The Role of Glutamate Neurotransmission in the Ventral Tegmental Area in the Expression of Conditioned Approach Learning

Priscila Hachimine-Merli

The Graduate Center, City University of New York

9-2017

How does access to this work benefit you? Let us know!

Follow this and additional works at: https://academicworks.cuny.edu/gc_etds

Part of the Behavioral Neurobiology Commons, Biological Psychology Commons, Cognitive Neuroscience Commons, and the Systems Neuroscience Commons

Recommended Citation

https://academicworks.cuny.edu/gc_etds/2249
THE ROLE OF GLUTAMATE NEUROTRANSMISSION IN THE VENTRAL TEGMENTAL AREA IN THE EXPRESSION OF CONDITIONED APPROACH LEARNING

by

PRISCILA HACHIMINE-MERLI

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2017
THE ROLE OF GLUTAMATE NEUROTRANSMISSION IN THE VENTRAL TEGMENTAL AREA IN THE EXPRESSION OF CONDITIONED APPROACH LEARNING

By

PRISCILA HACHIMINE-MERLI

This manuscript has been read and accepted by the Graduate Faculty in Neuropsychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

Date

Dr. Robert Ranaldi
Chair of Examining Committee

Date

Dr. Richard Bodnar
Executive Officer

Dr. Richard Bodnar
Dr. Carolyn Pytte
Dr. Jon Horvitz
Dr. John Robinson

Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK
THE ROLE OF GLUTAMATE NEUROTRANSMISSION IN THE VENTRAL TEGMENTAL AREA IN THE EXPRESSION OF CONDITIONED APPROACH LEARNING

by

Priscila Hachimine

Advisor: Robert Ranaldi, Ph.D.

Conditioned stimuli (CSs) come to function as CSs by acquiring the capacity to activate the same mesocorticolimbic dopamine (DA) neurons activated by primary rewards, producing conditioned activation of these neurons and their associated motivational states. This model stipulates that CSs activate mesocorticolimbic DA systems through the activation of glutamate receptors on DA neurons in the ventral tegmental area (VTA). We tested the hypothesis that glutamate receptor stimulation in the VTA is necessary for the expression of conditioned approach. Rats were tested in a conditioned approach protocol that consisted of 7 consecutive conditioning sessions (light presentations and food were paired), one session with no light or food and one test session with only light stimulus (CS-only) presentations. The number of head entries during the CS and pre-CS (baseline) periods was used to calculate difference scores. Bilateral VTA microinjections of glutamate receptor antagonists were made prior to the CS-only session. Kynurenic acid (ionotropic glutamate receptor antagonist; 1.125–4.5 µg/0.5 µl) significantly reduced difference scores compared to vehicle (0 µg), whereas MCPG (metabotropic glutamate receptor antagonist; 1.875–7.5 µg), AP-5 (NMDA antagonist; 0.03125–2.0 µg), and NBQX (AMPA antagonist; 0.5–4.0 µg) had no effects. When AP-5 and NBQX were administered simultaneously at doses of 0.25/4.0 and 2.0/4.0 µg, respectively, the combination significantly reduced the difference scores compared to 0/0 µg, indicating a reduction in the expression of conditioned approach. These findings indicate that either NMDA or AMPA receptor stimulation in the VTA is sufficient, but neither is necessary, for the expression of conditioned approach learning to occur.
Foreword

Portions of this manuscript have been submitted for publication.
Acknowledgements

I want and must first thank God for giving me the strength and perseverance during my graduate journey, for leading me all the way up to here where I am today.

There have been many people who have walked alongside me during these last seven years. They have guided me, placed opportunities in front of me, showed me which doors to close, which ones to open, and which doors could actually be the means to an end. I would like to thank each and every one of them, and in special I would like to sincerely thank Dr. Robert Ranaldi, without whose careful and patient supervision this dissertation would simply have not come to its conclusion.

Robert, your unerring conduct as a researcher combined with that of a professor inspired me ten folds more to keep it simple and carry on. You imparted in me ever valuable traits that permeated all areas of my life – changing who I am, for the better. Your pragmatic approach taught me how to focus on the solution, not on the problem; how to properly convey a rationale into words – few words 😊. As a researcher, you taught me efficient, clear and proper writing, how to carefully employ words, and meticulously question them. Beyond the scope of training me to be a good researcher, you inadvertently tutored me to cleverly be fluent in an idiom that is not my own, to intelligently and objectively advocate for a good argument and how to dismiss a bad one. You also instilled in me that it is ok to commit mistakes, patience and hard work pay off, attention to details matter, and that being steady and committed is more important than being perfect. Thank you for your supreme understanding and patience, and for allowing me time to reconstruct myself whenever my roads got rocky and winding during these seven years. All these together fostered in me the necessary tenacity to complete this degree when I did not think I was capable of. Your encouraging words helped me to be here today. I am very blessed and feel honored to have had you as my advisor on this journey. Thank you very much.

I want to also thank all the student members of Dr. Ranaldi’s laboratory who provided camaraderie and passed on much practical knowledge; in special I thank Michelle Saliba for teaching me all the technical skills necessary to initiate my graduate student research; Sandra Babic for your invaluable companionship and excellent and careful assistance; and Neal Seepersad for your steadfast
support, and for methodically exercising your best self towards the execution of my research. Thank you Neal for being my right arm all along.

My sincere gratitude to Dr. Ramsey for your confidence in me as a researcher when I first returned to school; to Dr. Pytte, Dr. Brumberg, Dr. Halperin, Dr. Croll, and Dr. Borod for your understanding, flexibility and not allowing me to “drop the ball” during my times of hardships; and to Dr. Brathewaite – New York City Alliance Project –for their financial and practical support.

My heartfelt thank you to my friends Kristine Bonacchi, Sofia Chabot and Jaqueline Castro for your ever so powerful assistance, shoulders, ears and prayers at different stages of my academic life.

My deepest love and thank you to my mother, the strongest and kindest and most humble soul I have ever come across, for your lifetime support and encouragement and your steadfast belief in me and all my endeavors throughout my life; to my father for modeling determination and perseverance on his character, and to my brother Esley for supporting me during the stages of my writing.

My profound thanks and love to my husband Peter, who patiently loved me, supported me in all possible ways, and so often “put up with me” every step of the way. Your unwavering belief that I could do this, your understanding, love and patience were critical elements for the conclusion of this degree, which I dedicate to you, our daughter Nyla Elise, and our son Noah David. Peter, thank you not only for enduring my physical, and at times mental, absence during my writing process, but mostly for respecting my time and allowing me to be a researcher, student, teacher, wife, friend, lover, housekeeper, and mom all at once. Thank you so very much, truly, “with all and from the bottom”.

Nyla Elise and Noah David, without you two, all this work would have no meaning. Thank you for being the core motivation that took me to the end.

Last, but not least, I want to sincerely thank the members of my dissertation committee: Drs. Richard Bodnar, Caroline Pytte, Jon Horvitz and John Robinson for your time, invaluable feedback along the way, and your willingness to participate in this dissertation defense. Thank you very much 😊.
# Table of content

Foreword ........................................................................................................................................... v

Acknowledgements ......................................................................................................................... vi

Table of Figures .............................................................................................................................. xi

List of Abbreviations ....................................................................................................................... xii

Chapter 1. .......................................................................................................................................... 1
  1. Introduction ................................................................................................................................ 1
  2. Background ............................................................................................................................... 2
     2.1. Fundamentals of Reward-Related Learning .......................................................................... 2
     2.2. The Importance of This Current Research .......................................................................... 3
     2.3. Learning and the “acquired” Relationship Between DA and CS ........................................ 4
     2.4. Dopamine – an Important Neurotransmitter Underlying Reward-Related Learning
         Mechanisms ............................................................................................................................... 6
     2.5. Our Model of Reward-Related Learning .............................................................................. 7
     2.6. The Primacy of the VTA in Reward-Related Learning Associative Processes ................. 8
  3. In Depth – Our Neurobiological Model of Reward-Related Learning .................................... 12
     3.1. The Premise .......................................................................................................................... 12
     3.2. From Our Model to Glutamate Focus – Why? .................................................................... 14
  4. Glutamate – an “Exciting” Way of Transmitting Information in the Brain ............................ 14
     4.1. Structure of Ionotropic Glutamate Receptors ...................................................................... 15
         4.1.1. Ionotropic Glutamate Receptors – NMDA and AMPA Receptors – in Reward-Related Learning .............................................................................................................................. 15
4.2. Structure of Metabotropic Glutamate Receptors ......................................................... 18
   4.2.1. Metabotropic Glutamate Receptors in reward-related learning ............................ 20

5. Overview of Specific Aims.................................................................................................. 22

Chapter 2. .......................................................................................................................... 25

2. Methodology .................................................................................................................... 25
   2.1. Subjects ....................................................................................................................... 25
   2.2. Surgical procedure ..................................................................................................... 25
   2.3. Experimental and testing apparatus ............................................................................ 26
   2.4. Conditioning experiments .......................................................................................... 26
   2.5. Conditioning procedure ............................................................................................. 26
   2.6. Microinjection procedure .......................................................................................... 27
   2.7. Drugs .......................................................................................................................... 27
   2.8. Histology .................................................................................................................... 28
   2.9. Data analysis ............................................................................................................... 28

3. Results ............................................................................................................................. 29
   3.1. Histology .................................................................................................................... 29
   3.2. Effects of intra-VTA kynurenic acid on expression of conditioned approach ............ 29
   3.3. Effects of intra-VTA MCPG on expression of conditioned approach .......................... 30
   3.4. Effects of intra-VTA AP-5 on expression of conditioned approach ............................. 30
   3.5. Effects of intra-VTA NBQX on expression of conditioned approach ......................... 30
   3.6. Effects of intra-VTA AP-5/NBQX combinations on expression of conditioned approach.. 31

Chapter 3. ............................................................................................................................. 43

Discussion ............................................................................................................................. 43
Reflections on Glutamate-Dopamine interactions in Reward-Related Learning and their Social Implications ................................................................. 51
Future directions ........................................................................................................ 53
Conclusion .................................................................................................................... 54

Bibliography. ............................................................................................................... 55
Table of Figures

**Figure 1:** A simplified illustration of Hebb’s postulate. .................................................. 4

**Figure 2:** A simplified schematic of the major dopaminergic, glutamatergic and GABAergic connections to and from the ventral tegmental area (VTA) and nucleus accumbens (NAcc) in the rodent brain. ______ 11

**Figure 3:** An illustration of the neurobiological model of reward-related learning in the VTA. ______ 13

**Figure 4:** Illustration of the methodology employed .................................................................. 27

**Figure 5:** Histological reconstruction of injection sites adapted from Paxinos and Watson (Paxinos & Watson, 1982). .......................................................... 32

**Figure 6:** Mean (±SEM) CS minus pre-CS difference scores for groups receiving no treatment prior to each conditioning session and kynurenic acid prior to the CS-only session. ____________________________ 33

**Figure 7:** Mean (±SEM) total number of food trough head entries emitted during the seven conditioning and the CS-only sessions for groups receiving no treatment prior to each conditioning session and kynurenic acid prior to the CS-only session. _________________________________ 34

**Figure 8:** Mean (±SEM) CS minus pre-CS difference scores for groups receiving no treatment prior to each conditioning session and MCPG prior to the CS-only session. ____________________________ 35

**Figure 9:** Mean (±SEM) total number of food trough head entries emitted during the seven conditioning and the CS-only sessions for groups receiving no treatment prior to each conditioning session and MCPG prior to the CS-only session. _________________________________ 36

**Figure 10:** Mean (±SEM) CS minus pre-CS difference scores for groups receiving no treatment prior to each conditioning session and AP-5 or AP-5/NBQX combination prior to the CS-only session. ____________________________ 37

**Figure 11:** Mean (±SEM) total number of food trough head entries emitted during the seven conditioning and the CS-only sessions for groups receiving no treatment prior to each conditioning session and AP-5 prior to the CS-only session. _________________________________ 38

**Figure 12:** Mean (±SEM) CS minus pre-CS difference scores for groups receiving no treatment prior to each conditioning session and NBQX prior to the CS-only session. _________________________________ 39

**Figure 13:** Mean (±SEM) total number of food trough head entries emitted during the seven conditioning and the CS-only sessions for groups receiving no treatment prior to each conditioning session and NBQX prior to the CS-only session. _________________________________ 40

**Figure 14:** Mean (±SEM) CS minus pre-CS difference scores for groups receiving no treatment prior to each conditioning session and AP-5 or AP-5/NBQX combination prior to the CS-only session. ______ 41

