by the Wake Forest University Institutional Animal Care and Use Committee. Before entering the study, rats were anesthetized with ketamine (100mg/kg) and xylazine (8mg/kg) and implanted with chronically indwelling Silastic® cannula (CamCaths,
Cambridgshire, UK) as previously described (Roberts and Goeders, 1989). Briefly, a 2.5 cm length of cannula tubing was implanted into the right jugular vein while exiting dorsally through the skin in the region of the scapulae.

Upon recovery, animals were housed individually in 30 x 30 x 30 cm experimental chambers. Each cannula was connected to Tygon® tubing enclosed within a stainless steel tether, which was then connected to a counterbalanced fluid swivel (Instech Laboratories Inc., Plymouth Meeting, PA, USA) mounted above the experimental chamber. An infusion pump (Razel Scientific Instruments Inc., Stamford, CT, USA) was connected to the opposite side of the fluid swivel using Tygon® tubing. Each cannula was flushed daily with heparinized saline to help maintain patency; although, a cannula related attrition rate resulted in the inclusion of data from 37 rats. Food and water were available ad libitum. Experimental chambers were housed in a temperature-controlled room (20-21°C) maintained on a 12-h light-dark cycle (lights on at 15:00-h).

**GENERAL SELF-ADMINISTRATION METHODS AND ACQUISITION**

Animals were given 3-5 days to recover from surgery before entering the study. Upon recovery, a lever was introduced into the experimental chamber which, when depressed, initiated an intravenous injection of cocaine. Sessions occurred 7 days/week and began in the middle of the dark cycle (1000 hours). The beginning of each session was signaled by the extension of the lever into the experimental chamber.

During acquisition, all animals were given daily access to cocaine (0.75 mg/kg) under an FR1 schedule of reinforcement. Sessions were terminated after a
maximum of 20 infusions or a period of 24 h had occurred. An animal was considered to have acquired if 20 injections were self-administered beginning at the onset of an experimental session and a stable pattern of post-infusion pauses between injections was apparent.

**WITHIN SESSION THRESHOLD PROCEDURE**

Following acquisition animals were given access to cocaine in the within-session threshold procedure. In this procedure rats are given access to a descending series of unit-injection doses of cocaine (421, 237, 133, 75, 41, 24, 13, 7.5, 4.1, 2.4 and 1.3 μg/injection) reinforced under an FR1 schedule. Doses were manipulated by the adjustment of pump duration as previously described (Oleson and Roberts, 2009). See supplemental material of Oleson and Roberts (2009) for a full characterization of this approach and validation that the appropriate quantity of drug is delivered across all pump durations. The duration of the pump infusion was the only experimenter-imposed inter-injection period. The available cocaine dose decreased every 10 minutes for all drug-pretreatments except d-amphetamine, for which the available dose decreased every 5 minutes.

**BEHAVIORAL ECONOMICS AND DATA ANALYSIS**

Behavioral economic theory has been successfully applied to drug self-administration (Hursh, 1991; Bickel, et al 1990). In most studies, the concept of unit price is manipulated by increasing the response requirement for a set drug dose across daily sessions (Wade-Galuska, et al 2007; Cosgrove and Carroll, 2002). However, as the unit price ratio (responses/mg of drug) might suggest, it is also valid to fix the response requirement and manipulate the available unit dose (Bickel, et al
Therefore, by reducing the available unit-injection dose throughout each session, rats were given access to cocaine across the following 11 ascending unit prices: 2.4, 4.2, 7.5, 13.3, 23.7, 39.9, 75, 133.9, 241.9, 416.7 and 750 resp/mg.

A behavioral economic analysis of data from the within-session threshold procedure provides two main dependent measures, the maximal price paid (Pmax) for cocaine and the animal’s average level of cocaine consumption. The mean level of consumption was derived by averaging cocaine intake across the second through fourth available unit dose (see gray ellipse Figure 1b). The maximal price expended to maintain consumption (Pmax) is defined as the point, or unit price, at which maximal responding occurs (Hursh, 1991). Pmax values were graphically determined in the present study, an approach that has been previously validated (Greenwald and Hursh 2006; Oleson and Roberts, 2009). Therefore, Pmax was determined to be the unit price corresponding to the apex of the price-response function. It should also be noted that Pmax coincided with the point at which cocaine consumption changes from being maintained (inelastic demand) to not being maintained (elastic demand). For all experiments consumption and Pmax values were reported as a percent change from the preceding 2-3 days.

STATISTICS

All statistics were performed using SigmaPlot (Version 11). Pmax and consumption data were analyzed using one-way ANOVA and Holm-Sidak post hoc analysis.

DRUGS

Cocaine HCl, obtained from the National Institute on Drug Abuse (Research Triangle Institute, NC, USA), was dissolved in a solution of sterilized saline 0.9% and passed
through a microfilter (0.45 µm pore size). A 5 mg/ml cocaine solution was used for threshold experiments; a 2.5 mg/ml solution was used for acquisition. Drug pretreatment times and doses for all drugs were chosen based on preliminary data. All injections were administered intraperitoneally and assigned using a latin-square design with a minimum of 3 days between treatments. Pre-dissolved injectable haloperidol (Bedford Labs, Bedford, OH), d-amphetamine (Sigma-Aldrich, St. Louis, MO) and a racemic mixture of baclofen (Sigma-Aldrich, St. Louis, MO) were dissolved, or diluted in the case of haloperidol, in sterile saline and injected prior to the beginning of self-administration sessions. Sterile saline was used for vehicle injections for these three drugs. Pretreatment times were 15 minutes for d-amphetamine and 30 minutes for haloperidol and baclofen. Fluoxetine (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile water and injected 30 minutes prior to self-administration sessions. Sterile water was used for vehicle injections in fluoxetine treated rats. All dosages are expressed as the salt.

**RESULTS**

Figure 1 illustrates a cumulative record, price-intake function and price-response function from one representative animal responding in the within-session threshold procedure. Note that the available unit dose of cocaine (lower x-axis) decreased every 10 minutes and this decrease in dose corresponds to an increase in the unit price of cocaine (upper x-axis). As shown in the cumulative record (Figure 1a), responding increased every 10 minutes as the available cocaine dose decreased until a threshold dose (7.5 mg/inj) was reached. These data are replotted in Figure
1b to demonstrate that a relatively stable level of cocaine intake is maintained at supra-threshold doses. Cocaine intake was higher in the first 10-minute interval (1.26 mg) than at any other dose, which is presumably due to the effect of drug loading. The level of cocaine intake then stabilized over the next several doses, and the mean level of consumption (0.61 mg) was taken by averaging cocaine intake across intervals 2-4. The maximal price paid (Pmax) for cocaine was determined by assessing the unit price at which maximal responding occurred to maintain a stable level of cocaine intake. This particular animal’s Pmax (133.9 resp/mg) can be visually distinguished by inspecting the apex of the price-response function (Figure 1c). Note that the apex of the price-response function coincides with the final point at which cocaine intake is maintained in figure 1b.
**FIGURE 1**

Representative within-session threshold data and explanation of behavioral economics measures. (a) A cumulative record from one animal (E293) responding in a single session (Jul-6, 2009) of the current threshold procedure is shown. Note that the available unit-injection dose (lower x-axis) decreases across eleven consecutive 10 minute bins. (b) The same animal’s data are replotted to show cocaine consumption (mg/10 min bin) as a function of unit price (upper x-axis). Mean cocaine consumption (0.61 mg; gray ellipse) is calculated by averaging intake across bins 2-4. (c) The same animal’s data are replotted to show responding (responses/10 min bin) as a function of unit price. The maximal price paid (Pmax; 133.9 resp/mg) can be distinguished by assessing the apex of the price response function. Note that Pmax also corresponds to the final unit price at which cocaine consumption is maintained in 1b.
FIGURE 1A
**Figure 1B&C**

**b**

- Consumption (mg)
- Consumption: 0.01 to 10
- Pmax = 133.9 resp/mg
- Avg. Consumption = 0.61 mg

**c**

- Responses
- Unit-Price (resp/mg)
- Unit-Dose (μg/inf)

Graph A: Consumption vs. Unit-Price (resp/mg)
Graph B: Consumption vs. Unit-Price (resp/mg)
Graph C: Responses vs. Unit-Dose (μg/inf)
The results of haloperidol pretreatment on Pmax and cocaine consumption are shown in Figure 2. Figure 2a illustrates the effect of haloperidol (56, 100, 178, or 310 µg/kg IP) on Pmax. A one-way ANOVA revealed a significant effect of haloperidol dose on Pmax (F_{(4,32)} =3.75, p=0.015). Holm-Sidak post hoc analysis revealed that doses of 100 (t=2.8, p=0.01), 178 (t=3.0, p=0.05) and 310 (t=3.1, p<0.05) µg/kg significantly decreased Pmax values relative to vehicle. Figure 2b illustrates the effect of haloperidol on cocaine consumption. A one-way ANOVA revealed a significant effect of haloperidol dose on consumption (F_{(4,32)} =4.90, p<0.01). Holm-Sidak post hoc analysis revealed that doses of 56 (t=2.4, p=0.02), 100 (t=3.9, p<0.05), 178 (t=3.2, p<0.05) and 310 (t=3.1, p<0.05) µg/kg significantly increased consumption relative to vehicle.
**FIGURE 2**

The effects of various doses of haloperidol on Pmax (top) and cocaine consumption (bottom). (a) Haloperidol produced a significant decrease in the maximal price paid (Pmax) for cocaine. (b) In contrast, haloperidol produced a significant increase in cocaine consumption. Data are expressed as mean (±SEM) Pmax or consumption and asterisks (*) indicate a significant difference between a haloperidol dose and vehicle ($p \leq 0.05$).
**Figure 2A**

![Graph showing consumption and Pmax (% change from baseline) against different dosages of Haloperidol (µg/kg). The graph includes error bars and asterisks indicating statistical significance.](image)
Figure 2B

Consumption (% Change from Baseline)

Haloperidol (μg/kg)

veh 56 100 178 310

Pmax (% Change from Baseline)

a
b
8 8 7 6 4
8 8 7 6 4

*
Figure 3 illustrates the effect of d-amphetamine pretreatment on Pmax and cocaine consumption. Figure 3a shows the effect of amphetamine (0.31, 0.56 and 1.0 mg/kg IP) on Pmax. A one-way ANOVA revealed a significant effect of amphetamine dose on Pmax ($F_{(3,30)} = 3.07$, $p=0.045$). Holm-Sidak post hoc analysis revealed that 1 mg/kg amphetamine significantly increased Pmax relative to vehicle ($t=2.7$, $p=0.01$). Figure 3b shows the effect of amphetamine on consumption. Amphetamine did not significantly change the rate of cocaine intake ($F_{(3,30)} = 0.88$, n.s.).
**Figure 3**

The effects of various doses of d-amphetamine on Pmax (top) and cocaine consumption (bottom). (a) Amphetamine produced a significant increase in the maximal price paid (Pmax) for cocaine. (b) However, amphetamine did not produce a significant change in cocaine consumption. Data are expressed as mean (±SEM) Pmax or consumption and asterisks (*) indicate a significant difference between amphetamine dose and vehicle ($p \leq 0.05$).
**Figure 3A**

![Graph showing Pmax (% Change from Baseline) vs. Amphetamine (mg/kg)](image)

- Y-axis: Pmax (% Change from Baseline) (%)
- X-axis: Amphetamine (mg/kg)

- Data points:
  - Vehicle (veh)
  - 0.31
  - 0.56
  - 1.00

- Bars indicate consumption (% Change from Baseline) with error bars for each dose level.

- Significant difference indicated with an asterisk (*) at the 1.00 mg/kg dose.
**Figure 3B**

![Graph showing consumption (% change from baseline) against amphetamine (mg/kg)]
The effect of fluoxetine pretreatment on Pmax and cocaine consumption is shown in Figure 4. Figure 4a illustrates the effect of fluoxetine (3.1, 5.6 and 10 mg/kg IP) on Pmax. A one-way ANOVA revealed a significant effect of fluoxetine dose on Pmax ($F_{(3,26)} = 4.82, p=0.01$). Holm-Sidak post hoc analysis revealed that 5.6 mg/kg ($t=2.5, p=0.02$) and 10 mg/kg ($t=3.5, p<0.05$) fluoxetine significantly decreased Pmax relative to vehicle. Figure 4b shows the effect of fluoxetine on consumption. A one-way ANOVA revealed a significant effect of fluoxetine dose on consumption ($F_{(3,26)} = 4.60, p=0.012$). Holm-Sidak post hoc analysis revealed that 10 mg/kg fluoxetine significantly decreased Pmax relative to vehicle ($t=2.8, p<0.011$).
FIGURE 4

The effects of various doses of fluoxetine on Pmax (top) and cocaine consumption (bottom). (a) Fluoxetine produced a significant decrease in the maximal price paid (Pmax) for cocaine. (b) Fluoxetine also produced a significant decrease in cocaine consumption. Data are expressed as mean (±SEM) Pmax or consumption and asterisks (*) indicate a significant difference between a fluoxetine dose and vehicle (p ≤ 0.05).
FIGURE 4A

Fluoxetine (mg/kg) veh 3.1 5.6 10.0
Consumption (% Change from Baseline)
-100
-50
0
50
100
150
200
Fluoxetine (mg/kg) veh 3.1 5.6 10.0
Pmax (% Change from Baseline)
-100
-50
0
50
100
150
200

7
6
7
7

Fluoxetine (mg/kg)

veh
3.1
5.6
10.0

*
**Figure 4B**

![Graph showing consumption (% change from baseline) vs. Fluoxetine (mg/kg). The graph displays data points for different doses of Fluoxetine (3.1, 5.6, 10.0 mg/kg) and indicates significant differences with an asterisk (*).](image-url)
Figure 5 illustrates the effect of baclofen pretreatment on Pmax and consumption. Figure 5a shows the effect of baclofen (1.0, 1.8 and 3.0 mg/kg IP) on Pmax. A one-way ANOVA revealed a significant effect of baclofen dose on Pmax ($F_{(3,25)} = 4.08$, $p=0.019$). Holm-Sidak post hoc analysis revealed that 1.8 mg/kg baclofen significantly decreased Pmax relative to vehicle ($t=3.4$, $p<0.05$). It should also be noted that despite failing to produce a significant change versus vehicle, the 3.0 mg/kg dose produced a 45% mean decrease in Pmax; however, the power of this dose comparison was limited because two rats did not start following pretreatment. Baclofen did not significantly change cocaine consumption ($F_{(3,25)} = 1.60$, n.s.).
The effects of various doses of baclofen on Pmax (top) and cocaine consumption (bottom). (a) Baclofen produced a significant decrease in the maximal price paid (Pmax) for cocaine. (b) However, baclofen did not significantly change cocaine consumption. Data are expressed as mean (±SEM) Pmax or consumption and asterisks (*) indicate a significant difference between a baclofen dose and vehicle ($p \leq 0.05$).
Figure 5B

Baclofen (mg/kg)
veh 1.0 1.8 3.0
Pmax (% Change from Baseline)
-100
-50
0
50
100
150
200

Baclofen (mg/kg)
veh 1.0 1.8 3.0
Consumption (% Change from Baseline)
-100
-50
0
50
100
150
200

*7 7 7 5
7 7 7 5

Consumption (% Change from Baseline)

Baclofen (mg/kg)
**DISCUSSION**

The effect of various drug pretreatments from a variety of pharmacological classes on cocaine consumption and price paid was investigated. The present results show that price and consumption are dissociable phenomena. While in some instances price and consumption might both be similarly or inversely affected by drug treatments this is not necessarily the case. For example, haloperidol dose-dependently increased cocaine consumption and decreased the price paid for cocaine. Conversely, acutely administered amphetamine increased the price paid for cocaine. The selective serotonin uptake inhibitor fluoxetine decreased both cocaine consumption and the price paid for cocaine. Importantly, however, consumption and price paid were also effected independently of each other. Baclofen (a GABA$_B$ agonist) decreased the price paid for cocaine while having no affect on consumption. These data suggest that the neurobiological substrates of price and consumption involve separate mechanism.

Applying behavioral economics to threshold data provides a valuable tool that allows for the assessment of two distinct concepts in addiction, drug consumption and price paid for drug. To date, much of our understanding of how neuropharmacology influences drug-taking behavior has been obtained using self-administration procedures that were designed to independently measure variables related to either price (eg. PR schedule) or consumption (eg. FR schedule). In the present study a new within-session threshold self-administration procedure was used to investigate the effect of compounds from different drug classes on cocaine consumption and price paid. The within session threshold procedure is an
improvement over a technical approach presented in a previous study (Oleson and Roberts, 2009), in which we performed behavioral economics analyses on data obtained using a threshold procedure occurring across eleven daily FR sessions. Providing access to cocaine at multiple unit prices within a single session allows for the investigation of drug pretreatments on both consumption and price paid simultaneously. Furthermore, this approach allows the experimenter to vary the exact timing that each unit price is available to accommodate for the pharmacokinetics and pharmacodynamics of the specific drug pretreatment. Aside from pharmacological studies, this tool will allow for the investigation of the roles of individual differences, behavioral histories and environmental influences on a rat’s preferred level of cocaine consumption and the maximal behavioral price paid for cocaine. Despite the many positives, several limitations are associated with the current technique. For example, providing access to cocaine at unit prices that ascend throughout each session in fixed intervals requires that all animals start at the beginning of the session. In the present study, this issue resulted in the exclusion of data from several animals that failed to start following high-dose pretreatments of either baclofen or haloperidol. A solution to this problem would be to provide access to cocaine at the first presented unit price until stable responding occurs. Another major issue is that each animal’s threshold (ie. Pmax) varies throughout testing; therefore, establishing stable baselines must be done immediately prior to drug pretreatments.

During the within-session threshold procedure animals maintain a preferred level of cocaine consumption until intake becomes confounded by price. It has long
been recognized that the rate of cocaine consumption is inversely related to the available unit dose along the descending limb of the FR dose-effect function (Pickens and Thompson, 1968; Wilson, et al 1971). This observation has led various investigators to speculate that cocaine intake is titrated around a preferred blood or brain level, variously referred to as a trigger point (Wise, et al 1995), set point (Ahmed and Koob, 1999) or priming threshold (Tsibulsky and Norman, 1999). Neuropharmacologically, this lower level reflects the maintenance of a preferred level of cocaine-induced dopamine uptake inhibition in the nucleus accumbens (Oleson, et al 2009), which provides the dopamine fluctuations observed in microdialysis studies that are thought to trigger responding for cocaine under an FR1 schedule (Pettit and Justice, 1989; Wise, et al 1995). When price becomes a factor, however, it becomes difficult for animals to maintain their preferred brain cocaine level. As the ability to maintain cocaine consumption becomes constrained by price, an increase in the firing rate of a specific neural population in the nucleus accumbens is observed which might provide a mechanism explaining how an animal’s maximal behavioral price is determined (Nicola and Deadwyler, 2000). If such mechanisms contribute to the maintenance of cocaine consumption and the maximal price that an animal might pay for cocaine, then altering neurochemical transmission should influence these concepts.