**Figure 15:** Mean (±SEM) total number of food trough head entries emitted during the seven conditioning and the CS-only sessions for groups receiving no treatment prior to each conditioning session and AP-5 or AP-5/NBQX combination prior to the CS-only session. ______ 42
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>serotonin</td>
</tr>
<tr>
<td>AC</td>
<td>anterior cyngulate</td>
</tr>
<tr>
<td>ACh</td>
<td>acetylcholine</td>
</tr>
<tr>
<td>ACPD</td>
<td>selective mGluR receptor agonist</td>
</tr>
<tr>
<td>AHP</td>
<td>after hyper-polarization</td>
</tr>
<tr>
<td>AMPA</td>
<td>alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>AMPH</td>
<td>amphetamine</td>
</tr>
<tr>
<td>Amyg</td>
<td>amygdala</td>
</tr>
<tr>
<td>AP-5</td>
<td>2-amino-5-phosphonovalerate or 2-amino-5-phosphopentanoate</td>
</tr>
<tr>
<td>BLA</td>
<td>basolateral nucleus of the amygdala</td>
</tr>
<tr>
<td>CA</td>
<td>conditioned approach</td>
</tr>
<tr>
<td>CaMK-II</td>
<td>Ca^{2+}/calmodulin-dependent protein kinase II</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic AMP</td>
</tr>
<tr>
<td>CCK</td>
<td>cholecystokinin</td>
</tr>
<tr>
<td>CFP</td>
<td>conditioned flavor preference</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CPP</td>
<td>conditioned place preference</td>
</tr>
<tr>
<td>CREB</td>
<td>cAMP response element-binding</td>
</tr>
<tr>
<td>CS</td>
<td>conditioned stimulus</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DNQX</td>
<td>AMPA receptor antagonist</td>
</tr>
<tr>
<td>EPSP</td>
<td>excitatory post-synaptic potential</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-aminobutyric acid</td>
</tr>
<tr>
<td>Glu</td>
<td>glutamate</td>
</tr>
<tr>
<td>iGluR</td>
<td>ionotropic glutamate receptor</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>IP</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>KA</td>
<td>kainate</td>
</tr>
<tr>
<td>LDTg</td>
<td>laterodorsal tegmental nucleus</td>
</tr>
<tr>
<td>LH</td>
<td>lateral hypothalamus</td>
</tr>
<tr>
<td>LTP</td>
<td>long-term potentiation</td>
</tr>
<tr>
<td>LY293558</td>
<td>AMPA receptor antagonist</td>
</tr>
<tr>
<td>LY379268</td>
<td>metabotropic - mGlu2/3 - receptor agonist</td>
</tr>
<tr>
<td>mAChR</td>
<td>muscarinic acetylcholine receptor</td>
</tr>
<tr>
<td>MCPG</td>
<td>metabotropic glutamate receptor antagonist</td>
</tr>
<tr>
<td>mGluR</td>
<td>metabotropic glutamate receptor</td>
</tr>
<tr>
<td>MK-801</td>
<td>NMDA receptor antagonist</td>
</tr>
<tr>
<td>MPEP</td>
<td>mGlu5 receptor antagonist</td>
</tr>
<tr>
<td>mPFC</td>
<td>medial prefrontal cortex</td>
</tr>
<tr>
<td>NAcc</td>
<td>nucleus accumbens</td>
</tr>
<tr>
<td>nAChR</td>
<td>nicotinic acetylcholine receptor</td>
</tr>
<tr>
<td>NBQX</td>
<td>AMPA receptor antagonist</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>PAG</td>
<td>periaqueductal gray</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>PKA</td>
<td>protein kinase A</td>
</tr>
<tr>
<td>PKC</td>
<td>protein kinase C</td>
</tr>
<tr>
<td>PLC</td>
<td>phospholipase C</td>
</tr>
<tr>
<td>PPTg</td>
<td>pedunculopontine tegmental nucleus</td>
</tr>
<tr>
<td>SCH-23390</td>
<td>dopamine - D1 receptor antagonist</td>
</tr>
<tr>
<td>US</td>
<td>unconditioned stimulus</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
</tr>
</tbody>
</table>
Chapter 1.

1. Introduction

A person formerly addicted to crack, getting a sudden craving for the drug by accidentally finding the bent spoon where he used to cook the drug; a man passing by an ATM machine and recalling he needs to get some money for the weekend; a little girl walking by McDonald’s and unexpectedly “feeling” hungry; a group of polar bears in the Arctic watching a particular set of ice blocks waiting for the seals to gather around to have some sun. All these scenarios appear very distinct from one another. But in fact, they hold something in common very powerful to their core – the neurobiological mechanisms by which at one point previously neutral stimuli – the bent spoon, ATM machine, McDonald’s, set of ice blocks – became associated with a reward (drug, money, food and food, respectively) expected by the organism in each situation. It is within this context that we set the importance of this literature review and discuss in more depth the role of glutamate receptor stimulation in reward-related learning. The neurobiology of reward-related learning is complex and remains to be fully understood.

Thus, this dissertation will seek to make a contribution to the body of knowledge pertaining to the neurobiological mechanisms involved in reward-related learning and highlight the role of glutamate (Glu) receptors in these processes. I will provide a neurobiological background in reward-related learning and the importance of this research. This will be followed by a presentation of how Hebb’s learning theory influenced our model. Next, a brief description of structural and physiological characteristics of dopamine (DA) will be given followed by a more detailed account of the ventral tegmental area (VTA). I will first review what is already known about the VTA, specifically the characteristics that make this brain area an ideal candidate node where the associative neuronal processes occur, as well as its embedded plasticity, in reward-related learning. In particular, I will also address a plethora of structural characteristics of Glu receptors – metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors (iGluRs) – and possible outcomes each kind brings about when implicated in the process of acquiring and expressing reward-related learned behaviors. Finally I will detail in specifics the hypothesis and the aims of this dissertation.
2. Background

2.1. Fundamentals of Reward-Related Learning

The ability to recognize biologically significant stimuli is one of the most important adaptive strategies for survival. A *stimulus* is a change in the environment that produces a behavioral response. It may be an object or an event perceived through an organism’s senses. Stimuli may include the sight of water, the smell of food, or the smell of a mate. Such stimuli function as *rewards*. As the organisms learn about these rewards’ patterns – when and where they will appear, how they smell, taste and what they look like – they develop behaviors that will place them in direct contact with such rewards. In the present dissertation, the stimulus/reward will be food and it will serve to bring about an incentive motivational effect as food is consumed. Such a stimulus will also be here designated as primary reward – in the Pavlovian scheme unconditioned stimulus (US) – for it is (1) crucial for the survival of the species and (2) capable of eliciting a response, such as approach, and reinforcing behaviors (Wise, 2004; Wise & Rompre, 1989). It should be noted that just because something is labeled as a reward, it does not necessarily imply that it is a reinforcer. A reward is an appetitive stimulus given to an organism to alter its behavior. Rewards typically serve as reinforcers. A reward stimulus can be defined as a reinforcer only if its subsequent delivery as a consequence of the behavior increases the probability of that behavior occurring again (Morse & Skinner, 1958; Skinner, 1938).

A myriad of studies involving different research paradigms show that one key component of reward-related learning is the acquisition of conditioned associations between rewards and stimuli that are simultaneously present in the environment. A research model on which environmental cues (e.g., stripes on the wall of a chamber) are repeatedly paired with an unconditioned stimulus (e.g., bowl of Lucky Charms) is called conditioned place-preference (CPP) only if these environmental cues, over time, lead the organism to prefer this side of the chamber as opposed to alternate sides that contained no appetitive stimuli associated with it. In CPP the initially neutral environmental cues become associated with the motivational properties of the unconditioned stimulus leading to approach toward the environment (Bardo, Neisewander, & Miller, 1986; Morency & Beninger, 1986; Spyraki, Fibiger, & Phillips, 1987).
Another model, called conditioned flavor-preferences (CFP), demonstrates that in flavor-flavor conditioning, a preference is acquired for an arbitrary flavor cue (e.g., grape flavor) paired with an already liked flavor (e.g., sweet flavor of saccharin). In this case, the sweet flavor is considered to be an US that reinforces the animal's preference for the added flavor, which represents the CS (Bodnar, 2004; Delamater, Sclafani, & Bodnar, 2000; Sclafani, Bodnar, & Delamater, 1998). In a conditioned approach (CA) model, a neutral stimulus (e.g., light) is paired with an US (e.g., food pellet). After a few pairings, the light (now CS) will be able to elicit an approach response similar to the one elicited by the food pellet itself.

These research models exemplify that despite the many different ways to form associations between rewards and stimuli the end result highlights a much bigger question – what underlies this conditioned activation mechanism; or better yet, once a CS acquires the capacity to act as such, can it be disrupted?

2.2. The Importance of This Current Research

Among the most powerful characteristics of a CS is the capacity that it acquires to control reward seeking (further discussed below), in addition to elicit conditioned approach responses similar to the ones caused by the primary rewards with which it is associated, thereby leading the organism to approach the primary reward. We (Ranaldi, 2014; Zellner & Ranaldi, 2010) and others (Beninger & Ranaldi, 1994; Bindra, 1974; Bolles, 1972; Wise, 2004) (Stuber et al., 2008) (Harris & Aston-Jones, 2003) suggest that reward-related stimuli become CSs because (1) they gain access to the same motivational neural circuits as the primary rewards, thus producing conditioned activation of the same neural pathways activated by the US and (2) they are able to elicit motivational states, such as approach behavior, similar to motivational states elicited by the primary reward. Exactly how this happens remains to be elucidated.

Elucidating the neurochemical mechanisms whereby a CS becomes a CS is important because it adds insight into understanding reward-related learning pathologies such as addiction. For example, in drug addiction, the strength of a CS extends to exerting a significant influence and control over our thoughts and our behaviors. Cues previously associated with the drug can trigger drug cravings (Ehrman, Robbins, Childress, & O'Brien, 1992; O'Brien, Childress, Ehrman, Robbins, & McLellan, 1992); (Childress
et al., 1999) which eventually may lead to drug-seeking (Ranaldi & Roberts, 1996) and subsequent relapse (Childress, McLellan, Ehrman, & O'Brien, 1988; Stewart, de Wit, & Eikelboom, 1984; Wallace, 1989). Thus, it is important to understand how CSs acquire control of the motivational/approach system. This knowledge will enable the identification of neural systems that can be manipulated to eliminate the control of CSs over reward-seeking and facilitate the discovery of novel and effective pharmacotherapeutic treatments and/or behavioral strategies in the treatment of neurobehavioral pathologies such as drug addiction.

2.3. Learning and the “acquired” Relationship Between DA and CS

The main pillar of the Hebbian learning theory describes a basic mechanism for synaptic plasticity where an increase in synaptic efficacy arises from the presynaptic cell’s repeated and persistent stimulation of the postsynaptic cell. Furthermore, according to Hebb, simultaneous activation of cells leads to pronounced increases in synaptic strength between those cells (Hebb, 1949). Hebb formulated what became the basis of the idea of hebbian learning “When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased” (Hebb, 1949).

![Figure 1: A simplified illustration of Hebb’s postulate. Here the graph shows the synaptic efficacy increasing over time from cell A after persistent stimulation of cell B.](image)

In reward-related learning, reward-associated stimuli acquire the capacity to function as such because they gain access to the same motivational neural circuits as primary rewards and become in this
way capable of activating these same neural circuits whereby activating DA neurons on their own (Kest, Cruz, Chen, Galaj, & Ranaldi, 2012) and elicit motivational states similar to primary rewards (Beninger & Ranaldi, 1994; Bindra, 1974; Bolles, 1972; Wise, 2004). We and others suggested that one way whereby a CS achieves this is through synaptic plasticity in the VTA (Bonci & Malenka, 1999; Harris, Wimmer, Byrne, & Aston-Jones, 2004; Sharf & Ranaldi, 2006; Zellner, Kest, & Ranaldi, 2009).

An extensive body of evidence documents a role for DA in the terminal regions of the mesocorticolimbic DA system in relation to CSs. Although this body encompasses several types of conditioning I will focus only on that which pertains directly to the conditioned approach type used here.

In the Pavlovian conditioning context, DA levels in the nucleus accumbens (NAcc) increase in relation to the presentation of CSs. Food-associated CSs increase DA utilization (Blackburn & Phillips, 1989) and DA release (Bassareo, De Luca, & Di Chiara, 2007; Bassareo & Di Chiara, 1999; Phillips, Atkinson, Blackburn, & Blaha, 1993) all in NAcc. Food deprived animals presented with a palatable meal, but prevented from eating it, demonstrate increased NAcc DA concentrations (Wilson, Nomikos, Collu, & Fibiger, 1995). CSs associated with cocaine (Kiyatkin & Stein, 1996), morphine or nicotine (Di Chiara & Bassareo, 2007) trigger DA release in the NAcc.

Sub-populations of NAcc neurons change their activity patterns in response to the presentation of CSs in a variety of contexts, with predominant changes being excitations. Although NAcc firing does not necessarily indicate VTA activity, it appears that the VTA nevertheless plays a crucial modulatory role in NAcc firing in relation to CSs. Inactivation of the VTA almost completely abolishes cue-evoked firing in the NAcc while preserving activity correlated with other task-related events such as an operant response and magazine training (Yun, Wakabayashi, Fields, & Niccola, 2004). In primates, a CS signaling of the beginning of a food-reward trial excites ventral striatal neurons (Schultz, Apicella, Scarnati, & Ljungberg, 1992). Similarly, in humans, presentations of cues predicting delivery or availability of a monetary reward activate the NAcc region (Knutson, Fong, Adams, Varner, & Hommer, 2001; Talmi, Seymour, Dayan, & Dolan, 2008).

Together, the evidence of the role of NAcc activity and NAcc DA in responding to CSs is extensive, and further shows that in reward-related learning mesocorticolimbic DA activity, initially elicited only by USs, can also come to be elicited by CSs.
As seen so far, DA plays an important role in the dynamics of reward-related learning. Hence, before we discuss what constitutes our neurobiological model of reward-related learning, I will devote the next section to DA, its neurochemical and neurophysiological properties.

2.4. Dopamine – an Important Neurotransmitter Underlying Reward-Related Learning Mechanisms

Dopamine is an organic chemical of the catecholamine and phenethylamine families that is critical for the proper functioning of the central and peripheral nervous systems. Its name stems from its chemical structure – it is an amine synthesized by removing a carboxyl group from a molecule of its precursor chemical L-DOPA, which is synthesized in the brain and kidneys. DA is also synthesized in plants and most multicellular animals. In the brain, DA functions as a neurotransmitter performing a modulatory role on the cellular level. Neurochemically speaking, DA is one of the catecholamine neurotransmitters, and the implicated dopaminergic cell groups project forward from the head of the midbrain to several forebrain structures. Subsets of these neurons are also implicated in other aspects of motivated behavior, and abnormal functioning of dopaminergic neurons has variously been suggested to account for aspects of Parkinson’s disease, schizophrenia, mania and depression.

DA acts via second-messenger systems to affect slow synaptic transmission (on the order of tens of milliseconds to seconds, compared to fast transmission on the order of milliseconds, mediated by Glu and GABA [gamma-aminobutyric acid]) (Greengard, 2001). Receptors for DA are grouped primarily according to their interaction with cyclic AMP (cAMP) (see Kebabian and Caine, 1979 for review). When coupled to the D1 family of DA receptors, DA initiates the activation of cAMP via a G_{olf} activation of adenylate cyclase, initiating a cascade of processes which result in phosphorylation of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors which increase their sensitivity to Glu, making the neuron more excitable by several mechanisms, including enhanced surface expression of AMPA and NMDA (N-methyl-D-aspartate) receptors (see Surmeier, et al., 2007 for review). On the other hand, when bound to the D2 family of receptors, DA usually produces inhibitory effects. D2 receptors couple to G_{i/o} proteins that inhibit adenylate cyclase when activated and have inhibitory downstream effects, including decreasing AMPA currents, reducing opening of Na+ channels, and promoting opening of K+ channels.
In addition, D1 and D2 receptors are present in different ratios in different brain regions, and exist in some areas as autoreceptors as well, leading to opposite effects on DA cells than postsynaptic neurons.

In addition to affecting how postsynaptic neurons respond to other input, there is also strong evidence of a positive correlation between mesolimbic DA and the expression of learned reward-related behavior (Ranaldi, Pocock, Zereik, & Wise, 1999; Richardson & Gratton, 1996; Wightman & Robinson, 2002). Furthermore, DA plays a crucial role in synaptic plasticity (see Beninger and Miller, 1998; Jay, 2003 for review). DA binding at the D1 receptors activates adenylate cyclase, increasing the amount of cAMP produced, which activates PKA (protein kinase A). PKA phosphorylates AMPA and NMDA receptors, as well as phosphorylates CREB (cAMP response element-binding), thereby boosting production of various proteins involved in synaptic activity; and DAARP-32, which increases CaMK-II (calmodulin-dependent protein kinase II) activity. These events, in conjunction with increased Ca^{2+} currents due to NMDA receptor stimulation, are thought to facilitate synaptic strengthening (Jay, 2003).

2.5. Our Model of Reward-Related Learning

In our proposed model of synaptic plasticity, one component of reward-related learning is the strengthening of CS-related synapses on which neuronal inputs previously associated with neutral environmental stimuli have now the capacity to activate VTA DA cells on their own, enhancing DA release at the terminal regions (Ranaldi, 2014).

Initially, the neutral environmental stimulus – we propose a Glu signal – is too weak to efficiently activate DA cells and elicit approach behavior. The consumption of food (US) in a given environment releases acetylcholine (ACh) into the VTA, which stimulates muscarinic acetylcholine (mACh) receptors on VTA DA cells leading to increased DA in mesocorticolimbic terminal regions (Miller & Blaha, 2005; Schilstrom, Svensson, Svensson, & Nomikos, 1998; Westerink, Enrico, Feimann, & De Vries, 1998) which stimulates increased approach behavior (Beninger, 1983; Berridge, 2007; Wise & Bozarth, 1987). While the VTA DA cells are firing, they are also receiving weak signals from Glu afferents conveying information about environmental stimuli. Recent evidence demonstrates that virtually all afferents to the VTA comprises some glutamatergic fibers, with the exception of the NAcc and the lateral septum (Geisler,
Derst, Veh, & Zahm, 2007), which corroborates earlier anatomical and physiological studies (e.g.,
(Sesack, Deutch, Roth, & Bunney, 1989; Sesack & Pickel, 1992). These afferents lead to Glu stimulation
of NMDA receptors on VTA DA cells. We presume that this convergence of signals onto the VTA DA cells
creates the conditions for long-term potentiation (LTP), which is an NMDA receptor dependent
phenomenon (Bonci & Malenka, 1999; Citri & Malenka, 2007; Stuber et al., 2008), and regarded as one
of the major intracellular mechanisms of synaptic plasticity underlying the learning process. Moreover, not
only has it been demonstrated that NMDA receptors are found in the VTA (Rodriguez, Doherty, & Pickel,
2008) but these synaptic alterations result in Glu being now capable of efficiently activating DA cells
causing consequently strong DA release. This increased DA release triggered by strengthened CS-
associated synapses in the VTA might result in increased levels of approach behaviors. The
strengthening of the CS signal may be due to one or more of several NMDA-dependent structural or
functional synaptic changes such as proliferation of AMPA receptors (Kessels & Malinow, 2009; Nicoll,
2003), growth of new synapses (Carlisle & Kennedy, 2005), increased presynaptic Glu release (Lisman &
Raghavachari, 2006) or other processes.

It is also proposed that these neuronal changes are responsible for the strengthening of CS-
related synaptic activity on VTA DA cells resulting this way in the acquisition by the reward-related
stimulus of the capacity to activate VTA DA neurons and elicit conditioned approach. And because the
VTA receives excitatory inputs from brain structures such as mesopontine cholinergic nuclei – likely
conveying primary reward signals – and from structures such as superior colliculus, prefrontal cortex
(PFC) and amygdala (Amyg) – most likely conveying information about environmental stimuli – it is
feasible to argue that the VTA is an ideal site in which, at least some of these neural associative
processes arising from this convergent stimulation occur.

2.6. The Primacy of the VTA in Reward-Related Learning Associative Processes

The VTA, also known as the ventral tegmental area of Tsai (Phillipson, 1979), is a group of
neurons located close to the midline of mesencephalon. The mammillary bodies and the posterior
hypothalamus extend rostrally from the VTA. The red nucleus and substantia nigra are situated laterally
and oculomotor fibers are situated ventromedial to the VTA. The pons and the rhombencephalon lie
caudally to the VTA. The VTA is the origin of the dopaminergic cell bodies of the mesocorticolimbic DA system and is widely implicated in the drug and natural reward circuitry of the brain.

The VTA projects axons to the medial prefrontal cortex (mPFC); olfactory tubercle and entorhinal cortex; Amyg; ventromedial striatum, particularly the NAcc; thalamus; posterior, lateral and preoptic areas of hypothalamus; bed nucleus of the stria terminalis; nucleus of the diagonal band; lateral septal nucleus; and brainstem areas including several raphe nuclei, parabrachial nucleus, and locus coeruleus (Beckstead, Domesick, & Nauta, 1979; Swanson, 1982) (Geisler et al., 2007; Geisler & Zahm, 2005). Projections to the PFC and NAcc as well as other structures such as the septum and inferior olive arise from separate, non-collaterized populations (Fallon, 1981) (Swanson, 1982) (Fallon, Schmued, Wang, Miller, & Banales, 1984). Afferents to the VTA will be reviewed further down in this section.

The VTA neuronal assemblages are modulated by a myriad of neurotransmitters and peptides, including Glu, GABA, serotonin (5-HT), ACh, norepinephrine, opioids, and peptides including CCK (cholecystokinin) and orexin (Kalivas, 1993; Meltzer, Christoffersen, & Serpa, 1997) (Mathon, Kamal, Smidt, & Ramakers, 2003). The VTA also contains both DA neurons (“principal” cells), which are distinguished by long duration action potentials and hyperpolarization to DA but not met-enkephalin, and GABA neurons (“secondary” cells), which have short duration spikes and are hyperpolarized by met-enkephalin but not DA (Johnson & North, 1992a). The GABAergic neurons project both within the VTA itself (as interneurons) and outside the VTA (Van Bockstaele & Pickel, 1995). The VTA also contains a third kind of cell that remains to be categorized, which does not respond to DA nor opioids (Johnson & North, 1992a). Recent research demonstrates the presence of glutamatergic neurons in the VTA, which are non-dopaminergic, and non-GABAergic, which may constitute this third type of VTA neuron (Yamaguchi, Sheen, & Morales, 2007). It should also be noted that research suggests that Glu is released by DA neurons in the NAcc (Chuhma et al., 2004) and PFC (Lavin et al., 2005).

The VTA receives cholinergic afferents from pedunculopontine (PPTg) and laterodorsal tegmental nuclei (LDTg) (Henderson & Sherriff, 1991) (Garzon, Vaughan, Uhl, Kuhar, & Pickel, 1999; Oakman, Faris, Kerr, Cozzari, & Hartman, 1995). VTA DA cells possess both mACh receptors and nicotinic acetylcholine (nACh) receptors (Gronier & Rasmussen, 1998). Application of ACh or its agonists depolarizes VTA neurons in vitro (Lacey, Calabresi, & North, 1990), causes burst firing (Gronier &
Rasmussen, 1998; Seutin, Verbanck, Massotte, & Dresse, 1990), DA release in PFC and NAcc (Seutin et al., 1990) (Miller & Blaha, 2005; Westerink et al., 1998). Our lab has previously demonstrated that VTA nACh receptors are not involved in acquisition nor expression of food-related operant learning (Sharf & Ranaldi, 2006), nor in food reward itself (Sharf & Ranaldi, 2006).

Evidence has shown that VTA ACh is strongly involved in reward-related behavior. VTA ACh concentrations increase during eating, drinking and self-stimulation (Rada, Mark, Yeomans, & Hoebel, 2000). Stimulation of mACh receptors in VTA enhances brain stimulation reward while mACh receptor antagonism reduces it (Kofman & Yeomans, 1988; Yeomans, Kofman, & McFarlane, 1985) (Yeomans, Mathur, & Tampakeras, 1993). Furthermore, mACh receptor antagonists in the VTA reduce eating and approach behavior (Ikemoto & Panksepp, 1996; Rada et al., 2000). These findings suggest that VTA mACh receptor stimulation is involved in mediating the unconditional motivational/approach effects of rewards, including food. Our lab has also shown that intra-VTA microinjections of a mACh receptor antagonist prevents the acquisition, but not expression, of food-reinforced instrumental conditioning (Sharf, McKelvey, & Ranaldi, 2006), suggesting a necessary role for VTA mACh receptor stimulation in reward-related learning.

The DA neurons of the VTA receive Glu afferents from the mPFC (Sesack & Pickel, 1992; Y. Smith, Charara, & Parent, 1996), Amyg nuclei and the bed nucleus of stria terminalis (Hopkins & Holstege, 1978; Phillipson, 1979), the PPTg (Charara, Smith, & Parent, 1996), and periaqueductal grey area (PAG) (Omelchenko & Sesack, 2009). Glu acts on NMDA, AMPA and mGlu (metabotropic glutamate) receptors on DA cells (Albin et al., 1992) to excite these cells (Christoffersen & Meltzer, 1995; Overton & Clark, 1992) (J. Zhang, Chiodo, & Freeman, 1994). The NMDA receptor conducts inward $\text{Ca}^{2+}$ currents, which activate CaMK-II and PKC (protein kinase C), protein kinases linked to gene transcription and the proliferation and phosphorylation of AMPA receptors, resulting in LTP of the postsynaptic response to Glu.
Figure 2: A simplified schematic of the major dopaminergic, glutamatergic and GABAergic connections to and from the ventral tegmental area (VTA) and nucleus accumbens (NAc) in the rodent brain. The primary reward circuit includes dopaminergic projections from the VTA to the NAc, which releases dopamine in response to reward-related stimuli. There are also GABAergic projections from the NAc to the VTA; projections through the direct pathway (mediated by D1-type medium spiny neurons (MSNs)) directly innervate the VTA, whereas projections through the indirect pathway (mediated by D2-type MSNs) innervate the VTA via intervening GABAergic neurons in the ventral pallidum (not shown). The NAc receives dense innervation from glutamatergic monosynaptic circuits from the medial prefrontal cortex (mPFC), hippocampus (Hipp) and amygdala (Amyg), as well as other regions. The VTA receives such inputs from the lateral dorsal tegmentum (LDTg), lateral habenula (LHb) and lateral hypothalamus (LH), as well as both GABAergic and glutamatergic connections from the extended amygdala (not shown). The dashed lines indicate internal inhibitory projections. RTMg, rostromedial tegmentum. License of the image was granted by Nature Publishing Group for reuse. From The brain reward circuitry in mood disorders (Russo & Nestler, Nature Reviews Neuroscience 14, 609-625 (2013)).

VTA Glu, just like VTA ACh, is also implicated in associative learning. Evidence has shown that cocaine conditioned locomotion fails to develop under VTA NMDA receptor antagonist treatment (Pert, 1998). In addition to that, simultaneous antagonism of VTA AMPA and NMDA receptors blocks the acquisition of cocaine conditioned place preference (Harris & Aston-Jones, 2003), and VTA NMDA receptor antagonism alone blocks acquisition of morphine conditioned place preference (Harris et al., 2004) and acquisition of conditioned approach (Ranaldi et al., 2011). You et al. (2007) demonstrated that context-induced cocaine seeking is positively correlated with VTA Glu release and reduced by intra-VTA administration of NMDA or AMPA receptor antagonists, suggesting a role for Glu receptors in the maintenance of contextual effects on cocaine-related behavior. Moreover, inhibition of Glu release in the VTA during training of heroin self-administration was found to later reduce context-induced reinstatement (Bossert, Liu, & Shaham, 2004). Our lab has shown that intra-VTA microinjections of AP-5, an NMDA
receptor antagonist, prevents the acquisition, but not expression, of food-reinforced instrumental responding (Zellner et al., 2009), and another team (Stuber et al., 2008) has also demonstrated that NMDA receptor antagonist can inhibit the acquisition of conditioned approach responses.

Altogether, this evidence points to the VTA as being a site where at least some of the synaptic modifications underlying the ability of previously neutral environmental cues to become associated with drug and food reward occur.