Dopamine manipulations inversely affect cocaine consumption and price paid. In the present study we found that increasing dopamine levels by pretreating rats with amphetamine increased the price paid for cocaine whereas antagonizing dopamine receptors with haloperidol decreased the price paid for cocaine.
Moreover, haloperidol dose-dependently increased cocaine consumption. Overall, the changes detected in the present study are in agreement with previous studies that investigated the role of dopamine transmission in regulating cocaine consumption and price paid. It is well accepted that dopamine antagonists increase the rate of cocaine consumption on an FR schedule (De Wit and Wise, 1977; Richardson, et al 1994), an effect that is theorized to be a compensatory response to pharmacological competition at post-synaptic dopamine receptors (Yokel and Wise, 1975; De Wit and Wise, 1977). Similarly, rate of intake is increased by dopamine receptor antagonists on a PR schedule although final ratios are decreased (Roberts, et al 1989; Depoortere, et al 1993), which provides further evidence that consumption and price and inversely effected by dopamine manipulations.

Currently investigators are beginning to focus on the exact role by which dopamine regulates behavioral interactions with price. One common theory is that dopamine is involved in overcoming response costs during operant behavior (Salamone, et al 2009). In support of this, it has been shown that dopamine antagonists and 6-hydroxydopamine-induced dopamine depletions selectively reduce the behavioral price paid for food reinforcement when the response cost is high, but can actually increase food consumption under free-access conditions (Aberman and Salamone, 1999; Salamone, et al 1991). Moreover, it has been shown that dopamine concentrations (DOPAC/dopamine) increase with the behavioral cost required of the animal during brain stimulation reward (Neill, et al 2002).

Neurochemical and pharmacological studies suggest that the concept of unit-price might be affected differently in cocaine self-administration studies depending
on whether price is manipulated by increasing the response requirement or decreasing the available unit dose. For example, Gan and colleagues (2009) reported the important distinction that dopamine encodes the individual variables that define unit price (ie. response cost vs. reinforce magnitude) separately. Specifically, it was shown that phasic dopamine encodes information about the magnitude of reinforcement rather than the required response cost as animals gain experience about the presented cost-benefit relationship (Gan, et al 2009). This latter finding suggests that behavioral responses to changes in unit price may vary depending on whether the response requirement is increased or reinforcer magnitude is decreased, a subject that has been behaviorally addressed but not completely resolved (cf. Nader, et al 1993; Woolverton and English, 1997). If the components of unit price are indeed neurochemically and behaviorally dissociable, the implication would be that final ratios and Pmax values determined by increasing response requirements are distinct from Pmax values determined by decreasing the available unit-injection dose.

Serotonin neurotransmission antagonizes both cocaine consumption and the price paid for cocaine. In the present study, we found that the selective serotonin uptake inhibitor fluoxetine decreased both cocaine consumption and the price paid for cocaine. These findings are in agreement with previous self-administration studies in which brain serotonin levels were manipulated using serotonin uptake inhibitors, dietary tryptophan and lesions. For example, fluoxetine decreases final ratios for cocaine on a PR schedule (Richardson and Roberts, 1991) and rate of cocaine consumption on an FR schedule (Carroll, et al 1990a). When brain serotonin
levels are increased by dietary tryptophan, decreases in cocaine maintained responding under both a PR schedule (McGregor, et al 1993) and an FR schedule (Carroll, et al 1990b) are observed. Importantly, dietary tryptophan decreases final ratios for cocaine on a PR schedule without decreasing final ratios for food reinforcement (McGregor, et al 1993), suggesting that the serotonin-induced decrease in the behavioral price paid for cocaine is not due to a generalized reduction in response rate. The pharmacological evidence that serotonin produces an inhibitory effect on cocaine reinforcement is further supported by lesion studies, which show that infusing the neurotoxin 5,7-dihydroxytryptamine into the intracerebroventricular space (Roberts, et al 1994), amygdala or medial forebrain bundle (Loh and Roberts, 1990) increases final ratios on a PR schedule of reinforcement. The finding that animals work harder for cocaine following depletion of brain serotonin levels contributed to the interpretation that serotonin systems produce aversive effects during cocaine self-administration (Roberts, et al 1994), and therefore antagonize the reinforcing effects of cocaine.

The GABA\textsubscript{B} agonist baclofen modulates cocaine reinforcement by selectively decreasing the behavioral price paid for cocaine. Here, we found that baclofen reduced the price paid for cocaine without affecting cocaine intake maintained by high unit injection doses. These findings are reminiscent of an explanation of baclofen’s effectiveness proposed by Carroll and colleagues (Campbell, et al 1999). These authors proposed that drugs like baclofen are most effective at suppressing cocaine self-administration at high unit prices – regardless of whether unit price is manipulated by increasing the response requirement or by decreasing the available
unit dose. This explanation is supported by several additional self-administration studies using PR and FR schedules. When high unit injection doses (1.5 mg/kg/inj) are available under an FR schedule baclofen does not affect the rate of cocaine self-administration (Roberts, et al 1996; Brebner, et al 2000). In contrast, the rate of cocaine intake is significantly decreased by baclofen (Campbell, et al 1999; Shoaib, et al 1998) when rats are given access to low unit injection doses (0.2-0.66 mg/kg/inj) under an FR schedule, an effect that is potentially explained by the unit price requirement imposed on animals to titrate cocaine intake at these low unit injection doses (Campbell, et al 1999). Furthermore, cocaine self-administration is reduced across a wide-range of doses (0.18-1.5 mg/kg/inj) (Roberts, et al 1996; Brebner, et al 2000) when rats are forced to work for cocaine under a PR schedule. While some evidence suggests that baclofen can produce sedation and suppress locomotor activity (Paredes and Agmo, 1989), baclofen only produces marginal effects on food-maintained responding under both FR (Shoaib, et al 1998) and PR schedules (Roberts, et al 1996) at doses that reduce cocaine self-administration (Roberts, et al 1996), which suggests that the reduction in the behavioral price paid for cocaine is not due to a generalized suppression of responding.

Consumption of cocaine and price paid for cocaine are dissociable concepts, both of which are regulated by multiple neurotransmitter systems. While titration of dopamine levels is important for cocaine consumption (Pettit and Justice, 1989), and changes in the firing rate of single units within the nucleus accumbens influences the maximal price an animal might pay for cocaine (Nicola and Deadwyler, 2000), the overall regulation of these distinct concepts is complex and
modulated by multiple neurotransmitters. For example, serotonin appears to antagonize the effects of dopamine in both the regulation of consumption and price, whereas other pharmacological targets such as GABA_B receptors and hypocretin/orexin 1A receptors (Borgland, et al 2009; Smith, et al 2009) appear to affect these concepts independently. These findings suggest that drug pretreatments can alter consumption and price paid differently because each concept involves distinct neural mechanisms; therefore, more encompassing screening tools should be used to assess the effectiveness of potential pharmacotherapies for drug addiction. Furthermore, caution should be used in drawing encompassing conclusions about drug-treatments from data obtained using a single schedule of reinforcement.

**ACKNOWLEDGMENTS**

Funding for this study was provided by Grants R01DA14030 (DCSR) and F31DA024525 (EBO). We would like to thank Leanne N. Thomas for technical assistance and Benjamin Zimmer for helpful comments in the preparation of this manuscript.
REFERENCES


Carroll ME, Lac ST, Asencio M, Kragh R (1990b) Intravenous cocaine self-administration in rats is reduced by dietary L-tryptophan. Psychopharmacology (Berl) 100: 293-300.


Oleson EB, Roberts DC (2009) Behavioral economic assessment of price and cocaine consumption following self-administration histories that produce escalation of either final ratios or intake. Neuropsychopharmacology 34: 796-804.


CHAPTER IV

DOPAMINE UPTAKE CHANGES ASSOCIATED WITH COCAINE SELF-ADMINISTRATION.

Erik B. Oleson, Sanjay Talluri, Steven R. Childers, Jim E. Smith, David C.S. Roberts, Keith D. Bonin, and Evgeny A. Budygin

The following manuscript was published by Neuropsychopharmacology 34(5):1174-84, April 2009.
ABSTRACT

The present study was designed to reveal the relationship between cocaine-induced dopamine uptake changes and patterns of cocaine self-administration observed under a fixed ratio schedule. Cocaine was intravenously infused into anesthetized rats, according to inter-infusion intervals obtained from self-administering animals, and dopamine uptake changes (apparent $K_m$) were assessed in the nucleus accumbens using voltammetry. The data demonstrate that cocaine-induced dopamine uptake inhibition accounts for the accumbal dopamine fluctuations, which are associated with the cyclic regularity of cocaine intake observed during self-administration. Specifically, the inter-infusion intervals that are maintained during cocaine self-administration correlate with the maintenance of a rapidly changing level of dopamine transporter (DAT) inhibition, which appears to be tightly regulated. Furthermore, this maintained level of dopamine uptake inhibition was found to shift upward using intervals from animals that had shown an escalation in the rate of cocaine self-administration. Although no significant change in the efficacy of cocaine for the DAT was revealed in animals that exhibited an escalation in the rate of cocaine intake, an increased dopamine uptake rate was found suggesting an up-regulation of DAT number in response to a history of high cocaine intake. This is the first demonstration of the tight correlation that exists between the level of dopamine uptake inhibition and rates of cocaine self-administration. Moreover, a new mathematical model was created that quantitatively describes the changes in cocaine-induced dopamine uptake and correctly predicts the level of dopamine uptake inhibition. This model permits a computational interpretation of cocaine-induced dopamine uptake changes during cocaine self-administration.

Keywords: dopamine transporter, addiction, psychostimulants, pharmacokinetics, Michaelis-Menten kinetics, tolerance
INTRODUCTION

It is commonly accepted that dopamine neurotransmission is essentially involved in the stimulating, reinforcing, and addictive effects of cocaine and other abused drugs (Koob and Bloom, 1988; Volkow, et al 2004). Acutely administered cocaine enhances extracellular dopamine concentrations in specific brain regions, including the caudate putamen and nucleus accumbens (Di Chiara and Imperato, 1988). The magnitudes of cocaine-induced psychomotor activation are positively and highly correlated with dopamine responses detected in these areas (Sabeti, et al 2002; Budygin, 2007). Subsecond dopamine fluctuations in the nucleus accumbens are associated with cocaine seeking behavior (Phillips, et al 2003; Stuber, et al 2005).

Levels of extracellular dopamine in the nucleus accumbens appear to regulate the rate of cocaine intake. Early studies showed that cocaine infusions are self-administered at regular intervals and that the inter-injection interval depends on the unit injection dose (Pickens and Thompson, 1968; Wilson, et al 1971). The timing of this behavior was initially suggested to be associated with fluctuating blood or brain levels of cocaine (Yokel and Pickens, 1974; Gerber and Wise, 1989) with responding being initiated when cocaine levels fall below a threshold level. This idea was extended to include brain dopamine levels by Justice and colleagues (1989) who used microdialysis to show that extracellular dopamine rapidly increases following each cocaine injection and drug seeking appears to be initiated when dopamine levels decline to some critical concentration (Pettit and Justice, 1989; Wise, et al 1995). This level has been variously called a trigger-point (Wise, et

While it is commonly believed that the inhibition of the dopamine transporter (DAT) by cocaine is the mechanism responsible for the elevation of extracellular dopamine in the nucleus accumbens (Wu, et al 2001; Garris and Rebec, 2002; Budygin, 2007); it remains unclear to what extent cocaine-induced DAT inhibition is involved in the timing of inter-infusion intervals or whether other mechanisms are involved. For example, subsecond dopamine release (i.e., dopamine transients) detected when an animal approaches a cocaine-paired lever (Phillips, et al 2003; Stuber, et al 2005) could play an essential role in cocaine self-administration. Moreover, the onset and time course of cocaine-induced dopamine uptake inhibition observed in some studies (Kiyatkin, et al 2000; Wakazono and Kiyatkin, 2008) is too slow and gradual to account for the rapid fluctuations in dopamine observed during cocaine self-administration. The fact that rats self-administer cocaine during the peak of dopamine uptake inhibition (Kiyatkin, 2000) is inconsistent with the hypothesis that decreases in DAT inhibition trigger responding. However, dopamine uptake inhibition has not been investigated during an actual time-course of cocaine self-administration.

In the present study we sought to establish the time course of cocaine-induced dopamine uptake inhibition by using fast scan cyclic voltammetry to assess changes in dopamine with high temporal resolution (milliseconds) following electrical stimulation. Measurements were taken every 1-minute to allow dopamine uptake
inhibition to be evaluated on a time-scale relevant to cocaine self-administration. Specifically, dopamine uptake was assessed in the nucleus accumbens of anesthetized rats using infusion rates obtained from two unique self-administration procedures, one which produces stable responding across sessions, and another which results in an escalation of the rate of cocaine intake over a two week period (Ahmed and Koob, 1998). A new mathematical model is proposed that accurately describes and predicts the changes of cocaine-induced dopamine uptake inhibition as they occur after cocaine administration.
MATERIALS AND METHODS

INTER-INFUSION INTERVAL DETERMINATIONS

These experiments were designed to allow in vivo voltammetric recordings to be performed while intravenous cocaine infusions were being administered in accordance with rates and patterns of responding observed during cocaine self-administration. In order to use patterns of self-administration that were representative of group data while performing voltammetry on individual animals, historical data were used to determine averaged rates of responding. The access conditions under which animals self-administer cocaine can change the rate at which infusions are self-administered. In the present study, two access conditions were of interest. Under short-access conditions (2 hr sessions) the rate of responding remains stable over daily sessions. Under long-access conditions (6 hr sessions) the rate of responding increases, or escalates, over daily sessions (Ahmed and Koob, 1998). For both conditions, rates were analyzed after 14 self-administration sessions had occurred. Rates from a short-access group (n=6) remained stable across sessions; therefore multiple sessions were averaged from the final self-administration sessions (30 total sessions). Rates from a long-access group (n=8) increased across sessions; therefore, only rates from the final day of self-administration were averaged across animals. Inter-infusion intervals were determined by averaging the time in seconds occurring between responses for each animal. Each response was considered separately – so, for example – the inter-infusion interval between response one and two would be different from the inter-infusion interval between response eight and nine. In order to investigate the effects
of a history of long-access to cocaine during self-administration on dopamine uptake, an experimental group (n=7) was given long-access to cocaine (see long-access training) before individual animals were infused with predetermined rates during voltammetric recording. Response rates from the experimental group did not differ from the response rates used to calculate the predetermined long-access inter-infusion intervals (F_{13,169} = 0.75; n.s.).

**General Cocaine Self-Administration**

Male Sprague-Dawley rats weighing approximately 350g at the start of the experiment were used as subjects. Rats were anesthetized via an IP injection of ketamine (100 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.) and implanted with a chronically indwelling Silastic® cannula (CamCaths, Cambridgeshire, UK) into the right jugular vein. The cannula exited through the skin on the dorsal surface in the region of the scapulae (Roberts and Goeders, 1989). Animals were then individually housed in 30 x 30 x 30 cm operant chambers. Following surgery, a stainless steel protective tether that enclosed Tygon® tubing was connected to a counterbalanced fluid swivel (Instech Laboratories, Inc., Plymouth Meeting, Pa., USA) mounted above the chamber. The swivel was connected to an infusion pump (Razel Scientific Instruments, Inc., Stamford, Conn., USA). The cannulae were flushed daily with heparinized saline to maintain patency. Following a recovery period (3-5 days), animals were given access to a cocaine-paired lever during an acquisition phase. During the acquisition phase, cocaine (0.75 mg/kg/infusion) was available on a fixed ratio 1 schedule of reinforcement. Rats were given access to cocaine in daily training sessions, which terminated after a maximum of 20 infusions or a period of
six hours had elapsed. An animal was considered to have acquired stable self-administration if 20 injections were self-administered during a single session and the pattern displayed consistent inter-infusion intervals. Upon completion of the acquisition phase animals began the long-access training procedure.

**LONG-ACCESS TRAINING**

One group of animals (n=7) was used for this experiment. Animals were given access to cocaine (0.75 mg/kg/infusion) under a fixed ratio 1 schedule of reinforcement during daily 6-hour session for 14 consecutive days. Under long-access conditions animals increase, or ‘escalate,’ cocaine intake and the rate of cocaine infusions over 14 days (Ahmed and Koob, 1998; 1999). In the present study, as expected, the long-access training procedure resulted in a significant increase in the rate of responding across sessions (F_{6,78} = 7.32; p < 0.01). Voltammetry (see voltammetric recordings) was performed 24 hours following the final self-administration session for each individual animal.

**VOLTAMMETRIC RECORDINGS**

All of the voltammetric experiments were performed on anesthetized rats. There were at least three important reasons why this design was chosen versus experiments on freely moving animals which self-administer cocaine. First of all, an electrical stimulation which is a necessary part of the procedure would change patterns of cocaine self-administration (Phillips, et al 2003). Secondly, anesthetized animals permit the application of larger electrical current than can be applied to awake rats. These stimulations permit larger dopamine efflux that allows more
accurate evaluation of dopamine uptake changes. Thirdly, the pure pharmacological effects of cocaine without any conditioned influence on dopamine neurotransmission that would be observed during cocaine self-administration in awake animals (Phillips, et al 2003; Stuber, et al 2005), was the main focus of this study. It should also be noted, urethane anesthesia was chosen because it does not alter dopamine uptake dynamics (Garris, et al 2003; Sabeti, et al 2003).

Rats were anesthetized with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic frame. A carbon fiber electrode was positioned in the nucleus accumbens core (AP + 1.3, L + 1.3, V - 6.6 mm from bregma) and a Ag/AgCl reference electrode was implanted in the contralateral hemisphere. A bipolar stimulating electrode was lowered to the ventral tegmental area ipsilateral to the working electrode at 5.2 mm posterior and 1.0 mm lateral to bregma. The stimulating electrode depth was optimized to evoke dopamine release in the nucleus accumbens which was monitored using a carbon fiber microelectrode. The reference and carbon fiber electrodes were connected to a head-mounted voltammetric amplifier (UNC Electronics Design Facility, Chapel Hill, NC) and voltammetric recordings were made at the carbon fiber electrode every 100 ms by applying a triangular waveform (-0.4 to +1.2 V, 300 V/s). The signals had an oxidation peak at + 0.6 V and a reduction peak at - 0.2 V versus Ag/AgCl reference, identifying the released species as dopamine. Data were digitized (National Instruments, Austin, TX) and stored on a computer. Dopamine release was evoked every 1 min with electrical stimulations (24 rectangular pulses, 60 Hz, 300 µA, 2 ms/phase, biphasic) and detected by a carbon fiber electrode. Importantly, with this
type of stimulation, physiological dynamics of dopamine release, which are not modified by anesthesia, were revealed (Montague, et al 2004). At least four stable stimulations of dopamine were collected, and then either a single (0.75, 1.5, 3.0 mg/kg, i.v.) or multiple injections (0.75 mg/kg i.v.) of cocaine (cocaine hydrochloride obtained from the National Institute on Drug Abuse, Rockville, MD, USA; the drug was dissolved in sterile 0.9% saline) was administered. The drug was administered as an experimenter-delivered bolus over 4 s in a volume of 0.10-0.13 ml.