3. In Depth – Our Neurobiological Model of Reward-Related Learning

3.1. The Premise

Our neurobiological model of reward-related learning is predicated on the assumption that conditioned approach occurs when the CS acquires the ability to activate the same neural system that produces unconditioned approach. We posit that at least some aspects of the neurophysiology underlying CS-US associative learning occur in the VTA. A Pavlovian conditioning model may suggest the representation of different aspects of a behavioral model in which the US consists of stimulation of mACh receptors on VTA DA cells and the CS consists of Glu stimulation of Glu receptors on VTA DA cells. The US-associated stimulus becomes a CS through concurrent stimulation of mACh and NMDA (representing the eventual CS) receptors on VTA DA neurons. This coincident stimulation leads the mACh receptor to strongly depolarize the DA cells, dislodging the Mg$^{2+}$ ion from the NMDA receptor channel allowing this channel to conduct Ca$^{2+}$ ions into DA cells. Intracellular Ca$^{2+}$ initiates the CaMKII and PKC intracellular cascades, resulting in several long-term changes that lead to a strengthening of the CS signal.

In our model then, the acquisition of conditioned approach is dependent on both NMDA and mACh receptor stimulation, since blockade of either will prevent one or both of the necessary steps (NMDA-conductance of Ca$^{2+}$ and initiation of CaMKII and or PKC) that initiate the neural plasticity for long-term change to happen. Once plasticity has occurred, the previously weak CS-related Glu signal is now strong enough to activate DA cells on its own to a level that produces approach behavior. In this case, NMDA and/or mACh receptor stimulation are no longer necessary for the expression of this
approach behavior, since the performance of this learning appears to be now maintained by whatever neuronal changes have resulted in the increased CS signal. In short, our model stipulates that the increased strength in CS signal is glutamatergic in nature and that the plasticity of how CS becomes CS involves Glu receptors in the VTA.

**Figure 3:** An illustration of the neurobiological model of reward-related learning in the VTA. Here are shown the components of the proposed neural mechanisms necessary for a neutral stimulus the ability to acquire the capacity to activate a VTA DA neuron on their own therefore acting as a conditioned stimulus that can cause a conditioned approach response. US, mACH receptor stimulation allows for activation of VTA DA neurons and then activation of the DA terminal regions that cause approach. CS, NMDA receptor stimulation, allows for the conditioned activation of VTA DA neurons and then conditioned activation of the DA terminal regions that cause conditioned approach. When there is coincident mACH and NMDA receptors stimulation in the VTA DA neurons, calcium flowing through the NMDA receptor initiates intracellular cascades resulting in long-term changes in neural activity. It is proposed that these changes in neural activity allow CS the ability to acquire the capacity to activate VTA DA neurons on their own and elicit conditioned approach. License of the image was granted by De Gruyter for reuse. From *Dopamine and reward seeking: the role of ventral tegmental area* (Ranaldi, Reviews in the Neurosciences 2014; 25 (5): 621-30.)
3.2. From Our Model to Glutamate Focus – Why?

In recent years, researchers have begun to focus on the neurochemical mechanisms underlying reward-related learning finding that many data suggest that DA afferents interact with glutamatergic afferents common to the same cell when reward-related learning occurs (Sutton & Beninger, 1999). Results from these studies further suggest that a number of signaling molecules activated by Glu and DA synaptic transmission interact to convey short and long-term modifications that mediate the neurochemical and molecular changes that form the basis of reward-related learning (Sutton & Beninger, 1999).

It is this platform of knowledge in the reward-related learning field that guided us to our hypothesis – we know that Glu neurotransmission, more specifically NMDA receptor stimulation, is crucial for the acquisition of approach learning, but what is it that maintains the approach-learned behavior once it is already been learned?

Thus, since it appears that glutamatergic neurotransmission is not only involved in acquisition (as we will see), but also during the plasticity event and now recently demonstrated (Hachimine 2016) in expression of reward-related learning, I will review in the next sections some of the studies focusing on glutamate receptors and their implications during acquisition and expression in reward-related learning studies.

4. Glutamate – an “Exciting” Way of Transmitting Information in the Brain

Glu is the major excitatory neurotransmitter in the mammalian central nervous system (CNS) and gates cation-permeable ionotropic receptors and activates metabotropic receptors coupled via G proteins to activation of phospholipase C (PLC) and inhibition of adenylate cyclase (AC) activity. iGluRs exert the rapid neuronal excitation characteristic of glutamate transmission, whereas mGluRs mediate relatively slow glutamate responses by coupling to intracellular signal transduction via G proteins (Nakanishi, 1992). However, glutamate is a common transmitter that acts on both the ionotopic and metabotropic receptors with similar efficiency. In what follows, a more detailed structural description and involvement of iGluRs and mGluRs in reward-related learning processes will be discussed.
4.1. Structure of Ionotrophic Glutamate Receptors

In general, iGluRs are glutamate-gated ion channels that, when activated, increase cationic flux (mainly Na$^+$ and K$^+$ ions and to a lesser extent Ca$^{2+}$ ions) across the neuronal membrane thereby increasing cellular excitability (Dingledine, Borges, Bowie, & Traynelis, 1999). There are three major types of iGluRs, NMDA containing NR1, NR2A – D and NR3A – B subunits, AMPA containing GluR1 – 4 subunits, and 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine (kainate) receptors containing GluR5 – 7, KA-1 and KA-2 subunits (Kew & Kemp, 2005; Nakanishi, 1992; Nakanishi & Masu, 1994).

IGluRs subunits possess an extra-cellular amino terminal domain which shows homology to mGluRs bi-lobed agonist binding domain, followed by a first transmembrane domain and then a pore forming membrane-residing domain that does not cross the membrane but creates a re-entry loop entering from and exiting to the cytoplasm (Kew & Kemp, 2005). The second and third transmembrane domains are connected by a large extracellular loop and the third transmembrane domain is followed by an intracellular carboxy-terminus (Dingledine et al., 1999; Mayer & Armstrong, 2004). The crystal structure of the iGluRs ligand binding domains, which consists of polypeptides in both the amino terminus (S1 domain) and the extracellular loop between transmembrane domains 3 and 4 (S2 domain) confirms this basic topology model for the iGluR family (Armstrong, Sun, Chen, & Gouaux, 1998; Mayer & Armstrong, 2004).

Even though NMDA and AMPA receptors are found to be under the same category, their basic molecular structures strongly differ in complex ways and, pharmacologically speaking, how they interact with agonists and antagonist agents (for a comprehensive review on NMDA and AMPA receptor structure and pharmacology see Kew & Kemp, 2005) is very distinct from one another. For the purposes of this dissertation, I will limit my discussion mostly to NMDA and AMPA receptors in pharmaco-behavioral research.

4.1.1. Ionotrophic Glutamate Receptors – NMDA and AMPA Receptors – in Reward-Related Learning

Current research shows that glutamate neurotransmission has been the target of in-depth study in reward-related learning mainly because of the interaction between glutamatergic and dopaminergic
afferents common to the same cell when reward-related learning is taking place, and the neurochemical synergistic effects that they generate. For example, Baldwin et al., 2002 and Smith-Roe & Kelley, 2000 studies showed that DA D1-like and Glu NMDA receptors produce synergistic effects in the NAcc core and mPFC by co-injecting sub-threshold doses of SCH 23390 plus AP-5 and observing that this procedure blocked acquisition but not expression of conditioned approach responses in these respective sites.

The exact mechanisms implicated in acquisition or expression of a behavior are not yet fully known. It is possible that synergistic effects result from a combination of different antagonists targeting different receptors. Co-infusion of low doses of both AP-5 and SCH-23390 into NAcc strongly impaired acquisition of lever pressing for food and caused a significant reduction in established responding, whereas when infused individually these doses showed no effect (Smith-Roe & Kelley, 2000). Infusion of the combined low doses had no effect on parameters of feeding and motor-activity suggesting that this specific effect is isolated to learning.

Extensive data, including from our lab, show the importance of NMDA receptors in the acquisition of conditioned approach. Bilateral injections of AP-5 in the VTA (Ranaldi et al., 2011) and NAcc core (but not shell) (Baldwin, Holahan, Sadeghian, & Kelley, 2000; Di Ciano, Cardinal, Cowell, Little, & Everitt, 2001; Kelley, Smith-Roe, & Holahan, 1997) blocked acquisition, but not expression, of approach behavior, whereas injections of AP-5 into the dorsal or ventral subiculum were without effect (Baldwin et al., 2000). Interesting to note, Di Ciano and colleagues (2001), using a discriminated approach to a lever that was extended to signal the delivery of a food pellet, further demonstrated that intra-NAcc core injections of LY293558 (AMPA receptor antagonist) did not block acquisition of approach behavior but impaired discrimination, so that approach was seen to levers that signaled either food or no food. The findings of this study suggest that NMDA receptors are implicated in the NAcc core, BLA and mPFC in the acquisition but not expression of conditioned approach responses. Furthermore, on a side note, it also demonstrates that AMPA receptors may play an important role in discriminative learning.

We (Zellner et al., 2009) and others (Kelley et al., 1997) further demonstrated that NMDA receptors are also important for the acquisition of lever pressing for food in rats. Intra-VTA injections of AP-5 impaired acquisition of lever pressing for food during learning sessions, whereas expression of
performance remained unaffected. Since AP-5 failed to reduce free feeding, food reward or motor activity, the observed impairment in acquisition cannot be attributed to reduced food motivation or locomotor activity (Zellner et al., 2009). Similar results were found when AP-5 was injected in the NAcc core and in the NAcc shell. Blockade of NMDA receptors in both regions abolished lever-press acquisition but not performance of operant responding (Kelley et al., 1997). Baldwin et al. (2000) also reported similar effects observing that not only was learning impaired by injections of AP-5 into NAcc core, but learning was also impaired by injections of AP-5 into BLA (basolateral Amygdala) or mPFC but not into dorsal or ventral subiculum on lever pressing acquisition. Taken together, these findings suggest that NMDA receptors are crucial for acquisition but not performance of learning.

The previous conclusion led research to alternatively investigate the role of AMPA receptors in the expression of lever pressing. For example, Pierce and colleagues (1997) demonstrated that MK-801, an NMDA receptor antagonist, decreased responding for cocaine but had no effect on responding for food, and that DNQX (AMPA receptor antagonist) similarly attenuated responding for cocaine and food. These findings indicate that NMDA receptors are implicated in the expression of lever press responding for cocaine but not food whereas AMPA receptors appear to be involved in the expression of both reinforcers. Moreover, blockade of AMPA receptors by infusion of CNQX (an AMPA receptor antagonist) into the NAcc abolished both reinstatement of lever pressing by cocaine priming (Cornish & Kalivas, 2000) and reinstatement of cocaine seeking induced by intra-mPFC cocaine (W. K. Park et al., 2002) demonstrating that the activation of AMPA receptors in the NAcc appears to be essential for the expression of drug craving induced by cocaine.

Accumulating evidence shows that ionotropic glutamatergic activation appears to be also critical in reward-related learning that involve drugs of abuse. Blockade of iGlu receptors in the VTA has been shown to dose-dependently decrease cocaine-primed reinstatement (Sun, Akins, Mattingly, & Rebec, 2005), attenuate cocaine-context mediated responding (You, Wang, Zitzman, Azari, & Wise, 2007) and impair acquisition and expression of CPP with amphetamine (Bespalov, 1996). Furthermore, Koob et al. (1992) demonstrated that injections of AP-5 into the NAcc produced an increase in cocaine (but not heroin) self-administration suggesting a decrease in cocaine reward (Pulvirenti, Maldonado-Lopez, & Koob, 1992). This data contrasts with other studies that show that systemic administration of Glu NMDA
receptor antagonists such as MK-801 augmented reward produced by cocaine (Pierce, Meil, & Kalivas, 1997; Ranaldi, French, & Roberts, 1996; Shoab, Shippenberg, Goldberg, & Schindler, 1995). Interesting to note, MK-801 was also found to impair acquisition of cocaine self-administration. Schenk et al. (1993) reported that rats treated with MK-801 pressed both the active and inactive lever indiscriminately even after discontinuation of the administration of the antagonist. (Schenk, Valadez, McNamara, et al., 1993) (Schenk, Valadez, Woryle, & McNamara, 1993). Recently, it has also been suggested that blockade of iGluRs in the VTA hindered the rat’s preference to the environment associated with cocaine. (Harris & Aston-Jones, 2003).

Research has indicated that iGluRs also interferes with the conveyance of the rewarding effects of opioids. For example, blockade of ionotropic glutamatergic transmission in the VTA reduces heroin reinforcement in rats (Xi & Stein, 2002) and AP-5 injections in the NAcc decreased ethanol reward (Rassnick, Pulvirenti, & Koob, 1992). Altogether, these findings suggest that iGluRs in the mesocorticolimbic region may also play an essential role in modulating opiate reinforcement.

Collectively, research appears to further favor the conclusion that ionotropic glutamatergic neurotransmission plays an essential role in the process of acquisition and expression of reward-related learning. More specifically, NMDA receptors are crucial for acquisition whereas AMPA receptors appear to be less important for acquisition than for expression of such learning processes. Further investigations into the role of NMDA and AMPA receptors in the brain regions that innervate the VTA will enhance our understanding of the neural substrates underlying the mechanisms of reward-related learning.