Carbon fiber microelectrodes were calibrated in vitro with known concentrations of dopamine (2-5 µM). Calibrations were done in triplicate and the average value for the current at the peak oxidation potential was used to normalize in vivo signals to dopamine concentration. Dopamine uptake was determined from the clearance rate of dopamine following the termination of the stimulus and was assumed to follow Michaelis-Menten kinetics (Garis and Rebec, 2002). A detailed description regarding a model characterizing changes in extracellular dopamine concentrations evoked by electrical stimulation as a balance between the opposing mechanisms of release and uptake was provided in previous publications (Wightman, et al 1988; Wightman and Zimmerman, 1990; Wu, et al 2001; Garis and Rebec, 2002). The changes in dopamine during and after electrical stimulation were fit using equation 1.

\[
\frac{d[D_A]}{dt} = (f) [D_A] - \left( \frac{V_{\text{max}}}{(K_m[D_A]) + 1} \right)
\]

\[ (1) \]
where \( f \) is the stimulation frequency (Hz), \([DA]_p\) is the concentration of dopamine released per stimulus pulse. \( V_{\text{max}} \) is a maximal velocity of dopamine uptake, which is proportional to the number of available DAT proteins. \( K_m \) is the substrate (dopamine) concentration at one half of \( V_{\text{max}} \). This second uptake parameter \((K_m)\) is a complex constant, related to the affinity of dopamine for the DAT and its rate of turnover. The baseline value of \( K_m \) was taken to be \( \approx 0.2 \, \mu \text{M} \), a value determined in rat brain synaptosomes (Near, et al 1988; Garis and Rebec, 2002). The derivative form of the above equation was used to simulate the dopamine response using single curve analysis. This method, which also agrees favorably with other analyses, has proven to be particularly convenient for the evaluation of dopamine uptake changes induced by competitive DAT inhibitors such as cocaine (Wu, et al 2001; Garis and Rebec, 2002; Budygin, 2007). Dopamine signals for each rat were fit individually at all time points before and after cocaine injections. The \( K_m \) was fixed and the other variables were determined, when predrug parameters were calculated. The calculation of \( K_m \) requires knowledge of \( V_{\text{max}} \) and \([DA]_p\), and a steady-state response of electrically evoked dopamine concentration (Wu, et al 2001). Therefore, when the cocaine effect was modeled, \( K_m \) became the subject of manipulation in order to obtain a best fit, while \( V_{\text{max}} \) was kept close to the predrug value.

**Statistical Analysis**

Data were analyzed in GraphPad Prism (GraphPad Software, San Diego, CA). A t-test, one-way repeated measures and two-way ANOVAs with Bonferroni post tests were used to determine statistical significance. The data are presented as mean ±
SEM and the criterion of significance was set at p < 0.05. For the mathematical model, which describes cocaine-induced dopamine uptake changes, MATLAB was used to fit the experimental data using a least-squares minimization procedure. The predicted curves were also generated using MATLAB.

**RESULTS**

**INTRAVENOUS COCAINE DOSE-DEPENDENTLY INCREASES APPARENT KM IN THE RAT NUCLEUS ACCUMBENS (EXPERIMENT 1)**

Electrically evoked dopamine concentrations in the nucleus accumbens were stable before drug injections. Saline administration (0.3 ml/inf, i.v.) did not significantly modify dopamine signals over the time course of this experiment (Figure 1a). The administration of single doses of cocaine elicited fast and robust (2-4 fold) increases in extracellular dopamine (Figure 1b). Kinetic analysis of the evoked dopamine signals indicated that the increases were associated with an increase in the apparent $K_m$ ($K_m(app)$) for dopamine uptake. This effect reached a maximum within one minute following all doses of cocaine and then gradually decayed. Figure 2a shows dose-dependent increases in $K_m(app)$ occurring one minute after an intravenous infusion when dopamine uptake inhibition was maximal [$F(3,16) = 52.3; p < 0.01$]. Bonferroni post tests indicated significant differences in $K_m(app)$ after 3.0 versus 1.5 mg/kg of cocaine ($p < 0.01$) and after 0 versus 0.75 mg/kg ($p < 0.01$). There was a trend toward significance between 1.5 and 0.75 mg/kg ($p = 0.056$). No significant changes in the maximal rate of dopamine uptake ($V_{max}$) were revealed following any cocaine dose (Figure 2b). The pre-drug value for $V_{max}$ was $1789 \pm 154$ nM/s, which is
consistent with the previously published $V_{\text{max}}$ values obtained in the nucleus accumbens core region (Mateo, et al 2004b). The amplitude of electrically evoked dopamine release was increased by cocaine in the same fashion as $K_m(app)$ [$F(3,16) = 9.1; p < 0.05$] (Figure 2c). Bonferroni post tests revealed significant differences in this parameter after 1.5 and 3.0 mg/kg of cocaine versus pre-drug values ($p < 0.05$).
FIGURE 1

Representative concentration-time plots of dopamine measured in the rat nucleus accumbens before and following a single intravenous infusion of saline (0.3 ml/inf) (a) and cocaine (1.5 mg/kg) (b). An infusion of saline did not induce any changes in dopamine peak height or in $K_m(app)$. In contrast, cocaine significantly increased dopamine peak height and $K_m(app)$. Maximal dopamine peak height occurred within approximately 1 min after cocaine administration and then gradually decayed (1331 ± 202 (pre-drug), 2820 ± 235 (1 min), 1744 ± 210 (5 min), 1424 ± 132 (40 min) nM (n=5)). $K_m(app)$ values were 180 ± 10 (pre-drug), 1131 ±172 (1 min), 936 ± 189 (5 min), 426 ± 68 (40 min) nM (n = 5).
FIGURE 1

a  saline

b  cocaine

Time after injection
**Figure 2**

Effect of single cocaine (i.v.) injection on dopamine release and uptake in rat nucleus accumbens. Electrically evoked dopamine concentrations and uptake parameters, reported as an $K_{m(app)}$ and $V_{max}$ were measured 1 min after a 4 s cocaine infusion. (a) $K_{m(app)}$ was dose dependently increased following a single infusion of intravenous cocaine (0.75, 1.5, or 3 mg/kg). (b) No changes in $V_{max}$ were detected. (c) Similarly to the effect on $K_{m(app)}$, cocaine significantly enhanced electrically evoked dopamine release. Data are means ± SEM of 5 rats per group. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$. 
Figure 2A

![Bar graph showing apparent $K_m$ (nM) vs. Cocaine dose (mg/kg, i.v.).](image)

- **0 mg/kg, i.v.**
- **0.75 mg/kg, i.v.**
- **1.5 mg/kg, i.v.**
- **3.0 mg/kg, i.v.**

Significance levels:
- **No significance**
- **P < 0.01**
- **P < 0.001**
Figure 2B

The graph shows the effect of different doses of cocaine (0, 0.75, 1.5, 3.0 mg/kg, i.v.) on $V_{max}$ (nM/s). The y-axis represents $V_{max}$, while the x-axis represents the different cocaine doses. The error bars indicate the standard error of the mean.
**Figure 2C**

![Graph showing the effect of different doses of cocaine on evoked DA release compared to the control (0 mg/kg, i.v.). The x-axis represents cocaine doses in mg/kg, i.v., and the y-axis represents evoked DA release in nM. There is a significant increase in DA release with 3.0 mg/kg, i.v. compared to the control.](image-url)
TWO DISTINCT PHASES OF DOPAMINE UPTAKE INHIBITION ARE OBSERVED WHEN COCAINE IS INFUSED USING INTERVALS OBSERVED DURING SELF-ADMINISTRATION (EXPERIMENT 2)

In this experiment, cocaine (0.75 mg/kg) was intravenously infused into naïve rats using inter-infusion intervals which were predetermined from rats that self-administered cocaine under a fixed ratio 1 schedule (2-hour session) of drug delivery (Figure 3). These intervals were 1.11, 2.18, 3.82, 4.52, 4.47, 4.67, 4.83, 4.70, 5.33, 5.37 and 5.18 min. Each cocaine infusion significantly increased $K_m(\text{app})$ compared to the drug pre-infusion value [$F(18,72) = 49.21; \ p < 0.01, \ n=5$]. Following the first four cocaine infusions (loading phase), the level of dopamine uptake inhibition reached a steady-state oscillation that persisted for the duration of the experiment (maintenance phase). During the maintenance phase upper and lower thresholds were distinguishable. The upper threshold was defined as the crest, whereas the lower threshold was defined as the trough, of the fluctuating level of dopamine uptake inhibition that occurs during the maintenance phase. There was a significant difference between the averaged upper and lower thresholds ($t=4.8; \ p < 0.01, \ n=6$, paired t test).

As expected, the amplitude of electrically evoked dopamine efflux was also significantly increased following cocaine infusions. The time course of the changes in evoked dopamine efflux paralleled the time course of the increase in $K_m(\text{app})$ (Figure 3, inset), suggesting that the effect of cocaine on dopamine peak height is preferentially driven by competitive antagonism of the DAT. However, multiple mechanisms are also involved in the effect of cocaine on electrically-evoked
dopamine. For example, cocaine can enhance dopamine release by mobilizing a synapsin-dependent reserve pool (Venton, et al 2006). Moreover, electrically-evoked dopamine release is also potentially subject to D$_2$ dopamine receptor-mediated autoinhibition (Schmitz, et al 2001; 2002; Wu, et al 2002), which takes place during cocaine-induced DAT blockade (Grace, 2000). Since the dopamine peak amplitude is affected by many factors, the interpretation of cocaine effects on dopamine neurotransmission using this parameter can be complicated.
**FIGURE 3**

Dopamine uptake changes obtained with inter-infusion intervals predetermined from rats self-administering cocaine. Dopamine uptake changes, which are measured as alterations in $K_m$(app) are indicated by empty circles. The solid line shows modeled changes in dopamine uptake inhibition. The time points when cocaine (0.75 mg/kg, i.v.) was injected over 4 s are depicted as arrows. Dopamine uptake changes associated with responding during the loading phase are indicated by the oval (A). A steady-state-oscillation of dopamine uptake inhibition that is observed throughout the maintenance phase (B) is indicated by dashed lines. Inset: time course of cocaine on evoked dopamine peak height. Data are means ± SEM of 5 rats per group.
**Figure 3**

![Graph showing time course of evoked DA release with cocaine treatment.

- A: Measurement points indicating changes in $K_m$ (nM) over time.
- B: Graph showing evoked DA release (nM) with time (min).
- Data points and model line illustrating the effect of cocaine 0.75 mg/kg.

*Time (min)*

-5 0 5 10 15 20 25 30 35 40

*Evoked DA release (nM)*

0 600 1200 1800 2400 3000 3600

**K_m (nM)**

0 500 1000 1500 2000 2500

*cocaine 0.75 mg/kg*

- Black line: model
- Circles: data
DYNAMICS OF DOPAMINE UPTAKE INHIBITION CAN BE MATHEMATICALLY MODELED AND PREDICTED (EXPERIMENT 3)

A new mathematical model was developed for the dynamics of dopamine uptake inhibition that quantitatively predicts the behavior of the apparent Michaelis constant $K_{m(app)}$ as a function of time. The model depends on three parameters: the initial cocaine concentration of each injection, the probability of dopamine diffusion and transport in the absence of DAT, and the rate of dopamine removal in the presence of DAT.

This model starts with a very simple form for the apparent Michaelis constant $K_{m(app)}$ based on a binary state representation of the dopamine transporter. A binary model means that the dopamine transporter is either occupied or unoccupied like a two-state system. In this case, the probability that a transporter is occupied by a cocaine molecule is represented by $p_o$. If $p_u$ represents the probability that a transporter is unoccupied by a cocaine molecule, then these two probabilities must sum to one, i.e. $p_o + p_u = 1$. This relation applies since the model is binary, and a transporter is either occupied or unoccupied by a cocaine molecule. Cocaine acts as an inhibitor, by attaching to a transporter that prevents a dopamine molecule from attaching to the same transporter. Thus, a physically reasonable model for these probabilities involves the ratio of the inhibitor (cocaine) concentration $[I]$ to the concentration of transporters $[T]$. If this ratio is defined to be

$$s = \frac{[I]}{[T]},$$

(2)
then the probability that cocaine will occupy a transporter is equal to \( s \), i.e. \( p_o = s \).

This last relation, that \( p_o = s \), is only true as long as the number of occupied transporters remains small, i.e. the occupation probability is much less than 1. However, when the inhibitor concentration becomes large, i.e. comparable to or higher than the transporter concentration, then the probability must be modified to account for this moderate to high concentration ratio of inhibitor to transporters. In the case where the transporters are basically saturated with inhibitors, then the occupation probability will reach its maximum value of 1. An appropriate model for such a system is similar to the two-state model from the structure of atoms, where the atoms can act like receptors for light particles or photons. Using this model it is assumed that the inhibitor occupation probability \( p_o \) is given by

\[
p_o = \frac{s}{1 + s}. \tag{3}
\]

This probability has the correct form in the two extreme cases of low inhibitor concentration (\( s \ll 1, p_o = s \)) and large inhibitor concentration (\( s \gg 1, p_o = 1 \)).

This inhibitor-occupied-transporter probability can be related to the apparent Michaelis constant. In enzyme reaction rate theory (see Voet and Voet, 2004), an apparent \( K_m \) in the presence of an inhibitor is given by

\[
K_m(app) = (1 + \frac{[I]}{K_1})K_m, \tag{4}
\]

where \( K_m \) is the Michaelis constant in the absence of inhibitor, and the constant \( K_1 \) is the equilibrium constant associated with the enzyme-inhibitor complex. To mimic this standard relation from enzyme reaction rate theory, it is assumed that a
Michaelis constant depends on the inhibitor-occupied-transporter probability in the following way

\[ K_{m(app)} = \left( \frac{1}{1 - p_o} \right) K_m. \tag{5} \]

The factor multiplying \( K_m \) in both of these cases can be related, in the case of small inhibitor concentration, using the geometric series expansion of the prefactor in the last equation. The geometric series \( 1 + p_o + p_o^2 + \ldots \) sums to the prefactor in Eq. (5) above. So if all the small numbers in the geometric series are dropped, such as \( p_o^2 \) and higher powers of \( p_o \), then for small \( p_o \) or \([I]\) Eq. (5) for \( K_{m(app)} \) can be written:

\[ K_{m(app)} = (1 + p_o) K_m = (1 + \frac{[I]}{K_i}) K_m. \tag{6} \]

Thus, this model, Eq. (5), gives the expected result in the extreme case of low inhibitor concentrations, Eq. (4). In the extreme case of high inhibitor concentrations the occupation probability \( p_o \) approaches 1, and the effective Michaelis constant approaches infinity – see Eq. (5). This means that inhibitors occupy all the available transporter sites and so the reaction with dopamine shuts down and dopamine is not taken up at all.

This last discussion, about the behavior of the transporter under high inhibitor concentration conditions, brings up one more issue that requires modification of the model. If the inhibitor completely binds to all transporter sites, the dopamine concentration will still decrease with time, through reactions with molecules other than transporters, and through diffusion. The apparent Michaelis
constant can never, in practice, reach infinity, which Eq.(5) would predict if $p_o=1$. Thus, a term must be included in the model that keeps $K_m(app)$ finite in the extreme case of very high inhibitor concentration. To implement this last important concept into the model, its final form becomes

$$K_m(app) = \frac{1}{1 - p_o + b}K_m.$$  

(7)

The constant $b$ is a small number with a value around $b \sim 0.005$. Physiologically, it accounts for dopamine diffusion and uptake in the presence of saturating amounts of cocaine, which means that the cocaine-responsive DAT transporters are effectively absent (actually occupied by cocaine). Note that under cocaine-saturating conditions, the apparent Michaelis constant becomes $K_m/b$. In the application of the final model, Eq. (7), to the experimental data, there are three parameters whose values are varied to fit the model to the data. Two of the constants are: 1) $s(0)$, the initial cocaine-to-transporter concentration ratio at the time of an injection, i.e. at time $t=0$, when cocaine first floods the probed region, and 2) the constant $b$. Note that the initial inhibitor-occupied-transporter probability $p_o(0)$ is directly related to $s(0)$. The third parameter needed is the decay rate $k_{\text{decay}}$ of the inhibitor concentration $[I]$ after an injection of cocaine. In this case it is assumed that $s$, which is proportional to $[I]$, decays exponentially in time after an inhibitor (cocaine) injection.

To explicitly describe the detailed time dependence of the model as it is used to fit the experimental data (see Figure 3), consider a set of data that has cocaine
injections at times \( t_0, t_1, t_2 \ldots \). This means that the inhibitor concentration \([I]\), and therefore \( s\), during the whole time series has the form

\[ s(t) = s(0) \sum_{i=1}^{N} e^{-k_{\text{decay}}(t-t_i)} \]  

(8)

where \( N \) is the total number of injections.

The model was used on the data from naïve rats repeatedly receiving a dose of cocaine (0.75 mg/kg), as described in the previous section and is shown in Figure 3. The model fits these data extremely well with the best fit values being: \( s(0) = 3.83 \), \( b = 0.0047 \), and \( k_{\text{decay}} = 0.0628/\text{minute} \). The reduced chi-square for this fit is: \( \chi_R = 0.177 \), with the number of degrees of freedom = 39.

To definitively test the predictive capability and efficacy of the model, it was used to blindly predict the values of \( K_m(\text{app}) \) in the case where the inter-infusion intervals of the maintenance phase were made very short (2 minutes) and very long (10 minutes). Note these intervals do not correspond to inter-infusion intervals selected by rats during the self-administration of cocaine, but were chosen outside of this range to determine if the model would predict outcomes of future experiments. An experiment was then initiated for the long inter-infusion intervals with naïve rats. The results demonstrated that the model can capture not only the important qualitative features of the dynamics of dopamine concentration, but the model successfully described the outcome of the experiment (Figures 3 and 4). Note that no parameters were adjusted in generating the model points – these points were generated prior to voltammetric experiments based on the model’s fit to rat
responses in the case where the inter-infusion intervals were much shorter (5 minutes).
Changes in cocaine-induced dopamine uptake inhibition can be predicted. Two lines show predicted changes in dopamine uptake inhibition using two different inter-infusion intervals. The two curves correspond to blind predictions for the case where the inter-infusion intervals for the maintenance phase are 2 min apart (upper curve) and for an inter-infusion interval of 10 min (lower curve). Dopamine uptake changes using 10 minute inter-infusion intervals, which were measured in animal experiments, are indicated by empty circles. Data are means ± SEM of 5 rats per group. The experimental measurements agree well with the blind prediction from the model.
Figure 4
DAT INHIBITION THRESHOLDS ARE SHIFTED UPWARD WITH ESCALATED INTER-INFUSION INTERVALS (EXPERIMENT 4)

Previous studies have demonstrated that providing long-access to cocaine (6-hr session) under a fixed ratio 1 schedule for 14 days results in animals increasing, or 'escalating' the rate of cocaine intake across sessions (Ahmed and Koob, 1998; 1999). As illustrated in figure 5, dopamine uptake inhibition ($K_m(app)$) was assessed while cocaine (0.75 mg/kg, i.v.) was infused into naïve rats using inter-infusion intervals which were predetermined from rats that either escalated (long-access) or did not escalate (short-access) cocaine self-administration rates. The escalated intervals were determined to be 1.00, 1.00, 1.48, 1.65, 3.00, 3.37, 3.88, 4.00, 3.00, 3.00, 3.20, 3.17, 4.00, 3.12 min (see inter-infusion interval determinations in the Material and Methods). Similarly to the previous experiment, cocaine-induced dopamine uptake inhibition (changes in $K_m(app)$) appeared in two distinct phases, which corresponded to the loading and maintenance phases observed during self-administration under a fixed-ratio schedule (Ahmed and Koob, 1999; Wee, et al 2007; Specio, et al 2007). The total level of DAT inhibition was extensively higher with escalated (long-access) inter-infusion intervals, compared with that from unescalated (short-access) animals (Figure 5). There was a significant main effect of the access condition from which rates were determined (i.e., long-access versus short-access) on $K_m(app)$ [$F(1,336) = 60.65; \ p < 0.01$]. There was also a significant main effect of time [$F(41, 336) = 5.45; \ p < 0.01$] on $K_m(app)$, while the interaction was not statistically significant. Upper and lower thresholds were compared between escalated (long-access) and unescalated (short-access) groups by
averaging the peaks and troughs of the oscillatory pattern of $K_m(app)$ changes observed during the maintenance phase. For the escalated (long-access) rats the upper threshold was $2809 \pm 31$ nM and the lower threshold was $2424 \pm 37$ nM ($n = 11$). For the unescalated (short-access) rats the upper threshold was $2282 \pm 26$ nM and the lower threshold was $1940 \pm 25$ nM ($n = 6$). There was a significant difference between the escalated (long-access) and unescalated (short-access) groups upper [$t = 11.33; p < 0.01$] and lower thresholds [$t = 8.976; p < 0.01$].
**FIGURE 5**

DAT inhibition thresholds are shifted upward during escalated cocaine self-administration. Dopamine uptake changes ($K_m(app)$) obtained using inter-infusion intervals that were predetermined from rats with escalated rates of cocaine self-administration are indicated by solid black circles. Empty circles show cocaine-induced $K_m(app)$ alterations, which were observed with non-escalated intervals. All of the experiments displayed in this graph were conducted using cocaine naïve rats. Data are means ± SEM of 5 rats per group.
Figure 5
**Escalated Cocaine Self-Administration Modifies the Maximal Rate of Dopamine Uptake (Experiment 5)**

In experiment 5, the dopamine uptake parameters before and after four consequent cocaine (0.75 mg/kg, i.v.) infusions were compared between the cocaine naïve group and rats which demonstrated escalated cocaine intake under a fixed ratio 1 schedule (long-access training). The experiment indicated that the basal (pre-drug) value of $K_{m(app)}$ was not different between cocaine-naïve and cocaine-exposed rats ($182.9 \pm 8.1$ versus $177.1 \pm 8.1$ nM; n.s.; n=7). However, there was a significant difference in the basal $V_{max}$ between these two groups (Figure 6). As was expected (see results with single cocaine injection), acute cocaine infusions did not significantly alter this parameter of dopamine uptake in both cocaine-naïve and drug exposed animals (data not shown). Cocaine-induced $K_{m(app)}$ changes were indistinguishable between these groups (Figure 7). A two-way ANOVA demonstrated that the effect of prolonged cocaine exposure was not significant [$F(1,40) = 0.043;\text{ n.s.}$], while the effect of acute cocaine was significant [$F(4, 40) = 0.043;\text{ p < 0.01}$].
FIGURE 6

Consequences of escalated cocaine self-administration on the dopamine uptake in rat nucleus accumbens. (a) $V_{\text{max}}$ is significantly increased following escalated cocaine intake (black bar) in comparison with naïve control (empty bar). Data are means ± SEM of 7 rats per group. **, $P < 0.005$. (b) No significant changes in the affinity of DAT for cocaine ($K_m(app)$) are found in rats following escalation in cocaine self-administration (black bars) compared with naïve control (empty bars). Four cocaine injections (0.75 mg/kg, i.v.) were performed to mimic loading phase. Data are means ± SEM of 5 rats per group.
Figure 6A

![Graph showing comparison of V_max between naive and cocaine-exposed groups. The bar graph indicates a significant difference (**).](image)
FIGURE 6B
DISCUSSION
The present study demonstrates that cocaine-induced DAT inhibition fluctuates over a time course which can account for patterns of cocaine self-administration reinforced under a fixed-ratio 1 schedule. Specifically, the inter-infusion intervals that are maintained during cocaine self-administration correlate with the maintenance of a rapidly changing level of DAT inhibition. These oscillating changes in dopamine uptake inhibition were modeled and can be predicted in a manner relevant to cocaine self-administration. Furthermore, this tightly maintained level of DAT inhibition was found to shift upward using intervals obtained after a history of escalated (long-access training) cocaine intake. Although, daily 6-hr access (long-access) during cocaine self-administration did not significantly alter the efficacy of cocaine for the DAT, it did result in a facilitated uptake of dopamine ($V_{\text{max}}$ changes) suggesting an up-regulation of DAT number in response to a history of high cocaine intake.

SINGLE INTRAVENOUS COCAINE ADMINISTRATION AND UPTAKE OF ENDOGENOUS DOPAMINE
The effect of intravenously injected cocaine on dopamine uptake occurs with a rapid onset and offset. The present data – together with previously published in vivo voltammetry data using intraperitoneal cocaine administration (Wu, et al 2001), as well as with in vitro drug application (Jones, et al 1995) – confirm that competitive inhibition of the DAT is the primary mechanism of acute cocaine action on increasing accumbal dopamine transmission. Indeed, intravenous cocaine dose dependently decreased the uptake of dopamine, acting through an alteration in
$K_m(app)$, while $V_{max}$ remained unaffected. According to single curve analysis, the maximum effect of cocaine on dopamine uptake inhibition ($K_m(app)$) was reached within 1-2 minutes after a single cocaine infusion and then gradually decreased (Figure 1b). Importantly, the maximal effect of cocaine on $K_m(app)$ is consistent with a peak in cocaine brain concentrations (Fowler, et al 1998; Ahmed, et al 2003) and the maximal inhibition of dopamine cells in the ventral tegmental area (Einhorn, et al 1988). These data should be contrasted to the results of Kiyatkin and colleagues who demonstrated that cocaine-induced changes in clearance of exogenous dopamine began two minutes following a cocaine infusion (Kiyatkin, et al 2000), when extracellular dopamine concentrations were clearly elevated (Wise, et al 1995; Ahmed, et al 2003; Heien, et al 2005) and peaked at 6-8 minutes (Kiyatkin, et al 2000). Therefore, the dynamics of dopamine uptake inhibition following intravenous cocaine administration (see also Mateo, et al 2004b; Samaha, et al 2004) appears to differ depending on whether stimulated dopamine release or exogenously applied dopamine is assessed.

**Levels of Dopamine Uptake Inhibition Are Tightly Linked to Cocaine Self-Administration**

The present study demonstrates a strong association between the pattern of cocaine self-administration and the level of dopamine uptake inhibition. Two stages of dopamine uptake inhibition are revealed in accordance with patterns of self-administration observed during cocaine self-administration under a fixed-ratio schedule. Previous reports, in addition to the data from the current study, have demonstrated that responding at the beginning of a self-administration session
(approximately 10 minutes) occurs at a faster rate than at any other period of the session (Ahmed and Koob, 1999; Wee, et al 2007; Specio, et al 2007). This initial high rate of responding, termed the loading phase, is also reflected by a rapid increase in $K_m(app)$ at the onset of a session (Figure 3). Following the loading phase, the rate of responding appears to subside and stabilize, presumably corresponding to the point in time at which an effective brain level of cocaine-induced DAT inhibition is reached. This level, which is reflected by a lower threshold of $K_m(app)$, is then sustained through a maintenance phase during which responses are separated by stable inter-infusion intervals (Ahmed and Koob, 1999; Specio et al 2007). During the maintenance phase, the level of DAT blockade fluctuates around a narrow range within lower and upper thresholds.

The present data suggest that responding maintained by cocaine occurs in association with a lower threshold of dopamine uptake inhibition. This lower threshold has also been referred to as a trigger-point, set-point, or priming threshold (Wise, et al 1995; Ahmed and Koob, 1998; Tsibulsky and Norman, 1999). In fact, a lower level of extracellular dopamine within the nucleus accumbens has previously been implicated as a ‘trigger’ to respond during cocaine self-administration (Wise, et al 1995). The data from the current study link the ‘trigger’ to a lower threshold of dopamine uptake inhibition, thereby demonstrating the mechanism that provides the trigger-dopamine concentration.

The upper threshold has also received speculative attention (Petitt and Justice, 1989; Tsibulsky and Norman, 1999; Lynch and Carroll, 2001). For example,
Petitt and Justice (1989) suggested that the upper threshold may be associated with aversive cocaine effects whereas Tsibulsky and Norman (1999) suggested this threshold occurs due to satiety mechanisms alone. Although the data from the present study can not reconcile why an upper threshold is observed, the observation that this threshold shifts upward when cocaine infusions are maintained at a high rate suggests that the upper threshold does not result from maximal DAT occupancy (see Figure 5).

Cocaine-induced dopamine uptake inhibition may alter phasic dopamine release through a $D_2$ dopamine autoreceptor-mediated feedback mechanism (Grace, 2000; Phillips, et al 2003). It was discovered that a lever press for a cocaine infusion appears to be associated with subsecond dopamine release in the nucleus accumbens (Phillips, et al 2003; Stuber, et al 2005). Importantly, these short-lived dopamine changes - which may be influential in an animal’s approach behavior toward a cocaine-paired lever or other drug-paired stimulus (Phillips, et al 2003; Stuber, et al 2005) - do not translate to a significant elevation in tonic dopamine levels. In contrast, the DAT inhibition has a pronounced effect on tonic dopamine levels (Budygin, et al 2000; Heien, et al 2005). An intriguing speculation is that subsecond dopamine release can be promoted through feedback mechanisms resulting from a decrease in extrasynaptic dopamine as the level of cocaine-induced DAT inhibition approaches the lower threshold. Future studies are necessary to determine whether this connection exists.
CONSEQUENCES OF ESCALATED COCAINE SELF-ADMINISTRATION ON ACCUMBAL DOPAMINE UPTAKE

Providing long access to cocaine during self-administration produces an increase, or 'escalation,' in the rate of cocaine self-administration (Ahmed and Koob, 1998), which is reflected by the maintenance of an increased level of dopamine uptake inhibition ($K_{m(app)}$). As illustrated in figure 5 (experiment 4), the inter-infusion intervals obtained from animals with a history of long-access training were applied to determine the effect of an increased rate of self-administration on dopamine uptake inhibition in naïve animals. It was found that cocaine-induced dopamine uptake inhibition reached a proportionally higher level during the loading phase consistent with an escalation of cocaine intake. Likewise, the dopamine uptake inhibition thresholds associated with the maintenance of responding during self-administration were shifted upward with 'escalated' intervals.

The neuroadaptations explaining the resulting changes in behavior during long-access training remain unknown. There is convincing evidence that changes in cocaine pharmacokinetics, and changes in baseline concentrations of dopamine in the nucleus accumbens, do not have a critical role in the escalation of cocaine intake observed during long-access training (Ahmed, et al 2003). Furthermore, no apparent sensitization or desensitization to the effect of cocaine on accumbal dopamine concentrations was observed in animals with a history of long-access cocaine self-administration (Ahmed et al. 2003). However, it may be argued that prolonged cocaine exposure could modify the DAT inhibiting efficacy of cocaine. Examination of Figure 6b (experiment 5) shows that long-access escalation training
did not result in significant changes in the efficacy \( (K_{m}(app)) \) of cocaine for the DAT. Therefore, the higher level of DAT blockade and subsequent maintenance of higher accumbal dopamine concentrations, which repeatedly take place during long-access cocaine self-administration, are not capable of significantly modifying the affinity of the DAT for cocaine. Thus, these data suggest that changes in the affinity of the DAT for cocaine are not involved in the escalation of cocaine intake.

Other possible neuroadaptations may occur during long-access training, such as post-synaptic adaptations and an up-regulation of DAT number, which may explain the escalation of cocaine intake. Several studies have reported post-synaptic adaptations following extended cocaine exposure demonstrating a down-regulation of both striatal \( D_1 \) (Graziella de Montis, et al 1998) and \( D_2 \) (Nader, et al 2002) dopamine receptors. Assuming post-synaptic changes occur during an escalation of cocaine intake, it can be hypothesized that increased dopamine uptake inhibition thresholds are maintained to produce a level of reinforcement equal to that occurring prior to escalation. One of the possible consequences of prolonged increases in extracellular dopamine concentrations following administration of addictive substances, including cocaine, is an increase in the maximal rate of dopamine uptake (Budygin, et al 2003; 2007; Mateo, et al 2005). The data presented here demonstrate the existence of facilitated dopamine uptake in the rat nucleus accumbens after a history of long-access during cocaine self-administration (Figure 6a). The increased rate of dopamine uptake is likely the result of an up-regulation in functional DAT number. Previous studies have reported increases in DAT availability following prolonged cocaine self-administration in both rats (Tella, et al
1996; but see also Ben Shahar, et al 1996) and non-human primates (Letchworth, et al 2001). Moreover, human postmortem analyses report an increase in striatal DAT binding sites in cocaine addicts (Little, et al 1998; Little, et al 1999). These presynaptic changes, which may occur following any regimen of prolonged cocaine exposure, may be a compensatory response of the dopamine system to persistently elevated dopamine concentration in the extrasynaptic space.

CONCLUSION
This study has implications for understanding the role of the DAT in regulating responding for cocaine during self-administration. The present study took advantage of fast-scan cyclic voltammetry, which provides high temporal and spatial resolution, and clearly demonstrates that a tight correlation exists between the level of dopamine uptake inhibition and the rate of responding for cocaine under a fixed-ratio schedule.

ACKNOWLEDGMENTS
We thank Dr. Caroline Bass and Dr. Jack Strandhoy for helpful comments and the UNC Department of Chemistry Electronics Facility for technical support. This work was supported by Wake Forest University Cross-Campus Collaborative Fund Award and National Institute of Health Grants DA021634 (EAB), F31DA024525 (EBO), and R01DA140309 (DCSR).
REFERENCES


CHAPTER V

INCREASED MOTIVATION TO SELF-ADMINISTER COCAINE IS DETERMINED BY THE PATTERN OF RESPONDING ASSOCIATED WITH FLUCTUATING BRAIN COCAINE LEVELS

Erik B. Oleson, Evgeny A. Budygin, Keith D. Bonin,
and David C.S. Roberts

The following manuscript is in preparation for submission to Neuropsychopharmacology
Abstract

Critical variables influencing whether the time and energy devoted to self-administer cocaine increase over time are beginning to be identified. For example, we have previously demonstrated that providing animals with access to high, rapidly delivered cocaine doses under a progressive ratio schedule produces an increase in final ratios reached for cocaine over time. However, providing animals with access to the same rapidly delivered cocaine dose under a fixed ratio schedule is not sufficient to produce an increase in final ratios. This led us to investigate the necessity of the interaction between patterns of behavioral responding and rapidly fluctuating brain cocaine concentrations resulting from high-dose cocaine injections to produce an increase in the motivation for cocaine. To investigate this interaction we took advantage of three very different self-administration procedures. Each procedure provides a unique interaction between the temporal characteristics of the behavioral response and fluctuating brain cocaine levels. It was found that only a self-administration history involving large brain cocaine fluctuations during periods of peak responding (i.e. progressive ratio training) produced an increase in the reinforcing strength of cocaine. These findings suggest that experiencing large brain cocaine fluctuations during periods of maximal responding contributes to an increased motivation for cocaine. These studies required the application of mathematical models which also allowed us to assess the role of brain cocaine fluctuations in the regulation of cocaine intake. The models revealed that animals maintain an upper brain cocaine level during self-administration until the cessation of responding occurs at a lower brain cocaine level.

Keywords: Cocaine, behavioral economics, titration, price, consumption, dopamine
INTRODUCTION

There is a growing interest in developing self-administration training procedures that address fundamental components of the addiction process (Deroche-Gamonet, et al 2004; Vanderschuren and Everitt, 2004). The development of such animal models will provide experimenters with the necessary tools required to study the neurobiological adaptations occurring through the addiction process. For example, specific neural changes associated with increased cocaine intake over time were identified (Ben Shahar, et al 2009; Briand, et al 2008) following the development of the long-access training procedure (Ahmed and Koob, 1998; Ahmed and Koob, 1999). Additionally, the neurobiological basis of cue-induced cocaine craving has been addressed (Conrad, et al 2008; Lu, et al 2009) using reinstatement self-administration models (Neisewander, et al 2000; Grimm, et al 2001).

Increased time and energy devoted to obtain cocaine is another feature of the addiction process that has been addressed. This aspect of the addiction process has been modeled using a progressive ratio (PR) schedule. Under a PR schedule animals are required to work for cocaine by meeting response requirements that are incremented throughout each self-administration session. The final response requirement that is completed, the final ratio, measures how hard an animal will work for a single, large bolus injection of cocaine (Richardson and Roberts, 1996). Recently, attempts have been made to identify critical factors that produce increases in final ratios. It is well documented that a pharmacological history of cocaine self-administration under a simple schedule of reinforcement does not produce an
increase in final ratios. For example, providing rats with access to cocaine under an FR1 schedule during limited sessions (1-2 hr) does not result in rats showing increased final ratios for cocaine (Paterson and Markou, 2003; Wee, et al 2009). However, providing rats with access to similar doses of cocaine under a PR schedule does produce an increase in final ratios over 14 days (Morgan and Roberts, 2004; Liu, et al 2005b). We have argued that this increase in final ratios over time models a specific aspect of the addiction process (ie. increased time and energy devoted to obtain cocaine; Roberts, et al 2007).

One fundamental question regarding the increase in final ratios observed during PR training is whether any history of high-rate responding is sufficient to increase the behavioral cost an animal might pay for cocaine, or whether the PR schedule encompasses some special feature. A unique characteristic of the PR schedule is that animals work hardest for high cocaine doses (Ward, et al 2005), which produce large fluctuations in brain cocaine levels (Nicola and Deadwyler, 2000). On first consideration, large bolus injections might not appear to be a critical factor. For example, providing animals with access to high doses of cocaine under an FR schedule, a condition which produces large brain cocaine fluctuations (Ahmed, et al 2003; Ahmed and Koob, 1999), for 1-2 hours (the duration of a PR session) does not produce increased final ratios (Paterson and Markou, 2003; Wee, et al 2009). Furthermore, we previously demonstrated that if rats are given access to the exact number of high-dose injections as PR trained rats, but responding is maintained under an FR schedule, they do not show increased final ratios for cocaine (Oleson and Roberts, 2009). On the other hand, it is intriguing that final ratios are most
likely to increase when a high cocaine dose is rapidly delivered (Liu, et al 2005b). One possibility is that for final ratios to increase over time – animals must emit patterns of high rate responding, as occurs when a final ratio is reached, while experiencing large brain cocaine fluctuations.

To investigate the interaction between patterns of responding during cocaine self-administration and brain cocaine fluctuations, we took advantage of two newly developed self-administration procedures. Both procedures engender high rates of responding in comparison to the PR schedule, but offer unique interactions between the pattern of the behavioral response and dose. Procedure 1: Under a hold-down schedule of reinforcement animals are given access to a lever, which when depressed turns on a syringe pump continuously until the lever is released. Depending on the available cocaine concentration, the rate of responding varies, but overall cocaine intake remains the same (Morgan, et al 2009). It appears, therefore, that under these conditions animals maintain a stable brain cocaine level by responding at high rates for very small unit-injection doses. Procedure 2: In the within-session threshold procedure animals are given access to a descending series of eleven unit doses (422 – 1.3 µg/inj) on an FR1 schedule of reinforcement during consecutive 10-minute intervals. Throughout each session response rate increases while overall cocaine intake remains relatively stable (Espana, et al 2009). As a result, early in the session animals respond minimally while experiencing large brain cocaine fluctuations, but later in the session responding occurs at maximal rates while a stable brain cocaine level is maintained. Both of these procedures produce different interactions between brain cocaine fluctuations and the temporal
characteristics of the behavioral response in comparison to those occurring under a PR schedule. Under a PR schedule, large brain cocaine fluctuations are observed throughout the entire session, even during periods of maximal responding (Nicola and Deadwyler, 2000).