4.2. Structure of Metabotropic Glutamate Receptors

In 1985 Glu was reported to stimulate PLC in cultured striatal neurons via a receptor that did not belong to the NMDA, AMPA or KA (kainate) receptor families (Pin & Duvoisin, 1995; Sladeczek, Pin, Recasens, Bockaert, & Weiss, 1985). Soon after, a similar effect of Glu was described in hippocampal slices (Nicoletti, Wroblewski, Iadarola, & Costa, 1986) and cultured cerebellar granule cells (Nicoletti, Wroblewski, Novelli, Guidotti, & Costa, 1986). These results suggested that Glu, like GABA, 5-HT and ACh, not only activated ligand-gated channel receptors but also receptors coupled to GTP-binding proteins (G-proteins). This novelty was then called mGluRs and it was confirmed using the Xenopus
To date, eight members of the G-protein-coupled mGluR family have been identified (mGluR1-8) which have been divided on the basis of sequence homology, second messenger coupling and pharmacology into three groups: group I (mGluR1 and 5), group II (mGluR2 and 3) and group III (mGluR4, 6, 7, 8) (Pin & Duvoisin, 1995; Tanabe, Masu, Ishii, Shigemoto, & Nakanishi, 1992) (Kew & Kemp, 2005). Group I mGluRs predominantly couple via Gq/G11 to phospholipase C, whereas the group II and group III receptors couple via Gi/Go to inhibition of adenylyl cyclase activity.

Although mGluR family members can mediate synaptic transmission via activation of slow excitatory postsynaptic potentials, they generally exert a more modulatory role, regulating neuronal excitability, synaptic transmission and plasticity. MGlur-mediated signaling is achieved both via the activation of intracellular second messenger pathways and subsequent regulation of downstream effectors and through the direct action of the βγ subunits of the heterotrimeric G protein, e.g., in the modulation of ion channel activity (Anwyl, 1999). With the exception of mGluR6 which is confined to the retina (Nakajima et al., 1993) all of the mGluR family members are expressed in the mammalian CNS in both neuronal and glial cells, with individual family members exhibiting distinct spatial and temporal expression profiles (Li et al., 2000) (Thomas et al., 2001).

MGlur family members all possess a large bi-lobed extra-cellular N-terminal domain, which has been demonstrated by site-directed mutagenesis (Malherbe et al., 2001) (Sato, Shimada, Nagasawa, Nakanishi, & Jingami, 2003) to contain the Glu binding site. The N-terminal bi-lobed domain is linked via an extracellular cysteine-rich region to a typical GPCR (G-protein coupled receptor) transmembrane heptahelical domain, which mediates G protein activation (Bhave et al., 2003; Galvez & Pin, 2003). The C-terminus is intracellular and plays an important role in the regulation of receptor activity and targeting through an interaction with proteins including calmodulin (Galvez & Pin, 2003).

Functional mGluRs are thought to comprise homodimers stabilized by both an intersubunit disulphide bond and hydrophobic interactions (Kunishima et al., 2000; Romano, van den Pol, & O’Malley, 1996) (Tsuchiya, Kunishima, Kamiya, Jingami, & Morikawa, 2002). In the absence of ligands or when occupied by competitive antagonists, the bi-lobed extracellular domains of the homodimer exist physically separated in an open conformation. Upon agonist binding they adopt a closed conformation, and move
together into direct contact. Rotation of the extracellular domains of the receptor dimer relative to each other is thought to trigger intracellular signal transduction by stabilizing the two transmembrane heptahelical domains in an active conformation (Kunishima et al., 2000) (Tsuchiya et al., 2002). Research has demonstrated that Ca\(^{2+}\) sensing receptor, isolated from a bovine parathyroid cDNA library, has been found to have about 30% sequence identity with mGluRs (Fujisawa et al., 1993). This receptor is also sensitive to Mg\(^{2+}\) and it is possible that there exist additional ion-sensitive receptors related to mGluRs. This scenario leads the way for future directions in a more in depth mGlu receptors molecular research.

4.2.1. Metabotropic Glutamate Receptors in reward-related learning

Since the development of (+) α-methyl-4-carboxyphenylglycine (MCPG) as a new competitive antagonist for mGluRs (Eaton et al., 1993), an extensive number of studies have examined the implications of mGluRs in the induction of LTP in the hippocampus and only a few were conducted in the midbrain. Collingridge and his colleagues first reported that MCPG blocked the induction of both NMDA receptor-dependent LTP at the CA1 region and NMDA receptor-independent LTP at the CA3 region in rat slice preparations (Bashir, Bortolotto, et al., 1993; Bashir, Jane, Sunter, Watkins, & Collingridge, 1993). Not only the same conclusion of the blocking effect of MCPG on the induction of LTP was reported by two other laboratories (Riedel & Reymann, 1993) (Richter-Levin, Errington, Maegawa, & Bliss, 1994), but Riedel et al. (1994) and Richter-Levin et al. (1994) also observed that MCPG disrupts spatial learning in a water maze task and a Y-maze task, both of which involve hippocampal-dependent learning. Furthermore, in contrast to Bashir et al. (1993) findings that MCPG antagonized both the mGluR-mediated blocking effect of ACPD (selective mGluR receptor agonist) on the slow AHP (after hyperpolarization) in hippocampal pyramidal cells – CA1 region – and the mGluR-mediated presynaptic inhibitory effect on EPSPs (excitatory postsynaptic potentials) in the CA1 and CA3 regions, Manzoni et al. (1994) reported that this antagonist was without effect on LTP in both the CA1 and CA3 regions. Chinestra et al. (1993) observed that MCPG neither prevented the induction of LTP nor antagonized the above mGluR actions in the CA1 region. These conflicting findings among different laboratories remain to be resolved. Collingridge and his colleagues extended their observation and showed that the role of mGluRs in LTP induction is different from that of NMDA receptors (Bortolotto, Bashir, Davies, &
and that MCPG reversibly blocked the induction of LTP in naive slices but failed to prevent the subsequent induction of LTP after LTP had already once been initiated.

Most information accrued regarding glutamatergic involvement in behavioral activation involves mostly ionotropic glutamate receptors (Burns, Everitt, Kelley, & Robbins, 1994; Pap & Bradberry, 1995; Pulvirenti & Koob, 1994). Thus, relatively few studies have evaluated the role of mGluRs in the mesoaccumbens projection to modulate motor activity. For example, Swanson and Kalivas (2000) showed in an intracranial bilateral cannulae microinjections study that Group I and Group II mGluR stimulation in the VTA and NAcc elicits motor activation. Kim and Vezina (1998) showed that rats pre-exposed to AMPH (amphetamine) in the VTA showed significantly higher locomotor activity when subsequently tested with a systemic AMPH challenge than rats pre-exposed to VTA saline. Rats pre-exposed to AMPH but co-injected with the selective mGluR antagonist RS-MCPG did not show this effect. These findings demonstrate that activation of mGluRs in the VTA is crucial for the induction of locomotor sensitization to amphetamine and further demonstrate the importance of excitatory amino acids in the VTA in the development of sensitization to amphetamine.

Even though all of the above studies were based on experiments using MCPG, it is noteworthy to mention that MCPG is a relatively weak antagonist and also reacts with different subtypes of mGluRs (Hayashi et al., 1994). Because circumstantial evidence has supported the involvement of mGluRs in the induction of LTP (Zheng & Gallagher, 1992) (Bortolotto et al., 1994) (Bortolotto & Collingridge, 1993) different approaches are also necessary to investigate the role and mechanisms of mGluRs in the induction of LTP and consequently reward-related learning. The exact mechanisms involved in LTP induction or potentiation by mGluRs are not known. Although the activation of PKC and the resulting potentiation of NMDA responses (O'Connor, Rowan, & Anwyl, 1994) may explain the facilitatory role played by mGluRs in the NMDA-mediated induction of LTP (M. A. Musgrave, Ballyk, & Goh, 1993), this action may not be enough for a pure mGluR-mediated induction of LTP. It is possible that an increase in cAMP induced by mGluRs may also be involved in this mechanism (Gereau & Conn, 1994; I. F. Musgrave, Genieser, Maronde, & Seifert, 1993).

mGluRs have been found to play an important role in regulating intra-cranial self-stimulation behavior in the VTA (Taber, Das, & Fibiger, 1995) (Kenny, Gasparini, & Markou, 2003; Meller, Harrison, &
Sharp, 2002). In addition to that, investigations have begun to elucidate the role of mGluRs in regulating drug use and abuse. Particularly, there appears to be an increased interest in the role of mGlu5 receptors based on the observations that mice in which the deleted gene encoding for the mGlu5 receptor did not acquire cocaine self-administration behavior (Chiamulera et al., 2001). Interestingly, responding for food under a similar schedule of reinforcement was not affected in these same mice (Chiamulera et al., 2001) demonstrating that the lack of responding for cocaine was not secondary to a deficit in learning or motor processes. Additional studies show that MPEP – an mGlu5 receptor antagonist – decreased cocaine self-administration in rats (Kenny et al., 2003) and nicotine self-administration in rats and mice (Kenny et al., 2003). Moreover, activation of mGluRs during pre-exposure to AMPH in the VTA appears to be essential for the enhancement of cocaine self-administration to develop (Kim & Vezina, 1998).

A large body of research also shows that alterations in glutamate-mediated transmission might be the driving force in relapse to drug-seeking behavior (Sutton et al., 2003; Vorel, Liu, Hayes, Spector, & Gardner, 2001). By the same token, but in the opposite direction, activation of Group II mGluRs in the NAcc shell has been shown to attenuate context-induced relapse to heroin seeking (Bossert, Gray, Lu, & Shaham, 2006). Bossert and colleagues (2004) also found, using a rat relapse model, that exposure to the heroin-associated context induced robust reinstatement of drug seeking and this effect was attenuated by systemic or intra-VTA injections of LY379268 – an mGlu2/3 receptor agonist. Moreover, Liechti and colleagues further demonstrated that acute systemic and intra-VTA or intra-accumbens application of the very same agonist decreased nicotine, but not food, self-administration in rats, and decreased both cue-induced reinstatement of nicotine and food-seeking behavior in rats (Liechti, Lhuillier, Kaupmann, & Markou, 2007).

Taken together, research seems to be further corroborating that glutamate transmission in the VTA plays a critical role in the mediation of the rewarding effects of not only drugs of abuse, but also in food reward context.

5. Overview of Specific Aims

This dissertation was designed to test a hypothesis arising from a model of reward-related learning which proposes that synaptic plasticity in the VTA constitutes at least one component underlying
the ability of conditioned stimuli to activate motivational circuits. This model proposes that glutamate synapses receiving signals associated with previously neutral stimuli become strengthened due to coincident excitation of VTA DA cells by cholinergic afferents transmitting a primary reward signal. This synaptic strengthening is mediated by NMDA receptors, whose ion channels are opened in the presence of depolarization, allowing for calcium influx that triggers second-messenger cascades leading to an increased excitability at those synapses, supposedly because of increased sensitivity of existing AMPA receptors or increased trafficking of AMPA receptors into the post-synaptic membrane. As these glutamate synapses are strengthened, glutamate signals associated with environmental signals begin to acquire the ability to activate VTA DA cells on their own, leading to an increase in approach behaviors, and thereby facilitating encounters with stimuli associated with reward.

Furthermore, this model stipulates that NMDA receptor stimulation is critical for the induction of this synaptic strengthening, in other words the acquisition of these conditioned associations, but that, once LTP has occurred, NMDA receptor stimulation, at least alone, is not important for the maintenance of VTA LTP – expression of reward-related associations. The latter appears to be, at least in part, dependent on AMPA receptor stimulation.

Because less is known about the neural mechanisms involved in the maintenance of an already learned behavior, it seems plausible that the maintenance of conditioned approach may be mediated by glutamate neurotransmission. Accordingly, this dissertation tested the hypothesis that blockade of one or more of NMDA, AMPA and mGlu receptors in the VTA impairs the expression of food-based conditioned approach learning.

Thus, the first specific aim of this dissertation was to assess if any kind of glutamate receptor stimulation in the VTA is crucial for a food-associated stimulus (CS) to elicit conditioned approach.

For specific aim 1, a paradigm was designed in which rats would be prepared with indwelling cannulae positioned so as to allow bilateral microinjections of kynurenic acid – (a non-selective ionotropic Glu R antagonist) – in the VTA. Male rats would be trained for seven consecutive sessions to retrieve a food pellet following a light presentation (the CS, a 3-s discrete light immediately preceding the US delivery) and have their head entries before, during and after the CS measured. After that a subsequent extinction session in which neither the CS nor pellets were presented, a final (test) session was
conducted in which rats would be tested for the expression of the food trough approach response with only CS presentations. Again, head entries before, during and after the CS were measured. Rats would receive bilateral injections of kynurenic acid immediately prior to the CS-only test session. Subsequently, all rats' brains would then be removed and processed for cannulae verification. If the number of CS minus pre-CS head entries for any kynurenic acid group did show a significant decrease during the test session in comparison with CS minus pre-CS head entries for rats receiving vehicle treatment, and, as expected, it did, this would indicate an impairment in the expression of conditioned approach. This would further corroborate that Glu receptor stimulation in the VTA is crucial for a food-associated stimulus (CS) to elicit conditioned approach.

The second specific aim of this dissertation is based upon the confirmation of our hypothesis delineated in specific aim 1. We predicted, in keeping with our hypothesis, that intra-VTA application of kynurenic acid would impair reward-related learning and thus rats would show significant decrease in conditioned approach responses when contrasted with vehicle rats. Hence, for specific aim 2, our role was to further investigate which subtype of Glu receptors in the VTA could be responsible for the impairment of the expression of this learned approach. For that we would use selective metabotropic and selective ionotropic Glu Rs antagonists (MCPG; AP-5 and NBQX, respectively) to test which specifically Glu Rs play an important role in the expression of conditioned approach.

For specific aim 2 the methodology is identical to the one delineated in specific aim 1 except that rats would receive bilateral injections of either MCPG, AP-5, NBQX or a combination of both types of ionotopic Glu Rs antagonists compounds immediately prior to the CS-only test session. And if the number of learned approach responses for any of these specific GluR antagonist groups was significantly smaller on the test session in comparison with the conditioned approach responses for rats receiving vehicle treatment, and, as expected, it was, this would presumably indicate which are the Glu receptors responsible for the significant impairment in the expression of conditioned approach response.
Chapter 2.