If any behavioral history of high-rate responding is sufficient to produce an animal that will pay a high behavioral price for cocaine, then providing access to cocaine under all three conditions (ie. PR, hold-down or within-session threshold) should produce rats that expend greater effort for cocaine in operant procedures. If, however, only PR training produces animals that pay a higher behavioral price for cocaine, a critical interaction between brain cocaine fluctuations and patterns of responding during self-administration would be revealed. To investigate the importance of this interaction we assessed final ratios and behavioral economic measures of reinforcement strength following cocaine self-administration histories under these three different conditions.

**METHODS**

**ANIMALS, SURGERY, AND HOUSING**

The Wake Forest University Institutional Animal Care and Use Committee approved all experiments before the study commenced. Male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA), weighing approximately 350 g at the time of surgery were used as subjects. Before entering the study, rats were anesthetized with ketamine (100mg/kg) and xylazine (8mg/kg) and implanted with chronically indwelling
Silastic® cannula (CamCaths, Cambridgshire, UK) as described before (Roberts and Goeders, 1989). Upon recovery animals were housed individually in 30 x 30 x 30 cm experimental chambers housed in a temperature-controlled room (20-21°C) maintained on a 12-h light-dark cycle (lights on at 15:00-h). A counterbalanced fluid swivel (Instech Laboratories Inc., Plymouth Meeting, PA, USA) mounted above the experimental chamber was used to connect an infusion pump (Razel Scientific Instruments Inc., Stamford, CT, USA) to the cannula using Tygon® tubing enclosed within a stainless steel tether. Cannulae were flushed daily with heparinized saline to help maintain patency. Food and water were available ad libitum.

**GENERAL SELF-ADMINISTRATION METHODS AND ACQUISITION**

Animals were given access to a cocaine-paired lever after a recovery period of 3-5 which, when depressed, initiated an intravenous injection of cocaine. Sessions occurred 7 days/week and began in the middle of the dark cycle (1000 hours). During acquisition, rats were given access to cocaine (0.75 mg/kg) under an FR1 schedule of reinforcement. Sessions were terminated after a maximum of 20 infusions or a period of 24 h had occurred. An animal was considered to have acquired if 20 injections were self-administered beginning at the onset of an experimental session, and a stable pattern of post-infusion pauses between injections was apparent.

**PROGRESSIVE RATIO SCHEDULE**

Following acquisition, one group of rats was given access to cocaine (1.5mg/kg/inj) for 14 consecutive days under a progressive ratio schedule of reinforcement. Under this PR schedule, cocaine reinforcement is contingent upon an increasing number of
lever responses that are incremented through the following progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603 (Richardson and Roberts, 1996). Final ratios were defined as the response requirement corresponding to the final cocaine injection. Each PR session lasted 6 hr, although rats typically reached a final ratio within 2-3 hr.

**Hold-down Schedule**

Following acquisition, one group of rats was given access to a single concentration of cocaine (5mg/ml) for 14 consecutive days under a hold-down schedule of reinforcement. Under this schedule, each rat is given access to a lever which, when depressed, continuously turns on an infusion pump until the lever is released. Therefore, each animal determines the magnitude of each unit-injection dose by manipulating the duration of each lever press. Each hold down session lasted 2 hr.

**Within-Session Threshold Procedure**

Following acquisition, one group of rats was given access to cocaine in the within session threshold procedure for 14 consecutive days. In the within session threshold procedure rats are given access to a descending series of eleven unit doses of cocaine (421, 237, 133, 75, 41, 24, 13, 7.5, 4.1, 2.4 and 1.3 μg/injection) in consecutive 10 minute bins under an FR1 schedule. Doses were manipulated by the adjustment of pump duration as previously described (Oleson and Roberts, 2009). See supplemental material of Oleson and Roberts, 2009 for a full characterization of this approach and validation that the appropriate quantity of drug is delivered across all pump durations. Each threshold session lasted 2 hr. The available dose during the final 10 minute bin was 0 mg/kg.
BEHAVIORAL ECONOMICS AND DATA ANALYSIS

Upon completion of training all rats were given access to cocaine in the within-session threshold procedure for 6 days to obtain data for behavioral economics analyses. Behavioral economic theory has been successfully applied to drug self-administration in general (Bickel, et al. 1993; Hursh, 1991) and threshold procedures in particular (Oleson and Roberts, 2009). In the current threshold procedure the descending series of doses (421, 237, 133, 75, 41, 24, 13, 7.5, 4.1, 2.4 and 1.3 μg/injection) resulted in rats receiving access to cocaine across the following 11 ascending unit-prices: 2.4, 4.2, 7.5, 13.3, 23.7, 39.9, 75, 133.9, 241.9, 416.7 and 750 resp/mg. Three dependent measures were analyzed in the present study: the maximal price paid for cocaine (Pmax), the maximal response output (Omax) at Pmax and cocaine consumption. Pmax was determined to be the unit-price corresponding to the apex of the price-response function as previously described (Espana, et al. 2009; Oleson and Roberts, 2009). It should be noted that the apex of the price-response function, and therefore Pmax, coincides with the point at which cocaine consumption changes from being maintained (inelastic demand) to not being maintained (elastic demand). Omax was measured by calculating the rate of responding occurring during the final 10-minute bin in which drug intake was maintained (ie. the bin in which Pmax is determined). Consumption was measured by averaging cocaine intake across unit-prices 4.2-13.3, a range at which cocaine intake was stable but minimally constrained by price. All three dependent measures were calculated by averaging data across days 4-6 in the
within-session threshold procedure. Each animal was given an initial 3 days to adjust to the contingencies of the procedure.

**Brain Cocaine Concentration Model**

Brain cocaine concentrations (μM) were calculated as described for cocaine self-administration by Nicola and Deadwyler (2000) using equations developed by (Pan, et al 1991). The brain concentration (C) of cocaine resulting from a single cocaine injection was calculated using the equation

\[ C = dA(e^{-\alpha t} - e^{-\beta t}) \]

The following constants, which were used in the current study, were originally reported by (Pan et al. 1991) using rats with a history of cocaine self-administration: \( A (9.673 \, \mu M \cdot kg^{-1} \cdot min^{-1} \cdot mg^{-1}) \), \( \alpha (0.642 \, min^{-1}) \), \( \beta (0.097 \, min^{-1}) \). The variables \( t \) (time in minutes following the injection) and \( d \) (unit-injection dose reported as mg/kg) varied depending on the self-administration protocol. As was done by Nicola and Deadwyler (2000), the cocaine concentrations following multiple cocaine injections (C) were calculated as the sum of C received before \( t \).

**Dopamine Uptake Inhibition Model**

Levels of dopamine uptake inhibition (nM) were calculated as described previously (Oleson, et al 2009). See the results of Oleson et al (2009) for a full description of how this model is derived and neurochemical validation that the model predicts changes in dopamine uptake inhibition. Briefly, the predicted level of dopamine uptake inhibition (\((K_{m})_{app}\)) was calculated using the equation
The constant $b$ (0.0047) represents the rate of dopamine diffusion and uptake in the presence of saturating amounts of cocaine. The value representing the probability that a dopamine transporter is occupied by a cocaine molecule ($p_o$) is calculated using the equation

$$p_o = \frac{s}{1 + s}$$

The ratio of available cocaine molecules to accessible dopamine transports ($s$) is adjusted to account for inter-trial intervals throughout the course of a self-administration session by accounting for multiple cocaine injections ($t_0, t_1, t_2...$) using the equation

$$s(t) = s(0) \sum_{i=1}^{N} e^{-k_{\text{decay}}(t-t_i)}$$

In this equation, the constant $s(0)$ (3.83) is the initial cocaine molecule to dopamine transporter ratio at the time of the first cocaine injection ($t=0$), $N$ is the total number of cocaine injections, and $k_{\text{decay}}$ (0.0628/min) is the decay rate of cocaine.

To assure conformity across schedule comparisons, modeled data were only shown across the first 160 min. For statistical comparisons, brain cocaine concentrations and levels of dopamine uptake inhibition were compared at each minute.
**Calculation of Inter-Trial Interval (t) and Average Dose (d)**

In order to calculate average unit-injection doses (d) and inter-trial intervals (t) 50 self-administration sessions were analyzed from each of the three procedures. To assess changes in the behavioral pattern of responding across time in the hold-down and threshold procedures, each session was broken into 5 min bins. Therefore, a distinct average inter-trial interval was calculated for each 5-minute bin occurring in these procedures. For the hold-down procedure these were: 0.17, 0.37, 0.40, 0.45, 0.42, 0.44, 0.48, 0.48, 0.47, 0.55, 0.48, 0.48, 0.50, 0.45, 0.57, 0.50, 0.47, 0.51, 0.49, 0.52, 0.51, 0.50, 0.51 min. Because each unit-injection dose varied in the hold-down procedure, but remained similar throughout each session (see figure 1c) the average unit-injection dose (0.1 mg/kg/inj), which was averaged across all 50 self-administration sessions, was used for this procedure. For the within-session threshold procedure the 50 sessions were analyzed separately based on Pmax values to account for extinction. Sessions corresponding to the 133.9 resp/mg Pmax value were compared because the greatest amount of animals (n=9) reached this particular Pmax. The inter-trial intervals for these rats were: 1.97, 1.42, 2.63, 1.18, 0.79, 0.83, 0.46, 0.45, 0.31, 0.36, 0.17, 0.23, 0.31, 0.45, 0.47, 3.1, 1.08, 0.53 min. Likewise, the 50 PR sessions were grouped based on final ratios and rats (n=8) reaching a final ratio of 188 were used for modeling. The average inter-injection interval occurring between injections for these rats were: 3.5, 6.8, 8.8, 8.0, 8.4, 8.4, 9.8, 9.4, 10.4, 8.6, 10.5, 8.9, 11.6, 11.6, 15.4, and 35.0 min.
**Drug**

Cocaine HCl, obtained from the National Institute on Drug Abuse (Research Triangle Institute, NC, USA), was dissolved in a solution of sterilized saline 0.9% and passed through a microfilter (0.45 µm pore size). A 5mg/ml cocaine solution was used for hold-down, PR, and within-session threshold experiments; a 2.5mg/ml solution was used for acquisition.

**Statistics**

All statistics were performed using SigmaPlot (Version 11). All final ratio, Pmax, Omax, consumption, and single session rate comparisons were performed using a one-way ANOVA with Holm-Sidak post hoc analysis. Changes in daily rate, final ratio, intake, Pmax, and hold-down time during training were assessed using a repeated measures one-way ANOVA with multiple pairwise comparisons post hoc analysis (Holm-Sidak method). Correlations are reported as a Pearson’s r.

**Results**

Figure 1 illustrates representative cumulative records from individual animals superimposed over modeled brain cocaine concentrations obtained using group data from each self-administration procedure. For all cumulative records, diagonal ticks depict when a cocaine injection was self-administered. The cumulative records move vertically for each response and horizontally as session time changes. Figure 1a shows the cumulative record from one rat (T117) reaching a final ratio of 188 on a PR schedule superimposed over the average brain cocaine concentrations from a
group of rats (n=8) reaching the same final ratio. Figure 1b shows the cumulative record from one rat (E264) reaching a Pmax of 133.9 resp/mg in the within-session threshold procedure superimposed over the average brain cocaine concentration from a group of rats (n=9) reaching the same Pmax. Figure 1c shows the cumulative record from one rat (E322) superimposed over the average brain cocaine concentration from a group of rats (n=50) responding on a hold-down schedule of reinforcement.
**FIGURE 1**

Representative cumulative records superimposed over modeled brain cocaine concentrations occurring in three different self-administration procedures. (a) PR schedule: A cumulative record from an individual rat responding under a PR schedule superimposed over the modeled brain cocaine concentrations from a group of rats reaching the same final ratio under the same conditions. (b) Within-session threshold procedure: A cumulative record from an individual rat responding in the within-session threshold procedure superimposed over the modeled brain cocaine concentrations from a group of rats reaching the same Pmax under the same conditions. (c) Hold-down schedule: A cumulative record from an individual rat responding under a hold-down schedule superimposed over the modeled brain cocaine concentrations from a group of rats responding under the same conditions.
FIGURE 1A

Modeled Brain Cocaine Concentrations (µM)

Time (Minutes)
Figure 1B

Modeled Brain Cocaine Concentrations (µM)

Time (Minutes)
FIGURE 1C

Modeled Brain Cocaine Concentrations (µM) vs. Time (Minutes)
Figure 2 illustrates how the dependent measures Pmax, Omax and consumption were determined in the present study. An individual animal’s daily response (left y-axis) and intake (right y-axis) data are plotted as a function of unit-price (responses/mg of cocaine; bottom x-axis) and unit-dose (top x-axis). The maximal price paid (Pmax) for cocaine (see arrow and dotted line) was determined by assessing the unit-price corresponding to the apex of the price response function as previously described (Oleson and Roberts, 2009; Espana, et al 2009). Note that this point also corresponds to the final unit-price at which cocaine intake is maintained, or the point at which demand shifts from being inelastic to elastic. Maximal response output (Omax) for cocaine (see gray rectangle) was reported as the total number of responses occurring in the final 10 minute bin at which Pmax occurred. Cocaine consumption (see ellipse) was determined by averaging cocaine intake across unit-prices 4.2-13.3 resp/mg as previously described (Espana, et al 2009).
**Figure 2**

Demonstration of $P_{\text{max}}$, $O_{\text{max}}$ and consumption determinations. An individual animal’s daily response (left y-axis; open squares) and intake (right y-axis; filled circles) data, obtained using the within-session threshold procedure, are plotted as a function of unit-dose (top x-axis) and unit-price (bottom x-axis). In this procedure, the available cocaine dose decreases every 10 minutes resulting in a reciprocal increase in the unit-price of cocaine. The maximal price paid for cocaine, or $P_{\text{max}}$ (75 resp/mg; arrow), is graphically distinguishable as the apex of the price-response function. Note that this point coincides with the unit-price at which the maintenance of cocaine intake begins to rapidly decline in the consumption-price function (dotted line). Maximal response output, or $O_{\text{max}}$ (48 resp/10min; gray rectangle) was calculated by assessing the rate of responding during the 10 minutes bin (or unit-price) at which $P_{\text{max}}$ was determined. The level of cocaine consumption that the animal is titrating around when intake is minimally constrained by price (0.68 mg; black ellipse) is calculated by averaging cocaine intake (mg/10min) across unit-prices 4.2-13.3.
**Figure 2**

- **Consumption** = 0.68 mg
- **Pmax** = 75 resp/mg
- **Omax** = 48 resp

Graph showing the relationship between cocaine dose and responses per 10-min bin, with consumption and unit-price on the y-axis and cocaine dose on the x-axis.
Changes occurring during a 14-day history of cocaine self-administration under a PR schedule of reinforcement are illustrated in figure 3. Figure 3a shows the mean daily final ratio reached for cocaine under a PR schedule over 14 days of training. As previously reported (Morgan, et al 2006; Liu, et al 2005b; Oleson and Roberts, 2009), PR training resulted in a significant increase in final ratios across sessions ($F_{(6,78)} = 4.475, \ p<0.01$). Figure 3b shows daily cocaine intake by rats responding on a PR schedule over 14 days of training. PR training resulted in a significant increase in cocaine intake across sessions ($F_{(6,78)} = 7.417, \ p<0.01$).
**Figure 3**

PR training increases the reinforcing strength of cocaine. The effects of PR training (filled circles) are illustrated over 14 days. Data are expressed as the mean (± SEM). (a) PR training resulted in final ratios increasing across sessions. (b) PR training resulted in daily cocaine intake increasing across sessions.
**Figure 3A**

![Graph showing the relationship between days and final ratio](image)

Days: 0, 2, 4, 6, 8, 10, 12, 14

Final Ratio: 50, 77, 118, 178, 268, 402, 603, 901

Days vs. Final Ratio graph with data points and error bars.
Figure 3B
Changes occurring during a history of cocaine self-administration in the within-session threshold procedure are illustrated in figure 4. Figure 4a shows the mean Pmax reached for cocaine in the within-session threshold procedure over 14 days of training. Pmax values significantly changed across sessions \( (F_{6,78} = 2.178, p < 0.01) \). Holm-Sidak post hoc analysis for pairwise comparisons revealed that Pmax was significantly higher on day 1 in comparison to day 6 \( (t=3.740) \), day 7 \( (t=3.913) \) and day 8 \( (t=3.954) \). Figure 4b shows daily cocaine intake by rats responding in the within-session threshold procedure over 14 days. Cocaine intake did not significantly change across sessions \( (F_{6,78} = 1.621; \text{n.s.}) \).
**FIGURE 4**

Within-session threshold training does not increase the reinforcing strength of cocaine. The effects a 14-day history of responding for cocaine in the within session threshold procedure are shown (filled triangles). Data are expressed as the mean (± SEM). (a) Training in the within-session threshold procedure did not result in maximal price paid for cocaine increasing across sessions. (b) Training in the within-session threshold procedure did not result in daily cocaine intake increasing across sessions.
**Figure 4A**

A line graph showing the daily intake (mg) of a substance over 14 days. The y-axis represents Pmax values, ranging from 23.7 to 750 mg, with data points marked at regular intervals. The x-axis represents days, from 0 to 14.

- Days: 0, 2, 4, 6, 8, 10, 12, 14
- Pmax values: 23.7, 39.9, 75, 133.9, 241.9, 416.7, 750 mg
Figure 4B
Changes occurring during a 14-day history of cocaine self-administration under a hold-down schedule of reinforcement are illustrated in figure 5 and supplementary figure S1. Figure 5a shows the mean duration of hold-down responses over 14 days of training. Hold-down times significantly increased across sessions ($F_{(6,78)} = 4.843, p<0.01$). Figure 4b shows daily cocaine intake by rats responding on a hold-down schedule over 14 days of training. Hold-down training resulted in a significant increase in cocaine intake across sessions ($F_{(6,78)} = 3.326, p<0.01$). Holm-Sidak post hoc analysis for pairwise comparisons revealed that cocaine intake was lower on day 1 (eg. $t=5.142$ vs day 12) and 2 (eg. $t=3.644$ vs. day 12), but remained stable after day 2. Supplemental figure S1 shows session response rate for cocaine maintained under a hold-down schedule over 14 days of training. Session response rate significantly decreased across sessions ($F_{(6,78)} = 1.883, p=0.045$).
FIGURE 5

Hold-down training results in rats learning to maximize their behavioral utility. The effects a 14-day history of responding for cocaine under a hold-down schedule are shown (filled triangles). Data are expressed as the mean (± SEM). (a) Training under a hold-down schedule resulted in rats increasing the duration of hold-down responses, thereby increasing the average unit-injection dose across session. (b) Despite the increase in the unit-injection dose per response, cocaine intake remained stable after the second day of training.
The results of PR, within-session threshold, and hold-down training are illustrated in figure 6 and supplementary figure S2. In all figures, the open bar represents the PR trained group (PR), the coarse bar represents the within-session threshold trained group (W/IN), and the filled bar represents the hold-down trained group (HD). Figure 6a shows final ratios determined from the three training groups. There was a significant effect of training history on final ratio ($F_{(2,18)} = 4.577$, $p=0.025$). Holm-Sidak post hoc analysis revealed the PR group reached significantly higher final ratios in comparison to either the within-session threshold group ($t=2.648$) or the hold-down group ($t=2.592$). Figure 6b shows Pmax valued determined from the three training groups. There was a significant effect of training history on Pmax ($F_{(2,18)} = 5.954$, $p=0.010$). Holm-Sidak post hoc analysis revealed the PR group reached significantly higher Pmax values in comparison to either the within-session threshold group ($t=2.752$) or the hold-down group ($t=3.179$). Figure 6c shows Omax valued determined from the three groups. There was a significant effect of training history on Omax ($F_{(2,18)} = 12.712$, $p<0.01$). Holm-Sidak post hoc analysis revealed the PR group reached significantly higher Omax values in comparison to either the within-session threshold trained ($t=4.135$) or the hold-down group ($t=4.566$). Figure 6d shows cocaine consumption determined from the three training groups. Training history did not effect cocaine consumption ($F_{(2,18)} = 1.230$, n.s.). Supplementary figure S2 shows total session response rate occurring on the first day of training for the three groups. There was not a significant effect of self-administration procedure on session response rate ($F_{(2,18)} = 2.947$, n.s.).
**FIGURE 6**

The effects of three different training histories on final ratios, Pmax, Omax and consumption are shown. Data are expressed as the mean (± SEM). PR trained (PR) animals are represented by the open bar. Within-session threshold trained (W/IN) animals are represented by the coarse bar. Hold-down trained (HD) animals are represented by the filled bar. Asterisks indicate significant differences between groups (p<0.05) (a) PR training increased final ratios for cocaine in comparison to HD and W/IN trained rats. (b) PR training increased Pmax for cocaine in comparison to HD and W/IN trained rats. (c) PR training increased Omax in comparison to HD and W/IN trained rats. (d) Cocaine consumption was similar following all training histories.
**Figure 6A**

[Graph showing final ratios for PR, W/IN, and HD conditions.]

- **Final Ratio**
  - PR: 901
  - W/IN: 603 (Starred)
  - HD: 268

*Note: The graph illustrates the final ratios for different conditions, with the highest value for PR and the lowest for HD, marked by a star to indicate statistical significance.*
**FIGURE 6B**

![Graph showing Pmax (resp/mg) for PR, W/IN, and HD]

- **Pmax (resp/mg)**
  - PR: 417
  - W/IN: 242
  - HD: 134

- Final Ratio:
  - PR: 77
  - W/IN: 118
  - HD: 178

Note: The graph indicates a significant difference at the 95% confidence level (asterisk).
Figure 6C

Omax (responses at Pmax)

<table>
<thead>
<tr>
<th></th>
<th>PR</th>
<th>W/IN</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Indicates statistical significance.
**Figure 6D**

![Bar chart showing consumption (mg) for different groups: PR, W/IN, HD. The bars for PR and W/IN are close in height, while the bar for HD is significantly lower.](image-url)
The data from the 21 rats reported in figure 6 were further analyzed to test for the homogeneity of the dependent measures final ratio, Pmax, Omax and consumption. These correlation analyses are illustrated in supplementary figures 3 and 4. The relationships between the dependent measures of reinforcement strength Pmax, Omax and final ratio are illustrated in supplementary figure S3. Figure S3a shows the relationship between Omax and Pmax. Omax was found to positively correlate with Pmax (r=0.914). Figure S3b shows the relationship between final ratio and Pmax. Final ratio was found to positively correlate with Pmax (r=0.488). Figure S3c shows the relationship between Omax and final ratio. Omax was found to positively correlate with final ratio (r=0.496). The relationships between the three dependent measures of reinforcement strength (final ratio, Pmax and Omax) and consumption are illustrated in supplementary figure S4. Figure S4a shows the relationship between final ratio and consumption. No significant relationship was found between final ratio and consumption (r=-0.320). Figure S4b shows the relationship between Pmax and consumption. No significant relationship was found between Pmax and consumption (r=0.318). Figure S4c shows the relationship between Omax and consumption. No significant relationship was found between Omax and consumption (r=0.415).

The models of brain cocaine concentrations and changes in dopamine uptake inhibition (Apparent Km) are illustrated in figure 7 and supplementary figure 5. Figure 7a shows modeled brain cocaine concentrations as a function of time for the three self-administration procedures. Figure 7b shows modeled levels of dopamine uptake inhibition as a function of time for the three self-administration procedures.
Supplementary figure S5a shows that the modeled brain cocaine concentrations were not significantly different from the levels of dopamine uptake inhibition for the PR schedule ($F_{(1,159)} = 1.054$, n.s.). Supplementary figure S5b shows that the modeled brain cocaine concentrations were not significantly different from the levels of dopamine uptake inhibition for the within-session threshold procedure ($F_{(1,159)} = 1.049$, n.s.). Supplementary figure S5c shows that the modeled brain cocaine concentrations were not significantly different from the levels of dopamine uptake inhibition for the hold-down schedule ($F_{(1,159)} = 1.074$, n.s.).
Regulation of cocaine intake is influenced by changing brain cocaine concentrations, which can also be described as fluctuations in dopamine uptake inhibition. Mathematical models of brain cocaine concentrations (a) and changes in dopamine uptake inhibition (apparent $K_m$; b) are shown for animals responding under a PR schedule (PR; light gray function), in the within-session threshold procedure (W/IN; black function), and under a hold-down schedule (HD; dark gray function).
Figure 7A

Modelled Brain Cocaine Concentrations (µM) vs. Time (Minutes)

- Modeled Brain Cocaine Concentrations
- Time (Minutes)
- Modeled Apparent Km (nM)

Graph showing modeled brain cocaine concentrations and apparent Km values over time for different conditions (HD, PR, W/IN).
Figure 7B
**DISCUSSION**

In the present study we confirmed our previous findings that final ratios increase over time when animals are provided with access to high unit-injection doses of cocaine under a PR schedule. (Liu, *et al* 2005b; Morgan, *et al* 2006; Oleson and Roberts, 2009). Importantly, the behavioral price paid for cocaine only increased under conditions in which an interaction between large brain cocaine fluctuations and patterns of maximal responding was present, which suggests this interaction is critical to observe an increase in the motivation for cocaine. Here, we allowed animals to self-administer cocaine under three very different protocols. We selected parameters so that session response rate was similar across conditions at the beginning of training (supplementary figure S2), but unique interactions between the temporal pattern of responding and brain cocaine fluctuations occurred (figure 1). It was found that increases in final ratios, Omax and Pmax, three heterogeneous measures of reinforcement strength (see supplementary figure S3), were most robust in animals exposed to a PR schedule (in confirmation of Liu, *et al* 2005b; Morgan, *et al* 2006), the only training history in which large brain cocaine fluctuations occur during periods of maximal response output. These data suggest that the behavioral change in the effort an animal might expend for cocaine is influenced by both the pattern of brain cocaine fluctuations during the session and the temporal characteristics of the behavioral response. To study the interaction between brain cocaine fluctuations and patterns of responding, we applied mathematical models that capably predict brain cocaine changes occurring during self-administration. The application of these models allowed us to examine the role
of fluctuating brain cocaine levels and levels of dopamine uptake inhibition in the regulation of cocaine intake using different self-administration procedures. The models revealed that under all tested conditions rats maintain an upper level of brain cocaine until the defense of this level becomes confounded by price at a lower level, which is associated with the cessation of responding.

Three factors that were previously determined to influence whether final ratios increase over time are unit injection dose, speed of drug injection and training history. Final ratios only increase over time when rats are given access to a high cocaine dose (eg. 1.5 mg/kg/inj; Liu, et al 2005b). It should be noted that high doses are also preferred by rats in choice procedures and support higher final ratios under a PR schedule (Ward, et al 2005). The observations that high cocaine doses have the greatest reinforcing strength in PR and choice procedures, and are more likely to facilitate an increase in final ratios over time, suggests that high cocaine doses are most likely to cause a transition through the addiction process. The speed at which cocaine is delivered affects the development of drug-related behaviors such as behavioral sensitization and increased final ratios over time. For example, it has been shown that the expression of behavioral sensitization and the accompanying neural adaptations are most robust when cocaine is delivered rapidly (Samaha, et al 2002; Samaha, et al 2004). Likewise, only rapidly (5s vs 25s or 50s) delivered cocaine produces an increase in final ratios over time under a PR schedule (Liu, et al 2005b). Taken together, these studies suggest that rapidly delivered high bolus cocaine injections are most likely to push an animal through the addiction process. An increase in final ratios over time is also influenced by the pharmacological
history of the animal. For example, we have shown that high dose training procedures that result in an increase in the rate of cocaine intake across sessions can prevent an increase in final ratios (Morgan, et al 2006; Oleson and Roberts, 2009; Liu, et al 2005a). These data suggest that more is not necessarily better, and the timeline along which drug exposure occurs is a critical factor in the transition through the addiction process.

A unique feature of the progressive ratio schedule is that peak response rate co-occurs with large brain cocaine fluctuations. Although self-administration maintained by high doses of cocaine under an FR1 schedule results in large brain cocaine fluctuations (Nicola and Deadwyler, 2000; Ahmed and Koob, 1999), a behavioral history of daily limited-access (1-2 hr) to cocaine under an FR1 schedule does not change the effort rats expend for cocaine (Paterson and Markou, 2003; Wee, et al 2009; Oleson and Roberts, 2009). One possibility, however, is that large brain cocaine fluctuations during periods of maximal response output are necessary to produce changes in the behavioral price animals pay for cocaine. To test this, we used three different self-administration procedures that engender high rates of responding but involve different interactions between the pattern of the behavioral response and brain cocaine fluctuations (figure 1). Importantly, only the PR schedule produced increases in the maximal price paid for cocaine (Pmax), the maximal response output for cocaine (Omax) and final ratios (figure 6 a-c). These findings suggest that the increased price paid for cocaine shown by PR trained rats in not merely due to an operant history of high rate responding. Instead, these findings suggest that a critical interaction between brain cocaine fluctuations and
the temporal pattern of responding is necessary to produce an increase in the time and effort animals devote to self-administer cocaine.

In comparison to the PR schedule, the within-session threshold procedure produces a different interaction between the pattern of behavioral responding and fluctuating brain cocaine levels. The within-session threshold and PR schedules are similar in that they both produce comparable rates of responding immediately following acquisition (supplementary figure S2). Moreover, the two procedures result in animals responding maximally, the point at which Pmax or final ratio is reached, at a specific time in each session (cf. figure 1a and b). However, the interaction between the timing of this maximal behavioral response and brain cocaine levels is quite different between the two procedures. Whereas large brain cocaine fluctuations occur throughout the duration of responding during a PR session (Nicola and Deadwyler, 2000; figure 1a and 7), similar fluctuations only occur during the beginning of a threshold session (figure 1b and 7), a period when the available unit dose is high and the behavioral response is low. At the time at which peak responding occurs in the within-session threshold procedure – these brain cocaine fluctuations are absent, and instead, a stable lower level of brain cocaine is observed. Unlike rats trained under the PR schedule, exposure to the within-session threshold procedure did not produce an increase in final ratios, Pmax, or Omax (figure 6a-c). On the contrary, an initial decrease in the price paid (Pmax) for cocaine was observed at the beginning of training (figure 4a), an effect which might be explained by the animals learning about the contingencies of the procedure, changes in extinction responding or perhaps a combination of both.
Under a hold-down schedule high response rates are consistently observed as rats maintain a stable brain cocaine level throughout each session, but the behavioral price paid for cocaine does not increase under these conditions. The hold-down schedule is unique in that rats are given continuous access to cocaine and allowed to determine the dosage administered by manipulating the duration of each response. Under these conditions, session response rate was similar to that observed under a PR schedule at the onset of training (supplementary figure S2). However, the interaction between the pattern of the behavioral response and brain cocaine fluctuations is unique in comparison to the PR schedule (cf. figure 1a and 1c; figure 7). It was found that after an initial loading period (approximately 10-min) a stable upper level of brain cocaine is maintained for the duration of the hold down session (figure 7). Therefore, fluctuating brain cocaine levels are absent under these conditions as is a period at which a maximal behavioral response occurs. These conditions did not result in an increase in final ratios, Pmax, or Omax in comparison to PR trained rats (figure 6a-c). The inability of these conditions to increase the behavioral price paid for cocaine further supports the importance of an interaction between large fluctuations in brain cocaine during periods of high response output to produce increases in the effort expended to self-administer cocaine over time. Although the behavioral price paid for cocaine did not increase following a history of responding under a hold-down schedule, overall changes in behavior were observed. It was found that response rate significantly decreased (supplementary figure S1) over training, whereas intake remained constant after the first two days (figure 5b). This latter finding is explained by rats beginning to hold down the lever
for longer pump durations over the course of training (figure 5a). The increased hold-down times suggest that under these conditions, rats learn to maximize their behavioral utility and begin to pay lower prices (responses/unit-injection dose) for cocaine while efficiently regulating a preferred level of cocaine intake.

Changes in the behavioral price paid for cocaine are dissociable from changes in cocaine consumption. A growing body of literature suggests that cocaine intake can increase over time without increasing dependent measures commonly associated with reinforcement strength, such as the preference for cocaine in choice studies (Banks and Negus, 2010), final ratios for cocaine in PR studies (Liu, et al 2005a), or the maximal price paid for cocaine in behavioral economics studies (Oleson and Roberts, 2009). Likewise, the behavioral price an animal might pay for cocaine can increase over time without producing increases in cocaine consumption (Oleson and Roberts, 2009). In the present study we show that three distinct dependent measures of reinforcement strength (ie. final ratio, Pmax and Omax) (Bickel, et al 2000; supplementary figure 3) increase independently of consumption following PR training (figure 6a-d). To further assess the relationship between consumption and price paid, each individual animal's average level of cocaine consumption was compared to their average final ratio, Pmax and Omax. It was found that consumption was not correlated with any of these values of reinforcement strength (supplementary figure 4), further supporting the conclusion that the concepts of consumption and price paid are dissociable. The implication of this dissociation is that cocaine consumption and price paid for cocaine are regulated by distinct neural mechanisms. It is likely, therefore, that as the addiction...
process progresses the dysregulation of cocaine intake can develop independently from an increased motivation for drug, and these changes in behavior involve separate neural adaptations.

In studying the interaction between patterns of behavioral responding and brain cocaine fluctuations, mathematical models were applied that allowed for a second question to be addressed: What is the role of brain cocaine concentrations in the regulation of cocaine intake under these difference self-administration procedures? It was found that during cocaine self-administration animals maintain an upper brain cocaine level, while a lower brain cocaine level is associated with the cessation of responding. The maintenance and regulation of cocaine intake has been previously theorized to be controlled by mechanisms related to: satiation (ie. an attempt to maintain an optimal drug level), direct drug effects (ie. a drug-induced disruption in responding), aversive effects (ie. punishing effect of high drug level) or stimulus-control (ie. interoceptive effects signal when cocaine would function as reinforcer) (Lynch and Carroll, 2001; Tsibulsky and Norman, 1999; Panlilio, et al 2008). The finding that an upper brain cocaine level is maintained under the hold-down schedule, and early in the within-session threshold procedure (figure 7), suggests that the regulation of cocaine intake is not solely controlled by satiety mechanisms. That is, if the regulation of cocaine intake were primarily controlled by a satiety threshold, then only a lower level of brain cocaine should be maintained under all access conditions. The rate disrupting effects (ie. direct drug effects) of cocaine also can not independently explain the upper brain cocaine level observed in the current study because rats continue to respond for brain stimulation reward
during inter-injection pauses during cocaine self-administration (Wise, et al 1977). One possibility regarding the occurrence of the upper brain cocaine level is that aversive interceptive stimulus effects associated with high brain cocaine levels produce a ceiling on drug intake (Roberts and Zito, 1987). Likewise, interceptive stimulus effects associated with the positive reinforcing effects of cocaine might trigger rats to respond between the upper and lower levels of brain cocaine observed across all access conditions in the current study (figure 7). It is also important to note that similar, lower brain cocaine levels are associated with the cessation of responding under both the PR and within-session threshold procedures (figure 7). The maintenance, or defense, of this lower brain cocaine level might contribute to the determination of the behavioral price an animal might pay (ie. when final ratio or Pmax occurs) for cocaine under these high effort schedules. However, the brain cocaine level associated with the cessation of responding in the PR and within-session threshold procedures was not found to be identical. As illustrated in figure 7, brain cocaine levels fall to a lower level during responding maintained under a PR schedule in comparison to responding maintained on the within-session threshold procedure. This could be due to differences between the behavioral histories of the two groups or the self-administration procedures themselves.

The brain cocaine levels that are highly correlated with responding during cocaine self-administration can be described as levels of dopamine uptake inhibition. We previously used a neurochemical approach to demonstrate that rapidly fluctuating levels of dopamine uptake inhibition are closely associated with
the rate and pattern of cocaine self-administration (Oleson, et al 2009). We further developed, and experimentally validated a model that quantitatively describes these rapidly changing levels of dopamine uptake inhibition (Oleson, et al 2009). In the current study we present this model (figure 7b and supplementary figure 5) along with a previously accepted model (figure 7a; Pan, et al 1991) that describes brain cocaine levels following cocaine administration to demonstrate the validity of our new model and to show that the brain cocaine levels commonly referred to when describing the regulation of cocaine intake (Pickens and Thompson, 1968; Lynch and Carroll, 2001; Ahmed and Koob, 1999; Tsibulsky and Norman, 1999) can be accounted for by levels of dopamine uptake inhibition.

**ACKNOWLEDGEMENTS**

The authors would like to thank Leanne Thomas for providing technical assistance. This work was supported by Wake Forest University Cross-Campus Collaborative Fund Award and National Institute of Health Grants F31DA024525 (EBO), DA021634 (EAB), and R01DA140309 (DCSR).
REFERENCES


Oleson EB, Roberts DC (2009). Behavioral economic assessment of price and cocaine consumption following self-administration histories that produce escalation of either final ratios or intake. *Neuropsychopharmacology* **34**: 796-804.