2. Methodology

The protocols used in the present experiments were in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Queens College Institutional Animal Care and Use Committee. This chapter describes procedures that were used for all experiments described in this dissertation.

2.1. Subjects

Subjects consisted of 178 male Long Evans rats, facility-bred from males and females obtained from Charles River Laboratories (Raleigh, NC), with initial free feeding weights between 350 and 375 g at the time of surgery. All rats were individually housed and maintained on a 12 h light: 12 h dark cycle (lights off at 6AM). All experimental sessions were conducted during the dark phase in order to test the rats during their active periods. All animals had unlimited access to food (LabDiet chow) until experimental sessions began, at which time access was restricted to daily rations that maintained their weights at 85% of their free-feeding values.

2.2. Surgical procedure

All animals received an intraperitoneal (IP) injection of atropine sulfate (0.1 ml) and were anesthetized with sodium pentobarbital (65 mg/kg). Stainless steel guide cannulae (0.635 mm outer diameter, 0.3302 mm inner diameter) were bilaterally implanted to a depth that allowed for microinjections into the ventral tegmental area (VTA) using the following coordinates: −5.6 mm caudal to bregma, ±2.0 mm from the midline at a 10 ° angle toward the midline and −8.4 mm below the surface of the skull. The cannulae were fixed in dental acrylic anchored to the skull by four stainless steel screws. Obturators (0.3048 mm diameter) were inserted at all times except during microinjections.
2.3. Experimental and testing apparatus

All behavioral testing was conducted in eight conditioning chambers each measuring 30 cm × 21 cm × 18 cm (l × w × h). One wall was equipped with a food trough and two white light stimuli, each situated 2.54 cm above and 2.54 cm to the right or left of the food trough. Each food trough was equipped with a photo-emitter and detector to detect head entries. Each chamber was housed in a ventilated, sound attenuating box. The chambers were controlled by a PC through a MED Associates interface.

2.4. Conditioning experiments

Four to seven days after surgery animals began the food restriction diet to reduce their weights to 85% of their free-feeding values where they were maintained for the duration of the experiments. For three consecutive days prior to the magazine training session all rats were given 20 food pellets (45 mg, Purified Formula, Bioserv, Frenchtown, NJ) in a bowl in their home cages.

2.5. Conditioning procedure

Subjects were given one 20-min magazine training session in the conditioning chambers in which 20 food pellets were delivered on a random time schedule, to allow rats to become acquainted with magazine delivery of food pellets. After that, the animals were exposed to 7 conditioning sessions, held one per day, consecutively. During each conditioning session 30 food pellets were delivered on a random time 120-s schedule. Each pellet delivery was preceded by a 3-s presentation of a light on the left side of the trough. One day following these sessions, all rats received one 30-min session during which no light or food presentations were made. This session occurred (1) in an attempt to bring all animals’ responses to the same level; and (2) to prevent a new experience of no food associated with light in the conditioning chambers prior to the test session. The next day was the CS-only test, a 60-min session in which only light presentations were made according to the same random time schedule as in the conditioning sessions and no food pellet deliveries occurred (see Figure 3). Just prior to the CS-only test session, all rats received intra-VTA vehicle or one of the glutamate receptor antagonist microinjections. For all rats the number of head entries during each session was counted and analyzed (see section 2.9
below for details). After the CS-only test session the animals were killed and their brains were extracted and prepared for histological cannula placement verification.

<table>
<thead>
<tr>
<th>S U R G E R Y</th>
<th>D A Y S</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days exposure to food pellets in home cage</td>
<td>7 days surgery recovery</td>
</tr>
<tr>
<td>7 days of 1h sessions where food is delivered right after a 3-s light (formation of light as CS)</td>
<td>7 days of 1h sessions where food is delivered right after a 3-s light (formation of light as CS)</td>
</tr>
<tr>
<td>30 reinforcements</td>
<td>1 day of no CS nor food is delivered</td>
</tr>
<tr>
<td>No microinjections</td>
<td>1 day where only CS is presented but food is not</td>
</tr>
<tr>
<td>Head entries are quantified</td>
<td>Animals receive Intra-VTA Microinjections prior the session</td>
</tr>
</tbody>
</table>

*Figure 4*: Summary of the main points elaborated in the text. During the seven days of one hour sessions, the environmental stimulus (light) was paired with the unconditioned stimulus (food pellet). On the last day – test session – the expression of the previously learned approach was measured.

### 2.6. Microinjection procedure

Immediately prior to the CS-only test session both obturators were removed from the guide cannulae and a stainless steel injector tube was inserted in each side to extend 1 mm beyond the end of each guide cannula. Each injector was connected by polyethylene tubing to a 10 µl Hamilton syringe (Reno, NV) preloaded with one of the glutamate receptor antagonists (kynurenic acid, MCPG, AP-5, NBQX, AP-5/NBQX combination) or its respective vehicle. The compound was injected using a pump (Harvard Apparatus) over a 90-s period. Each injector was kept in place for an additional 60 s before being removed and the obturator replaced. Next, the animals were placed in their respective conditioning chambers and the test sessions started.

### 2.7. Drugs

All drugs were purchased from Sigma–Aldrich, St. Louis, MO. For all drugs, each microinjection was delivered in a volume of 0.5 µl. Kynurenic acid (ionotropic glutamate receptor antagonist) was dissolved in NaOH and the doses used were 0.28125, 1.125 and 4.5 µg. MCPG (metabotropic glutamate receptor antagonist) was dissolved in DMSO and the doses used were 1.875, 3.75 and 7.5 µg. AP-5
(NMDA receptor antagonist), NBQX (AMPA receptor antagonist) and AP-5/NBQX combination were all dissolved in 0.9% saline. The doses of each of these latter compounds were 0.03125, 0.5, 1.0, 2.0 µg; 0.5, 1.0, 2.0, 4.0 µg; and 0.03125/4.0, 0.25/4.0, 2.0/4.0 µg, respectively. The doses of these compounds were chosen based on effective dose ranges observed in the literature.

2.8. Histology

Immediately after the end of the last session rats were anesthetized with an overdose of sodium pentobarbital, perfused with 0.9% saline followed by 4% formalin, and decapitated. The brains were removed and stored in 4% formalin for at least seven days before being sectioned in the coronal plane on a cryostat and inspected for cannulae implantations and injection sites. All rats included in the data analysis had verified cannulae placements in VTA.

2.9. Data analysis

The data consisted of the number of food trough head entries made during (1) the 6-s periods immediately preceding the onset of the CSs (pre-CS period), (2) the 6-s periods commencing with the onset of the CSs, (3) at all other times (non-CS period). For each session the total number of head entries during the CS periods and the total number of head entries during the pre-CS periods were used to calculate the difference scores between CS and pre-CS head entries. This difference score directly indicates the magnitude of the conditioned approach response (i.e., the degree to which food trough head entries were elicited by the CS). Separate one-way ANOVAs with dose as a between groups factor and session as a repeated measures factor were conducted on the total number of head entries data from sessions 1 to 7 for each test compound. Significant interactions were followed by tests of simple effect of session at each dose. Separate one-way ANOVAs were conducted on the difference scores (CS minus pre-CS food trough head entries) during the CS-only test session. Significant effects were followed by Tukey’s post hoc tests.
3. Results

3.1. Histology

Only the data from rats with verified VTA cannula placements were included in these results. The majority of microinjection sites were localized in the caudal portion of the VTA (-5.6 to -6.04 mm posterior to bregma) with some injections occurring in the central portion of the VTA (-5.2 to -5.3 mm posterior to bregma) (see Figure 4).

Although during conditioning all groups received no treatments prior to the sessions, the data were divided according to the treatment that they would receive in the CS-only test session and analyzed. All groups showed similar patterns of increasing difference scores across early conditioning days and remaining stable during the last few conditioning days. Statistical analyses showed that there were no significant differences among groups.

3.2. Effects of intra-VTA kynurenic acid on expression of conditioned approach

Figure 5 shows the difference score between CS and pre-CS food trough head entries in the CS-only test session after groups received vehicle or a dose of kynurenic acid. The 1.125 and 4.5 µg groups showed smaller difference scores than the 0.28125 µg and vehicle groups. A one-way ANOVA revealed a significant dose effect [F (3, 25) = 6.06, p < .01]. Tukey’s post hoc comparisons revealed that the animals receiving 1.125 or 4.5 µg kynurenic acid showed significantly smaller difference scores compared to animals receiving vehicle (p < .01). Figure 6 shows that all groups made similar amounts of total head entries during the CS-only test session except for the 4.5 µg kynurenic acid group which made a greater number of total head entries. A one-way ANOVA revealed a significant dose effect [F (3, 25) = 4.358, p < .05] and Tukey’s post hoc tests showed that the 4.5 µg group was significantly different from the 0.28125 and 1.125 µg groups.
3.3. Effects of intra-VTA MCPG on expression of conditioned approach

In Figure 7, CS minus pre-CS food trough head entries in the CS-only test session are shown after groups receiving vehicle or a dose of MCPG. All groups showed similar difference scores when compared to vehicle. Statistical analyses supported this observation with a one-way ANOVA failing to reveal a significant dose effect. Figure 8 shows that during the CS-only test session rats receiving 1.875 µg MCPG made fewer total head entries than the groups receiving vehicle, 3.75 µg or 7.5 µg MCPG. A one-way ANOVA revealed a significant dose effect \[F (3, 28) = 6.798, p < .01\]. Tukey’s post hoc comparisons among MCPG doses revealed that the animals receiving 1.875 µg MCPG made significantly fewer total food trough head entries compared to animals receiving vehicle, 3.75 or 7.5 µg MCPG \(p < .01\).

3.4. Effects of intra-VTA AP-5 on expression of conditioned approach

Figure 9 shows the difference score during the CS-only test session in rats receiving vehicle or a dose of AP-5. Although rats receiving AP-5 tended to show higher difference scores than the vehicle group the differences were not significant. In Figure 10, the CS-only test session shows that all groups receiving AP-5 appeared to make more total food trough head entries than the vehicle group but a one-way ANOVA failed to reveal a significant dose effect.

3.5. Effects of intra-VTA NBQX on expression of conditioned approach

Figure 11 shows the difference scores in the CS-only test session after groups received vehicle or a dose of NBQX. All groups demonstrated similar difference scores when compared to vehicle. Statistical analyses supported these observations with a one-way ANOVA failing to reveal a significant dose effect. In the CS-only test session, all groups appeared to make similar amounts of total head entries (Figure 12). A one-way ANOVA revealed a significant dose effect \[F (4, 36) = 2.640, P < .05\] but Tukey’s post-hoc comparisons failed to reveal significant differences among doses.
3.6. Effects of intra-VTA AP-5/NBQX combinations on expression of conditioned approach

Figure 13 shows the difference scores between CS and pre-CS food trough head entries in the CS-only test session after groups received vehicle or AP-5 alone or AP-5 combined with NBQX. Groups receiving the AP-5/NBQX combinations demonstrated a dose-related reduction in the difference score compared to vehicle. A one-way ANOVA on these data revealed a significant dose effect [F (4, 39) = 4.543, \( p < .001 \)]. Tukey’s post-hoc tests confirmed that 0.25 AP-5/4.0 \( \mu \)g NBQX and 2.0 AP-5/4.0 \( \mu \)g NBQX had significantly smaller difference scores than vehicle and AP-5-alone. Parallel to it, the group receiving 0.03125 AP-5/4.0 \( \mu \)g NBQX made a greater number of total head entries than all the other groups (Figure 14). A one-way ANOVA on these data revealed a significant dose effect [F (4, 39) = 4.035, \( p < .01 \)]. Tukey’s post-hoc tests indicated that the 0.03125 AP-5/4.0 \( \mu \)g NBQX group emitted a significantly greater number of total head entries in the CS-only test session compared to vehicle, \( p < 0.05 \). No significant difference was found between the vehicle group and four subjects under 2.0 AP-5/4.0 \( \mu \)g NBQX group whose site of injection “missed” the VTA (data not shown).
Figure 5: Histological reconstruction of injection sites adapted from Paxinos and Watson (Paxinos & Watson, 1982). Black circles represent intra-VTA injections. The numbers to the right of each section indicates the distance posterior to bregma.
Figure 6: Mean (±SEM) CS minus pre-CS difference scores for groups receiving no treatment prior to each conditioning session and kynurenic acid prior to the CS-only session. ♦ represents significant increase across the seven conditioning sessions. The arrows represent significant differences from the vehicle group, p < 0.01. kyn is kynurenic acid.
Figure 7: Mean (±SEM) total number of food trough head entries emitted during the seven conditioning and the CS-only sessions for groups receiving no treatment prior to each conditioning session and kynurenic acid prior to the CS-only session.
Figure 8: Mean (±SEM) CS minus pre-CS difference scores for groups receiving no treatment prior to each conditioning session and MCPG prior to the CS-only session. ♦ represents significant increase across the seven conditioning sessions.
Figure 9: Mean (±SEM) total number of food trough head entries emitted during the seven conditioning and the CS-only sessions for groups receiving no treatment prior to each conditioning session and MCPG prior to the CS-only session. (B)
Figure 10: Mean (±SEM) CS minus pre-CS difference scores for groups receiving no treatment prior to each conditioning session and AP-5 prior to the CS-only session. ♦ represents significant increase across the seven conditioning sessions.
Figure 11: Mean (±SEM) total number of food trough head entries emitted during the seven conditioning and the CS-only sessions for groups receiving no treatment prior to each conditioning session and AP-5 prior to the CS-only session.
Figure 12: Mean (±SEM) CS minus pre-CS difference scores for groups receiving no treatment prior to each conditioning session and NBQX prior to the CS-only session. ♦ represents significant increase across the seven conditioning sessions.
Figure 13: Mean (±SEM) total number of food trough head entries emitted during the seven conditioning and the CS-only sessions for groups receiving no treatment prior to each conditioning session and NBQX prior to the CS-only session.
Figure 14: Mean (±SEM) CS minus pre-CS difference scores for groups receiving no treatment prior to each conditioning session and AP-5 or AP-5/NBQX combination prior to the CS-only session. ♦ represents a significant increase across the seven conditioning sessions. The arrows represent significant differences from the vehicle and 0.03125 µg AP-5 groups, p < 0.05.
Figure 15: Mean (±SEM) total number of food trough head entries emitted during the seven conditioning and the CS-only sessions for groups receiving no treatment prior to each conditioning session and AP-5 or AP-5/NBQX combination prior to the CS-only session.
Chapter 3.