SUPPLEMENTARY FIGURE S1

Hold-down training results in rats learning to maximal their behavioral utility. The effect of a 14-day history of responding for cocaine under a hold-down schedule on the rate of responding is shown (filled circles). Data are expressed as the mean (± SEM). As reported in the manuscript, hold-down training results in animals increasing the duration of lever hold-down times (figure 5a) while maintaining a stable level of cocaine intake across sessions 3-14 (figure 5b). As the animals begin to hold down the level for extended durations and take larger unit-injections doses of cocaine, a significant decrease in response rate is across sessions also occurs ($F_{(6,78)} = 1.883$, $p=0.045$). Taken together, these data suggest that the animals are learning to respond less to receive the same amount of cocaine across session, and thereby maximize their behavioral utility.
SUPPLEMENTARY FIGURE S1
Session responding occurring on the first day of self-administration training under three different schedules of reinforcement (PR: Progressive ratio; W/IN: within-session threshold procedure; HD: Hold-down) are shown. We designed the current study so that the pattern of the behavioral response rather than total response rate would be a critical independent variable at the onset of training. Based on preliminary studies, session time and the available cocaine concentration were manipulated so that overall session response rate would be similar at the beginning of training under all schedules. As was expected, total session responding was not significantly different between the three schedules of interest on the first day of self-administration training $F_{(2,18)} = 2.947; \text{n.s.}$). These data support that the critical different between responding at the beginning of training was not the overall rate of responding, but rather the pattern of the behavioral response as illustrated by the cumulative records (figure 1 in manuscript).
**SUPPLEMENTARY FIGURE S2**

Day 1 Responses per Session

- **PR**
- **W/IN**
- **HD**

Day 1 Responses per Session range from 0 to 400.
SUPPLEMENTARY FIGURE S3A-C

The dependent measures final ratio, the maximal price paid (Pmax) and the maximal behavioral output at Pmax (Omax) are shown in relationship to one another. All three values have been argued to measure different aspects of reinforcement strength (Bickel, et al 2000). Therefore, the similarity of these values was compared using all rats from the present study. All data reported in the analyses shown in figure 6 (A-C) were used for these correlations. It was found that all three dependent measures were positively related. Pmax for cocaine was found to correlate with the Omax ($r_{21} = .91 \ p < 0.01$), Pmax was found to correlate with final ratio ($r_{21} = .49; \ p =0.025$), and final ratio was found to correlate with Omax ($r_{21} = .50; \ p < 0.022$). These data suggest that all three measures are related to one another and provide information that is generally related. However, these data also reveal that the values are not perfectly related. For example, Pmax and Omax are much more similar when obtained using the current experimental approach than final ratio and either Pmax or Omax. This supports the argument of (Bickel, et al 2000) that these values, typically used to assess relative reinforcing strength, are all dissociable, and therefore reinforcement strength is a heterogeneous concept. The implication of this finding is that caution should be used when making inferences changes in reinforcement strength, or the behavioral price an animal might pay for a drug, based upon results using a single self-administration approach.
SUPPLEMENTARY FIGURE S3A-C

(a) $r = .91$

(b) $r = .49$

(c) $r = .50$
Here we sought to determine whether a direct relationship exists between the levels of cocaine consumption that rats maintain and the various dependent measures that were used to measure price paid for cocaine in the current study (ie. final ratio, Pmax, or Omax). It was found that consumption was not significantly correlated to any dependent measure of price paid for cocaine. Consumption did not correlate with final ratio ($r_{(21)} = -0.032; p = 0.89$), Pmax ($r_{(21)} = .32; p = 0.16$), or Omax ($r_{(21)} = .42; p = 0.06$). These data suggest that the concepts of consumption and price paid are dissociable concepts (in confirmation of our previous studies (Oleson and Roberts, 2009; Liu, et al 2005b). The implication of this dissociation is that changes in consumption over time must not necessarily translate into changes in price paid for drug and vice versa. Moreover, caution should also be used in making broad conclusions regarding reinforcement from studies using self-administration procedures that provide information about only consumption or price paid.
SUPPLEMENTARY FIGURE S4A-C

(a) $r = -0.32$

(b) $r = 0.32$

(c) $r = 0.42$
**SUPPLEMENTARY FIGURE S5A-C**

Fluctuations in brain cocaine concentrations can be described as changes in dopamine uptake inhibition. We have previously (Oleson, et al 2009) demonstrated that the rate and pattern of cocaine self-administration is closely associated with changes in dopamine uptake inhibition. Moreover, we developed a computational model that capably predicts changes in dopamine uptake inhibition during cocaine self-administration based on the dosage available and the timing between injections. Here, we demonstrate that our new model (illustrated here in gray) provides similar predictions of brain cocaine dynamics during self-administration in comparison to an accepted model of brain cocaine fluctuations (illustrated here as a black function; Pan et al 1991). In order to compare the two models, predicted brain cocaine concentrations and levels of dopamine uptake inhibition were compared at every minute along a 160-min time course occurring in three different self-administration procedures. Figure S5a shows that the modeled levels of dopamine uptake inhibition are similar to those predicted by the accepted model of brain cocaine concentrations ($F_{(1,159)} = 1.054$, n.s.) using data from a PR schedule. Figure S5b shows that the modeled levels of dopamine uptake inhibition are similar to those predicted by the accepted model of brain cocaine concentrations ($F_{(1,159)} = 1.049$, n.s.) using data from the within-session threshold procedure. Figure S5a shows that the modeled levels of dopamine uptake inhibition are similar to those predicted by the accepted model of brain cocaine concentrations ($F_{(1,159)} = 1.074$, n.s.) using data from a hold-down schedule. These findings support the validity of our new model designed to predict changes in dopamine uptake inhibition occurring during cocaine self-administration, and suggest that the brain cocaine levels referred to when considering the regulation of cocaine intake can be described as levels of dopamine uptake inhibition.
SUPPLEMENTARY FIGURE S5A-C

(a) 
Modulated Brain Cocaine Concentrations (µM) vs. Time (Minutes)

(b) 
Modulated Brain Cocaine Concentrations (µM) vs. Time (Minutes)

(c) 
Modulated Brain Cocaine Concentrations (µM) vs. Time (Minutes)
CHAPTER VI

DISCUSSION

Erik B. Oleson
**General Summary**

Cocaine addiction is a serious social, medical and financial problem in modern society. Although historically regarded as a moral failing, a growing body of scientific evidence supports the view that drug addiction is actually a neuropsychiatric disorder involving changes in both brain and behavior (Cami and Farre, 2003; Gawin, 1991; Kalivas and Volkow, 2005). This perspective shift resulted in neuroscientists and behavioral pharmacologists alike developing an interest in how the brain and/or animal behavior changes during repeated exposures to an abused drug. As a result, many ongoing collaborative studies are devoting a great deal of time, energy and money in an attempt to understand the neurobiological basis of changes in addiction-like behavior using animal models. One fundamental question that must be addressed for such studies to be considered valid, however, is exactly how are these drug-induced changes in behavior observed in experimental animals related to the addiction process?

The studies presented herein suggest that investigators are tapping into multiple aspects of the addiction process using animal models. Our ability to discriminate at least two specific components of the addiction process will help direct scientists toward the specific neurobiological mechanisms involved in these distinct aspects of addiction. The two aspects that were addressed throughout all chapters of this dissertation are cocaine consumption and the price paid for cocaine. It is commonly assumed that changes in consumption will result in changes in price paid and *vice versa*. This assumption is particularly true in regard to self-administration models of addiction (Robinson, 2004; Ahmed, 2005). It might be
logical to reason; therefore, that if animals increase cocaine intake over time, and changes in cocaine intake co-occur with changes in the price paid for cocaine, then these same animals should also show an increase in the price paid for cocaine. If, however, these changes in behavior were found to be dissociable, the implication would be that there are multiple components of the addiction process, each involving distinct neural adaptations.

CHAPTER 1

A brief description of various models of addiction is provided from the perspective that each is unique and addresses a specific aspect of the addiction process.

Chapter one presents a review of the literature on animal models of addiction and specifically addresses how the various models are related to one another. Evidence is presented to support the theory that multiple components of the addiction process exist and can be addressed using distinct training procedures. Four main behavioral phenomena used to study the addiction process are covered, including an escalation in the price paid for cocaine (ie. PR training), the development of behavioral sensitization, an escalation in cocaine consumption (ie. LgA training) and increased cue-induced reinstatement responding (ie. incubation of craving). Importantly, evidence is presented showing that each of these behavioral phenomena can be dissociated from one another.
The realization that the common animal models used to study drug addiction are behaviorally distinct is an important, yet contentious, topic in the field. For example, it has been argued that increased cocaine consumption is due to the development of behavioral sensitization (Robinson and Berridge, 2001; Ferrario, et al 2005), although most empirical evidence it to the contrary (Ben Shahar, et al 2004; Ben Shahar, et al 2005). Others have suggested that the development of behavioral sensitization is the cause of animals paying higher prices for cocaine (Vezina, 2004; Vezina, et al 2002; Suto, et al 2002), although animals that pay a high price for cocaine do not necessarily show a sensitized behavioral response to cocaine (Lack, et al 2008). Another popular theory is that increased drug seeking behavior and reinstatement responding occurs due to the development of behavioral sensitization (Vanderschuren and Kalivas, 2000; De Vries, et al 1998; Vezina and Leyton, 2009) although, once again, multiple reports show that these two concepts are dissociable (Knackstedt and Kalivas, 2007; Ahmed and Cador, 2006). Finally, conflicting reports exist as to whether an escalation of cocaine intake produces an increase in the price paid to self-administer cocaine (cf. Paterson and Markou, 2003; Liu, et al 2005a).

**CHAPTER 2**

*The dissociation of two of these behavioral phenomena, an escalation in cocaine consumption and an escalation in price paid, was demonstrated using a newly developed self-administration approach.*
A new self-administration procedure was developed to test how cocaine consumption and price paid for cocaine change in two distinct addiction models. In keeping with many previous studies (Pickens and Thompson, 1968; Wilson, et al 1971), our newly developed self-administration procedure shows that animals adjust their rate of cocaine intake according to the dosage offered. As the available unit-dose approaches a minimally reinforcing dose (ie. threshold dose), remarkably high rates of responding are observed. Thus, animals appear to maintain a stable level of drug consumption across a wide range of unit-prices (ie. responses/mg of cocaine) as occurs with many commodities – such as gasoline, in a market economy – until a maximal price is reached. After this maximal price, or Pmax, has been passed cocaine consumption is no longer maintained. In behavioral economic terms, this is the point at which demand for cocaine transitions from being inelastic to elastic. An animal’s preferred level of cocaine consumption is determined by measuring cocaine intake at a low price. The maximal price an animal pays for cocaine is determined by measuring the highest unit-price that the animal pays to maintain their preferred level of consumption. This new self-administration approach provides a technique grounded in economic concepts that have been accepted by the field of behavioral pharmacology (Wade-Galuska, et al 2007a; Hursh, et al 2005; Woolverton and English, 1997) while catering to the needs of neuroscientists. For example, recent advances in neuroimaging (Sanfey, et al 2003) and in vivo neurochemistry (Gan, et al 2009) have allowed scientists to investigate the neural substrates involved in cost/benefit analyses and decision-making. Many future experiments could be designed to assess how these neural systems might
change in parallel with behavioral changes in cocaine consumption or the price paid for cocaine.

Two distinct self-administration training procedures have been developed to model the addiction process that produce either an increase in cocaine consumption or the price paid for cocaine over time. Ahmed and Koob (1998) developed the long-access training procedure, in which rats show an escalation in the rate of cocaine intake across daily sessions when provided extended (6-12 hr) access to cocaine under an FR1 schedule of reinforcement (Wee, et al 2007). The Roberts’ lab has developed a training procedure in which rats show an increase in the effort expended (ie. final ratios) for cocaine when provided access to a high, rapidly delivered dose of cocaine under a PR schedule of reinforcement (Morgan, et al 2006; Liu, et al 2005b). At question is whether the increase in cocaine consumption observed during long-access training results in an increase in the price paid for cocaine, and whether the increase in the effort expended for cocaine during PR training produces an increase in cocaine consumption?

In both addiction models, the primary behavioral effect (ie. changes in cocaine consumption or price paid) increased independently when assessed using the threshold procedure. That is, an escalation of cocaine consumption was not sufficient to produce an increase in the price paid for cocaine, and an increase in the price paid for cocaine was not sufficient to produce an increase in cocaine consumption. These results show that the behavioral economic concepts of consumption and price paid are dissociable in the context of cocaine self-administration. These findings suggest that increases in cocaine consumption and
price paid for cocaine involve independent neural adaptations. Our ability to parse these two aspects of the addiction process using self-administration models will allow for the investigation of the specific neurobiological changes involved in specific aspects the addiction phenomenon, such as increased consumption over time and increased time and energy devoted to obtain drug.

CHAPTER 3

The behavioral economic concepts of cocaine consumption and price paid for cocaine were further dissociated using a neuropharmacological approach. Next, we determined that not only does the price an animal might pay for cocaine change irrespective of the animal’s preferred level of consumption, but also the neurotransmitter systems that are involved in each of these two aspects of addiction are distinct. In chapter three data are presented describing the interaction between neuropharmacology, cocaine consumption and price paid for cocaine. It was found that different neurotransmitter systems are important in regulating cocaine consumption and price paid. In particular, the neurotransmitters dopamine and serotonin are important in regulating an animal’s preferred level of cocaine consumption. The price an animal pays for cocaine, on the other hand, is regulated by complex neural circuitry involved in motivated behavior. For example, we found that certain drug pre-treatments, which do not decrease rates of food-maintained responding, can decrease rates of cocaine maintained responding exclusively when the behavioral cost to maintain responding is high.
To investigate how drug pretreatments change cocaine consumption and price paid we had to adapt our previously developed threshold procedure to occur within a single session. We achieved this by providing rats with daily access to 11 descending cocaine doses presented in consecutive timed intervals under an FR1 schedule of reinforcement. This adaptation provides several advantages. First, two dependent measures can be examined daily, thereby reducing the attrition rates encountered in lengthy self-administration studies. Second, it allows for the investigation of pharmacological, hormonal and environmental variables that change daily. Third, the interval time during which each dose is presented to the animal can be manipulated to accommodate the pharmacokinetics and pharmacodynamics of specific drug-pretreatments.

A behavioral economic analysis of threshold data following various drug-pretreatments revealed that cocaine consumption and the price paid for cocaine are pharmacologically dissociable. Specifically, it was found that haloperidol, a dopamine receptor antagonist, dose-dependently increased cocaine consumption but decreased the price paid for cocaine. By contrast, acutely administered amphetmaine, a known dopamine release and uptake inhibitor (Jones, et al 1998), increased the price paid for cocaine. The selective serotonin uptake inhibitor fluoxetine decreased both cocaine consumption and the price paid for cocaine. Importantly, the GABA_B receptor agonist baclofen was found to selectively decrease the behavioral price paid for cocaine without affecting cocaine consumption. Likewise, as illustrated in appendix II, the hypocretin-1 receptor antagonist SB334867 decreased the price paid for cocaine without affecting cocaine
consumption. Taken together, these data show that cocaine consumption and the price paid for cocaine are neuropharmacologically dissociable concepts. The implications of these findings are that cocaine consumption and price paid are regulated by independent neural mechanisms, and that interpretations regarding the effects of drug-pretreatments on cocaine self-administration should be made cautiously when these concepts are studied independently.

**CHAPTER 4**

*It was found that the regulation of cocaine consumption is associated with a fluctuating pattern of dopamine uptake inhibition.*

It has long been recognized that animals adjust their rate of cocaine intake as the available unit-injection dose changes in order to titrate around blood/brain levels of cocaine (Lynch and Carroll 2001). As our pharmacological understanding of cocaine increased, the dopamine transporter was identified as the principle site of action responsible for cocaine's positive reinforcing properties (Ritz, et al 1987). It became apparent, therefore, that the brain level that is responsible for controlling the rate of cocaine intake is likely a level of dopamine uptake inhibition. Using fast-scan cyclic voltammetry, a method that offers the temporal and spatial resolution necessary to measure changes in dopamine uptake inhibition, we sought to study the relationship between rapid changes in dopamine uptake inhibition and the regulation of cocaine consumption.
To characterize the minute to minute fluctuations in dopamine uptake inhibition associated with patterns of cocaine consumption, IV cocaine was delivered into anesthetized rats in accordance with rates and patterns of self-administration observed under an FR1 schedule while voltammetric recordings were made in the nucleus accumbens every one-minute. We found that changes in dopamine uptake inhibition occurred in two distinct phases. The first, frequently referred to as a loading phase, was characterized by a rapid increase in dopamine uptake inhibition during approximately the first ten minutes. The second, referred to as a maintenance phase, was characterized by the emergence of a rapidly fluctuating, yet maintained level of dopamine uptake inhibition. These brain cocaine levels appear to be time-locked to the point at which responding occurs, which suggests that rats titrate around a set-point of dopamine uptake inhibition during cocaine self administration reinforced under a fixed ratio schedule. Moreover, it was found that this dopamine uptake inhibition set-point shifts upward using response rates from the long-access escalation model of cocaine addiction.

Obviously, studying neurochemical correlates of behavior is best done in behaving animals. We next, therefore, began attempting to conduct voltammetric recording in freely-moving and behaving rodents. One of our more fruitful attempts involved adapting fast-scan cyclic voltammetry for the freely-moving mouse. The data presented in appendix I, show the first published time-course of cocaine-induced changes in dopamine uptake inhibition and evoked dopamine release per stimulus pulse occurring in freely-moving mice. It was found that in the freely-moving animal, changes in evoked dopamine release paralleled changes in
dopamine uptake inhibition in the presence of cocaine. Two different interpretations, which can not be resolved from the present data, might account for the parallel changes in these two variables. First, the parallel changes in evoked dopamine release and dopamine uptake inhibition could be due to cocaine increasing dopamine release. This interpretation might seem paradoxical because dopamine release should be decreased in the presence of a dopamine uptake inhibitor due to increased dopamine D2 auto-receptor inhibition (Einhorn, et al 1988). However, others have shown that cocaine might actually stimulate vesicular release of dopamine from synapsin dependent stores (Venton, et al 2006). Moreover, even in the absence of external conditioned cues, non-contingently administered cocaine can produce an increased frequency of dopamine transients in the nucleus accumbens (Stuber, et al 2005). Therefore, it remains theoretically possible that the correlation in between evoked dopamine release and dopamine uptake inhibition that was observed in the freely-moving mice is due to an increase in cocaine-induced dopamine release. A second possibility is that the parallel changes in evoked dopamine release and dopamine uptake inhibition could be explained by evoked dopamine release being a completely artificial parameter. That is, the increased dopamine concentrations following electrical stimulation in the presence of cocaine may not reflect increased release at all, but rather simply reflect increased extracellular dopamine concentrations that are a direct result of increased dopamine uptake inhibition. The use of genetically modified freely-moving mice could help resolve this issue. For example: would cocaine still increase evoked dopamine release in freely-moving mice following genetic deletion of the DAT?
Would cocaine still increase evoked dopamine release if this study were repeated in freely-moving mice following genetic disruption of vesicular transport machinery? Indeed, many questions concerning the genetic influences on cocaine-induced changes in dopamine signaling during ongoing behavior, even those involving the role of dopamine in regulating cocaine consumption and price paid, could be addressed by conducting fast-scan cyclic voltammetry in freely-moving mice – a possibility that now exists due to the advances reported in appendix I.

**CHAPTER 5**

Large brain cocaine fluctuations during periods of maximal responding for cocaine was identified as a critical interaction involved in increasing the price paid for cocaine over time.

The observation that the price an animal might pay for cocaine can escalate over time has only recently been reported (Liu, *et al* 2005b) and is still not completely understood. Chapter five focuses on the specific conditions that must be made available to an animal to observe an increase in the price paid for cocaine over time. Previous studies demonstrated that high, rapidly infused doses of cocaine must be provided to observe an increase in the effort expended for cocaine (Liu, *et al* 2005b). The data presented in chapter five builds on these initial findings by demonstrating that the escalation in price paid for cocaine requires a behavioral history of responding for cocaine at maximal rates while experiencing large fluctuating brain cocaine levels.
During PR training the behavioral price paid for cocaine only increases under certain conditions. For example, final ratios only increase when a rapidly delivered high dose of cocaine is made available under a PR schedule (Liu, et al 2005b). However, providing access to the exact same number of rapidly-delivered cocaine injections under an FR schedule does not result in an increase in the behavioral price paid for cocaine (Oleson and Roberts, 2009). Taken together, these observations led us to consider the importance of the interaction between the pattern of the behavioral response (ie. responding under a PR vs. FR schedule) and brain cocaine fluctuations resulting from rapidly-delivered large bolus injections of cocaine.

In order to investigate the necessity of this interaction to produce an increase in the behavioral price paid for cocaine, we took advantage of three different self-administration procedures that provide unique combinations of behavioral response patterns and fluctuating brain-cocaine levels. The three self-administration procedures were: a PR schedule which produces periods of maximal responding during large fluctuations in brain cocaine concentrations, a within-session threshold procedure (Espana, et al 2009) which produces periods of maximal responding during the maintenance of steady brain cocaine concentrations, and a hold-down schedule (Morgan, et al 2009) which produces stable high rates of responding during the maintenance of steady brain cocaine concentrations. It was found that only a behavioral history of cocaine self-administration under a PR schedule produced an increase in the behavioral price paid for cocaine. This suggests that an interaction between the pattern of the
behavioral response and fluctuating brain cocaine levels influences whether the motivation to self-administer cocaine increases over time. Moreover, it was found that this increase in the maximal price paid for cocaine occurred independently from changes in cocaine consumption when all groups were compared after training.

**OVERALL CONCLUSIONS**

Taken together, these studies suggest that the concepts of consumption and price paid are dissociable phenomena that are regulated by distinct neural circuitry. Indeed, these two aspects of cocaine taking behavior were found to be both behaviorally and neuropharmacologically distinct. Importantly, both concepts were found to increase independently of one another during behavioral transitions reminiscent of an addiction process.
REFERENCES


Lack CM, Jones SR, Roberts DC (2008). Increased breakpoints on a progressive ratio schedule reinforced by IV cocaine are associated with reduced locomotor activation and reduced dopamine efflux in nucleus accumbens shell in rats. *Psychopharmacology (Berl)* **195**: 517-525.


Oleson EB, Roberts DC (2009). Behavioral economic assessment of price and cocaine consumption following self-administration histories that produce escalation of either final ratios or intake. *Neuropsychopharmacology* **34**: 796-804.


APPENDIX I

REAL-TIME VOLTAMMETRIC DETECTION OF COCAINE-INDUCED DOPAMINE CHANGES IN THE STRIATUM OF FREELY MOVING MICE

Erik B. Oleson, Jonathan Salek, Keith D. Bonin, Sara R. Jones, and Evgeny A. Budygin

The following manuscript was published by Neuroscience Letters 467(2):144-6, December 2009.
ABSTRACT

In the present voltammetric study, we have characterized cocaine-induced changes in evoked dopamine release and uptake in the striatum of freely moving mice in real time. Cocaine induced marked dopamine uptake inhibition measured as apparent $K_m$ changes, producing a maximal effect 20 minutes following a single injection (15 mg/kg i.p.). Changes in uptake were paralleled by increases in evoked dopamine release per stimulus pulse, revealing a high correlation between these two parameters following cocaine administration. This initial characterization of cocaine effects on striatal dopamine transmission in the commonly used C57BL/6 mouse strain provides a basis for future voltammetric studies using genetic mouse models.

*Keywords: Cocaine, freely-moving mouse, voltammetry, dopamine*
INTRODUCTION

Cocaine increases extracellular dopamine levels by inhibiting the uptake of dopamine through dopamine transporters located on presynaptic terminals (Ritz et al., 1987). Many of the characteristic behavioral effects associated with cocaine, such as psychomotor activation (Sabeti et al., 2002), stereotypic movements (Budygin, 2007) and reinforcement (Oleson et al., 2009) are tightly linked to levels of dopamine uptake inhibition. Although several studies have documented the time-course of cocaine-induced changes in dopamine uptake in vivo using rats (Espana et al., 2008; Budygin, 2007), a time-course of this effect has never been reported in mice.

Advances in molecular biology have allowed the production of many different strains of genetically modified mice, which provide researchers with animal models to study different human diseases, including psychiatric disorders such as drug addiction. These mouse models can help to further clarify the neurochemical mechanisms of addictive drugs. Here, we applied fast-scan cyclic voltammetry to study the time-course of cocaine-induced changes in dopamine uptake and stimulated dopamine release in the striatum of freely moving C57BL/6 mice, a commonly used mouse strain. Our approach clearly demonstrates that real time dopamine measurements can be conducted in freely moving mice, and opens the door to future analyses in transgenic and knockout mice.
METHODS

All voltammetric recordings were performed in freely moving male mice (C57BL/6, 8-12 weeks old, n=5). The experimental protocol adhered to National Institutes of Health Animal Care guidelines and was approved by the Wake Forest University Institutional Animal Care and Use Committee.

Surgery for implantation of a stimulating electrode, a reference electrode and a guide cannula for the micromanipulator was carried out as previously described in rats (Budygin, 2007). Mice were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and placed in a stereotaxic frame. A hole for the guide cannula (Bioanalytical Systems, West Lafayette, IN) was drilled according to coordinates from a mouse brain atlas (AP +1.0, L +1.3 mm from bregma). A Ag/AgCl reference electrode was implanted in the contralateral superficial cortex. A bipolar stimulating electrode was lowered to the VTA/SN area ipsilateral to the guide cannula at 3 mm posterior and 1.0 mm lateral to bregma. A newly designed lightweight micromanipulator (0.95 g) capable of inserting large diameter (1.2-mm) glass capillary carbon-fiber electrodes in the mouse brain through the guide cannula was constructed for this study. The head-mounted voltammetric amplifier (UNC Electronics Design Facility, Chapel Hill, NC) was miniaturized for use with mice. Dopamine was evoked by electrical stimulation of the VTA/SN and monitored in the dorsal striatum using fast-scan cyclic voltammetry. Voltammetric recordings were made at the carbon-fiber microelectrode every 100 ms by applying a triangle waveform (-0.4 to +1.3 V, 300 V/s). Upon establishment of stable baseline signals, stimulation (24 pulses, 60 Hz, 120 µA, 2 ms/phase, biphasic) was applied every 10
minutes for 20 minutes before and 2 hours after cocaine (15 mg/kg, i.p.) administration. Cocaine HCl, obtained from the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, N.C., USA), was dissolved in a solution of sterilized 0.9% saline, passed through a microfilter (0.45 μm pore size) and diluted to a solution of 2.5 mg/ml for these experiments. All statistics were performed using SigmaPlot (version 11). Evoked dopamine levels and uptake parameters were statistically analyzed using an ANOVA with repeated measures. Correlations are reported as Pearson’s r values.

**RESULTS**

Stimulated extracellular dopamine efflux was detected in the dorsal striatum of freely-moving mice as first reported by Yavich and Tiihonen (Yavich and Tiihonen, 2000). As illustrated in Figure 1, electrical stimulation of the VTA/SN area resulted in a rapid increase in striatal extracellular dopamine (~1 µM) prior to cocaine administration. The observed maximal amplitude of the evoked dopamine signal approximately doubled (~2 µM) 10 minutes after a single cocaine injection (15 mg/kg i.p.). Figure 2 shows changes in the parameters of apparent $K_m$ (filled circles) and $DA_p$ (open squares) over a 2 hour time-course after a single cocaine injection (15 mg/kg i.p.). Cocaine resulted in a significant increase in $DA_p$ ($F_{(12,64)} = 7.895; p<0.01$), which reflects the concentration of dopamine released per stimulus pulse, and apparent $K_m$ ($F_{(12,64)} = 25.685; p<0.01$), which represents the affinity of dopamine for the dopamine transporter. Increased apparent $K_m$ values represent
greater uptake inhibition. Maximal apparent $K_m$ (1006.2 nM ± SEM 23.0) and $DA_p$ (182.4 nM ± SEM 36.9) values were recorded 20 minutes following cocaine administration and then gradually returned to baseline values. The apparent $K_m$ and $DA_p$ data are replotted in figure 3 to emphasize the relationship between these two parameters. As illustrated in Figure 3, apparent $K_m$ and $DA_p$ are highly correlated ($r = 0.91$) in the presence of cocaine. Consistent with competitive uptake inhibition, cocaine did not significantly change $V_{max}$ (data not shown), which reflects the maximal velocity of dopamine uptake. The average $V_{max}$ in the dorsal striatum was 3427 nM/s ± SEM 353.5.
**Figure 1**

(top) Representative concentration-time plots of electrically evoked dopamine measured in the dorsal striatum before (left) and 10 minutes after (right) a single injection of cocaine (15 mg/kg i.p.). Arrows indicate the onset of electrical stimulation.

(bottom) Representative color plots – which topographically depict the voltammetric data with time on the x-axis, applied scan potential on the y-axis and background-subtracted faradaic current shown on the z-axis – are illustrated before (left) and after (right) cocaine administration.
FIGURE 1
**Figure 2**

time-course of dopamine uptake inhibition and evoked dopamine release after cocaine administration. Changes in apparent $K_m$ (filled circles) and $DA_p$ (open squares) following administration of cocaine (15 mg/kg, i.p.) are shown over 2 hours. Data are expressed as means ± SEM. Maximal apparent $K_m$ (1006.2 nM ± SEM 23.0) and $DA_p$ (182.4 nM ± SEM 36.9) occurred 20 minutes following cocaine administration.
Figure 2

The graph shows the apparent $K_m$ (nM) on the y-axis and time (min) on the x-axis. There are two curves represented by filled circles and open squares, with error bars indicating variability. The [DA]_p (nM) is also plotted on the y-axis on the right side of the graph.
**Figure 3**

Dopamine uptake inhibition and evoked release are correlative parameters in the presence of cocaine. A high correlation coefficient was found ($r = 0.91$) between apparent $K_m$ and $DA_p$ when compared across all time-points following a single injection of cocaine (15mg/kg i.p.). Data were grouped into 10-minute bins of time following cocaine administration and are expressed as means ± SEM.
Figure 3
**DISCUSSION**

While measuring dopamine dynamics in the striatum of freely moving mice we observed that cocaine markedly decreased the uptake of dopamine by increasing apparent $K_m$ without significantly changing the maximal uptake rate ($V_{\text{max}}$). When maximal dopamine uptake inhibition (apparent $K_m$) and maximal dopamine per pulse ($DA_p$) were observed, the uptake rate ($V_{\text{max}}$) remained unchanged (3427 nM/s ± SEM 353.5). These data are in agreement with previous *in vitro* studies performed in striatal slices from mice (John and Jones, 2007) and *in vivo* studies using anesthetized and freely moving rats (Budygin, 2007; Greco and Garris, 2003). Dopamine uptake inhibition (apparent $K_m$) reached a maximum at 20 minutes, which coincides with previous reports showing maximal behavioral activation and extracellular dopamine concentrations occur between 20-30 minutes after a single intraperitoneal injection of 15 mg/kg cocaine in both mice and rats (Kalivas and Duffy, 1993; Frank et al., 2008). The apparent $K_m$ gradually returned to baseline values within approximately 2 h. These changes were accompanied by a parallel decrease in behavioral activity. These data further confirmed the critical role of the dopamine transporter in cocaine-induced psychomotor activation.

In this study both apparent $K_m$ and $DA_p$ were determined using a model developed by Wightman and colleagues, in which electrically stimulated dopamine concentrations are described as a delicate balance between release and uptake (Wightman et al., 1988). One aspect that merits additional discussion is the possibility that the changes in electrically-evoked dopamine concentrations observed after cocaine can be influenced by alterations in both dopamine uptake
and release. In addition to delaying uptake, cocaine can increase the amount of dopamine detected during the stimulus train by promoting dopamine release from reserve pools of dopamine-containing vesicles (Venton et al., Additionally, electrically-stimulated dopamine release is also subject to D$_2$ dopamine receptor-mediated autoinhibition (Schmitz et al. 2001; Schmitz et al. 2002; Wu et al. 2002) which would have the opposite effect of reducing evoked dopamine concentrations during dopamine transporter inhibition. In light of this complicated action of cocaine on electrically-evoked dopamine release, the observation of a high correlation between changes in an apparent $K_m$ and DA$_p$ during the drug time course is obviously important. The strong temporal association between these parameters suggests that the effect of cocaine on the evoked dopamine release can be preferentially attributed to changes in dopamine uptake. The fact that the increase in electrically-evoked dopamine levels following cocaine was not observed in mice with a genetic deletion of the dopamine transporter (Jones et al. 1998b; Budygin et al. 2002) supports this notion. However, the transporter knockout mice have many other alterations in dopamine storage and release (Jones et al. 1998a), which make direct comparisons to wild-type mice difficult.

In conclusion, the present data provide the first characterization of cocaine-induced changes in dopamine uptake and evoked dopamine concentrations in freely moving mice. A time-course of cocaine induced changes in apparent $K_m$ and DA$_p$ was documented, revealing a tight correlation between the two parameters. This work provides both a methodology and a baseline standard for future pharmacological studies using genetic mouse models of drug addiction.
ACKNOWLEDGEMENTS

This work was supported by Wake Forest University Cross-Campus Collaborative Fund Award (EAB, KDB) and National Institute of Health grants F31DA024525 (EBO) DA021634 (EAB) DA018815, AA014091 and AA013900 (SRJ).
REFERENCES


APPENDIX II

ADDITIONAL EFFECTS OF DRUG PRETREATMENTS ON COCAINE CONSUMPTION AND PRICE PAID

FIGURE 1

Rodrigo A. España, Erik B. Oleson, Jason L. Locke, Bethany R. Brookshire, David C.S. Roberts and Sara R. Jones

Figure 1 was published by European Journal of Neuroscience (currently in Press; Spring 2010).
FIGURE 1

SB-334867 does not affect cocaine consumption but reduces responding as the unit price of cocaine is increased. (A) The mean number ± standard error of the mean of 0.75 mg / kg cocaine injections taken per hour following intraperitoneal (i.p.) injection of vehicle (Veh) (n = 6) or 30 mg / kg SB-334867 (n = 6) on an fixed ratio (FR) schedule. (B) An individual event record showing how the dependent measures of consumption (open circles) and price are extracted from these same data. Consumption is calculated by averaging cocaine intake across three bins (demarcated by dashed oval; see Materials and methods). Pmax is defined as the point at which maximal responding occurs on the price-response function (filled squares). (C) The mean ± standard error of the mean percentage baseline (BL) consumption of cocaine following i.p. injection of vehicle (n = 9) or SB-334867 (7.5, 15 or 30 mg / kg; n = 9) on the threshold schedule. (D) Event records from an individual rat that received an i.p. injection of vehicle or 30 mg / kg SB-334867. Dashed lines denote times in which cocaine doses were reduced (every 10 min; only every other dose is shown, for clarity). Note that the rate of responding increases as the dose of cocaine is lowered throughout the session. (E) Shown are the mean ± standard error of the mean Pmax values, expressed as a percentage of baseline, following i.p. injections of vehicle or SB-334867. *P < 0.05 and **P < 0.01 relative to vehicle.
FIGURE 1
CURRICULUM VITAE

Erik B. Oleson
Medical Center Blvd.  
Winston-Salem NC 27103  
eoleson@wfubmc.edu
864-871-4156 (cell)  
336-716-8697 (office)

Education

<table>
<thead>
<tr>
<th>Year</th>
<th>Degree</th>
<th>Institution</th>
<th>Major</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>BS</td>
<td>Presbyterian College</td>
<td>Biology</td>
</tr>
<tr>
<td>2005</td>
<td>BA</td>
<td>Presbyterian College</td>
<td>Psychology</td>
</tr>
<tr>
<td>2009</td>
<td>PhD</td>
<td>Wake Forest University School of Medicine</td>
<td>Neuroscience Program, Department of Physiology and Pharmacology</td>
</tr>
</tbody>
</table>

Citizenship

United States of America

Technical Skills

• IV Drug Self-Administration
• \textit{in vivo} Electrochemistry
• Stereotaxic Surgery
• Rodent Behavior
• Behavioral Pharmacology
• Behavioral Economics

Research and Professional Experience

<table>
<thead>
<tr>
<th>Year</th>
<th>Position</th>
<th>Institution</th>
<th>Duties</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005-2010</td>
<td>Graduate Student/Research Assistant</td>
<td>Wake Forest University School of Medicine</td>
<td>Primary Mentor: Dr. David CS Roberts</td>
</tr>
</tbody>
</table>
| 2009 | Research Mentor to MARC USTAR (Minority Access to Research Careers - Undergraduate Student Training for Academic Research) student |  | Student: Jasmine Richardson  
Duties: Instructed and supervised a research project |
| 2007-2009 | Instructor at Winston-Salem State University |  | Duties: Instructed Physiology and Pharmacology Classes to Physical Therapy Students  
Supervisor: Dr. Allyn Howlett |
| 2006 | Course Coordinator of Professional Development Class |  | Duties: Coordinated Class Contrasting Traditional vs. Non-traditional Post-doctoral Fellowships  
Supervisor: Dr. Dwayne Godwin |
2004  Adjunct Professor (fall semester)  
Converse College (Spartanburg, SC)  
Duties: Instructed Cell Biology and Human Biology  
Supervisor: Dr. Douglas Jensen

**Honors and Awards**

<table>
<thead>
<tr>
<th>Year</th>
<th>Award</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007-present</td>
<td>Ruth L. Kirschstein National Research Service Award (NRSA-F31)</td>
</tr>
<tr>
<td>2009</td>
<td>Travel Award to Attend Gordon Research Seminar on Catecholamines</td>
</tr>
<tr>
<td>2006</td>
<td>Travel Award to Attend South East Nerve Net Conference</td>
</tr>
<tr>
<td>2005</td>
<td>Degree Honors: B.A. in Psychology with Honors &amp; B.S. in Biology; (cum laude)</td>
</tr>
<tr>
<td>2004-2005</td>
<td>Order of Omega (Greek Leadership Honors Fraternity of Presbyterian College)</td>
</tr>
<tr>
<td>2003-2005</td>
<td>Psi Chi (Psychology Honors Fraternity of Presbyterian College)</td>
</tr>
<tr>
<td>2003-2005</td>
<td>Omicron Delta Kappa (Leadership Honors Fraternity of Presbyterian College)</td>
</tr>
<tr>
<td>2002-2005</td>
<td>Beta Beta Beta (Biology Honors Fraternity of Presbyterian College)</td>
</tr>
</tbody>
</table>

**Publications**

*Published Papers (11 career total):*

España RA, **Oleson EB**, Locke JL, Brookshire BR, Roberts DC, Jones SR. Hypocretin/Orexin neurotransmission is necessary for cocaine self-administration and dopamine signaling in the nucleus accumbens 2009 Dec 23; Epub Ahead of Press.


Papers is submission (1):


Papers in Preparation (2):

Oleson EB, Espãna RA, Jones SR. Diphenylpyraline, an antihistamine drug, exhibits psychostimulant but not rewarding effects. In Preparation for Pharmacology Biochemistry and Behavior.

Oleson EB, Bonin KD, Budygin EA, Roberts DC. Increased motivation to self-administer cocaine is determined by the pattern of responding associated with fluctuating brain cocaine levels. In Preparation for Neuropsychopharmacology.

Published Abstracts and press releases (2004-present; 8 career total):


**Invited Conference Seminars**


**Selected Conference Presentations**


**Oleson EB**, Richardson JM, Budygin EA, Jones SR and Roberts DC (2009) Rapid changes in dopamine uptake inhibition are associated with patterns of cocaine self-administration. Gordon Research Conference (and Seminar) on Catecholamines, Biddeford ME.


Oleson, EB (2004) The effects of restoring Homer 2 on extracellular basal glutamate levels following repeated ethanol exposure. Student Undergraduate Research Program (SURP) presentation day, Charleston, SC.

Oleson, EB (2003) The role of homer proteins in the rewarding and psychomotor activating effects of cocaine. Student Undergraduate Research Program (SURP) presentation day, Charleston, SC.

Professional Memberships and Activities

2009 Contributing Author to the Neurotransmitter (Newsletter of the Western North Carolina Chapter of the Society for Neuroscience) editor: Stephanie Willard

2006-present Student Member of the Society for Neuroscience

2006-present Student Member of the Western North Carolina Chapter of the Society for Neuroscience

2008-2009 Teacher Advancement Program Participant Wake Forest University School of Medicine

2006-present Brain Awareness School Visit Volunteer, Forsyth County NC

2006-present Brain Awareness Week Volunteer, Wake Forest University School of Medicine

Research Interests

- Neurobiological basis of drug abuse and dependence
- Drug addiction
- Comorbidity between schizophrenia and drug addiction
- Neural mechanisms of reinforcement and reward
- Neurochemical correlates of behavior