Discussion

This dissertation tested the hypothesis that blockade of either metabotropic or NMDA and/or AMPA receptors in the VTA impairs the expression of food-based conditioned approach learning. The findings here described support this hypothesis. More specifically, bilateral microinjections of the metabotropic glutamate receptor antagonist MCPG into the VTA did not alter the expression of learned approach whereas the injection of the combined NMDA and AMPA receptor antagonists compound AP-5/NBQX into the VTA significantly impaired the expression of reward-related learning. In short, these findings indicate that at least a part of the neural changes critical for the maintenance of an already learned reward-related behavior do occur in the VTA, and that these neural changes do involve NMDA and AMPA receptor stimulation.

The findings of this dissertation summarize as follows: both the non-selective receptor antagonist kynurenic acid and the selective glutamate receptor antagonist AP-5/NBQX combination caused a dose-related reduction in the CS minus pre-CS head entries difference scores – that is, they caused a reduction in the number of conditioned approach responses. When rats were treated with intra-VTA injections of the highest and medium doses of kynurenic acid, the CS minus pre-CS difference score decreased significantly. Intra-VTA injections of the highest and second to highest AP-5/NBQX combination in rats produced similar results where the difference scores showed significant decreases. These findings indicate a decrease in the effectiveness of the CS to elicit conditioned approach and support our hypothesis that glutamate neurotransmission in the VTA is necessary for the expression of conditioned approach and reward-related learning in general (Zellner & Ranaldi, 2010).

The observed impairment in the expression of conditioned approach after kynurenic acid or combined AP-5/NBQX treatments cannot adequately be explained as a general reduction in motoric activity. First, rats treated with kynurenic acid or AP-5/NBQX combinations emitted as many total head entries as their respective vehicle groups during the CS-only test session (under treatment). This indicates that treatment with these glutamate receptor antagonists did not impair the rats’ abilities to enter
the food trough during the CS presentation. The observed reduction in conditioned approach after kynurenic acid or combined AP-5/NBQX treatment suggests a reduced efficiency of the CS to keep on eliciting approach. In other words, such findings support the notion that the key element impaired in our studies during the expression of conditioned approach was the CS ability to continue controlling behavior. Specifically, it appears that not only NMDA receptor stimulation in the VTA is necessary for the CSs to elicit incentive-motivational behavior (Zellner et al., 2009) (Ranaldi et al., 2011), but it also appears, at least in part, crucial for the maintenance of an already learned behavior.

We also found that neither intra-VTA injections of the non-selective metabotropic glutamate receptor antagonist MCPG, nor AP-5 or NBQX alone caused reductions in the number of food trough head entries during the CS presentation. Although in general the magnitude of learning increased for all the rats in all treatment groups across the conditioning days, some distinctions among test compounds during the expression tests were noted.

Animals in any of the four doses of MCPG tested showed on average minimal differences in their difference scores among the doses. Thus, MCPG did not produce any behavioral effect pertinent to the expression of conditioned approach suggesting that stimulation of metabotropic glutamate receptors in the VTA is presumably not necessary for the performance of this behavior. Indeed, there is some evidence that further corroborates that idea. Intraventricularly pre-injected rats with MCPG demonstrated to take longer time to reach the platform than vehicle rats in a Morris water maze paradigm (Bordi, Marcon, Chiamulera, & Reggiani, 1996). However, once the pre-treated rats learned the task, they reached the same level of performance as control animals (Bordi et al., 1996). Not only these findings suggest that MCPG disrupts the acquisition of Morris water maze learning, but it further supports our findings showing that once learning occurred, metabotropic glutamate receptors are not critical for the expression of an already learned behavior.

Similarly to MCPG manipulation findings in our study, the different doses of NBQX produced minimal differences showing a slight trend toward a dose-related decline in approach responding. Thus it appears likely that NBQX attenuated, though minimally and non-significantly, the CS signal carried by glutamatergic inputs to the VTA. Consideration of the neurochemistry and neuroanatomy of the VTA suggests an interesting and possible explanation that may account for this event.
The VTA receives input via a variety of neurotransmitter systems including glutamatergic, GABAergic, serotonergic and cholinergic (Goldner, Dineley, & Patrick, 1997; Korotkova, Ponomarenko, Brown, & Haas, 2004). Assuming that AMPA receptor pool on DA cells and AMPA receptor pool on GABA cells are differentially sensitive to NBQX blockade, we can conjecture that a higher dose of NBQX, by sufficiently blocking AMPA receptor pools in both DA and GABA cells, would, by blocking excitation of GABAergic cells, indirectly disinhibit DA cells, and by blocking excitation of dopaminergic cells, directly inhibit DA cells. These two effects could hypothetically partially cancel each other out, leaving mesolimbic dopaminergic tone closer to baseline levels. This would result in less behavioral activation with levels similar to vehicle levels. And this is exactly what we observed (fig. 12).

Furthermore, a study conducted by You et al. (You et al., 2007) demonstrated that glutamate release in the VTA might play a critical role in cocaine seeking. They further reported that cocaine seeking was attenuated by intra-VTA irrigation with AP-5 alone, CNQX – a purported AMPA receptor antagonist – alone, or both antagonists simultaneously, suggesting that cocaine seeking is dependent on the stimulation of one or both NMDA and AMPA receptors. Contrary to their findings, in our study, blockade of AMPA receptors alone with NBQX did not reduce the expression of conditioned approach. Noteworthy to point that cocaine seeking is a learned behavior just as conditioned approach is. One possible explanation that may account for such discrepancy between these two studies is that they used CNQX whereas we used NBQX as AMPA receptor antagonist. Research shows that NBQX is a more selective blocker of AMPA receptors than CNQX (Yu & Miller, 1995); (Mead & Stephens, 1999); and that CNQX, although considered an AMPA receptor antagonist, has also a significant blocking action at the glycine site of NMDA receptors (Mead & Stephens, 1999; Sheardown, Nielsen, Hansen, Jacobsen, & Honore, 1990; Yu & Miller, 1995). Interestingly, evidence has documented that NBQX failed to block the expression of place-preference in an amphetamine-conditioned place preference study, whereas CNQX did not (Mead & Stephens, 1999) showing to effectively block the expression of place preference via its glycine antagonism action (Mead & Stephens, 1999). Thus, it appears plausible to infer that You et al. (You et al., 2007) observed significant decrease in cocaine seeking with CNQX because of its antagonistic action at the NMDA site receptor rather than AMPA receptor. Therefore, we can state with more assurance our argument that both NMDA and AMPA receptors are important for the expression of
conditioned approach, since in our study we used AP-5/NBQX treatment, which are both specific and selective NMDA and AMPA antagonists respectively.

In a contrary pattern to our NBQX treatment findings, rats treated with AP-5 alone demonstrated an increasing trend in the learned approach responding. One possibility that may explain this is that NMDA receptor antagonist treatment in the VTA results in increasing DA neurotransmission in the NAcc (Johnson & North, 1992b), subsequently elevating motoric activity levels.

While blockade of neither VTA NMDA nor VTA AMPA receptors alone reduced CS head entries during the CS-only session, there occurred significant reductions in CS minus pre-CS difference scores when animals were treated with NMDA and AMPA receptor antagonists combined, suggesting that under the condition when both of these ionotopic receptors are blocked the food-related CS is less effective to elicit conditioned approach responses. So, the relevant question here is why was conditioned approach reduced only when both NMDA and AMPA receptors were simultaneously blocked? The development of LTP, including in the DA cells in the VTA, has been shown to be NMDA receptor dependent (Bonci & Malenka, 1999; Stuber et al., 2008). Under normal physiological conditions, the synaptic plasticity induced by NMDA receptor stimulation allows environmental signals to activate VTA DA cells. This stimulation of VTA neurons elicits an appetitive motivational state and triggers approach behaviors, which are both important for engaging the organism in reward-directed behavior, thereby increasing the animal’s encounters with reward-related stimuli. Simultaneously, increased DA release at the terminal regions allows for downstream associative processes to occur which underlie additional associative processes necessary in reward-related learning, such as stimulus-response and response-outcome dependent associations.

As we discussed above, whereas the initiation of LTP is shown to be NMDA receptor dependent, the expression of LTP, on the other hand, appears to crucially involve AMPA receptors (Muller, Joly, & Lynch, 1988; M. Park, Penick, Edwards, Kauer, & Ehlers, 2004) (Lee et al., 2003). Based on the neurophysiology of LTP, and assuming that the expression of learning requires the expression of LTP, one might expect that blockade of AMPA receptors, which would consequently block the expression of LTP, would have inhibited, or at least impaired, the expression of conditioned approach in the present studies. However, that is not the case. We found that AMPA receptor antagonism significantly reduced
conditioned approach only when it was accompanied by antagonism of NMDA receptors. One possible explanation for this is that our NBQX treatments did not fully antagonize the relevant AMPA receptor pools and that an effective antagonism of the pool was only achieved by simultaneous blockade of NMDA receptors with AP-5. Alternatively, we could also presume that at its highest dose – 4 µg/0.5 µl – AMPA receptors were fully blocked by NBQX and therefore reached a saturation point. Once that happened, other surrounding receptor types were left to function normally – possibly NMDA receptors in our case. Next, the blockade of a small pool of these NMDA receptors at their glutamate site would be the missing piece of the puzzle that once it occurred, it could be the reason why impairment of the expression of conditioned approach was observed only when antagonism of AMPA receptors was accompanied by the antagonism of NMDA receptors in the VTA.

This would suggest that at least one component of AMPA receptor postsynaptic effects require NMDA receptor stimulation, an idea that is supported by other work (Shi et al., 1999) (Lopez, Gamache, Schneider, & Nader, 2015). Clearly, more research is needed to investigate and understand the complex interplay between NMDA and AMPA receptors in the VTA in the mediation of food reward-related learning processes.

Here, NMDA receptor stimulation in the VTA, at least alone, proved, again, not to be necessary for expression of reward-related learning. This finding is supported by other studies in our lab (Zellner, 2008) (Ranaldi et al., 2011), and is also similar to other studies which blocked NMDA receptors in the NAcc (Kelley et al., 1997) (Di Ciano et al., 2001) (Hernandez, Andrzejewski, Sadeghian, Panksepp, & Kelley, 2005). This was indicated by the fact that animals did not significantly reduce their learned approach responses on the final session after pre-treatment with AP-5. The fact that isolated NMDA receptor antagonism did not impair the expression of conditioned approach is consonant with part of the rationale that led to our hypothesis – once glutamatergic synapses carrying environmental stimuli-related signals have been strengthened, in other words acquisition of learning occurred, activation of VTA DA cells is no longer dependent on NMDA receptor stimulation (at least not alone) during the expression phase. Thus, the performance of conditioned approach learning would more likely be dependent on some other mechanism, possibly AMPA receptor stimulation, as we have previously discussed.

Other studies have assessed the involvement of glutamate receptors in reward-related learning.
Our lab tested the effects of intra-VTA AP-5 in animals acquiring or maintaining a learned food-reward operant response and found that it blocked acquisition, but not expression, of this response (Zellner et al., 2009). Furthermore, in a conditioned approach response procedure identical to the current one, intra-VTA AP-5 blocked the acquisition of learning but, again, not the expression of the behavior (Zellner et al., 2009) (Ranaldi et al., 2011). Others have found a comparable effect; for example, an NMDA receptor antagonist manipulation into the VTA blocked the development of morphine place preference (Harris et al., 2004), whereas only a concomitant treatment of NMDA and AMPA receptor antagonists effectively interfered with the expression of conditioned place preference (Harris et al., 2004). Not only this indicates that NMDA receptors are crucial for the acquisition of reward-related learning, but also emphasizes that NMDA receptors alone do not account for the expression of reward-related learning processes to occur.

You and colleagues (You et al., 2007) demonstrated that irrigation of the VTA with AP-5 through a microdialysis probe attenuated context-induced cocaine-seeking, suggesting that by itself, NMDA receptor stimulation may play a significant role in expression of reward-related learning. As mentioned previously, this study also demonstrated the same effect when an AMPA antagonist was applied and when NMDA and AMPA antagonists were applied simultaneously (You et al., 2007). Similarly, a combination of AMPA and NMDA receptor antagonists reduced reinstatement of cocaine seeking elicited by cues in a Pavlovian cue-triggered drug-seeking paradigm (Backstrom & Hyytia, 2006; Mahler, Smith, & Aston-Jones, 2013). In contrast, Solecki and colleagues (Solecki et al., 2013) showed that in a cue-induced cocaine-seeking paradigm, VTA infusion of AP-5 failed to affect either cocaine seeking or phasic DA release in terminal regions. As one can note, the precise roles of VTA AMPA and VTA NMDA receptors, specifically in the expression of reward-related learning phenomena, as well as the complex interaction between these ionotropic receptors, remains to be fully understood.

Other neurotransmitters in the VTA may also be involved in the expression of reward-related learning. For example, intra-VTA injections of scopolamine, a muscarinic acetylcholine receptor antagonist, significantly reduced lever pressing during acquisition of this task, but once the task was learned, had no effect on its maintenance (Sharf & Ranaldi, 2006) (Sharf et al., 2006). Also, a new stream of studies involving the manipulation of orexin – a neuropeptide that regulates arousal, wakefulness and appetite (Halford & Blundell, 2000) (Wise, 2006) – has demonstrated similar effects to the ones previously
reported. Orexin antagonism significantly attenuated the expression of cue-induced reinstatement of cocaine-seeking in rats (R. J. Smith, See, & Aston-Jones, 2009), reduced heroin self-administration and heroin seeking elicited by cues (R. J. Smith & Aston-Jones, 2012), decreased the expression of amphetamine sensitization (Quarta, Valerio, Hutcheson, Hedou, & Heidbreder, 2010), and also reduced the acquisition and expression of cocaine-conditioned reinforcement and the expression of amphetamine-conditioned reward (Hutcheson et al., 2011).

This would suggest that other neurochemicals may also play an important role in the expression of reward-related learning. Evidently, more research is necessary to investigate and better comprehend the convoluted interaction between other neurochemicals and glutamate receptors in order to shed light on which elements are crucial for the expression of conditioned approach in reward-related learning. Besides indicating a decrease in the effectiveness of the CS to elicit conditioned approach, our findings could also possibly indicate that antagonism of NMDA and AMPA receptors in the VTA can inhibit the capacity of a light CS to elicit conditioned approach responses. This suggests that an environmental glutamate signal acting at ionotropic glutamate receptors in the VTA mediates the control of food associated CSs to activate approach-eliciting neural substrates, perhaps through phasic activation of VTA DA cells.

Another possibility is that the antagonism of both ionotropic glutamate receptors in the VTA reduced the performance of the conditioned approach behavior not because it blocked a glutamate signal (i.e., phasic DA activation) but because it reduced tonic DA activity. However, if this was the case one might have expected reduced activity in general which would have been manifested in our procedure as a reduced number of total head entries. We found that total head entries were not significantly lower in antagonist-treated groups as opposed to vehicle groups making this explanation not likely.

In a more specific setting such as the neurochemical level under which the impairment of this conditioned approach performance occurred, it is possible that interference with the expression of learning was more due to effects on GABA neurons than on DA neurons. Conceivably, AP-5 and NBQX could have blocked synaptic transmission therefore disrupting synaptic plasticity on GABA cells, which in turn would have affected the behavioral expression of the learned response. DA neurons and GABA neurons in the VTA exhibit similar AMPA/NMDA current ratios (Bonci & Malenka, 1999) indicating that
GABA cells also possess NMDA and AMPA receptors. However, while DA cells demonstrate LTP following paired pulse stimulation, GABA cells did not. In contrast, they showed LTD (Bonci & Malenka, 1999). This is in accordance with studies done in the hippocampus showing that excitatory synapses, at least on majority of GABA cells, do not express LTP (Nugent, Hwong, Udaka, & Kauer, 2008). As previously noted, based on the neurophysiology of LTP which accounts NMDA receptors for the induction of LTP (M. A. Musgrave et al., 1993) (Muller et al., 1988) and AMPA receptors for the expression of LTP (Mead & Stephens, 2003) (Nicoll, 2003) (Lee et al., 2003) (Malinow & Malenka, 2002), and assuming that the expression of learning requires the expression of LTP, it is plausible to argue that blockade of NMDA and AMPA receptors on GABA cells would also block the expression of LTP. However, because NMDA and AMPA receptor antagonism would most likely not affect LTP on GABA cells, a blockade of GABA neuron potentiation cannot account for our findings.

In summary, intra-VTA microinjections of kynurenic acid or AP-5/NBQX combinations significantly reduced conditioned approach responding but similar treatments with MCPG, AP-5 alone and NBQX alone did not. These results suggest that performance of reward-related learning, such as conditioned approach, is necessarily dependent on the stimulation of NMDA or AMPA receptors in the VTA.

Thus, the present findings contribute to extend our knowledge and further deepen our understanding of the neural mechanisms underlying reward-related learning associations in general, and also in regards to pathological behaviors based on stimulus-stimulus association disorders such as drug and food addiction. As such, additional research into intracellular processes critical for the establishment and performance of reward-related learning behaviors appear to be deemed necessary and will enable us to answer the question that we posed initially – what is critical for the occurrence of expression of reward-related learning once glutamate signals have already been strengthened? As discussed earlier, a wealth of evidence from different lines of research demonstrates that LTP is likely to be dependent on AMPA receptors, and that once the stimulus-stimulus association is formed and learned by the organism, an alternative neural mechanism appears to “take charge” and be responsible for the expression of such learning. Gathering evidence and theorizing plausible outcomes in light of our findings will increase our understanding of brain mechanisms that contribute to the maintenance of drug or food conditioned approach and lead to novel pharmacotherapeutic treatment strategies.
Reflections on Glutamate-Dopamine interactions in Reward-Related Learning and their Social Implications

Traditionally, reward-related learning research in neuroscience has focused on mechanisms involving dopamine. It is only more recently that it has been realized that glutamate also plays a central role in processes underlying the acquisition and performance of conditioned associations in the addiction field including reinforcement, sensitization, habit learning and reinforcement learning, context conditioning, craving and relapse.

In the past few years, major progress has been made towards understanding how glutamate acts and interacts with other transmitters (in particular, dopamine) in the context of processes underlying reward-related learning.

It appears that while many actions of glutamate derive their importance from a stimulatory interaction with the dopaminergic system, there are some glutamatergic mechanisms that contribute to learning independent of dopaminergic systems. Among those, context-specific aspects of behavioral determinants (i.e. control over behavior by conditioned stimuli) appear to heavily depend on glutamatergic transmission, and it is these glutamatergic mechanisms that may be responsible for plastic changes in the brain that lead to learned associations.

It is clear that neither dopamine nor glutamate alone mediate processes underlying the acquisition and expression of learned approach. While the consideration of other potentially important transmitters such as the endogenous opioids, GABA, acetylcholine, noradrenaline, or even cholecystokinin are beyond the scope of this dissertation topic, it is worth to briefly consider the interactions between dopamine and glutamate in the context of reward-related learning processes.

The mesocorticolimbic dopamine system is intricately connected with glutamatergic structures or their efferents. Both the cell body region in the VTA and the terminal region in the nucleus accumbens receive massive glutamatergic input from several corticolimbic structures such as PFC, amygdala and hippocampus (Christie, Summers, Stephenson, Cook, & Beart, 1987; Kelley, Domesick, & Nauta, 1982), structures that have been implicated in aspects of reward evaluation, conditioning and learning (Everitt, Morris, O'Brien, & Robbins, 1991; Everitt et al., 1999). The interaction between glutamate and dopamine
in VTA is rather complex, but in more simplified terms, glutamatergic input to the VTA increases the activity of dopaminergic cells and enhances dopamine release in the nucleus accumbens (Tzschentke, 2001; Tzschentke & Schmidt, 2000).

Although dopamine affects glutamatergic transmission by modulating glutamatergic signals in the nucleus accumbens originating from the amygdala and hippocampus (Floresco, Blaha, Yang, & Phillips, 2001), some glutamatergic mechanisms appear to be independent of dopaminergic mechanisms. For example, it has been demonstrated that in mice lacking mGluR5, cocaine does not produce locomotor-stimulant effects and lacks rewarding effects, as evidenced by an absence of cocaine self-administration behavior (Chiamulera et al., 2001). This suggests that the deficits in the mutant mice were not secondary to changes in the dopaminergic system but were solely due to changes in glutamatergic signaling.

As previously mentioned, glutamate neurotransmission appears to be involved in a series of reward-related learning processes, including drug addiction. After sensitizing treatment schedules with drugs of abuse, repeated cocaine or amphetamine administration was shown to enhance the responsiveness to glutamatergic stimulation of mesolimbic dopamine neurons and to reduce the responsiveness to glutamatergic stimulation of nucleus accumbens neurons (White, Hu, Zhang, & Wolf, 1995, X. F. Zhang, Hu, White, & Wolf, 1997), to alter the expression of glutamate receptor subunits/splice variants, in particular, in the mesolimbic system (Churchill, Swanson, Urbina, & Kalivas, 1999, Fitzgerald, Ortiz, Hamedani, & Nestler, 1996), and to result in increased glutamate releasability in the accumbens (Pierce, Bell, Duffy, & Kalivas, 1996, Reid & Berger, 1996). Taken together, this shows that repeated psychomotor stimulant administration can alter responsiveness of the mesoaccumbens DA system to glutamate suggesting the sensitization phenomenon to be intrinsically associated with alterations in glutamate transmission of the mesoaccumbens DA pathway. This further explains that the behavioral sensitization developed under drug addiction and certain of its neuronal correlates can be prevented by glutamate antagonists, such as AP-5 or NBQX or both, suggesting not only an integral role for glutamate systems in the sensitization processes, but also highlighting the importance of glutamate in the expression of reward-related learning.

In reinforcement learning for example, glutamatergic mechanisms in the nucleus accumbens core appear to be involved in response-reinforcement learning in the acquisition of a lever-press task to obtain
food reward (Kelley et al., 1997). For example, injection of the NMDA receptor antagonist AP-5 into the accumbens core impaired the acquisition (but not the expression) of this task, while leaving feeding and locomotor responses and the formation of stimulus–reward associations intact.

In addition, recent brain imaging studies in human addicts have shown that the presentation or mental representation of US-related cues can trigger craving and is associated with increased activity in the amygdala and prefrontal cortex (Childress et al., 1999, Wexler et al., 2001). The increased activity in these areas could result in increased glutamatergic transmission in the accumbens, which nicely fits the emerging picture on the role of nucleus accumbens glutamate in reinstatement of drug-seeking behavior derived from animal studies. Another line of evidence showing the role of glutamatergic transmission in reinstatement of drug-seeking behavior comes from the work of Vorel et al. (Vorel et al., 2001) who showed that electrical stimulation with physiologically relevant parameters of the ventral subiculum of the hippocampal formation potently reinstates cocaine-seeking behavior. Moreover, it was further shown that comparable reinstatement effects could be obtained by intra-VTA infusion of NMDA, and that stimulation-induced reinstatement was completely blocked by microinfusion of kynurenic acid into the VTA (Vorel et al., 2001). This is in agreement with our findings, which showed that performance of learning was significantly impaired by intra-VTA kynurenic acid treatment.

Altogether, these studies highlight the importance to expand our knowledge in the reward-related learning field, and most importantly, they further corroborate our hypothesis that the role of glutamate neurotransmission is critical for the expression of conditioned approach learned behavior.

**Future directions**

It is important to take into consideration that differences exist between different types of NMDA and AMPA receptor antagonists, as future work is suggested to further explore the role of glutamate neurotransmission in the VTA during the reward-related learning process. The studies cited herein have generally been those which used primarily AP-5 as a competitive NMDA antagonist, because different types of NMDA antagonists have different and at times opposite effects (Meltzer et al., 1997). For example, non-competitive NMDA antagonists including MK-801 and phencyclidine (PCP) both operate through a different neurochemical mechanism – they block the ion channel pore of the receptor (Kew &
Kemp, 2005), thus only having effects when the channels have been activated – increasing firing rate and bursting VTA DA cells, which competitive antagonists CGS 19755 and (±) CPP either have no effect or non-significantly reduce activity (French, Mura, & Wang, 1993). Another example is the non-competitive AMPA receptor antagonist CNQX, which is found to operate at both on the glutamate and the glycine site of NMDA receptors (Mead & Stephens, 1999), besides acting on glutamate AMPA receptor sites. Thus, for future research, it becomes necessary to keep the antagonists in different categories – simply because findings for one type of antagonists do not necessarily imply similar and valid findings for another.

Furthermore, as future work is conducted on the role of VTA NMDA and VTA AMPA receptors in the expression of reward-related learning, it will be interesting to determine if the same treatment effect can also be found in other regions that comprise the mesolimbic system, namely the NAcc core and shell, lateral habenula or amygdala for example.

In short, these are alternate directions that could branch out from our originating hypothesis, and eventually lead to further and deeper understanding of the reward-related learning mechanisms.

Conclusion

Altogether, the results of the experiments described in this dissertation contribute to the reward-related learning field being evidence supporting the hypothesis with which this study originated. The results herein suggest that glutamate neurotransmission in the VTA is critical for the expression of conditioned approach in reward-related learning to occur, more specifically NMDA and AMPA receptors. It would be interesting to see future studies that can extend from these experiments, thereby adding to a growing body of literature which we hope will eventually lead us to a fuller and more comprehensive knowledge of the plasticity that underlies the neural mechanisms of the expression and maintenance of learned approach. This would potentially support the development and contribute to pharmacotherapeutic strategies for disorders based on the pathophysiology that arises from maladaptations in this neural circuit.
Bibliography


Bespakov, A. Yu. (1996). The expression of both amphetamine-conditioned place preference and pentyleenetetrazol-conditioned place aversion is attenuated by the NMDA receptor antagonist (+/-)-CPP. *Drug Alcohol Depend, 41*(1), 85-88.


Mead, A. N., & Stephens, D. N. (1999). CNQX but not NBQX prevents expression of amphetamine-induced place preference conditioning: a role for the glycine site of the NMDA receptor, but not AMPA receptors. *Journal of Pharmacology and Experimental Therapeutics, 290*(1), 9 - 15


Zellner, M. R. (2008). The role of NMDA receptors in the ventral tegmental area in the acquisition of reward-related learning. The Graduate Center of the City University of New York.

